Environment and Natural Resources Trust Fund
2012-2013 Request for Proposals (RFP)

Project Title: **ENRTF ID: 153-I**

Understanding Novel Microorganisms to Enhance Cleanup of Pollutants

Topic Area: I. Water Resources

Total Project Budget: $ 194,000

Proposed Project Time Period for the Funding Requested: 3 yrs, July 2013 - June 2016

Other Non-State Funds: $ 0

Summary:

Water and sediment contaminated with chlorinated industrial pollutants is a significant problem in Minnesota. Novel organisms that “breathe” chlorinated pollutants will be studied, directly enabling development of new remediation technologies.

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Sponsoring Organization: U of MN

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Location

Region: Statewide

County Name: Statewide

City / Township:

_____ Funding Priorities _____ Multiple Benefits _____ Outcomes _____ Knowledge Base

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

_____ Capacity Readiness _____ Leverage _____ Employment _______ TOTAL ______%

PROJECT TITLE: Understanding novel microorganisms to enhance cleanup of pollutants

I. PROJECT STATEMENT

Water and sediment contaminated with chlorinated industrial pollutants is a significant problem in Minnesota. In addition to being very common, chlorinated industrial pollutants are thought to cause the most significant risk to human health. The Minnesota Pollution Control Agency has 77 contaminated sites listed on the Minnesota Permanent List of Priorities (so called state Superfund sites). Of these sites, over half are contaminated with chlorinated pollutants that are known or suspected to cause serious human health effects such as cancer, kidney and liver damage, reproductive damage, or birth defects. This problem is also expensive, with almost $59 Million of state funds spent from 2006-2010 on the cleanup of state Superfund sites. In many current remediation efforts, pollutants are dug or pumped above ground and either moved into a different medium (air or solid) or landfilled. In this way they might impact future generations or be expensive to treat. Research is needed to develop ways to affordably detoxify chlorinated pollutants, safeguarding current and future human and economic health. Common bacteria may play an important role in this endeavor.

Interestingly, bacteria have been identified that can “breathe” toxic chlorinated pollutants, generating energy for growth and removing chlorines from the pollutants (dechlorinating them) in the process. These bacteria are called halorespirers and this process often results in the formation of less- or non-toxic compounds. Although halorespirers have been shown to be marginally effective for remediation at some contaminated sites, the potential that these microorganisms hold remains to be fully realized. This is because, by definition, halorespirers require the presence of chlorinated pollutants to live, with higher concentrations of chlorinated pollutants typically sustaining these organisms more effectively. During remediation, however, we are interested in removing chlorinated pollutants to very low concentrations, which can in turn make it difficult to sustain halorespirers. If compounds could be found, perhaps even natural compounds, that halorespirers could breathe while simultaneously breathing chlorinated pollutants, this problem might be solved.

We have recently discovered that in addition to living in contaminated areas where there are a lot of pollutants to breathe, a different type of halorespirer also lives in uncontaminated environments. In fact, the halorespirers that live in uncontaminated Minnesota lake sediments are numerous and closely related (genetically) to those halorespirers that thrive by breathing chlorinated pollutants. This makes these bacteria a potential resource in the search for remediation technologies and suggests that it might be possible to add them to contaminated sites where they can grow on natural chlorinated compounds in sediment and soil while simultaneously degrading chlorinated pollutants. Although we know that halorespirers exist in uncontaminated Minnesota lakes and that they appear to be closely related (genetically) to pollutant degraders, we *do not* know:

- Whether halorespirers from uncontaminated environments can detoxify chlorinated pollutants, or
- How, genetically, these organisms detoxify these chlorinated pollutants.

In essence, we do not know the value of this bacterial resource. This research will allow us to determine whether these common bacteria can be useful in remediating our state’s contaminated sites. In addition, if we understand how they detoxify pollutants genetically, we can quickly and easily detect whether halorespirers are present and actively remediating pollutants without time-consuming experiments.

II. DESCRIPTION OF PROJECT RESULTS

Activity 1: Dechlorination potential of selected halorespirers **Budget: $64,000**

Sediment samples will be collected from Moose Lake, MN. Our previous research showed that halorespirers in the sediment of this lake were numerous and genetically similar to known pollutant-degrading halorespirers. Sediment will be added to reactors and fed either natural chlorinated compounds or one of three chlorinated pollutants commonly found in Minnesota. Reactors will be monitored to determine halorespirer growth and dechlorination rate and extent. Natural chlorinated compounds will be made in the laboratory using fungus; this is the way that they are naturally generated in the environment.

Outcome	Completion Date
1. Determination of halorespirer growth and dechlorination rates when fed natural chlorinated compounds	6/30/14
2. Determination of halorespirer growth and dechlorination rates when fed one of three common chlorinated pollutants (perchloroethene, polychlorinated biphenyls, and pentachlorophenol)	6/30/15

Activity 2: Understanding how halorespirers "signal" active dechlorination Budget: $130,000

We are currently developing an approach to determine the genes used by halorespirers for dechlorination. Genes are the code "telling" organisms which functions to perform (such as breathing chlorinated compounds). By analyzing genes, we can quickly and easily understand what organisms are capable of doing. The genes used to dechlorinate most chlorinated pollutants and all natural chlorinated compounds are not currently known. Samples will be taken from the reactors described above (fed both natural chlorinated compounds and chlorinated pollutants) and the halorespirers' genetic material will be extracted and analyzed. This analysis will show the dechlorinating capability of the halorespirers (can they perform a particular reaction?) but also which genes are actively being used by the halorespirers (*i.e.*, are they actually performing a particular reaction?). From this we will learn which genes are used to detoxify natural chlorinated compounds and chlorinated pollutants. By understanding how to "read" the genes used to detoxify chlorinated pollutants (reading the "signals" of dechlorination) we will be able to quickly and easily test sites for dechlorination potential or determine if dechlorination is occurring without having to perform labor-intensive and expensive experiments with soil or sediment from polluted sites.

Outcome	Completion Date
1. Determine which genes are used for dechlorinating natural compounds	6/30/15
2. Determine which genes are used for dechlorinating pollutants	6/30/16

III. PROJECT STRATEGY

A. Project Team/Partners

The project team consists of the Principal Investigator Paige Novak (UMN) and a graduate student researcher. Novak has the appropriate skills and background to perform the research.

B. Timeline Requirements

The proposed project will be completed in the allotted three-year period.

C. Long-Term Strategy

Minnesota has impressive resources in terms of the lakes and rivers of the state. The state also has potential bacterial resources. The halorespirers in Minnesota lake sediments are a resource that will be better utilized if understood. Novak has worked on the halorespiration of chlorinated pollutants for about 15 years. She is the first to perform research on the existence of halorespirers in uncontaminated environments. The goal of this project is to draw connections between the organisms that exist in uncontaminated Minnesota lakes and those that detoxify chlorinated pollutants. This will demonstrate how halorespirers from uncontaminated sites can be used to remediate polluted sites. This research should directly 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.

2012-2013 Detailed Project Budget

IV. TOTAL ENRTF REQUEST BUDGET: 3 years

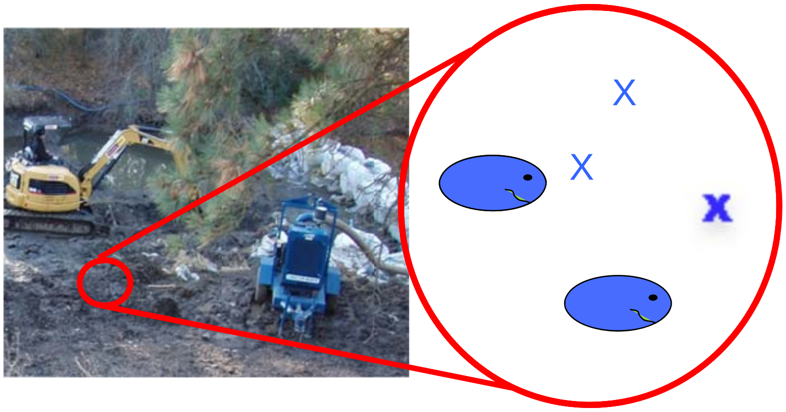
11	AMOUNT
Personnel: Novak (PI, 6% time per year, salary 73.5% of cost, fringe benefits 26.5% of cost). Project supervision, provide guidance on laboratory techniques and data analysis, prepare reports and manuscripts.	$ 40,000
Personnel: Graduate student (50% time per year, 56% salary, 33% tuition, 11% fringe benefits). Conducting laboratory experiments.	$ 128,500
Equipment/Tools/Supplies: Laboratory supplies including, but not limited to: chemicals for culturing bacteria and making the natural chlorinated compounds; analysis needs such as standards, solvents, gas tanks, needles, septa, supplies for bacterial enumeration and identification; supplies for genetic work; consumables such as gloves and solvents ($7,000/yr). Additional funds are budgeted for equipment repair and maintenance ($4,000).	$ 25,000
Travel: Mileage charges for sampling Moose Lake in Minnesota. Mileage will be reimbursed $0.55 per mile or current U of M compensation plan.	$ 500
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 194,000

V. OTHER FUNDS

SOURCE OF FUNDS	AMOUNT	Status
Other Non-State $ Being Applied to Project During Project Period: none	$ -	
Other State $ Being Applied to Project During Project Period: none	$ -	
In-kind Services During Project Period: In-kind Services During Project Period: Novak will provide unpaid time to the project (including 1% 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 52%.	$ -	
Remaining $ from Current ENRTF Appropriation (if applicable): no prior projects directly related to proposed project	$ -	
Funding History: none	$ -	

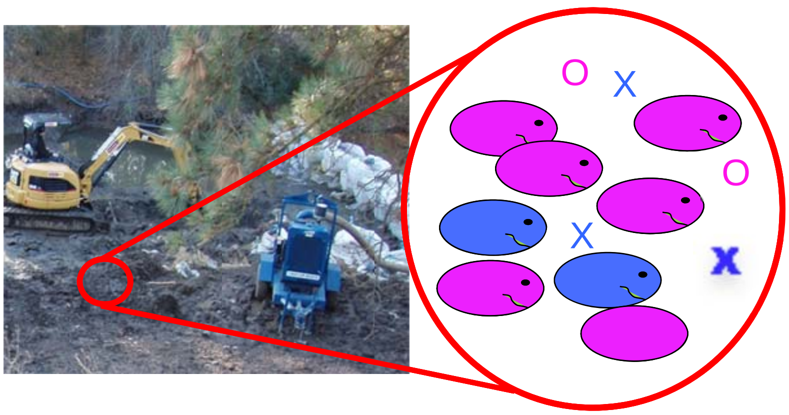
Use of novel bacteria for remediation

The problem



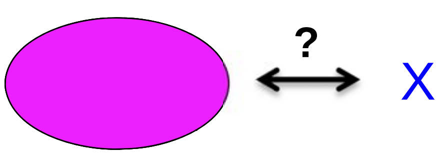
- Bacteria exist that "breathe" chlorinated contaminants ("**X**")
- Bacteria need higher concentrations of contaminants to thrive
- There is often too little contamination to support the bacteria, but too much to consider the site safe

A potential solution



- Bacteria exist that "breathe" natural chlorinated compounds ("**O**")
- We could add these bacteria to contaminated sites
- Once added, they may degrade the contaminants along with any natural chlorinated compounds, cleaning the site

What we need to know



- Do the bacteria that "breathe" natural chlorinated compounds also "breathe" contaminants <u>efficiently</u>?
- How do they do this, so that we may monitor their progress without expensive and time-consuming experiments?

Project Manager Qualifications and Organization Description

Paige J. Novak

Professor, Environmental Engineering, Department of Civil 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. Paige Novak will be responsible for overall project coordination. She has been studying anaerobic bacteria and halorespirers for almost 20 years. She is the first person to study halorespirers 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 halorespirers to environments to enable the dechlorination of polychlorinated biphenyls, very persistent and now banned, pollutants.

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 contain all of the necessary fixed and moveable equipment and facilities needed for the proposed studies.