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NEVADA WATER NEWS

NWRRI NEVADA WATER RESOURCES RESEARCH INSTITUTE

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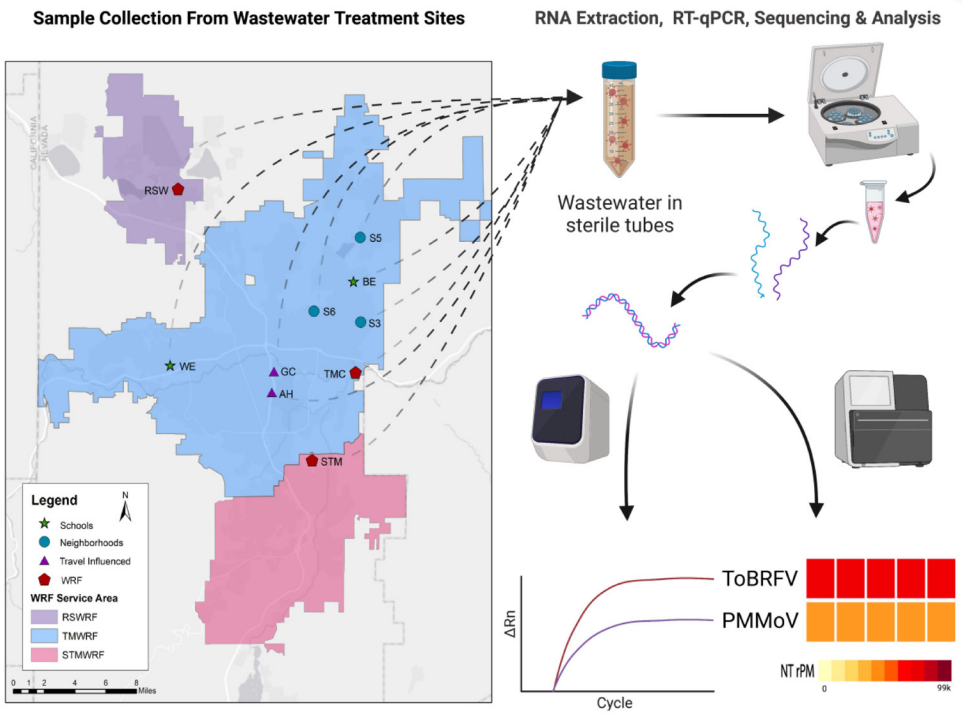
PROJECT SPOTLIGHT

"Use of Viral and Genetic Marker Surrogates to Assess Transport of Human Enteric Viruses in Water Reclamation Systems"

This project is funded through the National Institutes for Water Resources (NIWR) 104(g) grant.



The wastewater collection sites (left) in Washoe County, Nevada, used for the study and the steps of the wastewater virome analysis process (right). Once the samples were collected, the RNA and DNA of the target viruses were sequenced so that they could be identified in the column experiments. RSWRF = Reno Stead Water Reclamation Facility, TMWRF = Truckee Meadows Water Reclamation Facility, STMWRF = South Truckee Meadows Water Reclamation Facility (figure by Subhash Verma).

With increasing demands on water resources and droughts related to decreased precipitation, finding ways to augment drinking water supplies is a pressing concern for water researchers throughout the Southwest. But before recycled water can be used as a drinking water source, it is important to ensure that the treatment processes have effectively treated any pathogens, particularly human enteric viruses such as noroviruses and adenoviruses, and the water is safe for human consumption. Human enteric viruses are widely persistent in wastewater, are responsible for waterborne gastrointestinal diseases, and are also infectious at low doses. Therefore, robust viral surrogates or genetic markers are needed for effective viral monitoring of treated water intended to augment drinking water supplies.

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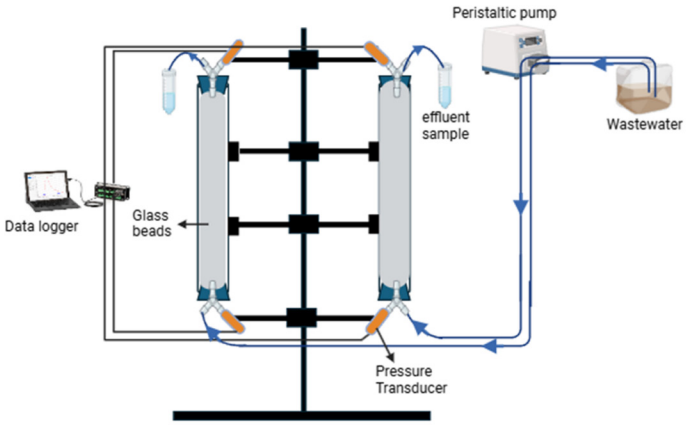
If you have questions about submitting a NWRRI proposal, email Suzanne Hudson at Suzanne.Hudson@dri.edu. Visit the NWRRI website at www.dri.edu/nwrri for current RFP information.



(Left) Emmanuel Cobbinah running a soil column experiment for the study. (Right) Setup of the soil column experiments used in the project to study virus transport and removal in a spreading basin (photo and figure courtesy of Emmanuel Cobbinah).

The goal of the project "Use of Viral and Genetic Marker Surrogates to Assess Transport of Human Enteric Viruses in Water Reclamation Systems" was to improve the viral monitoring techniques used in wastewater treatment plants to ensure that pathogens are effectively inactivated. The project was funded through the NIWR 104(g) National Competitive Grant and addressed the NIWR research priority area of water-related hazards and public health. This is a highly competitive grant that is awarded nationally to only six projects each year.

Human enteric viruses are widely persistent in wastewater. Therefore, robust viral surrogates or genetic markers are needed for effective viral monitoring of treated water intended to augment drinking water supplies.

Although wastewater treatment processes successfully remove most virus species, human enteric viruses can persist in treated effluents. "Enteric viruses can be difficult to detect and quantify, particularly through advanced water treatment processes and soil aquifer treatment systems, because of their occurrence at relatively low concentrations," explains Dr. Rishi Parashar, one of the principal investigators of the project. "A stringent requirement of a reduction of up to 12-log levels from the raw wastewater is often stipulated before it can be considered for potable reuse, but this level can be difficult for water treatment authorities to demonstrate by measuring virus concentrations."

The project team—which included Drs. Parashar and Lazaro Perez of Desert Research Institute (DRI), Drs. Subhash C. Verma and Krishna Pagilla of the University of Nevada, Reno (UNR), and Dr. Todd Caldwell of the United States Geological Survey (USGS)—was comprised of experts in virology, water reclamation for potable reuse, and modeling flow and transport processes in soil aquifer systems. First, they extracted samples from raw and reclaimed wastewater and concentrated the samples to determine the types of viruses present. They then collected RNA and DNA from the concentrated samples to create a sequence of viral gene markers to use as viral surrogates.

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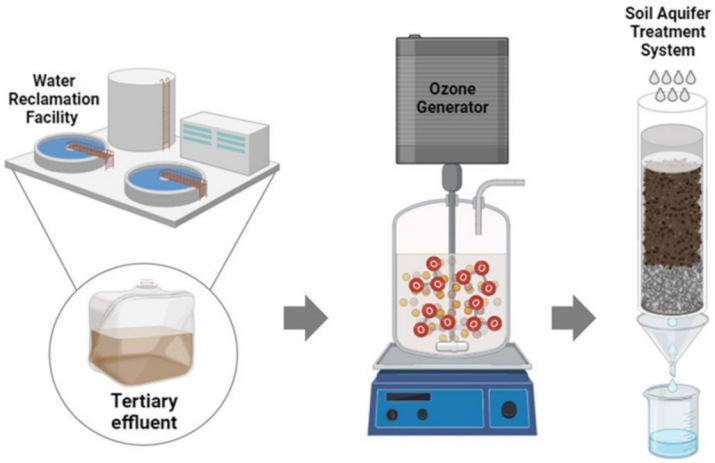
Once the viral surrogates were characterized, the team constructed bench-scale soil aquifer treatment (SAT) columns to determine the viral removal efficiency of the water treatment process. The columns replicated the soil, water, and temperature of spreading basins, which are used to recharge groundwater aquifers with recycled water by allowing large amounts of water to soak into the aquifers below the surface. Using these SAT columns, the researchers collected valuable data on the log removal values for the targeted virus species, including norovirus and the surrogate pepper mild mottle virus (PMMoV). Because PMMoV is commonly present in human waste, it is a good indicator for determining water quality. "Different infiltration rates were assessed to investigate the impact of various operational conditions on removal efficiency, and water parameters were monitored to identify and evaluate the involvement of other microorganism in removing viruses," Parashar explains. "Overall, the methodology used in this project allowed us to demonstrate that spreading basins are essential for treating water intended for reuse applications, which provides valuable insights into the efficiency and mechanisms involved in removing viruses."

The team conducted virus transport experiments in both glass-bead columns (to create a nearly homogenous and inert environment) and natural soil columns under saturated and unsaturated conditions. They then used sorption isotherms, which are curves that reflect how substances stay on a solid surface at different concentrations, to determine viral transport through the columns. "We are currently using the sorption and SAT column data to produce calibrated models of filtration and transformation kinetics," Parashar adds. "We are also quantifying the removal of viral particles in column studies using colloid filtration theory, which allows us to separate out the effects of flow and diffusion from our models by expressing the attachment rate of viruses in terms of collision and sticking efficiencies."



Bench-scale ozonation followed by the soil aquifer treatment (SAT) process with reclaimed water collected after denitrification and treated in a semi-batch ozone system (figure by Madeline Carine).

The results of this project also provided insights into both the efficiency of current water treatment processes and possible advancements in treatment processes. The data collected from the SAT columns demonstrated the effectiveness of porous media filtration in removing surrogate viruses, such as PMMoV, at different infiltration rates. "Specifically, our results showed that a 2.0 log removal value for PMMoV was obtained in the bench-scale SAT columns with no observable difference in virus removal at various infiltration rates," Parashar says. "Norovirus concentrations were only detected in influent wastewater, indicating that removal occurred in the columns. Furthermore, water quality parameters indicated a microbial presence within the soil columns, suggesting that predation may be a mechanism of virus reduction." These results highlighted that spreading basins are efficient at removing additional viruses and demonstrated that the technology is effective for water reuse applications.

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"Overall, the methodology used in this project allowed us to demonstrate that spreading basins are essential for treating water intended for reuse applications, which provides valuable insights into the efficiency and mechanisms involved in removing viruses."

–RISHI PARASHAR

The team also had some surprising results over the course of the project. While they were identifying and sequencing the viruses present in the municipal wastewater samples, the team found multiple plant viruses that can be used as genetic markers to determine water quality. "Samples collected over an extended period of time were subjected to genomic material extraction and sequencing to identify all the viruses through a microbe identification pipeline," Parashar explains. "Relative copies of the viral genome in Reno's wastewater showed the presence of novel tomato brown rugose fruit virus at concentrations higher than the well-studied surrogate PMMoV." This result is significant because it provides other potentially useful viral surrogates that are naturally occurring in high concentrations for researchers to use to assess the quality of water intended for reuse.

A key component of the National Competitive Grants is to build collaboration between university scientists and USGS. This project provided researchers from DRI and UNR the opportunity to work closely with Dr. Caldwell of USGS. "Dr. Caldwell worked closely with the team and provided expert guidance on the design, instrumentation, and construction of the column experiments," Dr. Parashar says. "Through regular biweekly meetings and working closely with the graduate student on the project, Dr. Caldwell helped troubleshoot several challenges with experiments, such as ensuring a stable flow system over long durations, and contributed to the development of the overall modeling plan." The results of this project also provide other opportunities for further research on topics such as managing aquifer recharge, storage, and recovery and collaboration with various stakeholders.

Another important aspect for all NWRRI research projects is to provide the next generation of scientists opportunities to gain skills in water resources research. This project supported graduate students Emmanuel Cobbinah, who worked on the column experiments and transport modeling, and Madeline Carine, who worked on virus detection and quantification in wastewater. "By participating in this project, the students were trained in a highly interdisciplinary setting with frequent interactions with virologists, water quality scientists, and transport modelers," Parashar adds. "The student training to be a modeler learned how to extract genetic materials from wastewater samples and conduct virus quantification studies. The student focusing on treatment processes became more aware of the building blocks of transport modeling in porous media. Through frequent interactions in group meetings, they also developed skills to engage with scientists from diverse backgrounds."

The Walker River in Nevada (photo courtesy of USGS).

NWRRI Program Announcements

We are excited to announce the newly funded NWRRI 104(b) project "Comparative Analysis of Climate Driven Shifts in Streamflow Trends for the Upper Carson and Walker River Basins." In this project, Dan Saftner (PI) and Dr. Rishi Parashar (co-PI) of DRI will study the effects of climate change on streamflow in the Carson and Walker River Basins. Under a warming climate, streamflow starts earlier in the spring. This shift makes it difficult for farmers and water managers to plan for changing water availability. The project will focus on rivers in the Sierra Nevada that flow from California into Nevada, where the water is used primarily for agriculture.

The researchers will analyze historical streamflow trends from the two basins to determine changes in timing and magnitude, and they will use historical weather and water data to assess the impact of temperature on surface water flows. They will also use observational streamflow and climate data to identify the factors in the basins that are affecting these trends. Using this information, they will then examine the connections between streamflow, temperature, precipitation, and snow water equivalent, as well as the interdependencies of hydrometeorological variables and watershed characteristics. The results of this project will be shared with farmers, water managers, and other stakeholders, which will give them data that can be used to make informed decisions and improve community resilience to climate change.

NWRRI GRAD STUDENT INTERVIEW:

Emmanuel Cobbinah

We asked Emmanuel Cobbinah, a third year PhD student in the Graduate Program of Hydrologic Sciences at UNR and DRI who worked on the NWRRI project "Use of Viral and Genetic Marker Surrogates to Assess Transport of Human Enteric Viruses in Water Reclamation Systems," about his current studies and plans for the future. Here's what he had to say:

Photo by Emmanuel Cobbinah

1) What are you currently studying and what sparked your interest in that field?

My research focuses on contaminant transport within hydrological systems. My interest in this field was inspired by a deep commitment to environmental protection, sparked by firsthand exposure to pollution caused by small-scale mining in my home community, Ghana. Through my work, I aim to mitigate potential health and environmental impacts by contributing to sustainable water management solutions and fostering a healthier ecosystem.

2) You worked on the NWRRI project "Use of Viral and Genetic Marker Surrogates to Assess Transport of Human Enteric Viruses in Water Reclamation Systems." What did you learn from your experience?

Working on this project has provided me with invaluable insights and allowed me to develop highly marketable skills, such as conducting viral RNA/DNA extraction and using quantitative polymerase chain reaction (qPCR) for viral enumeration. A significant takeaway from this project was that I gained a deeper understanding of the differing transport behaviors of plant-based viruses compared to pathogenic viruses, specifically adenovirus. The project also highlighted the critical role of interdisciplinary collaboration in tackling public health and environmental challenges, which has further strengthened my commitment to advancing sustainable water treatment solutions.

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>
> **–EMMANUEL COBBINAH**

3) What do you find most interesting about water resources research, particularly working in Nevada?

Working in an arid/semiarid environment like Nevada offers a unique platform to engage in sustainable water resource management. In these

View of Lake Mead from Hoover Dam.

water-scarce regions, ensuring the sustainability of water resources is a top priority. This presents numerous opportunities for me to both contribute meaningfully to working toward this goal and continuing to learn. The challenges inherent in these environments drive innovation and reinforce the importance of developing effective strategies for water conservation and public health protection.

4) What are some of your goals for the next steps in your studies and career?

In the near term, my goal is to investigate the mechanisms of virus transport under varying conditions by integrating experimental and modeling studies. Additionally, I plan to scale up these models for applications in both environmental and industrial contexts.

My long-term aspiration is to become a professor who conducts interdisciplinary, collaborative, and innovative research focused on advancing sustainable water management practices and promoting public health, particularly in arid and semiarid regions and across Africa. I am also deeply committed to mentoring the next generation of scientists, inspiring their interest in the field, and involving them in impactful projects that address critical global water challenges.

5) If you could go on vacation anywhere in the world, where would you go and what would you want to do?

If I could go on vacation anywhere, I would want to go to London, England. I have been a fan of Chelsea Football Club since childhood. Their home stadium is in West London and it would be great to see them play live. Also, I would have the opportunity to visit iconic landmarks such as Buckingham Palace and the Tower of London.

6) What is something that we might not know about you (hobbies, interests, etc.)?

My hobbies are mostly centered around soccer. For fun, I'm usually either playing soccer on the field, playing soccer games on my computer, or watching matches on TV—I rarely do anything else. However, I recently developed an interest in basketball, so I've also started going to the gym to play occasionally.

7) If you were shipwrecked on a deserted island, but all of your human needs were met, what two items would you want to have with you?

I would want a PlayStation 5 and a basketball, no doubt!

NWRRI UNDERGRAD INTERNSHIP INTERVIEW:

Rachel Eves

Rachel Eves participated in the NWRRI Undergraduate Internship Immersion Program in the summer of 2024. She worked on the project "Are Beaches a Source of Litter to Lake Tahoe?" and was mentored by Dr. Monica Arienzo of DRI. The focus of the project was to measure and categorize the litter on Lake Tahoe's beaches in collaboration with ECO-CLEAN Solutions to identify sources of litter in Lake Tahoe. We asked Rachel about her experience during the internship, current research, and plans for the future. Here's what she had to say:

1) What are you currently studying and how did you find out about the internship?

I'm currently working on getting my bachelor's degree in environmental science with a specialization in soil science. I found out about the internship last semester through my geology teacher, Roger Putnam, at TMCC.

2) The project you're working on is "Are Beaches a Source of Litter to Lake Tahoe?" What does this project entail and in what ways are you participating?

This project is about studying how litter on Lake Tahoe beaches can persist in the environment long enough to make it under the top layer of sand, making it more likely to be transported into the lake. Our team has been working closely with ECO-CLEAN Solutions and their BEBOT to collect, categorize, count, and catalog the litter found roughly two inches under the surface of the sand. I've been able to participate in the project by helping sort the trash as well as attending meetings with local government officials, Clean Up The Lake, and The League to Save Lake Tahoe.

3) What have you learned about the presence of litter in Lake Tahoe? How does this research improve our understanding of the sources of litter and the potential effects on freshwater lakes and the surrounding environment?

There's so much more litter than you would expect! Each beach has a different litter personality, giving us clues as to how humans are interacting with the environment. Most of the plastic found under the

Photo by Rachel Eves

> *"In preparation for presenting our findings, there have been so many more questions that have come up. […] One of my favorite things about science is that there is always more to discover!"*
>
> **–RACHEL EVES**

surface has begun to degrade and once those small plastic pieces make their way into the water, they become virtually impossible to remove. Lake Tahoe has a much higher concentration of microplastics than anyone thought it would, in part due to the plastics that get buried in the beach sand.

4) What have you learned so far from your experience on the project? Is there anything you've found particularly interesting or surprising?

My mentors on this project have been absolutely incredible. They have helped me understand how to compile and present data through a creatively scientific lens. I've learned that there are more things that count as litter than I originally thought (for example, pistachio shells), challenging my idea of the kinds of litter that people leave in natural settings.

5) Has participating in this internship given you any ideas for your future studies that you may not have thought about otherwise?

Absolutely! In preparation for presenting our findings, there have been so many more questions that have come up. I wonder how the grain size of the sand on each beach affects the degradation of litter in Lake Tahoe. Another big question has been about the demographics that visit different beaches and how those demographics contribute to the litter on Lake Tahoe's beaches. Yet another question is about how long a piece of litter generally spends on the beach prior to being transported to the water. One of my favorite things about science is that there is always more to discover!

6) What are your goals for the next steps in your studies and what career direction are you pursuing?

I'm looking forward to my first year at UNR and gaining more knowledge about the environment and how to better care for it. I would love to have the opportunity to continue helping with research at DRI and hope to one day be able to help fight climate change through working with soil. I'm considering pursuing a graduate degree in soil science, but I'm trying to take things one step at a time. I'll just have to wait and see!

NWRRI undergraduate interns (from left to right) Sabbathiel Greene, Lauren Broncho, and Rachel Eves sorting litter collected from Lake Tahoe's beaches (photo by DRI Science).

EVENTS

Please keep an eye on the event websites for changes in conference schedules.

Ocean Visions Biennial Summit
March 25–27, 2025; Vancouver, British Columbia
www.agu.org/events/meetings/ocean-visions-2025

Aquifer Testing Workshop
March 25, 2025; Las Vegas, NV
www.nvwra.org/aquifer-testing-workshop

2025 Amargosa Valley Tour
March 27 & 28, 2025; Las Vegas, NV
www.nvwra.org/2025-amargosa-valley-tour

2025 Cordilleran Section Meeting
April 1–4, 2025; Sacramento, CA
www.geosociety.org/GSA/Events/Section_Meetings/GSA/Sections/cd/2025mtg/home.aspx?hkey=88411fd7-3278-41be-aa78-f451032e17f3

AEG Southern Nevada Chapter: "70 Years of Flood Control in the Las Vegas Valley: Historical Floods and the District's Response" by Andrew Trelease
April 8, 2025; Las Vegas, NV
www.aegsnv.org/meetings

AEG Southern Nevada Chapter: Field trip to Tropicana Detention Basin led by Andrew Trelease
April 12, 2025; Las Vegas, NV
www.aegsnv.org/meetings

Webinar: Digital Map Submittal with Jay Dixon
April 16, 2025; Virtual
www.nvwra.org/webinars

2025 Gold Quarry Mine Tour
April 28–29, 2025; Elko, NV
www.nvwra.org/2025-gold-quarry-mine-tour

AWRA 2025 Spring Conference
April 28–30, 2025; Anchorage, AK
www.awra.org/Members/Events_and_Education/Events/2025%20Landing%20Pages/01_SPRING/Spring2025.aspx

21st Annual Truckee River Field Study Course
May 1–2, 2025; Reno, NV
www.nvwra.org/2025-truckee-river-tour

AEG Southern Nevada Chapter: "Threats to Western Water Security as Viewed from Space" by Dr. Jay Famiglietti
May 13, 2025; Las Vegas, NV
www.aegsnv.org/meetings

Borehole Geophysical Logging for Water Resources/ Water Supply Applications Workshop
May 13 & 14, 2025; Reno, NV
www.nvwra.org/borehole-geophysical-logging-workshop-2025

Well Design, Construction & Rehab Workshop
May 15, 2025; Reno, NV
www.nvwra.org/2025-well-design-workshop

UCOWR/NIWR 2025: Water. Place. People
June 3–5, 2025; Minneapolis, MN
ucowr.org/conference/

Sampling Field Methods, Environmental Project Planning and Data Management: A Holistic Approach
June 9–11, 2025; Westerville, OH
www.ngwa.org/detail/event/2025/06/09/default-calendar/25jun226

2025 Legislative Updates
July 23, 2025; Virtual
www.nvwra.org/2025-legislative-updates

People fishing at Pyramid Lake, northeast of Reno, Nevada (photo by Sayantan Majumdar).

Success and dedication to quality research have established DHS at DRI as the Nevada Water Resources Research Institute (NWRRI) under the Water Resources Research Act of 1984 (as amended). The continuing goals of NWRRI are to develop the water sciences knowledge and expertise that support Nevada's water needs, encourage our nation to manage water more responsibly, and train students to become productive professionals. The work conducted through the NWRRI program is funded through the National Institutes for Water Resources (NIWR), which is supported by the U.S. Geological Survey under Grant/Cooperative Agreement No. G21AP10578. DRI administratively houses and logistically supports the operations of NWRRI.

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