Environment and Natural Resources Trust Fund

2024 Request for Proposal

General Information

Proposal ID: 2024-175

Proposal Title: Enabling Nature to Destroy Environmental PFAS Contaminants

Project Manager Information

Name: Romas Kazlauskas

Organization: U of MN - College of Biological Sciences

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Project Basic Information

Project Summary: Low-levels of perfluoroalkyl substances (PFAS) contaminate water and soil in Minnesota. We propose to identify enzymes and microbes that break down PFAS, making them non-toxic.

Funds Requested: $378,000

Proposed Project Completion: June 30, 2026

LCCMR Funding Category: Methods to Protect, Restore, and Enhance Land, Water, and Habitat (F)

Project Location

What is the best scale for describing where your work will take place?

Region(s): Metro

What is the best scale to describe the area impacted by your work?

Statewide

When will the work impact occur?

In the Future

Narrative

Describe the opportunity or problem your proposal seeks to address. Include any relevant background information.

Perfluoroalkyl substances (PFAS) comprise nearly 5,000 different synthetic chemicals that are used widely in products and industrial processes. A few of the most studied PFAS are hazardous to human health. PFAS contamination has been found in water, sediment, and soil throughout Minnesota. The Environmental Protection Agency recently ruled that even low-level contamination with PFAS is harmful.

Most PFAS contamination is low-level contamination over wide areas. A mild, environmentally compatible, in-situ decontamination of PFAS is needed for these large areas in Minnesota.

In nature, microbes degrade fallen branches and trees and recycle the material. The recycling is done by enzymes - special proteins within these microbes that digest the material as food. We have used these natural digestive processes to degrade chemical contaminants, but not PFAS, which have sometimes been called forever chemicals. We hypothesize that PFAS are a poor food source for microbes. For this reason, PFAS-degrading microbes are not common in nature.

What is your proposed solution to the problem or opportunity discussed above? Introduce us to the work you are seeking funding to do. You will be asked to expand on this proposed solution in Activities & Milestones.

We propose to accelerate the formation of PFAS-degrading microbes. First we propose to identify an enzyme that starts the degradation of PFAS and second to identify a microbe that produces such an enzyme.

PFAS have been shown to degrade using harsh chemical conditions that clip the end of the PFAS molecules and make non-toxic products. This cannot be done naturally in the environment but must be done in a factory environment. We hypothesize that enzymes that naturally carry out similar reactions can be used in natural settings. Identifying such an enzyme would eliminate the need for harsh chemical conditions and allow PFAS to degrade at the contamination site. One half of the project is to find such enzymes. Manufacturing and testing the enzyme on contaminated materials will come next.

To reduce the cost of treatment, we hypothesize that a microbe could make the enzyme at the contamination site, similar to the way natural degradation of trees works. We propose to identify microbes that grow well in the presence of PFAS. These microbes will be tested for their ability to grow and degrade PFAS contaminants to restore the environment to its original clean state.

What are the specific project outcomes as they relate to the public purpose of protection, conservation, preservation, and enhancement of the state's natural resources?

- Identification of ten enzymes that clip the ends of PFAS to start their degradation.
- Laboratory tests of decontamination of PFAS-contaminated soil with enzymes.
- Identification of twenty microbes that grow well in the presence of PFAS.
- Identification of a PFAS-tolerant microbe that produces the enzymes.
- Laboratory tests of decontamination of PFAS-contaminated soil and water.

Activities and Milestones

Activity 1: Decontamination of PFAS-contaminated soil with enzymes

Activity Budget: $189,000

Activity Description:
First, we propose to identify enzymes that can clip the ends of the PFAS molecules. This step weakens the molecules so they fragment into non-toxic pieces. We will detect the clipping by adding a reagent that turns the solution purple when it encounters the non-toxic pieces. We previously developed this method with funding from a Fortune 50 company. We will start the current project by searching public databases of millions of enzymes to find those that clip the ends of molecules similar to PFAS. We will narrow the results to about one hundred candidates. We will experimentally test these candidates by first making the enzymes and then measuring if they can clip PFAS molecules. We expect that approximately ten of the 100 candidates will clip the PFAS molecules.
Second, we will make larger amounts of the most promising five enzymes and test their ability to decontaminate soil collected in Cottage Grove. We will mix enzymes and contaminated soil, allow them to sit a room temperature, and then remove a sample, mix it with our reagent and look for the purple color. The intensity of the purple color will reveal how much PFAS has been been decontaminated.

Activity Milestones:

Description	Approximate Completion Date
Identification of ten enzymes that clip the ends of PFAS to start their degradation.	June 30, 2025
Laboratory test of decontamination of PFAS-contaminated soil with enzymes.	December 31, 2025

Activity 2: Decontamination of PFAS-contaminated soil with microbes

Activity Budget: $189,000

Activity Description:
First, we propose to identify microbes that grow well the presence of PFAS. We will start by searching public databases of millions of microbes to identify those that may grow in the presence of PFAS. We will narrow the list to 100 candidates and request samples of the microbes from public repositories of microbes like the American Type Culture Collection. We will test the microbes by growing them with and without PFAS and choosing the ones that show little or no difference. The cloudiness of the solutions reveals how well the microbes grow. We expect to find about twenty. Next, we propose to identify which of these twenty contain an enzyme identified in Activity 1 to clip the ends of PFAS molecules. Sequencing the DNA of the microbe will reveal what enzymes it contains. Finally, we will test the enzyme-containing microbes for their ability to decontaminate PFAS-contaminated soil and water. The reagent from Activity 1 will turn purple as the non-toxic fragments form.

Activity Milestones:

Description	Approximate Completion Date
Identification of twenty microbes that grow well in the presence of PFAS	March 31, 2025
Identification of a PFAS-tolerant microbe that produces the enzymes	September 30, 2025
Laboratory test of decontamination of PFAS-contaminated soil and water	June 30, 2026

Project Partners and Collaborators

Name	Organization	Role	Receiving Funds
Lawrence Wackett	U of MN - College of Biological Science	Co-Investigator. Lawrence Wackett is best known for the Biodegradation Database, which predicts how microbes degrade man-made chemicals in the environment. Prof. Wackett's role is to identify microbes suitable for the biodegradation of polyfluorinated substances (PFAS) in the environment.	Yes

Long-Term Implementation and Funding

Describe how the results will be implemented and how any ongoing effort will be funded. If not already addressed as part of the project, how will findings, results, and products developed be implemented after project completion? If additional work is needed, how will this work be funded?

This proposal will identify a path by which the forever PFAS chemicals will no longer last forever. The next stage of the project would be a scaling up from the laboratory to field trials to estimate costs and demonstrate effectiveness. We will expand the team to include engineers and environmental experts, including those at the Minnesota Pollution Control Agency, and community participants for field testing. We are exploring additional funding from companies and from BioMADE, a national center for furthering bio-manufacturing that involves companies such as Cargill and is headquartered at the University of Minnesota.

Project Manager and Organization Qualifications

Project Manager Name: Romas Kazlauskas

Job Title: Professor of Biochemistry

Provide description of the project manager's qualifications to manage the proposed project.

Romas Kazlauskas is a biochemist trained at MIT and Harvard, whose research focuses on using enzymes for sustainability. One example of enzymes for sustainability is the addition of proteases to laundry detergents. These enzymes break down food, blood, and sweat stains making clothes cleaner without the need for excess soap, hot water or harsh bleaching chemicals. Kazlauskas is best known for the 'Kazlauskas rule' which predicts how enzymes can be used to make pharmaceuticals. This application makes pharmaceutical manufacture more efficient and generates less toxic waste. He has supervised over twenty graduate students and numerous undergraduates in his lab. He holds six patents for enzyme-related inventions. Prof. Kazlauskas's role is to identify enzymes that can break down polyfluorinated substances (PFAS) in the environment.

Organization: U of MN - College of Biological Sciences

Organization Description:

The University of Minnesota Twin Cities is a public land-grant research university and the flagship institution of the University of Minnesota System. Among US universities, the University of Minnesota ranked 20th in research activity in the fiscal year 2020. The College of Biological Sciences is one of seven freshman-admitting colleges in the University of Minnesota. The College of Biological Sciences faculty conducts basic research on a range of applications in human health, agriculture, biotechnology and environmental sciences.

Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	$ Amount
Personnel								
Postdoctoral Research Fellow		Identification of enzymes that degrade PFAS			21%	2		$151,000
Postdoctoral Research Fellow		Identification of microbes that support PFAS degradation			21%	2		$151,000
Undergraduate research student		Measurement of enzyme properties			0%	0.2		$8,000
Undergraduate research student		Measurement of microbe properties			0%	0.2		$8,000
							Sub Total	**$318,000**
Contracts and Services								
							Sub Total	**-**
Equipment, Tools, and Supplies								
	Tools and Supplies	Supplies and services for enzyme and microbe discovery	Chemicals for measurement of PFAS fragmentation and for the growth of microbes and preparation of enzymes. Services include samples of microbial strains, synthesis of DNA, sequencing of DNA.					$54,000
							Sub Total	**$54,000**
Capital Expenditures								
							Sub Total	**-**
Acquisitions and Stewardship								

							Sub Total	**-**
Travel In Minnesota								
							Sub Total	**-**
Travel Outside Minnesota								
							Sub Total	**-**
Printing and Publication								
	Publication	Open access fees for three peer-reviewed publications	Permit everyone to access the publications without the need for a subscription to the journal.					$6,000
							Sub Total	**$6,000**
Other Expenses								
							Sub Total	**-**
							Grand Total	**$378,000**

Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or Type	Description	Justification Ineligible Expense or Classified Staff Request

Non ENRTF Funds

Category	Specific Source	Use	Status	Amount
State				
In-Kind	University of Minnesota	Indirect costs of research	Secured	$208,000
			State Sub Total	**$208,000**
Non-State				
			Non State Sub Total	**-**
			Funds Total	**$208,000**

Attachments

Required Attachments

Visual Component

File: 1ba96cb5-f78.pdf

Alternate Text for Visual Component

Map of PFAS contamination in Minnesota and structure of PFAS showing the weak link...

Optional Attachments

Support Letter, Photos, Media, Other

Title	File
UMN Sponsored Projects Authorization to submit	83b11c76-14f.pdf

Administrative Use

Does your project include restoration or acquisition of land rights?

No

Does your project have potential for royalties, copyrights, patents, or sale of products and assets?

No

Do you understand and acknowledge IP and revenue-return and sharing requirements in 116P.10?

N/A

Do you wish to request reinvestment of any revenues into your project instead of returning revenue to the ENRTF?

N/A

Does your project include original, hypothesis-driven research?

Yes

Does the organization have a fiscal agent for this project?

No

Does your project include the design, construction, or renovation of a building, trail, campground, or other capital asset costing $10,000 or more?

No

Do you propose using an appropriation from the Environment and Natural Resources Trust Fund to conduct a project that provides children's services, as defined in Minnesota Statutes section 299C.61 Subd.7?

No