Environment and Natural Resources Trust Fund

2023 Request for Proposal

General Information

Proposal ID: 2023-068

Proposal Title: Source Tracking of Bacterial Contamination in Minnesota Waters

Project Manager Information

Name: Satoshi Ishii

Organization: U of MN - College of Biological Sciences

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Project Basic Information

Project Summary: This project will identify the sources of fecal contamination in Minnesota's watersheds to improve surface water quality

Funds Requested: $488,000

Proposed Project Completion: June 30, 2026

LCCMR Funding Category: Water Resources (B)

Project Location

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

Statewide

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

Statewide

When will the work impact occur?

During the Project and In the Future

Narrative

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

Many Minnesotans enjoy and benefit from Minnesota's waters; however, some watersheds do not meet water quality standards and are therefore included in the impaired list. Bacteria contamination is one of the major causes of impairment of Minnesota's waterways across the states, making the water unsafe for swimming and other recreational activities. Currently, Minnesota Pollution Controlling Agency (MPCA) monitors the levels of fecal contamination by using fecal indicator bacteria (FIB) such as fecal coliforms and Escherichia coli (E. coli). While FIB monitoring is practical and convenient, it cannot tell the sources of fecal contamination. Various point and non-point sources can contribute to the bacteria loads, including humans (discharge from wastewater treatment plants, leakages from septic systems), pets (dogs and cats), farm animals (cows, pigs, and poultry), and wildlife (deer, beavers, geese, ducks, etc.). Therefore, identifying the sources of fecal contribution is essential to assess associated health risks and establish effective control strategies to improve water quality.

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.

The overall goal of this project is to improve water quality by identifying the sources of fecal contamination in Minnesota waters. We will use state-of-the-art microbial source tracking (MST) methods to determine the sources of fecal contamination in urban, agricultural, and natural watersheds in Minnesota. Water samples will be collected from multiple tributaries within each watershed over time and both under low and high flow conditions to analyze the temporal and spatial dynamics of fecal contamination sources. In addition to fecal contamination sources, basic water quality parameters and the occurrence of human pathogens will be also quantified.

To achieve this goal, the following three activities are planned. In Activity 1, we will collect water samples from six watersheds (two urban, two agricultural, and two natural environments) and measure FIB in water samples along with ancillary water quality parameters over two years. In Activity 2, we will quantify various MST markers and pathogens in the water samples by using a high-throughput gene quantification tool. In Activity 3, we will analyze the water quality, MST marker, and pathogen data together to establish the relationship between them.

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?

The specific outcomes are (1) the levels of FIB in urban, agricultural, and natural watersheds in Minnesota, (2) temporal and spatial dynamics of fecal contamination sources in these watersheds, and (3) the relationship between fecal contamination sources and the occurrences of pathogens. These outcomes will be used to establish strategies (e.g. TMDL management recommendations) to improve Minnesota's water quality and protect public health.

Activities and Milestones

Activity 1: Monitor the levels of FIB in urban, agricultural, and natural watersheds in Minnesota

Activity Budget: $180,000

Activity Description:
The objective of this activity is to monitor the levels of FIB (E. coli) in multiple impaired watersheds over two years. Six watersheds will be selected in consultation with MPCA and local watershed management organizations. Target watersheds include two urban (Twin Cities metro areas), two agricultural (southern MN), and two natural environments (northern MN). Water samples will be collected from multiple tributaries within each watershed over two years and both under low and high flow conditions (n = 80 per watershed; total 480 samples). The levels of FIB and other water quality parameters (temperature, pH, turbidity, suspended solids, nutrients, flow rate, etc.) will be measured by the standard methods. These data will be compared with the historical data managed by MPCA. DNA will be also extracted from the water samples for Activity 2.

Activity Milestones:

Description	Completion Date
Identify the target watersheds	December 31, 2023
Monitor the levels of FIB in urban, agricultural, and natural watersheds	December 31, 2025
Extract DNA from the water samples	December 31, 2025

Activity 2: Determine the temporal and spatial dynamics of fecal contamination sources in the impaired watersheds

Activity Budget: $205,000

Activity Description:
The objective of this activity is to determine the temporal and spatial dynamics of fecal contamination sources in the impaired watersheds. DNA samples collected in Activity 1 will be used for microbial source tracking (MST). Two MST methods will be employed: a microfluidic quantitative PCR (MFQPCR) and a high-throughput DNA sequencing (SourceTracker). We previously developed an MFQPCR tool to simultaneously quantify multiple MST markers (for humans, dogs, cows, pigs, horses, poultry, geese, ducks, deer, beavers, and muskrats) as well as human pathogens (pathogenic E. coli, Salmonella, Campylobacter, etc.) for many samples. We will use this MFQPCR tool in this activity. In addition, the same DNA samples will be amplified, sequenced, and analyzed with SourceTracker software to identify the potential sources of bacteria in the water samples. These methods are economical, high-resolution, and practical new tools for the determination of fecal contamination sources. The results obtained with MFQPCR and SourceTracker will be compared and used to identify the sources of fecal contamination in the watersheds. Spatiotemporal dynamics of fecal contamination sources will be analyzed. In particular, the impacts of land-use patterns, precipitation, and other environmental factors (temperature, etc.) on fecal contamination sources will be analyzed.

Activity Milestones:

Description	Completion Date
Quantify various MST markers and human pathogens by MFQPCR	December 31, 2025
Determine the potential sources of bacterial contamination by SourceTracker	December 31, 2025
Analyze the spatiotemporal dynamics of fecal contamination sources	June 30, 2026

Activity 3: Establish the relationships between fecal contamination sources and the occurrences of pathogens

Activity Budget: $103,000

Activity Description:

The objective of this activity is to establish the relationship between fecal contamination sources and the occurrences of pathogens. While human fecal contamination is generally of primary concern, contamination with animal feces can also pose human health risks because some animals harbor human pathogens more frequently than others. For example, ruminants (cows, deer, etc.) can serve as reservoirs of Shiga toxin-producing E. coli, including E. coli O157, whereas avians frequently carry pathogenic Campylobacter species. Therefore, the occurrence of these pathogens in water may depend on the sources of fecal contamination. In this activity, we will use statistical modeling to analyze the data collected in Activities 1 and 2 to establish the relationships among the water quality parameters (E. coli, flow, etc.), fecal contamination sources (MST marker concentration, etc.), and pathogen occurrence. In addition, potential human health risks associated with recreational activities (swimming, canoeing, etc.) in the target watersheds will be analyzed.

Activity Milestones:

Description	Completion Date
Statistically analyze the relationships between fecal contamination sources and the occurrences of pathogens	June 30, 2026
Analyze the potential human health risks associated with the recreational activities in the target watersheds	June 30, 2026

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?

The data will be shared with MPCA so that they can use them for future decision-making. If the approach described in this project is proven useful, we will expand the target watersheds by closely working with MPCA and other watershed management organizations. The longer-term benefit of this work will be refining environmental management plans and regulations by government agencies like MPCA, MDH, and MDA. Additionally, the improvement of microbiological water quality will provide economic benefits to state and local communities through less beach closure, ecosystem services, and reduction of health costs due to waterborne illnesses.

Other ENRTF Appropriations Awarded in the Last Six Years

Name	Appropriation	Amount Awarded
Assessment of Water Quality for Reuse	M.L. 2017, Chp. 96, Sec. 2, Subd. 04f	$148,000

Project Manager and Organization Qualifications

Project Manager Name: Satoshi Ishii

Job Title: Associate Professor

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

Satoshi Ishii is Associate Professor at the BioTechnology Institute (BTI) and the Department of Soil, Water, and Climate (SWC) at the University of Minnesota. Dr. Ishii's research focuses on environmental microbiology and biotechnology, including water quality and public health microbiology. He has over 18 years of experience working on microbial source tracking and pathogen detection. Dr. Ishii has managed multiple projects related to the proposed subject, some of which were done in collaboration with the Minnesota Pollution Control Agency and local watershed management organizations.

Organization: U of MN - College of Biological Sciences

Organization Description:

The University of Minnesota is the main research and graduate teaching institution in the state of Minnesota. The BioTechnology Institute provides advanced research, training, and university-industry interaction in biological process technology. In the Department of Soil, Water, and Climate, we seek to improve and protect the quality of soil, air, and water resources in natural and managed ecosystems, through research, teaching, and extension.

Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	$ Amount
Personnel								
Satoshi Ishii		Project manager			25%	0.24		$46,100
Chan Lan Chun		Technical support			25%	0.12		$20,000
Postdoctoral Research Associate		Perform experiments and analyze data for Activity 1, 2, and 3			17%	3		$188,700
Postdoctoral Research Associate		Analyze data for Activity 2 and 3			17%	0.45		$28,300
Research Technician		Collect and process samples for Activity 1			22%	2		$120,300
							Sub Total	**$403,400**
Contracts and Services								
University of Minnesota Genomics Center	Internal services or fees (uncommon)	UMGC provides DNA sequencing services with a discounted fee for U of M researchers				0		$9,600
							Sub Total	**$9,600**
Equipment, Tools, and Supplies								
	Equipment	Colilert sealer	Colilert sealer is necessary to quantify E. coli in accordance with the EPA's standard method.					$5,000
	Tools and Supplies	DNA extraction kits	Extract and purify DNA from surface water samples					$5,000
	Tools and Supplies	Reagents for water quality testing	Reagents necessary to measure water quality parameters (E. coli, nutrients, suspended solids, etc.)					$10,000
	Tools and Supplies	Reagents and comsumables for high-throughput qPCR	Necessary to quantify various MST markers and pathogens in the water samples					$30,000

	Tools and Supplies	Miscellaneous laboratory supplies and consumables	Various supplies and consumables are needed to collect and process water samples (bottles, filters, gloves, pipette tips, plates, etc.)					$10,000
							Sub Total	**$60,000**
Capital Expenditures								
							Sub Total	**-**
Acquisitions and Stewardship								
							Sub Total	**-**
Travel In Minnesota								
	Miles/ Meals/ Lodging	A total of 60 trips (13,600 miles) are planned (60 miles/trip for Twin Cities metro areas and 300 miles/trip for the rest of MN) at a rate of $0.585/mile.	Necessary to collect water samples					$8,000
	Conference Registration Miles/ Meals/ Lodging	Trips and registration fees to present our research results at a conference in MN ($500/person x 4 researchers)	To disseminate the results					$2,000
							Sub Total	**$10,000**
Travel Outside Minnesota								
							Sub Total	**-**
Printing and Publication								
	Publication	Open access publication fee	Necessary to make our results publicly available					$3,000
							Sub Total	**$3,000**
Other Expenses								
		Equipment repair and maintenance	Necessary to cover part of the annual maintenance contract for the DNA					$2,000

			extraction machine ($1,000/year x 2 years).					
							Sub Total	**$2,000**
							Grand Total	**$488,000**

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	The University of Minnesota is not allowed to charge the State of Minnesota its typical overhead rate of 55% of the total modified direct costs. We are listing our unrecoverable indirect cost as in-kind contribution.	Secured	$265,650
			State Sub Total	**$265,650**
Non-State				
			Non State Sub Total	**-**
			Funds Total	**$265,650**

Attachments

Required Attachments

Visual Component

File: 8635bdfc-6bd.pdf

Alternate Text for Visual Component

Sources of bacterial contamination will be identified using microbial source tracking approaches. Water samples will be collected from 2 urban (Twin Cities metro areas), 2 agricultural (southern MN), and 2 natural watersheds (northern MN)....

Optional Attachments

Support Letter or Other

Title	File
Letter of support from MPCA	ac6b716a-bf4.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?

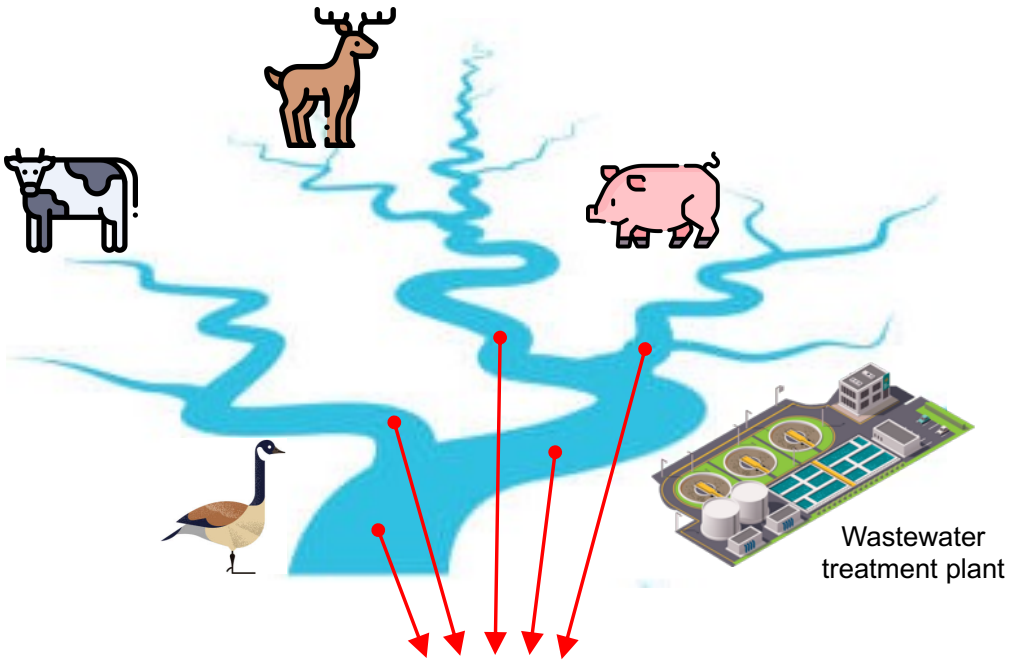
Yes

Does the organization have a fiscal agent for this project?

Yes, Sponsored Projects Administration

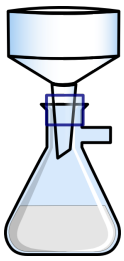
Problem: Current water quality monitoring cannot tell the sources of bacteria contamination

Proposed approach: Microbial source tracking (MST) and pathogen detection



Watershed-wide sample collection

2 Urban (Twin Cities metro), 2 Agricultural (Southern MN), and 2 Natural watersheds (Northern MN) over 2 years

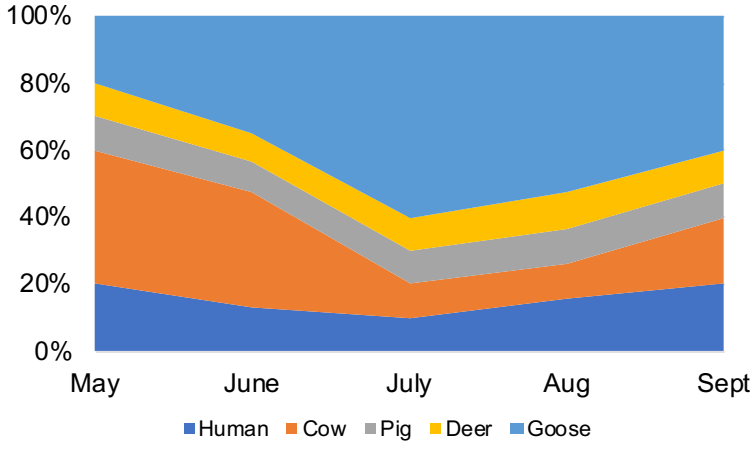


Water filtration & DNA extraction

High-throughput gene quantification for MST markers and pathogens

High-throughput DNA sequencing (SourceTracker)

Expected Results



Questions to be addressed:

- What is the major sources of bacteria contamination?
- How they change over time?
- If pathogens occur, is the water safe for recreational activities?

These results will be used to establish strategies to improve MN's surface water quality.