Environment and Natural Resources Trust Fund
2017 Request for Proposals (RFP)

Project Title: **ENRTF ID: 037-B**

Antibiotic Resistance Assessment in St. Louis River Watershed

Category: B. Water Resources

Total Project Budget: $ 254,576

Proposed Project Time Period for the Funding Requested: 3 years, July 2017 - June 2020

Summary:

This project will determine the fate of antibiotic resistance from sources through sewer systems, wastewater treatment, and finally into environment for prioritization of their source control in St. Louis Watershed.

Name: Chanlan Chun

Sponsoring Organization: U of MN - Duluth NRRI

Address: 5013 Miller Trunk Hwy

Duluth MN 55811

Telephone Number: (218) 788-2613

Email chun0157@d.umn.edu

Web Address www.nrri.umn.edu

Location

Region: Northeast

County Name: St. Louis

City / Township: Duluth

Alternate Text for Visual:

Visual of the potential fate of antibiotics, with hospitals, the residential community, improper disposal, and industrial facilities as sources of antibiotics that enter the St. Louis River Watershed through sewer systems and wastewater treatment.

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

PROJECT TITLE: Antibiotic Resistance Assessment in St. Louis River Watershed

I. PROJECT STATEMENT

Antibiotics are often found in Minnesota's water bodies. The continuous release of antibiotics to environment potentially results in an increase in natural resistance background levels which leads the rising numbers of bacterial pathogens becoming resistant against single or multiple antibiotics. Accordingly, the expansion of antibiotic resistance from hospital settings into environment is recognized as critical ecological and public health concerns. Antibiotics taken by people in hospital and at home are diluted and transported through sewer systems into wastewater treatment and finally released to environment. Various reports have revealed elevated levels of antibiotic resistance genes in wastewater effluents. Thus, it is best is to reduce the amount of antibiotics and their resistance that reach a treatment plant in the first place (source control). This project aims at identifying the fate of antibiotic resistance from sources through sewer system, wastewater treatment, and finally into surface water for prioritization of source control of antibiotic resistance.

St. Louis River is the largest watershed on Lake Superior and is currently receiving intense investments to recover water quality from generations of impairment. Addressing antibiotic resistance as potential new impacts on this watershed is of great importance for the state of Minnesota and the citizens of Duluth who value in these waters. In this project, we will 1) determine resistance levels of antibiotics in characteristic (e.g. domestic, institutional, hospital, and industrial) sewages which enter Western Lake Superior Sanitary District wastewater treatment, 2) examine the fate and evolution of antibiotic resistance through wastewater treatment process. This project will be collaboratively conducted with health care providers, pharmacy, and City of Duluth to achieve the objectives.

With the ultimate goal of improving water quality and protecting human health, results of the project will identify sources of antibiotic resistance as environmental stressors in the St. Louis River watershed, which is essential to assess associated ecological and health risk as well as to develop effective source control strategies. The findings will be disseminated to public sectors, health care providers, and public through programs addressing the antibiotic stewardship and controlling antibiotic release into the environment and community.

II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1: Collect characteristic sewage and wastewater samples **Budget: $25,600**

We will collect residential, hospital, industrial sewer samples in consultation and collaboration with City of Duluth. The sampling points include upstream residential sewer pipes prior to the pipe receiving hospital wastewater, the hospital wastewater discharge pipes, and industrial (e.g. paper mil) outlet pipes. To examine the fate and evolution of antibiotic resistance during sewage treatment, wastewater samples (influent, effluent of each unit process, and final effluent) will be collected from WLSSD. The sample collection will be conducted monthly for two years. With the consultation with hospitals, additional samples will be obtained for certain periods when prescription and use of antibiotics increase. The samples will be processed for chemical and microbiological characterization (Activity 2 and 3)

Outcome	Completion Date
1. Collection of sewages and wastewater samples	September 2019
2. Sample processes for chemical and microbiological characterization	September 2019

Activity 2: Determination of the levels of antibiotics in wastewater samples **Budget: $80,195**

We will measure levels of commonly-prescribed antibiotics including penicillin, ampicillin, vancomycin, quinolones (nalidixic acid, norfloxacin) and linezolid. A volume of 0.1-1L wastewater sample will be extracted and the level of target antibiotics will be quantified using liquid chromatography tandem mass spectrometry.

Outcome	Completion Date
1. Optimize extraction and analytical methods to target antibiotics	September 2017

2. Measure the concentration of target antibiotics in wastewater samples	December 2019

Activity 3: Determination of the levels of antibiotic resistance **Budget: $140,281**

Genomic DNA will be extracted from the samples and used for quantification of antibiotic resistance levels. The resistance level will be measured using the quantification of specific resistance genes with high resolution and next-generation DNA sequencing for collection of all antibiotic resistance gene. These results will be correlated with the level of antibiotics determined in Activity 2.

Outcome	Completion Date
1. Genomic DNA extraction and quantification of specific resistance genes using qPCR	October 2019
2. Identification of dominant antibiotic resistance genes in sewage and wastewater using DNA sequencing	December 2019
3. Correlation analysis of antibiotic resistance with antibiotic levels	June 2020

Activity 4: Project data dissemination and public outreach **Budget: $ 8,500**

Results of this project will be disseminated to interested public agencies (City of Duluth, St. Louis County, MPCA and MDH) and groups (WLSSD and health care providers) through presentations (Minnesota Water Conference), written reports, and peer-reviewed publications including open access journal. We will engage the public about antibiotic stewardship and controlling antibiotic release into the environment and community. Our outreach efforts include development of educational courses to general public, WLSSD staffs, and health care professionals in collaboration with health care providers.

Outcome	Completion Date
1. Development of educational materials and community discussions	December 2019
2. Dissemination of project data and results via seminars and workshops	June 2020
3. Publish results in the peer reviewed literatures	June 2020

III. PROJECT STRATEGY

A. Project Team/Partners

The project team include Chan Lan Chun, Adrian Hanson, and Grant Anderson at the University of Minnesota Duluth (Civil Engineering and Pharmacy) in collaboration with Steve Waring and Dan Dauner at Essential Health and Todd Carlson at City of Duluth. Chun is an expert in chemical and microbiological water quality and quantification of genes including pathogens and resistances. Hanson will provide his expertise on wastewater treatment and Anderson, Waring and Dauner will provide expertise on the use and metabolites of antibiotics in hospitals. Carlson will advise the research team for selection and sampling of sewages and wastewater.

B. Project Impact and Long-Term Strategy

This project will provide key information on sources and fate of antibiotics and their resistance in St. Louis River watershed. This information will be made available to government agencies and hospitals in order to assess associated ecological and human health risks and to develop goals and strategies for prioritization of source control of antibiotic resistance. Such potential actions will have positive impacts on protection of Minnesota's surface waters in the long term. Our research approach can be applied to other MN's watershed for assessment and prioritization of antibiotic resistance as potential impacts on the watershed.

C. Timeline Requirements

The project will be completed in 3 years. Samples will be collected over a 2-year period which allow us to determine the temporal variability of fate of antibiotic resistance from sources to environment through sewer and wastewater systems. Chemical and microbiological analyses will be conducted throughout 3 years.

2017 Detailed Project Budget

Project Title: Antibiotic Resistance Assessment in St. Louis River Watershed

IV. TOTAL ENRTF REQUEST BUDGET: 3 years

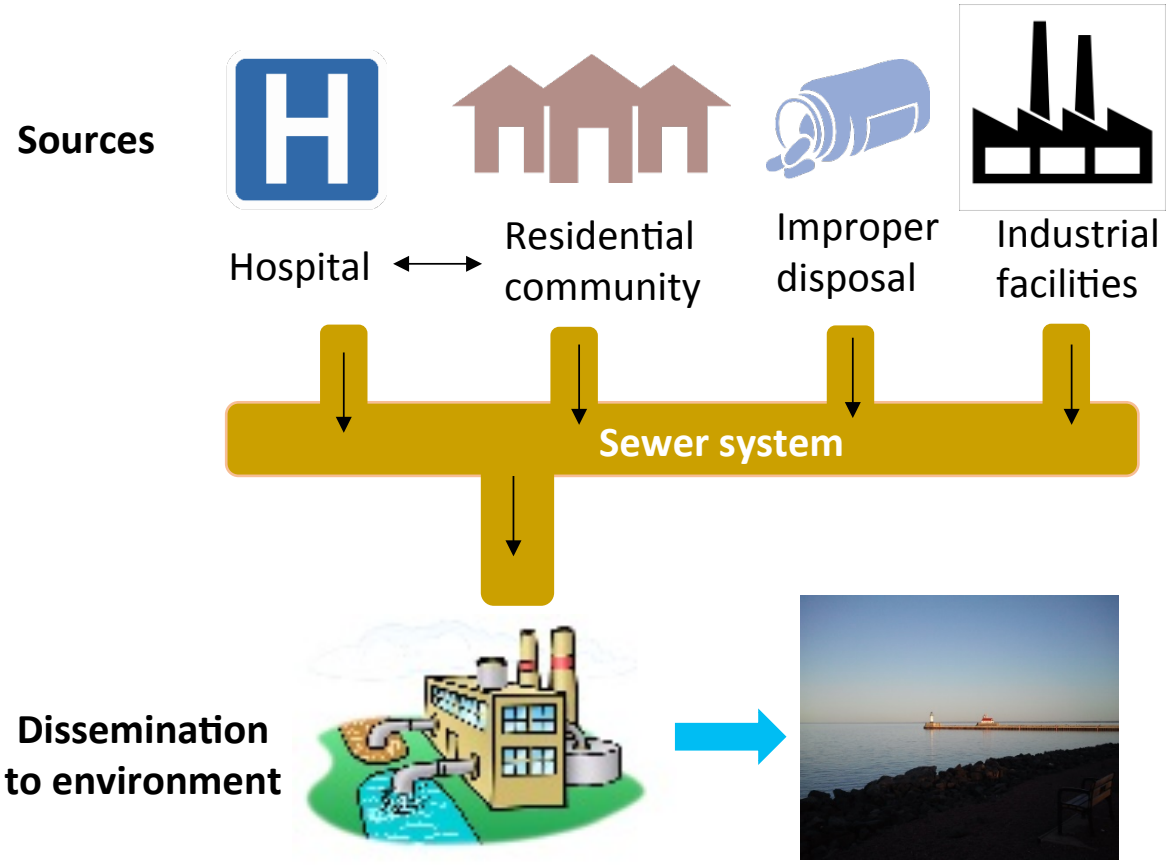
BUDGET ITEM	AMOUNT
Personnel:	
Chan Lan Chun, Principal Investigator (66.3% salary, 33.7% benefits); 7.4% each year for 3 years	$ 36,044
Adelle Schumann, Research Technician (72.6% salary, 27.4% benefits); 40% FTE each year for 3 years	$ 61,830
Graduate Research Assistant (82.4% salary, 17.6% benefits); 50% FTE each year for 3 years	$ 91,728
Equipment/Tools/Supplies:	
Sewer and wastewater sampling supplies	$ 8,000
Chemicals and expendable lab supplies	$ 9,500
DNA extraction and molecular biological agents	$ 10,000
Travel:	
In-state sampling: 500mi/yr*3yrs*$0.54/mi=$810 + vehicle rental $10*45=$450	$ 1,260
In-state conference attendance: Registration 2 people: $750; lodging $89/night *3nights*2rooms=$1,284; per diem/meals for 3 days $38.25+$51+$38.25=$127.50* 2people = $255	$ 2,289
Additional Budget Items:	
Antibiotic chemical anlayses $70/sample x 200 samples	$ 14,000
Illumina sequencing and supercomputer usage fee: UMN Genomic Center (UMGC): Illumina Squencing and library preparation.~ $3975/lane + $10 library prep/sample: ~800 samples per project = $8,000 (sample prep) + 3 lanes ($11,925) = $19,925	$ 19,925
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 254,576

V. OTHER FUNDS

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: Unrecovered indirect (53% MTDC 7/1/17-6/30/18; 54% MTDC 7/1/18-6/30/20)	$ 131,106	Secured
Funding History:	N/A	
Remaining $ From Current ENRTF Appropriation:	N/A	

Objective: To reduce the amount of antibiotics and their resistance released to environment with the ultimate goal of improving water quality and protecting human health

Potential fate of antibiotics



Antibiotic resistance assessment in St. Louis River watershed

Determine fate of antibiotic resistance from sources through sewer systems and wastewater treatment to environment

Research outcomes

- **Assessment of associated ecological and health risks**
- **Prioritization and development of effective source control strategies (e.g. satellite pretreatment)**

PROJECT MANAGER QUALIFICATIONS AND ORGANIZATION DESCRIPTION

Chan Lan Chun is an Assistant Professor of Civil Engineering and Natural Resources Research Institute at the University of Minnesota Duluth (UMD). She completed a Ph.D. in Environmental Engineering at the University of Minnesota (UMN) and did postdoctoral work at Medical University of South Carolina. She was a research assistant professor at UMN before joining faculty at UMD.

Dr. Chun will have chief management responsibilities for overseeing the proposed project. She will be responsible for working with co-investigators, Dr. Adrian Hanson (Environmental Engineering) and Dr. Grant Anderson (Pharmacy) and research partners (Essentia Health and City of Duluth) to ensure that project goals, results and timelines are met. Dr. Chun is an environmental microbiologist with research experiences in the analysis and use of microorganisms in natural and engineered environments. Dr. Chun has studied the distribution and diversity of microorganisms in aquatic and soil environments to understand the roles microbes play in water quality and human health using a combination of cultivation-dependent and cultivation-independent techniques (e.g. quantitative PCR and DNA sequencing approach). Particularly, she has worked on fecal contamination and bacterial pathogens including antibiotic-resistant bacteria in aquatic ecosystem. In addition, her work focuses on the development of management strategies to improve water quality. She has published over 22 scientific journal articles and book chapters. The collective research and organizational 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.

ORGANIZATIONAL DESCRIPTION

The University of Minnesota is a non-profit, state-funded educational institution of the State of Minnesota. Dr. Chun’s research laboratory is located in Natural Resources Research Institute (NRRI) at the University of Minnesota Duluth campus. Dr. Chun's laboratory is equipped for research in the areas of microbial ecology, geochemistry, and molecular biology and includes computers and special software for genetic and phylogenetic analyses. Equipment specific to Chun’s lab includes culturing, benchtop electron microscopy, incubators, thermocycler, real-time PCR, general DNA/RNA electrophoresis, autoclave, and centrifuges. In addition to her laboratory, UMD and NRRI also provides substantial shared laboratory space with state-of-the-art specific function-based core laboratories under the direction of a Core Laboratory Director with expertise in that research area. The shared space and its associated equipment are provided for the supervised use of all who reside at UMD-NRRI. Core laboratories at UMD and NRRI include: research instrumentation laboratory, central analytical laboratory, microscopy laboratory, aquaculture laboratory, natural product and modification laboratory, and paleolimnology laboratory. This project have access to DNA sequencing facilities at the University of Minnesota Biomedical Genomics Center and the Minnesota Supercomputing Institute for analysis of DNA sequence data generated by this project.