Environment and Natural Resources Trust Fund
2020 Request for Proposals (RFP)

Project Title: **ENRTF ID: 089-B**

Mitigating Insecticide Toxicity for Safer Waters in Minnesota.

Category: B. Water Resources

Sub-Category:

Total Project Budget: $ 370,000

Proposed Project Time Period for the Funding Requested: June 30, 2022 (2 yrs)

Summary:

We propose to harness the potential of newly discovered proteins from Minnesota ecosystems that turns environmental, toxic pollutants into harmless compounds to protect our state waters quality.

Name: Mikael Elias

Sponsoring Organization: U of MN

Job Title: Assistant professor, PhD

Department: Biochemistry, Molecular Biology and Biophysics

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Location:

Region: Statewide

County Name: Statewide

City / Township:

Alternate Text for Visual:

Newly discovered proteins from Minnesota ecosystems will be used to develop prototype water filters to remove environmental, toxic pollutants.

_____ Funding Priorities	_____ Multiple Benefits	_____ Outcomes	_____ Knowledge Base
_____ Extent of Impact	_____ Innovation	_____ Scientific/Tech Basis	_____ Urgency
_____ Capacity Readiness	_____ Leverage	_____ TOTAL	_____%

PROJECT TITLE: Mitigating Insecticide Toxicity for Safer Waters in Minnesota.

PROJECT STATEMENT: The health of all Minnesotans is threatened by the presence of toxic compounds found in everyday places such as soil, air, food, and even water. In a recent breakthrough, we have discovered a protein which can transform harmful toxins into harmless compounds, all within a matter of seconds. It is urgent that we take advantage of this discovery, and design products which will increase the safety and cleanliness of Minnesota's water supply to protect both the environment and the health of its residents.

Extensive documentation has shown the presence of man-made toxic chemicals, such as hazardous pesticides, in the Minnesota water sheds. Among the most dangerous are organophosphorus compounds. These types of compounds have been linked to serious diseases and disorders such as: heart disease; neurologic developmental disorders in children including autism; and—especially in the state of Minnesota—a higher risk of leukemia among men. The use of organophosphorus compounds as the active ingredient in pesticides is startling. In 2012, more than 20 million pounds of this dangerous compound were used in the United States alone (EPA, Pesticides Industry Sales and Usage, 2008-2012). In Minnesota, they are used on all major crops (2013, 2016 Pesticide Usage Report, MDA). As a direct result, these compounds are contaminating Minnesota's water, air and fresh produce (Pesticide Drift Monitoring in Minnesota Report, 2006-2009).

Organophosphorus pesticides are neurotoxic compounds, dangerous even in small amounts, which can accumulate in the body over time. These toxic compounds also have deleterious effects on aquatic life. Currently, the best solution to deal with these compounds is simply to limit exposure. However, due to the presence of the toxins in our immediate environment (air, water and food), this suggestion does little to prevent human mortality, intoxications, and diseases. Therefore, efficient solutions to remediate these toxins is one of the Holy Grails in environmental sciences. This research proposal is a direct outcome of an outstanding discovery in our laboratories. We recently found, for the first time, proteins that destroy organophosphorus insecticides in mere seconds. We have successfully infused these harmless biologicals into materials such as fabrics (view video here).

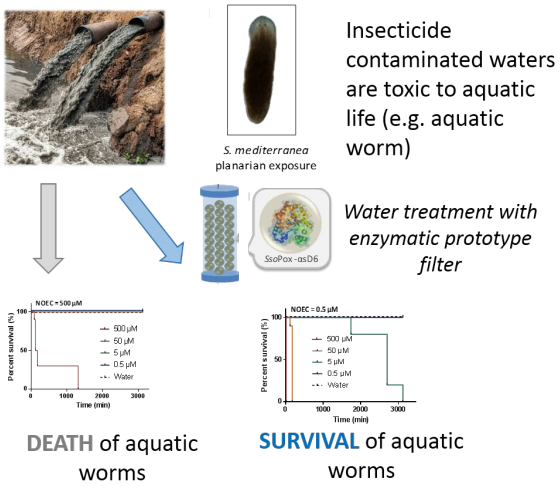


Figure 1: How a prototype enzymatic filter protects aquatic worms (planarians) from death from the toxic induced by the toxic insecticide (parathion).

Beyond these achievements, these proteins unveiled the existence of entirely new families of proteins produced by Minnesota mushrooms that could complement and add to our growing arsenal of detoxifiers. This is a unique opportunity to use the extraordinary diversity of our ecosystems to develop much needed tools to protect our children, and the aquatic environments. **These achievements give our team an undisputable advantage and a distinct edge to develop prototype products based on these 100% biological molecules to clean water in real testing conditions.** In order to achieve these goals, we propose to:

1. Study and develop prototype products for cleaning water. We will develop and optimize water filtration prototypes with our active biologicals and measure their ability to clean water samples.
2. Expand the decontamination ability of our biologicals toolbox by tapping into the hidden biological reservoir of the state of Minnesota.

This proposal will provide biological prototypes to solve serious contaminations issues (pollution of ground, surface, and drinking water) for which there are no satisfactory solutions. Moreover, these biologicals will serve as an ever-expanding and adaptable platform to neutralize other pollutants for which there are no effective solutions, such as the contamination of food products by man-made and natural toxins.

II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1: Develop detoxification prototypes for cleaning water **ENRTF BUDGET: 243,100$**

We will take advantage of our recent discovery and success to affix our biologicals on surfaces in coatings, on fabrics, as well as in clay beads. We propose to develop and optimize prototypes of water filters containing active biologicals, as well as filtration resins, and evaluate their ability to clean lab-monitored water samples. We anticipate >90% reduction of the toxic chemicals after filtration, including the removal of degradation products.

Outcome	Completion Date
1. Production of water filters containing active biologicals.	December 1, 2020
2. Optimization of the water filters.	June 30, 2021
3. Measure the activity of filtration beads in ground and surface water samples.	November 30, 2021

Activity 2: Expand the decontamination ability of our biologicals toolbox **ENRTF BUDGET: 126,900$**

Outcome	Completion Date
1. Identify new biologicals for major emerging water pollutants	January 30, 2022
2. Production and optimization of water filters containing active biologicals	June 30, 2022

Besides organophosphorus pesticides, other compounds are present in Minnesota waters, such as Roundup (glyphosate) and other emerging contaminants. We have discovered new types of our biologicals that we will collect and harness from Minnesota resources (enormous variety of mushroom species) to degrade Roundup, as well as emerging pollutants such as pharmaceuticals and compounds from birth control pills.

III. PROJECT PARTNERS:

The assembled team is highly complementary, and has the unique set of skills required for this highly innovative project, as well as all the necessary resources to successfully perform the proposed research. **Dr. Elias** (PI, UMN, BTI) is an expert in organophosphorus degradation (> 20 research articles). Dr. Elias also has acquired experience in field testing using biologicals and has founded and lead biotechnology companies. **Dr. Michael Freeman** (co-PI, UMN, BTI)) is an expert in natural products. He has extensive experience in the isolation of new biologicals, especially from unusual sources such as fungi.

A. Partners receiving ENRTF funding

Name	Title	Affiliation	Role
Mikael Elias	Assistant Professor, PhD	University of Minnesota	Principal Investigator
Michael Freeman	Assistant Professor, PhD	University of Minnesota	Co-investigator

IV. LONG-TERM- IMPLEMENTATION AND FUNDING: After consulting with various stakeholders, including water treatment plants, local farmers, cooperatives, and packaging companies, we propose to evaluate the usefulness of our technologies in two different translational spaces. The water filtration matrices will be evaluated for their ability to be effective in conditions similar to existing water treatment steps to facilitate its translation. The biologicals-embedded filters will be evaluated for their ability to clean drinking water and eliminate all insecticides residues. Our existing IPs, and new IP generated from this study will be used to kick-start the creation of a startup company for the development, funding and translation of this research.

V. TIME LINE REQUIREMENTS: This project will take 24 months to carry out as described above. Thereafter, it is expected that the products of the project to be handed off to state agencies and the private sector.

Attachment A: Project Budget Spreadsheet
Environment and Natural Resources Trust Fund
M.L. 2020 Budget Spreadsheet
Legal Citation:
Project Manager: Mikael Elias
Project Title: Mitigating Insecticide Toxicity for Safer Waters in Minnesota.
Organization: University of Minnesota
Project Budget: 370,000
Project Length and Completion Date: 2 years; june 30th 2022
Today's Date: 3/30/2019

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Budget	Amount Spent	Balance
BUDGET ITEM			
Personnel (Wages and Benefits)	$ 288,000	$ -	$ 288,000
Mikael Elias, project manager, PI: 8% time (75% salary; 25% benefits) 1 month/year (summer) for 2 years . Dr Elias will be in charge of the completion of all project activities.			
Michael Freeman, co-PI: 4% time (75% salary; 25% benefits) 0.5 month/year (summer) for 2 years .			
Postdoc Researcher 1: (82% salary, 18% benefits); 100% FTE for 2 years. Funds are requested for 2.0 year to support a postdoctoral fellow to fulfill activity 1 and 2.			
Postdoc Researcher 2: (82% salary, 18% benefits); 100% FTE for 2 years. Funds are requested for 2.0 year to support a postdoctoral fellow to fulfill activity 1 and 2.			
Professional/Technical/Service Contracts			
Core facility costs (DNA sequencing and protein production): will cover costs associated with the production of our biofouling inhibitor and with 'reading' the DNA of microbial communities in experiments to determine success of experiments. $16,000/year x 2 years = $32,000.	$ 32,000	$ -	$ 32,000
Equipment/Tools/Supplies			
Funds are for producing and optimizing presticide-degrading materials for lab testing, as well as routine lab supplies (chemicals, flasks, pipetters, disposable plasticware, for example test tubes and petri plates, as well as media needed for production of molecular biology reagents made in the lab). $25,000/year x 2 years=$50,000.	$ 50,000	$ -	$ 50,000
Capital Expenditures Over $5,000			
	$ -	$ -	$ -
Fee Title Acquisition			
	$ -	$ -	$ -
Easement Acquisition			
	$ -	$ -	$ -
Professional Services for Acquisition			
	$ -	$ -	$ -
Printing			
	$ -	$ -	$ -
Travel expenses in Minnesota			
	$ -	$ -	$ -
Other			
	$ -	$ -	$ -
COLUMN TOTAL	$ 370,000	$ -	$ 370,000

SOURCE AND USE OF OTHER FUNDS CONTRIBUTED TO THE PROJECT	Status (secured or pending)	Budget	Spent	Balance
Non-State:		$ -	$ -	$ -
State:				$ -
In kind: The University of Minnesota does not charge the State of Minnesota its typical overhead rate of 54% of the total modified direct costs.	secured	$ 199,000	$ -	$ 199,000

Other ENRTF APPROPRIATIONS AWARDED IN THE LAST SIX YEARS	Amount legally obligated but not yet spent	Budget	Spent	Balance
		$ -	$ -	$ -

Mitigating Insectide Toxicity for Safer Waters in Minnesota

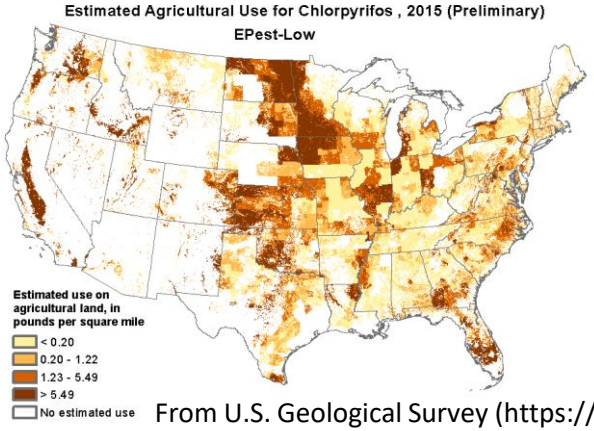
Mikael Elias, Michael Freeman

Insecticide toxicity is a serious health concern

For example, Chlorpyrifos, the pesticide most strongly linked to autism in a 2014 study, is applied in large amounts in Minnesota. Solutions are currently unavailable.

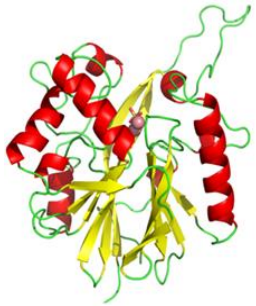
Toxic to both humans and aquatic life.

Organophosphorus pesticides are contaminants of waters, air and fresh produce in Minnesota



From U.S. Geological Survey (https://www.usgs.gov/)

Our proposal: **<u>Water filters infused with ecological proteins, isolated from Minnesota ecosystems, that destroys pollutants and clean water</u>**



Ecological protein cleaning insecticides

Prototyped ecological water filters

Optimized ecological water filters for water plants and personal uses

Management: The research team will include Prof. Mikael Elias, Prof. Michael Freeman. Prof. Elias will be the project manager. The team assembled has unique, and complementary, skills necessary to achieve the goals of the project. The specific expertise of each team member is described below.

Prof. Mikael Elias, PI, is an Assistant Professor in the Department of Biochemistry, Molecular Biology and Biophysics at the University of Minnesota. Elias has over 10 years of research experience on insecticide biodegradation, producing 4 patents and >25 research articles on this topic alone (total of 55), including in prestigious journals (*JACS*, *Nature, PNAS*) and extensive know-how in protein engineering where he pioneered methods, such as the use of ancestral methods. He will invest most of his time on the project, and perform experiments and data analysis. Additionally, he reviews data and meets with laboratory personnel on a daily basis to promote the projects. He also prepares the dissemination of results, such as the proposed conference and publications. As the PI of the project, Dr. Elias will oversee the entire project, design the experiment plans, and draft the project reports.

Prof. Michael Freeman, co-PI, is an assistant professor in the Department of Biochemistry, Molecular Biology and Biophysics at the University of Minnesota. For over 15 years, Dr. Freeman has studied the biosynthesis of natural products and has characterized dozens of enzymes originating from soil bacteria, marine sponges, and mushrooms. He is currently an inventor on three patents and has published >15 papers on these topics in prestigious journals including *Science*, *Nature Chemistry*, and *Nature Chemical Biology*. Dr. Freeman has extensive expertise in identifying unique genes from complex biological samples (targeted metagenomics) and heterologous expression and mass spectrometric analysis of proteins. As a Co-PI, Dr. Freeman will spearhead the enzyme discovery aspects of this project and will oversee, along with Dr. Elias, the design, implementation, and analysis of all experiments related to this topic, as well as help writing project reports and publishing manuscripts.

Organization: the University of Minnesota has several missions: improve lives through research, education, and outreach. The University possess extensive facilities that ensure high research performance. In particular, for this project:

• Biotechnology Resource Center: (http://www.bti.umn.edu/brc/index.html) A wide variety of bench-scale to pilot scale fermenters is available, up to 500L, and will be used in this project to produce cost-effective biomaterials.

Elias Lab: 1,800 sq. ft. of renovated research space is dedicated to Dr. Elias. This space is located on the 1st floor of the GortnerLab Building, on the St Paul campus. Elias's office space is adjacent to the laboratory. The lab contains all of the necessary equipment for molecular biology, biochemistry, protein production and purification, enzyme kinetics, and crystallography. Numerous facilities are available, such as microplate readers, spectrophotometers, scintillation counters, fplc, liquid nitrogen storage, -80 freezers, incubators/shakers, autoclave, as well as 4 and -20 rooms.

Freeman Lab: The PI's laboratory, located in newly renovated lab space in Gortner Laboratory, occupies ~1800 square feet within the Department of Biochemistry, Molecular Biology, and Biophysics at the University of Minnesota's St. Paul campus. The lab has desk and bench space for eight researchers and ample accessible storage space for equipment and supplies; the PI's office is located within the laboratory in an adjacent room with direct access to the lab. The PI's lab is outfitted with all required molecular biological and biochemical equipment necessary for the described experiments. This lab shares the floor with three additional senior researchers studying natural products and/or anaerobic microorganisms, with shared centrifuges, gel-doc, fluorescent microscope, autoclaves, an ultrapure water source, glass-washing station, walk-in 4 ºC room, and a communal meeting area/lunch room. As a BioTechnology Institute member, the PI has direct access to Biotechnology Resource Center (housed in the same building), which provides a variety of microbiological services including a wide-range of fermentation and heterologous expression technologies.

The collective research, organizational, and administrative experiences of the project team members and the resources available to this project from the University of Minnesota should ensure the successful completion of the proposed project goals.