Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2016 Work Plan

Date of Report:

Date of Next Status Update Report: January 1, 2017

Date of Work Plan Approval:

Project Completion Date: June 30, 2019

Does this submission include an amendment request? No

PROJECT TITLE: Bacterial Assessment of Groundwater Supplies Used for Drinking Water

Project Manager: Raymond M. Hozalski

Organization: University of Minnesota

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Location: Statewide

Total ENRTF Project Budget:	**ENRTF Appropriation:**	**$299,000**
	Amount Spent:	**$0**
	Balance:	**$299,000**

Legal Citation: M.L. 2016, Chp. xx, Sec. xx, Subd. 04l

Appropriation Language:
$299,000 is from the trust fund to the Board of Regents of the University of Minnesota to characterize and analyze bacterial communities in Minnesota groundwater used as drinking water supplies and link the microbiological data to other water quality indicators for drinking water supply safety. This appropriation is available until June 30, 2019, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Bacterial Assessment of Groundwater Supplies Used for Drinking Water

II. PROJECT STATEMENT:

Use of quantitative PCR (qPCR) targeting DNA or RNA provides a rapid, powerful, and specific screening tool that can be used to directly enumerate pathogenic organisms of concern. Investigations of the microbiological safety of groundwater supplies in the neighboring state of Wisconsin using such a technique found many contaminated wells. Unfortunately, we know virtually nothing about the microbiological quality of Minnesota's groundwater supplies. This is particularly problematic because groundwater is used as the source for more than 97% of Minnesota's public water supplies serving about 75% of the state's population; often that water is provided without any disinfection. Thus, the goal of this study is to characterize and quantify all of the bacteria, including the direct quantification of several disease-causing organisms, in selected drinking water systems supplying groundwater throughout the state of Minnesota. We plan to sample approximately 15-20 water systems overall including systems in the Karst region in the southeast, the agricultural areas to the west and southwest, forested areas to the north, as well as a few systems in the Twin Cities Metro area. We predict that the majority of Minnesota's groundwater supplies from deep aquifers are free of bacterial pathogens but that supplies from shallow aquifers and those in Karst regions are likely to be impacted by agriculture, septic systems, etc. and contain unsafe levels of pathogens. Our secondary hypothesis is that the microbiological safety of Minnesota's groundwater will correlate to other chemical measures of drinking water quality, such as nitrate levels (an indication of agricultural impacts) and elevated tritium levels (tritium is measured to assess well vulnerability). The specific goals of this work are to:

1. Quantify and characterize the microorganisms in groundwaters around the state
2. Attempt to link the microbiological data to conventional or chemical water quality indicators, including nitrate and tritium
3. Disseminate the information gained from this work to stakeholders by working with the Minnesota Department of Health (MDH), Minnesota Rural Water Association (MRWA), and by presenting at the Minnesota section of the American Water Works Association (AWWA) Annual Conference.

The selection and sampling of the water systems will occur first (Activity 1). The collected samples will then be analyzed for bacterial community composition and to quantify specific bacterial pathogens using state-of-the-art DNA-based technologies (Activity 2). Water samples will also be evaluated for chemical indicators of contamination and for microbial contamination using conventional culture-based assays (Activity 3). Finally, we will disseminate the research results to sampled utilities directly, as well as to the general public and to interested water professionals. We will preserve the anonymity of participating utilities in our press releases and publications upon their request and work with them and the MDH to determine how best to communicate the results from their individual systems to their customers.

This research would compliment on-going efforts by the Minnesota Department of Health to evaluate the safety of Minnesota's public water supplies with respect to pathogenic viruses and a recent state report showing increased nitrate contamination of water supplies.

III. OVERALL PROJECT STATUS UPDATES:

Project Status as of January 1, 2017:

Project Status as of July 1, 2017:

Project Status as of January 1, 2018:

Project Status as of July 1, 2018:

Project Status as of January 1, 2019:

Overall Project Outcomes and Results:

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Select and sample 15 to 20 groundwater utilities across the state of Minnesota.

Description:

Approximately 15-20 water groundwater utilities across the state of Minnesota will be selected for sampling that represent a variety of aquifer types (e.g., fractured bedrock, shallow unconfined, deep confined) and overlying land uses (e.g., agricultural, residential, forested). Each of the water systems will be sampled three times throughout a 1-year period to evaluate temporal (i.e., seasonal) variations in water quality. We will also sample from the source, after any treatment (if present), and from taps in the distribution system in order to evaluate whether the groundwater supply is contaminated, the effectiveness of the treatment (if any), and whether any contamination occurs during distribution.

Summary Budget Information for Activity 1:

ENRTF Budget: $ 51,000
Amount Spent: $ 0
Balance: $ 51,000

Outcome	Completion Date
1. *Work with MDH personnel to select and contact water utilities*	September 1, 2016
2. *Collect samples from 15-20 water utilities across MN 3 times each*	August 1, 2017

Activity Status as of January 1, 2017:

Activity Status as of July 1, 2017:

Activity Status as of January 1, 2018:

Activity Status as of July 1, 2018:

Activity Status as of January 1, 2019:

Final Report Summary:

ACTIVITY 2: Characterize the bacterial communities in Minnesota's groundwaters that are used as drinking water supplies.

Description:

We will make detailed characterizations of the microorganisms in the sampled waters using state-of-the-art, next-generation DNA sequencing technology to generate between 50,000 and 100,000 sequences per sample. We will also directly (as opposed to indirectly, like the fecal coliform assay) quantify the presence of about a dozen organisms (e.g., *Salmonella*, *Legionella,* etc.) known to cause disease in humans. Finally, we will use software that is freely available to University researchers at the Minnesota Supercomputing Institute to statistically analyze the data and correlate our microbiological data to other water quality data (see Activity 2).

The composition of the bacterial communities in the sampled waters will be tracked by using the polymerase chain reaction (PCR) to amplify the V3-region of the 16S rRNA gene, purifying these PCR products using a commercially-available kit, and then directly sequencing these PCR products using an Illumina MiSeq instrument. The Illumina MiSeq data will be processed using Mothur to trim for sequence quality and to

determine the diversity of the bacterial communities (Chao estimator, Shannon Index, etc.) and to identify the individual members of the bacterial community. The structure of the bacterial communities will be statistically compared by weighted Unifrac. We can multiplex as many as 256 samples simultaneously.

Quantitative PCR (qPCR) will be used to quantify the 16S rRNA gene (a measure of bacterial biomass) as well as for genes that are specific to pathogens of interest (*Campylobacter*, *Legionella*, etc). We anticipate performing qPCR on ~15 to 20 water systems × 5 samples/system × 3 times each = 225 to 300 samples in total.

Summary Budget Information for Activity 2:

ENRTF Budget: $ 173,000
Amount Spent: $ 0
Balance: $ 173,000

Outcome	Completion Date
1. *Extraction and purification of DNA from water samples*	September 1, 2017
2. *Sequencing of 16S rRNA genes for bacterial community analysis*	March 1, 2018
3. *Quantify total bacteria and pathogens in water samples via qPCR*	June 1, 2018
4. *Statistical analysis of data*	December 1, 2018

Activity Status as of January 1, 2017:

Activity Status as of July 1, 2017:

Activity Status as of January 1, 2018:

Activity Status as of July 1, 2018:

Activity Status as of January 1, 2019:

Final Report Summary:

ACTIVITY 3: Analysis of Conventional Water Quality Indicators.
Description:

At the same time that we collect samples for microbiological analysis, we will also collect samples for analysis of conventional water quality parameters. Of these conventional parameters, we will measure temperature, pH, chlorine, chloride, sulfate, nitrate, and carbemazepine (a conservative chemical tracer for sewage). We will also measure tritium (a radioactive form of hydrogen), as this is used to assess aquifer vulnerability during the development of wellhead protection plans. We will also quantify fecal coliforms, *Escherichia coli*, and *Enterococcus* spp. using cultivation-based methods.

Summary Budget Information for Activity 3:

ENRTF Budget: $ 75,000
Amount Spent: $ 0
Balance: $ 75,000

Outcome	Completion Date
1. *Perform "conventional" culture-based microbiological assays for comparison with qPCR results obtained in Activity 2*	September 1, 2017
2. *Quantify chemical parameters and correlate with microbiological results obtained in Activity 2*	December 1, 2017

Activity Status as of January 1, 2017:

Activity Status as of July 1, 2017:

Activity Status as of January 1, 2018:

Activity Status as of July 1, 2018:

Activity Status as of January 1, 2019:

Final Report Summary:

V. DISSEMINATION:

Description:

Findings will be disseminated directly to each of the sampled utilities as a written report and an in-person presentation. Findings will also be disseminated and archived via reports to LCCMR, peer-reviewed publications, presentations at conferences, and city council meetings (if appropriate). We will also, when appropriate, disseminate results via press releases to the media and via the MDH website. The audience is not only the scientific community, but also the public, policymakers, and practitioners. The work will also be of interest to the medical community and we will seek avenues to share the results with this community. We will preserve the anonymity of participating utilities in our press releases and publications upon their request and work with them and the MDH to determine how best to communicate the results from their individual systems to their customers.

Status as of January 1, 2017:

Status as of July 1, 2017:

Status as of January 1, 2018:

Status as of July 1, 2018:

Status as of January 1, 2019:

Status as of July 1, 2019:

Final Report Summary:

VI. PROJECT BUDGET SUMMARY:
A. ENRTF Budget Overview:

Budget Category	$ Amount	Explanation
Personnel:	$ 251,500	For Drs. Hozalski ($52,899) and LaPara ($48,809) for directing the project and for a graduate ($139,494) and an undergraduate student ($10,298) at the University of Minnesota.
Professional/Technical/Service Contracts:	$20,000	University of Minnesota Genomics Center for DNA sequencing (~300 samples) and other services
Equipment/Tools/Supplies:	$22,500	General laboratory supplies ($12,000)

		qPCR reagents ($5,000) DNA extraction kits, purification kits ($5,000)
Travel Expenses in MN:	$5,000	Miscellaneous travel within MN. This will include travel to 15-20 treatment facilities across Minnesota 3 times each for sampling ($3,000) and travel to water utilities and MN-section AWWA conference in Duluth in 2017 and 2018 for dissemination of results ($2,000).
TOTAL ENRTF BUDGET:	**$299,000**	

Explanation of Use of Classified Staff: N/A

Explanation of Capital Expenditures Greater Than $5,000: N/A

Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: 1.75

Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: N/A

B. Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
Non-state			
		$	
State			
U of MN	$127,807	$	In-kind contribution; indirect costs not charged to this project
TOTAL OTHER FUNDS:	**$127,807**	**$**	

VII. PROJECT STRATEGY:

A. Project Partners:

The Minnesota Department of Health will aid in selecting water utilities to be sampled and facilitate access to the systems for sampling.

B. Project Impact and Long-term Strategy:

The long-term goal of the proposed research is to gain a better understanding of the microbiological quality of groundwater in Minnesota. This research is especially important because more than 90% of Minnesota's public water supplies use groundwater as their source, often providing this water without disinfection. Numerous new laboratory techniques have been developed over the last decade to enable microbiologists to analyze the microbes in drinking water with astounding detail and precision. The proposed study, therefore, will generate novel and critically important knowledge on the microbiological quality of Minnesota's public water supplies and will provide sound guidance for protecting the public health of Minnesotans.

There is increasing evidence that groundwater supplies can be contaminated by pathogenic microorganisms including viruses. Groundwater has typically been considered a safe water source that can be consumed with little or no treatment because the water is naturally filtered by the porous media it passes through. Some groundwaters, such as those in unconfined aquifers or in Karst regions, can become contaminated with pathogens from anthropogenic activities on the overlying land surface including septic systems and agricultural activities. Further, contamination of water supplies can also occur during water

distribution. For example, negative pressure events resulting from valve or pumping changes can draw water into the water mains from the surrounding soil. Also, bacteria present in biofilms that have colonized the interior of the water mains can be shed into the water and end up at the taps of water consumers.

Sampling the source, post-treatment, and distribution system followed by DNA-based quantification and characterization of the microbial communities present will allow us to determine the extent of contamination and contaminant origin (i.e., groundwater or distribution system). With this information, we can recommend changes to systems with evidence of contamination such as installing or improving water treatment (e.g., UV irradiation) or changing distribution system operation and maintenance protocols (e.g., increase residual disinfectant concentration or add/increase distribution system flushing program). Such changes are expected to improve the quality and microbiological safety of Minnesota's groundwater systems that have shown evidence of contamination. This research project will sample 15 out of the 947 community groundwater supplies (1.6%) and provide a preliminary assessment of the safety of these representative supplies throughout the state. Based on the results of this investigation, an expanded investigation might be warranted to sample a broader array of systems to fully document the extent of the problem. Future efforts might also be directed at sampling and evaluating the quality of water supplied by private wells, as 20% of Minnesotans receive their water from private wells. Finally, in systems that implement changes to address contamination issues, a follow-up study could be done to determine the effectiveness of those changes.

C. Funding History: N/A

VIII. FEE TITLE ACQUISITION/CONSERVATION EASEMENT/RESTORATION REQUIREMENTS: N/A

IX. VISUAL COMPONENT or MAP(S): See attached visual

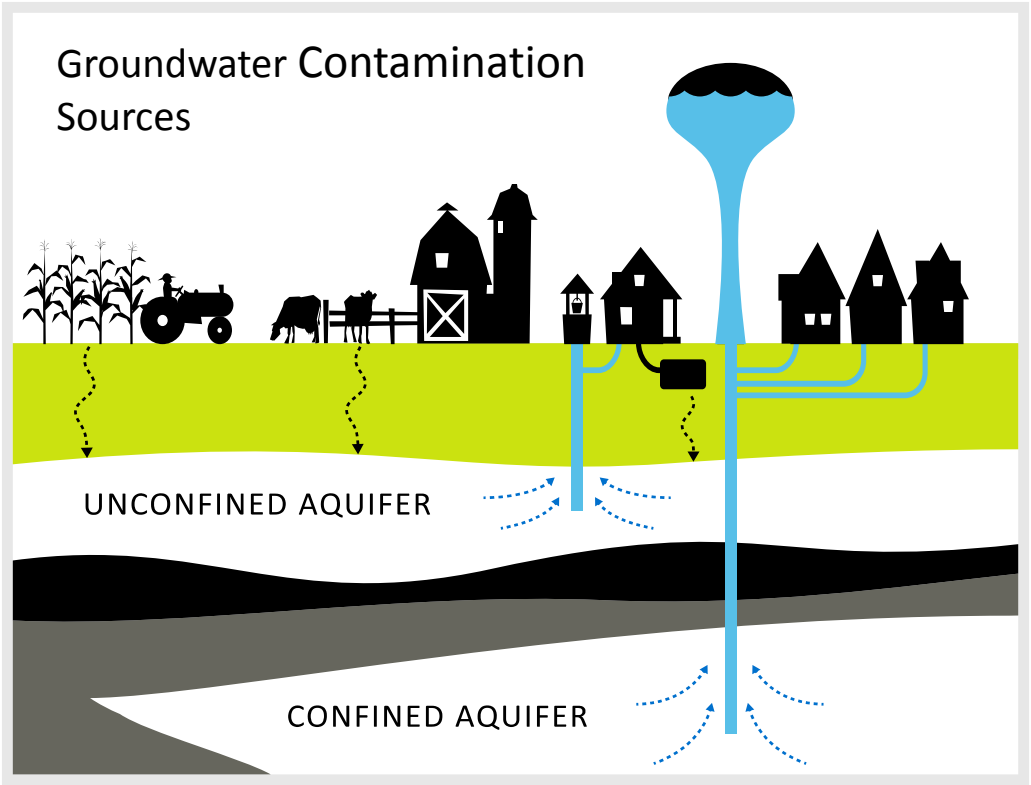
X. RESEARCH ADDENDUM: See attached

XI. REPORTING REQUIREMENTS:
Periodic work plan status update reports will be submitted no later than January 1, 2017, July 1, 2017, January 1, 2018, July 1, 2018, and January 1, 2019. A final report and associated products will be submitted between June 30 and August 15, 2019.

Environment and Natural Resources Trust Fund
M.L. 2016 Project Budget

Project Title: *Bacterial assessment of groundwater supplies used for drinking water*
Legal Citation: *Fill in your project's legal citation from the appropriation language - this will occur after the 2016 legislative session.*
Project Manager: *Raymond M. Hozalski*
Organization: *University of Minnesota*
M.L. 2016 ENRTF Appropriation: $*299,000*
Project Length and Completion Date: *3 Years, June 30, 2019*
Date of Report: *Fill in the date of report submission (this will be updated for each status update report)*

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Activity 1 Budget	Amount Spent	Activity 1 Balance	Activity 2 Budget	Amount Spent	Activity 2 Balance	Activity 3 Budget	Amount Spent	Activity 3 Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM	***Water Utility Selection and Sampling***			***Groundwater microbiome analysis***			***Analysis of Conventional Water Quality***				
Personnel (Wages and Benefits)	$41,216	$0	$41,216	$147,199	$0	$147,199	$63,085	$0	$63,085	$251,500	$251,500
Project Management, Raymond M. Hozalski ($52,899; 8% of time; 75% to salary, 25% to fringe benefits)											
Project Management, Timothy LaPara ($48,809;8% of time; 75% to salary, 25% to fringe benefits)											
Graduate Student at U of M ($139,494; 50% of time; 59% to salary, 41% to benefits)											
Undergraduate Student at U of M ($10,298; 25% of time during academic year; 100% to salary, 0% to benefits)											
Equipment/Tools/Supplies					$0						
General lab supplies ($12,500), reagents for qPCR ($5,000), use of UMGC's facilities for qPCR and Illumina sequencing ($20,000), DNA extraction kits ($3,000), PCR purification kits ($2,000)	$6,965	$0	$6,965	$24,875	$0	$24,875	$10,661	$0	$10,661	$42,501	$42,501
Travel expenses in Minnesota											
Travel to approximately 15-20 water utilities across Minnesota three times each to collect water samples; travel to annual AWWA water conference in Duluth to disseminate results; travel to the sampled water utilities to discuss results	$3,000	$0	$3,000	$1,000	$0	$1,000	$1,000	$0	$1,000	$5,000	$5,000
COLUMN TOTAL	**$51,181**	**$0**	**$51,181**	**$173,074**	**$0**	**$173,074**	**$74,746**	**$0**	**$74,746**	$299,001	**$47,501**



Adapted from: www.mn.gov/governor/images/nitrate_sources_infographic.pdf

http://pixshark.com/happy-person.htm

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