[Insert Appropriation Year of Legal Citation] Project Abstract
For the Period Ending June 30, 2019

PROJECT TITLE: Assessment of Water Quality for Reuse
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2017, Chp. 96, Sec. 2, Subd. 04f

APPROPRIATION AMOUNT: $148,000
AMOUNT SPENT: $148,000
AMOUNT REMAINING: $0

Sound bite of Project Outcomes and Results

The outcome of this project will help expand the water reuse in Minnesota, which can reduce demands on groundwater aquifers and improve surface water quality.

Overall Project Outcome and Results

Reusing water will reduce demands on groundwater aquifers and improve surface water quality. However, public perception of health risks associated with microbiological contaminants remains a key barrier to the expansion of water reuse. The goal of this project is to maximize the potential of water reuse in Minnesota by eliminating barriers to water reuse implementation. In this project, water quality of 25 water reuse systems around Minnesota was assessed by quantifying potential human pathogens. At each reuse facility, water samples were collected at the source and when available at the distribution site such as an irrigation tap. When treatment steps were in place, water samples were also collected before and after the treatment. Samples were collected more than once for some reuse facilities. As a result, 90 water samples were collected from the 25 sites. Bacterial and viral pathogens in these water samples were quantified using a high-throughput method. Most of the water samples did not contain detectable levels of pathogens. Some pre-treatment wastewater samples, contained potential human pathogens such as norovirus. Based on a preliminary quantitative microbial risk assessment (QMRA) for norovirus, the risk for illness and infection is considerable for these samples. However, advanced water treatment removed these pathogens to the levels considered low risk of infection and illness for reuse. Due to the complexity of QMRA analyses and the variability of the results, the risk assessment is only done for norovirus. Potential health risks associated with pathogens other than norovirus should be analyzed in the future.

Project Results Use and Dissemination
This project has produced two presentations: one at the EPA's webinar on "Water Reuse and Reclaimed Water" and one at a national conference (Association of Environmental Engineering and Science Professors [AEESP] Conference). Two publications are being prepared: one as a peer-review journal publication and one as a white paper published from Minnesota Department of Health. These publications will be freely available to the public.

The outcomes of this research have been used to expand our water reuse research. MN Stormwater Research Council has provided additional funding to continue and expand the water reuse research. In addition, the outcome obtained in this project will be shared with other state and federal agencies (EPA, MPCA, etc.) as well as private sectors to establish safe water reuse in MN and other states.

Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2017 LCCMR Work Plan Final Report

Date of Submission: August 16, 2019

Final Report

Date of Work Plan Approval: 6/7/2017

Project Completion Date: June 30, 2019

PROJECT TITLE: Assessment of Water Quality for Reuse

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Location: Statewide

Total ENRTF Project Budget:	**ENRTF Appropriation:**	**$148,000**
	Amount Spent:	**$148,000**
	Balance:	**$0**

Legal Citation: M.L. 2017, Chp. 96, Sec. 2, Subd. 04f

Appropriation Language:
$148,000 the first year is from the trust fund to the Board of Regents of the University of Minnesota to collect and analyze pathogen data for evaluation of water reuse in order to maximize water reuse and protect groundwater and surface water quality.

I. PROJECT TITLE: Assessment of Water Quality for Reuse

II. PROJECT STATEMENT:

The goal of this project is to maximize the potential of water reuse in Minnesota by eliminating barriers to water reuse implementation. Reusing water will improve water quality through better stormwater management and reduce demands on groundwater aquifers. Quality of reclaimed water (i.e., treated wastewater/stormwater for reuse purposes) needs to be maintained to assure safe water reuse. However, there is no water quality standard for water reuse for various purposes such as toilet flushing, vehicle washing, irrigation and final product rinse. On some occasions, sophisticated and expensive treatment is used to create reclaimed water with quality possibly higher than necessary for flushing a toilet. The cost of water reuse systems may be reduced by utilizing design components that minimize pathogens. By quantitatively detecting multiple human pathogens in Minnesota water reuse systems, we will have data for use in setting water quality standards and making design recommendations.

The specific goals of this work are to:

1. System Design: Collect information on design elements (including water source, storage, and treatment devices) for the 24 water reuse systems within the state (see Activity 1 for detail).
2. Pathogens: Quantitatively detect multiple human pathogens in the 24 water reuse systems. Compare pathogen data among the reuse systems.
3. Risk Assessment: Relate the pathogen data to health risk through quantitative microbial risk assessment (QMRA) in order to ensure the health and safety of the public is protected.
4. Recommendations: Make recommendations about water quality standards and treatment design to set a clear path for water reuse in Minnesota.

This project will be done with a team of scientists/engineers from the University of Minnesota (U of M) and the Minnesota Department of Health (MDH). The U of M previously developed innovative tools to quantify multiple pathogens (both bacteria and viruses) in many water samples. By taking advantage of these tools, the team can comprehensively analyze the safety of water reuse and relate system design to pathogen occurrence. Based on these results, the MDH can set a standard for water reuse and make system design recommendations.

The need for this work is supported by national reports that conclude understanding the occurrence and fate of human pathogens in graywater and stormwater is a primary research need. Thus, we have a unique opportunity in Minnesota to contribute to advancing water reuse both in Minnesota and across the country.

III. OVERALL PROJECT STATUS UPDATES:

Project Status as of Status as of January 1, 2018:

We started contacting water reuse sites in the Twin Cities Metro areas. From four of these sites, we collected water samples and measured their physical and chemical characteristics. Microbial cells were concentrated by filtering large volume of water samples, and stored for future use.

We will continue collecting water samples in 2018. Once we have enough samples, we will quantify pathogens in these samples by microfluidic quantitative PCR.

Project Status as of Status as of July 1, 2018:

The project is going well as scheduled. We contacted water reuse sites in the Twin Cities Metro and Duluth areas, and collected water samples and measured their physical and chemical characteristics. Microbial cells were concentrated by filtering large volume of water samples, and stored for future use. We will continue collecting water samples in 2018. The sample collection is expected to complete by December 2018.

We have established the DNA and RNA extraction methods. We will use these methods to extract DNA and RNA from samples collected in Activity 1, and use to quantify pathogens by microfluidic quantitative PCR.

Project Status as of Status as of January 1, 2019:

The project is going well as scheduled. We have completed Activity 1. Activity 2 is still ongoing but expected to complete in 1-2 months. After we get Activity 2 results, we plan to start Activity 3.

Project Status as of Status as of July 1, 2019:

The project is going well as scheduled. We have completed Activity 1 and Activity 2, and are finishing up the analysis for Activity 3. We are preparing the final report to present our outcomes.

We also presented some of our results at a national conference and through EPA's webinar to disseminate the results. We are also preparing a manuscript for journal publication to disseminate the research outcomes.

AMENDMENT REQUEST JULY 1, 2019

We are requesting funds be shifted from the personnel and travel budget lines to lab supplies.

- Personnel budget would be reduced by $581 to a revised budget of $122,419.
- Travel budget would be reduced by $3,991 to a revised budget of $1,009.
- Supply budget would be increased by $4,572 to a revised budget of $24,572.

These changes are being requested because more supplies are necessary to process increased number of samples (n=270) to accomplish Activity 2. Accordingly, adjustment for the budget amount for each activity is also being requested as follows:

- Activity 1 budget would be increased by $1,419 to a revised budget of $48,419.
- Activity 2 budget would be increased by $861 to a revised budget of $72,861.
- Personnel in Activity 3 budget would be decreased by $2,280 to a revised budget of $26,720.

Amendment Approved by LCCMR 7/18/2019

Overall Project Outcomes and Results:

Reusing water will reduce demands on groundwater aquifers and improve surface water quality. However, public perception of health risks associated with microbiological contaminants remains a key barrier to the expansion of water reuse. The goal of this project is to maximize the potential of water reuse in Minnesota by eliminating barriers to water reuse implementation. In this project, water quality of 25 water reuse systems around Minnesota was assessed by quantifying potential human pathogens. At each reuse facility, water samples were collected at the source and when available at the distribution site such as an irrigation tap. When treatment steps were in place, water samples were also collected after the treatment. Samples were collected more than once for some reuse facilities. As a result, 90 water samples were collected from the 25 sites. Bacterial and viral pathogens in these water samples were quantified using a high-throughput method. Most of

the water samples did not contain detectable levels of pathogens. Some pre-treatment wastewater samples contained potential human pathogens such as norovirus. Based on a preliminary quantitative microbial risk assessment (QMRA) for norovirus, the risk for illness and infection is considerable for these samples. However, water treatment removed these pathogens to levels considered to result in a low risk of infection and illness for reuse. Due to the complexity of QMRA analyses and the variability of the results, the risk assessment for all pathogens is not yet complete.

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Water sample collections

Description:

The goal of this activity is to collect various recycled water samples. Samples collected in this activity will be used to quantify pathogen concentrations in Activity 2.

Water samples will be collected from 24 water reuse systems around the state. Final site selection will depend on the owner's agreement to participate in the study. Most of the water reuse systems are located in the metro area (18 research sites), but there are examples in Northeast (3 sites), Central (2 sites), and Southern MN (1 site). The reused water at these systems is used for various purposes such as toilet flushing (4 sites), vehicle washing (2 sites), irrigation (11 sites), and other industry usages (7 sites).

The preferred sample sites include a mixture of public and privately owned locations with various designs, treatment techniques, and end uses. For example, one potential site is located on the University of Minnesota campus in the Twin Cities. At this site, precipitation is collected from the 17th Avenue Residence Hall roof and surrounding sidewalk for use primarily in flushing toilets, but also for irrigation. Taking samples from this site under different weather conditions will help expand our understanding of microbial dynamics at this site.

Other potential systems to include are locations where precipitation is collected for various purposes, such as flushing toilets, turf irrigation, washing vehicles or washing stadiums. For example, the St. Paul Saints stadium in St. Paul, MN and Twins stadium in Minneapolis, MN could provide examples of systems located at similar venues but with differing collection systems, treatment trains, and end uses. Additional examples of water reuse systems that could be studied include several municipally-owned systems that collect stormwater for irrigating city properties or park areas. Possible examples include irrigation systems in the cities of Centerville, Cottage Grove, St. Anthony Village, or Woodbury, MN. A vehicle washing facility at St. Louis County, MN, that uses rainwater in the washing process could provide data about this additional type of end use. Further, a new graywater system at Lake Vermillion State Park would provide an excellent opportunity to learn more about microbial populations in a graywater system in Minnesota. The diversity of the systems' designs and treatment techniques will help to broaden understanding of microbial populations in water reuse systems in Minnesota's diverse settings and variable weather conditions.

We anticipate collecting samples from each reuse system on two different occasions, sampling at the source, after treatment and distributed water as appropriate for the site (24 locations x 2 time points x 3 types = 144 samples). Tap water collected from several drinking water treatment facilities will be used as negative control samples.

Physical and chemical properties of the water samples (temperature, pH, turbidity, odor, biomass and chlorine concentration, etc.) will be recorded. We will also inventory and record the types of water reuse design components such as source of reuse water, storage and treatment devices used in each water system. Sources will include rainwater (from roofs), stormwater, graywater and industrial process water. Storage includes

cisterns, stormwater ponds and underground storage. Treatment devices include first flush devices, disinfection such as chlorine or ozone, or filters. These components can affect water quality by affecting the ability of pathogens to collect, survive, and multiply.

Water samples (10-1,000 L depending on water quality) will be filtered on site through membrane filters to capture bacteria and viruses, and brought back to the lab in the University of Minnesota. Bacterial cells and viral particles will be detached from the membranes and pelleted by centrifugation. These pellets will be frozen and stored until used for Activity 2.

Summary Budget Information for Activity 1:

ENRTF Budget: $ 48,419
Amount Spent: $ 48,419
Balance: $ 0

Outcome	Completion Date
1. *Sample collection and bacteria/virus concentration (144 samples)*	September 30, 2018
2. *Physical and chemical properties of the water samples*	September 30, 2018
3. *Documentation of water reuse system design*	September 30, 2018

Activity 1 Status as of January 1, 2018:

Operators of 14 water reuse sites through the Twin Cities Metro areas and Duluth were contacted: CHS Field of St Paul, Minneapolis Convention Center, 1st St and 10th St site in Waconia; Eagle Ridge, Health East, Bielenberg Gardens and Summit Pointe in Woodbury; Mystic Casino and Tewapa at Shakopee Mdewakanton Sioux; Fireman's Park, Club West and Founder's Ridge in Chaska; and St. Louis County Garage in Duluth. Descriptions of design elements used in water reuse systems were collected and recorded including source water(s), storage, transport, age, treatment and ultimate use.

Water samples and physical and chemical characteristics were collected from four sites; Health East Sports Complex in Woodbury, 1st St in Waconia, Mystic Casino in Shakopee Mdewakanton Sioux, CHS Field in St. Paul, and St. Louis County Garage in Duluth. Collected and processed 2-4 samples at each site by filtering approximately 500L water through a membrane filter to collect bacterial and viral cells. Biological samples were processed by removing from the membrane, concentrating and pelleting and freezing for further use.

Activity 1 Status as of July 1, 2018:

Water Samples and physical and chemical characteristics were collected from nine sites: GNP, 17th St Residence Hall, Minneapolis Convention Center, Oneka Ridge Golf Course, Beaver Pond in Hugo, 1st St and 10th St in Waconia, Fireman's Park and Harvest in Chaska. 1-3 samples from each site were collected, processed and concentrated in the method described above.

Operators from an additional four reuse sites were contacted: Westminster Church, Minneapolis Downtown Improvement District, Bell Museum, Forest Lake High School.

Activity 1 Status as of January 1, 2019:

Activity 1 (water sample collection) was completed. In total, we collected 90 samples from 24 sites, including rainwater systems (water collected from roofs), stormwater systems (water collected from ponds), and domestic and industry wastewater treatment systems. From each site, one to three samples (pre and post treatment if any, and at distribution site) were collected, processed and concentrated in the method described above. Twelve of the sites have duplicates, which were collected in a different season.

Activity 1 Status as of July 1, 2019:

As described in the previous updates, Activity 1 was completed in early 2019. All expected outcomes as shown above were successfully obtained, including collecting samples that reflect:

- A variety of water reuse systems (n=24) including:
    - rainwater,
    - stormwater, and
    - domestic and industrial wastewater treatment systems
- Environmental conditions from different seasons of the year at 13 sites.
- A variety of land use locations
- Pre and post treatment water quality

At each reuse facilities, water samples were collected at the distribution site such as irrigation pipes. When treatments (filtration, UV, ozonation, etc.) were taken place, water samples were also collected before and after the treatments. Therefore, the total number of water samples collected became 90 from 24 locations. The number of water samples (n=90) was smaller than initially planned because some reuse facilities did not do any treatment, or did not operate their facilities in the second year of the project period. In addition to the reuse water samples, tap water samples (n=2) were also collected for reference.

All the samples were processed and concentrated in the lab according to the process described in the Activity 1 description. See Activity 2 for more information about sample analysis.

Final Report Summary:

In this project, we collected 90 water samples from 25 water reuse systems around the state. They reflect different source water types (rainwater, stormwater, and domestic and industrial wastewater), environmental conditions from different seasons of the year, a variety of land use locations, and presence/absence of water treatment. At each reuse facility, water samples were collected at the source and when available at a distribution site such as an irrigation tap. When treatment (filtration, UV, ozonation, etc.) was in place, water samples were also collected after the treatment. Therefore, the total number of water samples collected became 90 from 25 locations. In addition to the reuse water samples, tap water samples (n=2) were also collected for reference. One of these tap water samples was from an irrigation system. Description of the sites is summarized in Table 1.

All the samples were processed and concentrated in the lab according to the process described in the Activity 2 description. In addition, physical and chemical properties of the water samples were recorded, including pH, turbidity, UV transmittance, conductivity, chlorine concentration, total coliform counts, and *E. coli* counts. Water reuse system design was also documented and summarized (Table 1).

Therefore, all expected outcomes as shown above were successfully obtained.
Table 1. Description of the sampling sites

Site No.	Source Water Type	Treatment Description	Microbial treatment (y/n)	Reuse Purpose	Containmment for Non-treated water	Type of Drainage Area
1	Stormwater	Coarse Filtration, Ozone recirculation and Microfiltration	y	Toilet flushing	35,000 Underground Concrete Cistern, 2-1000 gal indoor polyethylene containers	Residential
2	Stormwater	Coarse Sediment Filtration	n	Lawn Irrigation	Outdoor Pond	Residential
3	Stormwater	Coarse Sediment Filtration	n	Lawn Irrigation	Outdoor Pond	Residential
4	Stormwater	Coarse Sediment Filtration	n	Lawn Irrigation, Vehicle Washing	Outdoor Pond	Commercial
5	Wastewater	Industrial Wastewater treatment, including: Activated Sludge, Flocculation, phosphorus and ammonia removal. Hollow membrane bioreactor, sand filtration and UV disinfection	y	Industrial Use, Cleaning floors, Washing, Equipment and Lawn Irrigation	Direct to Treatment	Commercial
6	Rainwater	Coarse Sediment Filtration	n	Lawn Irrigation	392,000 gal Underground Steel Cistern	Commercial
7	Stormwater	Coarse Sediment Filtration	n	Lawn Irrigation	Outdoor Pond	Commercial
8	Stormwater	No treatment	n	Lawn Irrigation	Outdoor Pond	Residential
9	Stormwater	Coarse Sediment Filtration	n	Lawn Irrigation	120,000 gal Underground Tank	Commercial
10	Stormwater	No treatment	n	Lawn Irrigation	Outdoor Pond	Residential
11	Rainwater	Screen filter, Coarse Sediment Filtration,UV disinfection	y	Lawn Irrigation, Toilet, Washing	Underground 27,000 gal Polyethylene tank	Commercial
12	Stormwater, Wastewater	Coarse Sediment Filtration	n	Lawn Irrigation	Outdoor Pond	Commercial
13	Stormwater	Coarse Sediment Filtration	y	Lawn Irrigation	Outdoor Pond	Residential
14	Stormwater	Chlorine Disinfection	y	Lawn Irrigation, Vehicle Washing	Outdoor Pond	Commercial
15	Stormwater	Shock chlorination disinfection, as needed	n	Lawn Irrigation	5 Underground Tanks	Residential
16	Stormwater	Coarse Sediment Filtration, UV disinfection	y	Lawn Irrigation	Outdoor Pond	Residential
17	Wastewater	Disc filters, Chlorine disinfection	y	Lawn Irrigation, Cooling, Vehicle Use, Industrial Use	direct to treatment	Commercial
18	Rainwater	Coarse Sediment Filtration, UV disinfection	y	Garden Irrigation	2 Indoor 5000L Polyethylene Tanks	Commercial
19	Stormwater	No treatment	n	Lawn Irrigation	Outdoor Pond	Commercial
20	Rainwater	No treatment	n	Lawn irrigation	4 Underground Tanks with 111, 000 gal capacity	Residential
21	Stormwater	UV disinfection	y	Flushing Toilet, Irrigating lawns, decorative fountain	6-4000 gal Indoor Polyethylene Tanks (24,000gal total)	Commercial
22	Rainwater	No treatment	n	Lawn Irrigation	5 Polyproplene Underground Tanks (60,000 gal total)	Commercial
23	Stormwater	Biofiltration basin	n	Garden Irrigation	206,575 gal Underground Tank	Commercial
24	Stormwater	No treatment	n	Concrete Manufacture, Vehicle Wash	190,000 gal Underground Cistern	Commercial
25	Stormwater, filter backflush	Disc Filtration	n	Lawn Irrigation	500,000 gal Underground Tank	Residential

ACTIVITY 2: Quantitatively detect multiple human pathogens in reuse systems

Description:

In this activity, we will quantitatively detect multiple human pathogens (both bacteria and viruses) in the 144 water samples collected in Activity 1. We will target all major waterborne pathogens including *E. coli* O157, *Salmonella*, *Campylobacter*, *Shigella*, *Clostridium perfringens*, *Legionella pneumophila*, *Listeria monocytogenes*, human adenovirus, Astrovirus, Enterovirus, human Norovirus (GI, GII, and GIV genotypes), Hepatitis A virus, Hepatitis E virus, Rotavirus A, and Sapovirus. The project manager (Dr. Ishii at the U of M) has developed and optimized the microfluidics quantitative PCR chip system to simultaneously quantify these pathogens in water samples. All necessary equipment is present in the U of M, and will be used in this project.

DNA and RNA will be extracted from the cell pellets prepared from the 144 water samples in Activity 1. Pathogen-specific genes will be amplified and quantified in the microfluidic quantitative PCR chip. Based on these results, we will calculate the concentrations of pathogens per liter water. These concentration data will be used for the risk assessment in Activity 3.

Summary Budget Information for Activity 2:

ENRTF Budget: $ 72,861
Amount Spent: $ 72,861
Balance: $ 0

Outcome	Completion Date
1. *DNA and RNA samples (144 samples)*	December 31, 2018
2. *Concentrations of multiple pathogens in water samples*	December 31, 2018

Activity 2 Status as of January 1, 2018:

Not yet started.

Activity 2 Status as of July 1, 2018:

DNA and RNA extraction method was selected. Process controls for the DNA and RNA extractions were established. We will use these methods to extract DNA and RNA from samples collected in Activity 1.

Activity 2 Status as of January 1, 2019:

DNA and RNA extraction process was initiated. Protocol for microfluidic quantitative PCR chip system has been established. We will run microfluidic qPCR once we complete DNA/RNA extractions from all samples.

Activity 2 Status as of July 1, 2019:

DNA and RNA extraction process was completed. We did DNA and RNA extractions in triplicate to overcome the expected data variations by statistical analyses. Therefore, number of DNA and RNA samples became 270 (90 water samples x 3 replicates), which is larger than originally planned (n=144).

Microfluidic quantitative PCR was performed to quantify multiple bacterial and viral pathogens in the DNA/RNA samples (n=270). We detected fecal indicator bacteria *E. coli* and several pathogens in the samples. These data are being used for Activity 3 to assess potential health risks. Outcomes 1 and 2 shown above were successfully obtained.

Final Report Summary:

A total of 270 DNA/RNA samples were successfully obtained from 90 water samples collected in Activity 1 from 25 water reuse systems around the state. Bacterial and viral pathogens were quantified using a microfluidic quantitative PCR approach. Major waterborne pathogens were quantified, including pathogenic *E. coli*, *Salmonella*, *Campylobacter*, *Shigella*, *Clostridium perfringens*, *Legionella pneumophila*, *Listeria monocytogenes*, human adenovirus, Astrovirus, Enterovirus, human Norovirus (GI, GII, and GIV genotypes), Hepatitis A virus, Hepatitis E virus, Rotavirus A, and Sapovirus. In addition, we also quantified fecal indicator bacteria (total *E. coli* and enterococci), human-specific genetic marker (HF183), and sample process controls (NH8B-1D2 and murine norovirus).

Most samples did not contain detectable levels of pathogens, although many samples contained fecal indicator bacteria. Some water samples, especially those before treatment, contained potential human pathogens such as enteropathogenic *E. coli*, *Clostridium perfringens*, *Legionella pneumophila*, sapoviruses, and noroviruses. However, in some cases, advanced water treatment reduced the levels of these pathogens. These pathogen concentration data were used for Activity 3 to assess potential health risks.

Therefore, all expected outcomes as shown above were successfully obtained.

ACTIVITY 3: Quantitative microbial risk assessment

Description:

In this activity, we will assess the potential health risks associated with the water samples through quantitative microbial risk assessment (QMRA). Risk of pathogen infection is a function of pathogen concentration in water, infectious dose of the pathogen, and a probability of pathogen exposure. We will use the pathogen concentration data obtained in Activity 2. Infectious dose is the concentration of pathogens that cause pathogen infection to 50% of human population. The infectious dose differs by pathogen and is available in the literature. The probability of pathogen exposure differs by water reuse purpose. For example, when reclaimed water is used to for irrigation to golf green, people may ingest mist generated by sprinklers. We will calculate the volume of mist people may ingest and then calculate the probability of pathogen exposure by taking pathogen concentration in the mist into account.

Based on the QMRA results (Activity 3) and water reuse system design assessment (Activity 1), we will analyze how system design components affect the microbial pathogen populations in the water. We will then recommend water quality standards and make system design recommendations using best public health and engineering practices. Benefits of water reuse will be maximized due to elimination of barriers.

Summary Budget Information for Activity 3:	**ENRTF Budget:**	**$ 26,720**
	Amount Spent:	**$ 26,720**
	Balance:	**$ 0**

Outcome	Completion Date
1. *Potential health risks of water samples*	March 31, 2019
2. *Analysis of microbial data in relation to system design*	March 31, 2019
3. *Recommend water quality and design standards for water reuse systems*	June 30, 2019

Activity 3 Status as of January 1, 2018:

Not yet started.

Activity 3 Status as of July 1, 2018:

Not yet started.

Activity 3 Status as of January 1, 2019:

Not yet started. We will start this activity soon after we get pathogen data (in early 2019).

Activity 3 Status as of July 1, 2019:

We started analyzing the potential health risks of water samples based on the pathogen data obtained in Activity 2. Relationship between microbial data and system design is also being analyzed. These results will be used to create design recommendation for safe water reuse. Expected outcomes as shown above will be obtained by the time of final report submission.

Final Report Summary:

Quantitative microbial risk assessment (QMRA) was done to evaluate the quality of water samples for reuse. QMRA consists of four parts: (1) hazard identification, (2) dose response, (3) exposure and (4) risk assessment.

(1) Hazard Identification

Several potential pathogens were detected based on the Activity 2 results. However, we focused on norovirus in Activity 3 for the following reasons:

- Norovirus is a food and waterborne virus that results in illness, hospitalization and fatalities in humans.
- Norovirus has been shown to have high infectivity, where exposure to small numbers of viral particles is associated with high rates of infection.
- The results from qPCR give three positive detections for Norovirus GI. All three samples are pretreatment, from the same site.
- Dose-response relationship is well published for norovirus (see below).

(2) Dose-Response

Dose is the quantity of pathogenic microorganisms ingested or inhaled. Dose is exposure multiplied by concentration of hazard. A dose-response model is a mathematical way (i.e., an equation) that helps predict the

likelihood of an adverse effect after exposure to a certain number of microbes. Many dose-response models estimate the probability of infection, not of illness. While there are multiple models available, each with its own assumptions, this QMRA uses two different published dose-response models for norovirus to give either a conservative or liberal risk estimate. Viral aggregation and population immunity are two fairly controversial parameters which affect the risk probability. An aggregation model will consider the extent to which viral particles "stick" together. The opposite would be a disaggregation or single-hit model, which does not consider any viral aggregation. Another potential variable to consider is to what extent the general population is immune or fully susceptible due to genetic factors. In both cases the data is fit to an exact Beta Poisson model. Teunis' 2008 model uses a single hit model and no immunity. Schmidt's 2015 model considers disaggregation and 27.5% immunity among the population.

(3) Exposure Assessment

Exposure is the actual dose, or the quantity of microbe that an individual within a population was exposed to. It also includes the route and number of exposure incidents over a given time period. In this study exposure is assumed to be 1 mL for accidental exposure during spray irrigation in an area with unrestricted access. This estimate considers either work or recreation-related exposure by hand-to-mouth contact.

(4) Risk Characterization

Risk characterization combines the data from exposure, dose-response and hazard identification to determine health risk. Risk is exposure times dose. The model is shown below.

$P_{infection} = a * (1 - {}_1F_1 (\alpha, \alpha+\beta, -dose)$

For Teunis model: a=1, α=0.04, β=.055

For Schmidt's model: a=0.7246, α=2.910, β=2734

The results showing the risk of infection are given in Table 3

It is important to note that the dose response used in our study uses infection as an endpoint, rather than illness. Infection is identified by multiple means, such as elevated immune response or shedding of virus. Illness will not occur for every infection and is dependent on multiple factors, however, the risk is dose dependent. One study puts to risk of illness for those who have been infected at 68%. This is the value that is used here. The risk of illness, given a rate of 68% is shown in Table 2.

For the given samples, regardless of model, the risk for illness and infection is considerable (>90 illness out of 10,000 individuals; Table 3). This risk varies significantly depending on the model. There is much uncertainty in these models surrounding viral aggregation and population immunity and other characteristics. The Teunis model may overestimate risk and Schmidt model may underestimate risk; the actual risk may lay in the middle. Given that no samples collected after treatment, and during actual reuse had results above detection limit for norovirus. No samples other than untreated wastewater had greater than half of the replicates above detection limit for norovirus.

Table 2. Risk of infection and risk of illness calculated based on the QMRA. Two dose response models (Schmidt model and Teunis model) were used.

Sample Name	Average Conc. (log copies/L)	Exposure (L)	Dose (copies)	Risk of Infection		Risk of Illness	
				Schmidt	Teunis	Schmidt	Teunis
A139	4.9	.001	79.4	.0575	.523	.0391	.356
A140	4.69	.001	49.4	.0365	.513	.0248	.349
A187	4.3	.001	20.1	.0152	.495	.0103	.337

In conclusion, of the 90 samples collected only 3 showed detectable levels of norovirus in replicates. Most samples collected from the water reuse systems in MN did not contain detectable levels of norovirus. Even when some pathogens such as norovirus were detected, water treatment removed these potential pathogens to the levels considered low risk of infection and illness for reuse. Due to the complexity of this dataset, and the unique nature of each reuse system, the outcomes for this activity are not yet complete. Norovirus was used as an example for risk assessment, but potential health risks associated with pathogens other than norovirus should be analyzed in the future. Many of the pathogens identified in the samples need more careful examination to be assessed for risk to human health, and overall, additional future research will be needed to answer some of the new questions that this research raised. U of M and MDH researchers will continue to work on these outcomes.

V. DISSEMINATION:

Description:

Findings will be disseminated and archived via reports to LCCMR, peer-reviewed publications, and presentations at conferences. We will also publish recommended water quality and design standards for water reuse purposes on the Minnesota Department of Health's website or other locations as appropriate.

Status as of January 1, 2018:

Status as of July 1, 2018:

MDH has released a report about water reuse on March 7, 2018.
http://www.health.state.mn.us/news/pressrel/2018/water030718.html

Status as of January 1, 2019:

Anita Anderson at MDH presented at the EPA's webinar on "Water Reuse and Reclaimed Water" on Oct. 31, 2018, and introduced our research effort.
https://www.epa.gov/water-research/water-research-webinar-series

Status as of July 1, 2019:

We have presented our results at the Association of Environmental Engineering and Science Professors (AEESP) Conference at Tempe, AZ, in May 2019. We are also preparing a manuscript for journal publication to disseminate the research outcomes.

The outcomes of this research have been used to expand our water reuse research. We received funding from MN Stormwater Research Council to continue and expand our water reuse research. In addition, we are working with other state and federal agencies (EPA, MPCA, etc.) as well as private sectors to disseminate and leverage the research outcomes.

Final Report Summary:

Presentations:

- Anita Anderson at MDH presented at the EPA's webinar on "Water Reuse and Reclaimed Water" on Oct. 31, 2018, and introduced our research effort. https://www.epa.gov/water-research/water-research-webinar-series
- Val Dooling, Anita Anderson, Nancy Rice, Tim LaPara, Satoshi Ishii. 2019. How safe is our reclaimed water: A QMRA Approach. Association of Environmental Engineering and Science Professors (AEESP) Conference, Tempe, AZ. May 14-16, 2019.

Publication:

- Val Dooling, Anita Anderson, Nancy Rice, Tim LaPara, Satoshi Ishii. 20XX. Safety assessment of various water reuse systems in Minnesota. Manuscript in preparation
- MDH is preparing a white paper regarding water reuse that will reference the data obtained from this study. The white paper will be published and become available online at MDH's website.

VI. PROJECT BUDGET SUMMARY:

A. Preliminary ENRTF Budget Overview:

***This section represents an overview of the preliminary budget at the start of the project. It will be reconciled with actual expenditures at the time of the final report.**

Budget Category	$ Amount	Overview Explanation
Personnel:	$ 122,419	1 project manager at 8% FTE for two years; one graduate research assistant at 50% FTE for two years; 1 undergraduate research assistant at 100% FTE for 5 months.
Equipment/Tools/Supplies:	$ 24,572	General lab supplies ($3,000), membrane filters ($3,000), water sample analysis ($2,000), DNA/RNA extraction kits ($3,572), reagents for qPCR ($7,000), use of UMGC's facilities for microfluidic qPCR ($6,000)
Travel Expenses in MN:	$ 1,009	In-state travel to collect water samples: Mileage $1,009
TOTAL ENRTF BUDGET:	**$148,000**	

Explanation of Use of Classified Staff: N/A

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

Total Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: 1.5

Total 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			
N/A		$	
State			
University of Minnesota	$56,000	$56,000	In-kind contribution of indirect costs not charged to this project
Minnesota Department of Health	$32,000	$32,000	In-kind salary for Anita Anderson and Nancy Rice
TOTAL OTHER FUNDS:	**$88,000**	**$88,000**	

VII. PROJECT STRATEGY:

A. Project Partners:

Partners receiving ENRTF funding: N/A

Partners NOT receiving ENRTF funding

- Timothy LaPara (Professor at the Department of Civil, Environmental, and Geo- Engineering, the University of Minnesota): Providing technical support (Activities 1, 2, and 3)
- Anita Anderson (Principal Engineering Supervisor at the Drinking Water Protection Unit, Minnesota Department of Health): Providing advice on water sample collection (Activity 1) and recommending water quality and design standards for water reuse systems (Activity 3)
- Nancy Rice (Research Scientist at the Health Risk Assessment Unit, Minnesota Department of Health): Providing advice on QMRA (Activity 3)

B. Project Impact and Long-term Strategy:

This project will maximize the potential of water reuse to conserve Minnesota's groundwater and improve surface water quality by providing the pathogen data needed to eliminate barriers to water reuse. This project will provide assurances to the public, regulators and system owners that water reuse can become common practice without negative effects on public health and safety and provide design information to reduce cost.

C. Funding History:

Funding Source and Use of Funds	Funding Timeframe	$ Amount
Clean water fund	September 1, 2014 to December 31, 2016 (no-cost extension being requested to June 30, 2017)	$200,000
Total		$200,000

VIII. REPORTING REQUIREMENTS:

- **The project is for 2 years, will begin on July 1, 2017, and end on June 30, 2019.**
- **Periodic project status update reports will be submitted January 1 and July 1 of each year.**
- **A final report and associated products will be submitted between June 30 and August 15, 2019**.

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

Environment and Natural Resources Trust Fund
Final M.L. 2017 Project Budget

Project Title: *Assessment of Water Quality for Reuse*
Legal Citation: *M.L. 2017, Chp. 96, Sec. 2, Subd. 04f*
Project Manager: *Satoshi Ishii*
Organization: *University of Minnesota*
M.L. 2017 ENRTF Appropriation: **$** *148,000*
Project Length and Completion Date: *2 Years, June 30, 2019*
Date of Report: *7/1/2019*

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Activity 1 Budget [07/18/19]	Amount Spent	Activity 1 Balance	Activity 2 Budget [07/18/19]	Amount Spent	Activity 2 Balance	Activity 3 Budget [07/18/19]	Amount Spent	Activity 3 Balance	Revised TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM	***Water sample collections***			***Quantitatively detect multiple human***			***Quantitative microbial risk assessment***				
Personnel (Wages and Benefits)	$43,588	$43,588	$0	$52,111	$52,111	$0	$26,720	$26,720	$0	$122,419	$0
Satoshi Ishii, Project Manager: $27,000 (75% salary, 25% benefits) 8% FTE each year for 2 years											
Graduate research assistant: $87,000 (55% salary, 45% benefits); 50% FTE for 2 years											
Undergraduate research assistant: $9,000 (100% salary, 0% benefits); 100% FTE in summer for 5 months											
Equipment/Tools/Supplies											
General lab supplies ($3,000), membrane filters ($3,000), water sample analysis ($2,000), DNA/RNA extraction kits ($2,000), reagents for qPCR ($4,000), use of UMGC's facilities for microfluidic qPCR ($6,000)	$3,822	$3,822	$0	$20,750	$20,750	$0				$24,572	$0
Travel expenses in Minnesota											
In-state travel to collect water samples: Mileage $4000; meals $1,000	$1,009	$1,009	$0							$1,009	$0
COLUMN TOTAL	**$48,419**	**$48,419**	**$0**	**$72,861**	**$72,861**	**$0**	**$26,720**	**$26,720**	**$0**	**$148,000**	**$0**