Environment and Natural Resources Trust Fund

M.L. 2021 Draft Work Plan

General Information

ID Number: 2021-121

Staff Lead: Corrie Layfield

Date this document submitted to LCCMR: March 8, 2021

Project Title: Monitoring Emerging Viruses In Minnesota's Urban Water Cycles

Project Budget: $416,000

Project Manager Information

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Project Reporting

Date Work Plan Approved by LCCMR:

Reporting Schedule: December 1 / June 1 of each year.

Project Completion: June 30, 2024

Final Report Due Date: August 14, 2024

Legal Information

Legal Citation:

Appropriation Language:

Appropriation End Date: June 30, 2024

Narrative

Project Summary: This project will address the presence and fate of enveloped viruses (e.g. coronaviruses) and their survivability in aqueous environments with emphasis on wastewater and drinking water treatment processes.

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

Over the last month MPCA and Minnesota Department of Health have been receiving several questions related to the risk that COVID-19 poses to wastewater professionals. This project will develop new detection methods and quantitative risk assessment (QRA) models that MPCA and the Department of Health can use to better quantify future risks related to the role of the urban water cycle in the spread of enveloped viruses. Past research efforts have focused mainly on nonenveloped human enteric viruses such as human noroviruses. However, avian influenzas, SARS, MERS, and the ongoing COVID-19 pandemic, have been caused by enveloped viruses. These viruses have direct connection to wastewater and drinking water purification when they are excreted in feces or urine. Recent reports show that SARS-CoV-2 has been detected in stool samples of COVID-19 cases. Increasing circulation of viruses such as SARS-CoV-2 in a population will increase virus loads in to sewer systems of our cities. It is important to collect information about the occurrence and fate of enveloped viruses in sewage to understand if there is a risk to sewage workers, but also to determine if sewage surveillance could be used to monitor the circulation of enveloped viruses in our communities.

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

SARS-CoV-2 (the virus that causes COVID-19), as well as any other disease-causing viruses in wastewater, are currently impossible to detect by wastewater professionals as they conduct their day-to-day work. The currently unknown role of the environment, specifically the urban water cycle, in the spread of enveloped viruses highlights the need for the development of rapid testing methods and risk assessment model proposed in this project. In order to effectively control outbreaks and pandemics of novel enveloped viruses in the future we need to understand what conditions influence their environmental persistence. In this project we will study the fate of enveloped viruses in the urban water cycle and identify locations of potential human exposure. In order to achieve this goal, we will develop new, cost-efficient, molecular screening methods that will allow the rapid detection and quantification of enveloped viruses in environmental water samples. The new testing methods will be useful to municipalities and state agencies, and health departments to estimate the risk of infection and illness when a population is exposed to enveloped viruses in the environment. Sewage surveillance can also serve as early warning of (re-)emergence of COVID-19 in the Twin Cities and local communities connected to central sewage treatment.

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 proposed project will have the following specific project outcomes:

(1) Development and optimization of methods for extracting, purifying, and quantifying enveloped and non-enveloped viruses and their nucleic acids from complex sample matrices such as wastewater, residual biosolids, and surface waters.

(2) Quantification of presence and survivability of enveloped and non-enveloped viruses during wastewater and drinking water treatment processes, as well as when they are released into the environment via wastewater effluent or land-applied biosolids.

(3) Quantitative risk assessment will be conducted to better characterize exposure and transmission pathways for enveloped viruses through the urban water cycle environment.

Project Location

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

Region(s): Metro

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

Statewide

When will the work impact occur?

During the Project

Activities and Milestones

Activity 1: Development of molecular assays for extracting, purifying, and quantifying enveloped viruses in complex aquatic samples.

Activity Budget: $157,187

Activity Description:
We will sample five of the nine wastewater treatment plants operated by Metropolitan Council Environmental Services in the Twin Cities metropolitan area and one wastewater treatment plant operated by Brainerd Public Utilities in Brainerd, Minnesota. Detecting and quantifying viruses in environmental samples requires concentrating the virus in the samples into a smaller volume to improve detection limits. Standard concentration methods will be carefully evaluated with respect to their efficiency for enveloped viruses because their outer lipid layer makes them more sensitive to temperature, pH, and organic solvents. We will evaluate if flow cytometry can be used for viral particle enrichment from wastewater and surface waters. In this activity we will also develop assays for the reliable and fast quantification of enveloped coronaviruses and SARS-CoV-2 using reverse transcription quantitative PCR. Strict process and quality control protocols for RNA extraction, recovery, and quantification using appropriate virus surrogates will be performed to ensure assay reliability and to quantify the efficiency of virus detection in water and wastewater samples. The process controls will include inoculating viral surrogates into a wastewater samples before virus concentration, adding viral surrogate concentrate before RNA extraction, and adding known amounts of a viral RNA/cDNA standard before RT-qPCR.

Activity Milestones:

Description	Completion Date
Comparative metagenomic sequence analysis of virus populations in collected water and wastewater samples	June 30, 2022
Quality control and quality assurance of extraction and detection methods using appropriate virus surrogates	June 30, 2022
Development of RT-PCR methods for the detection of enveloped viruses in environmental samples	July 31, 2023

Activity 2: Quantification of presence and survivability of enveloped viruses during wastewater treatment processes.

Activity Budget: $162,174

Activity Description:
In this part of the project we will use the new tests developed in Activity 1 to answer specific questions regarding the fate of viruses in different compartments of the urban water cycle. The study will characterize how different stages of wastewater treatment will impact virus particle partitioning between solids, liquid, and air and how engineering design of water treatment plants will impact viral persistence in wastewater, in wastewater effluent, biosolids, and aerosols. We plan to analyze the partitioning of viral particles in sludge settling experiments quantifying viral particles in the supernatant vs. the settle sludge. Non-Infectious, intact viral particles of SARS-Cov-2 will be used in sorption experiments with wastewater to determine their partitioning between solids and liquid phases. Modeling of viral decay and wastewater flow rates through the samples urban sewage systems will be performed to accurately relate viral concentration at the point of sampling to the presence of virus within the catchment population. Positive RT-qPCR signals for SARS-CoV-2 RNA in wastewater will be confirmed by sequencing analysis to validate our assay specificity for the tested environmental samples. Sequence analysis will be performed using the Gopher-Pipeline of the University of Minnesota Supercomputing Institute.

Activity Milestones:

Description	Completion Date
Quantification of enveloped viruses in raw sewage, wastewater effluent, biosolids, and surface waters	July 31, 2023
Quantification of virus particle partitioning between solids, liquid, and aerosols during different water treatment processes	December 31, 2023
Comparison of different water treatment plant designs (continuous flow, sequencing batch reactors, anaerobic digestion	December 31, 2023

Activity 3: Quantitative risk assessment to characterize exposure and transmission pathways for enveloped viruses in the urban water cycle environment.

Activity Budget: $96,639

Activity Description:

In this part of the project we will characterize risks associated with occupational exposures to wastewater during operation and maintenance of sewage treatment infrastructure. Results from this project will inform QRA models to understand how much an impact viruses in the environment will have on the health of wastewater professionals. The QRA framework will consist of four steps, including hazard identification, dose−response, exposure assessment, and risk characterization. The quantified virus gene copy numbers (Activity 2) will be analyzed to quantify their statistical signatures. The estimated risk will be evaluated using available EPA health benchmarks for microbial pathogen exposure scenarios. Monte Carlo simulations will provide a range of uncertainty in infection risks to human health from exposure to wastewater and other virus containing environmental water samples. We plan to set up membrane air filters at the Brainerd wastewater treatment plant to quantify the concentration of enveloped coronaviruses and SARS-CoV-2 in different indoor facilities of the plant. Filter sampling is an easy-to-use method, relatively inexpensive, and the samples are suitable for many types of downstream analyses such as nucleic acids-based analysis. Samples will be taken over long periods of time to increase total aerosol mass collected.

Activity Milestones:

Description	Completion Date
Statistical data analysis of virus concentrations in aqueous samples	December 31, 2023
Characterization of occupational and public exposure scenarios	June 30, 2024
Quantitative risk assessment and development of guidelines to manage virus exposure risks through wastewater	June 30, 2024

Project Partners and Collaborators

Name	Organization	Role	Receiving Funds
Leisa Thompson & George Sprouse	Metropolitan Council, Environmental Services, Wastewater & Water	Facilitation and support of sample collection efforts. Data discussion and communication with policy-makers, planning agencies, and water service providers in the Twin Cities metropolitan region. Please also see attached letter of support from MCES.	No
Prof. Dr. Timothy LaPara	University of Minnesota	Dr. LaPara is a full professor in the Department of Civil, Environmental, and Geo-Engineering. His area of expertise are environmental engineering and environmental microbiology. Dr. LaPara will serve as co-PI on the proposed project. He will co-advise the graduate student and postdoc, and support data analysis and discussion.	Yes

Dissemination

Describe your plans for dissemination, presentation, documentation, or sharing of data, results, samples, physical collections, and other products and how they will follow ENRTF Acknowledgement Requirements and Guidelines.
The target audience for research results from this project are professionals in all areas of water treatment including employees of public water works and distribution system. The quantitative test to detect enveloped viruses in environmental water samples that will be developed in this project is critical to ensure the health and safety of workers servicing sewage system and employees at wastewater and drinking water treatment plants.
We will work closely with Leisa Thompson and George Sprouse from the Metropolitan Council Environmental Services (MCES, Department of Wastewater and Water, see letter of support), to ensure knowledge transfer and communication with policy-makers, planning agencies, and water service providers in the Twin Cities metropolitan region. Results will be shared with state agencies and health departments as soon as they become available to ensure instant public access to new testing methods and recorded water quality data. Upon completion of the project results will be disseminated through scholarly publications in peer-reviewed scientific journals. Highlights and project updates will be published on the Behrens lab and University websites. Talks and poster presentations will be given at local water conferences. The Environment and Natural Resources Trust Fund will be acknowledged through use of the trust fund logo or attribution language on project print and electronic media, publications, signage, and other communications per the ENTRF Acknowledgment Guidelines.

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 be funded?
The research questions addressed in this project are critical to prepare environmental engineers and public health workers in Minnesota in the event that enveloped viruses causing a deadly outbreak or pandemic enter the urban water cycle. Results will be published and shared with state agencies and health departments as soon as they become available to ensure instant public access to new testing methods and recorded water contamination data. Upon completion of the project publications and outcomes will be summarized in a final report downloadable from our University website. Talk and poster presentations will be given at local conferences.

Other ENRTF Appropriations Awarded in the Last Six Years

Name	Appropriation	Amount Awarded
Wastewater Treatment Process Improvements	M.L. 2016, Chp. 186, Sec. 2, Subd. 04k	$398,000
Engineered Biofilter for Sulfate and Metal Removal from Mine Waters	M.L. 2016, Chp. 186, Sec. 2, Subd. 04p	$440,000

Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	$ Amount
Personnel								
Post Doctoral Researcher		TBD			20.25%	2		$123,257
1 Graduate Student		tbd			43.61%	1.5		$152,155
Faculty		CO-PI			26.7%	0.09		$14,000
Faculty		PI			26.74%	0.33		$54,588
							Sub Total	**$344,000**
Contracts and Services								
							Sub Total	**-**
Equipment, Tools, and Supplies								
	Tools and Supplies	Consumable and supplies: $18K for years 1 & 2 and $15K for year 3. Cost for consumable and supplies include $ 3K for flow cytometry supplies (dyes, standard beads, buffer, filters); $ 5K per year for water chemistry analysis (nutrients, DOC, ions, metals); $11K for each year for general lab supplies and reagents for DNA/RNA extraction, cDNA synthesis, primer and quantitative PCR. Lab services: $ 5K for each year to run the flow sorter and use of the UMGC DNA sequencing services.	for DNA/RNA extraction, cDNA synthesis, chemical and reagents for flow cytometry and quantitative PCR, water chemistry analysis, lab services to use UMGC DNA sequencing services, lab services to run the flow sorter					$66,000
							Sub Total	**$66,000**
Capital Expenditures								
							Sub Total	**-**
Acquisitions and Stewardship								

							Sub Total	**-**
Travel In Minnesota								
	Miles/ Meals/ Lodging	Mileage in Minnesota	sample collection from wastewater treatment plants and drinking water facilities around the state					$2,000
	Conference Registration Miles/ Meals/ Lodging	meals and lodging for students	meals and lodging to allow students to participate in local conferences and meetings to present research on this grant. Travel would be to formally present of project findings(e.g. Minnesota Wastewater Operators Association or the Minnesota Section of the American Water Works Association).	X				$1,000
							Sub Total	**$3,000**
Travel Outside Minnesota								
							Sub Total	**-**
Printing and Publication								
	Publication	Sponsored Publications $1500 per year based on current rates	Publication costs for research results on this project					$3,000
							Sub Total	**$3,000**
Other Expenses								
							Sub Total	**-**
							Grand Total	**$416,000**

Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or Type	Description	Justification Ineligible Expense or Classified Staff Request
Travel In Minnesota	Conference Registration Miles/Meals/Lodging	meals and lodging for students	meals and lodging to allow students to participate in local conferences and meetings to present research on this grant. Travel would be to formally present of project findings(e.g. Minnesota Wastewater Operators Association or the Minnesota Section of the American Water Works Association).

Non ENRTF Funds

Category	Specific Source	Use	Status	Amount
State				
			State Sub Total	**-**
Non-State				
In-Kind	Indirect costs for this proposal, though not allowed, are listed as in-kind contribution of 55% MTDC which is the Federally Negotiated rate with the U of MN. The indirect is proportionate to the awarded funds at a rate 55% so if the award is reduced the F&A would be reduced.	To pay for administrative and facility expenses for this project	Secured	$201,696
			Non State Sub Total	**$201,696**
			Funds Total	**$201,696**

Attachments

Required Attachments

Visual Component

File: 2c30b554-09f.pdf

Alternate Text for Visual Component

The fate of infective viruses in the urban water cycle and locations of potential human exposure. Viruses that are excreted in feces, urine, and vomit enter the sewage system. Toilet flushing or problems with indoor plumbing systems may form virus-laden aerosols that could result in human exposure. Viruses are transported through the municipal sewage system to the wastewater treatment plant (WWTP). Workers servicing sewage systems could be exposed to infective viruses. Combined sewage overflo...

Optional Attachments

Support Letter or Other

Title	File
Letter of support MCES	3d489c91-23e.pdf
Research_Addendum_2021-121_Behrens_approved	7a3532b9-198.docx

Difference between Proposal and Work Plan

Describe changes from Proposal to Work Plan Stage

Revisions 03/04/2021: Thank you for pointing out that there was a word missing in a sentence in the description of Activity 3. The missing word has been added. The sentence now reads: "Results from this project will inform QRA models to understand how much an impact viruses in the environment will have on the health of wastewater professionals."

Revision 02/15/2021: Approved research addendum has been uploaded and is now attached to this work plan.

Revisions 02/13/2021: Work plan activity descriptions have been updated to reflect approved research addendum revisions following peer review. Activities 1-3 have been reordered to match the approved timeline of the project.

Revisions 01/30/2021: A statement has been added to the Dissemination section about how the Environment and Natural Resources Trust Fund will be acknowledged on any publications resulting from this project.

The main changes between the work plan and the initial proposal are pertaining to the project budget. The LCCMR recommended funding for this project is $416,000, while the initial proposal request for funding was for $489,000. In order to lower the overall project cost by $73,000 the following adjustments have been made to the budget:

1) 3rd-year funding for the postdoctoral research fellow has been cut
2) Co-PI summer salary has been cut in half
3) Expenses for consumable and supplies has been slightly increased in order to match the recommended total funding amount of $416,000

As consequence of the personnel budget reduction we will have to limit the number of sampling locations and overall number of samples that can be processes during the funding period. We will therefore have to focus our work on water treatment facilities in the metro region. However, project outcomes and impact of the proposed research from this

project will continue to be applicable statewide.
Dissemination efforts and publication of the project outcomes are now described in detailed in a dissemination plan.

Additional Acknowledgements and Conditions:

The following are acknowledgements and conditions beyond those already included in the above workplan:

Do you understand and acknowledge the ENRTF repayment requirements if the use of capital equipment changes?

N/A

Do you agree travel expenses must follow the "Commissioner's Plan" promulgated by the Commissioner of Management of Budget or, for University of Minnesota projects, the University of Minnesota plan?

Yes, I agree to the UMN Policy.

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

Environment and Natural Resources Trust Fund (ENRTF)
2021 Main Proposal VISUAL
Project Title: Monitoring emerging viruses in Minnesota's urban water cycles