Reclaimed Riddle

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USF Assistant Professor Mya Breitbart (left) and graduate student Karyna Rosario gather samples of treated wastewater at a local treatment plant for their on-going studies of viruses that are present in reclaimed water.

(PhysOrg.com) -- It was the "yuck factor" of reclaimed water that got Karyna Rosario thinking. As communities increasingly turn to reclaimed water as a source for irrigation - and some communities consider using it for drinking water - Rosario, a PhD student at USF's College of Marine Science, became increasingly curious about exactly what viruses are present in reclaimed water.

What intrigued her were not the viruses the labs at sewage treatment facilities would know to go look for, but the more obscure or exotic ones that aren't identified in routine testing.

So Rosario waded - quite literally - into the question at water treatment plants in Pinellas and Manatee counties and began cataloging the viruses she found with a novel technique designed to identify a full-range of unknown viruses. Her analysis - which found a full complement of animal, plant and human viruses in wastewater - has been published recently in the journal Environmental Microbiology.

The reassuring news, Rosario reports, is that none of the viruses she found in treated reclaimed water locally are human pathogens, putting to rest the most serious of fears about humans using treated wastewater. But her study, nonetheless, provides an important starting point for future research on viruses which survive the human body and are discharged into reclaimed water and how they might impact the environment when treated wastewater is used, whether for irrigation or drinking water supplies.

"There is a yuck factor when we think of reclaimed water," said Rosario, who conducted her study under the tutelage of USF Professor Mya Breitbart, whose laboratory has used a new technique for identifying previously unknown viruses based on their genetic material (DNA or RNA). "But the reality is we need this alternative water supply and, thus, we need to know what is in the water, including viruses. You say the word 'virus' and people freak out, but not all viruses are dangerous to humans."

Reclaimed water is currently used in Florida for non-potable public water supply, crop irrigation, lawn watering, industrial uses and groundwater recharge. But an increasingly serious drought that has forced government to severely curtail water use has led to some new

considerations for using reclaimed water as a potential source of drinking water. This summer, the Tampa City Council voted to ask residents in a 2010 ballot question to consider whether highly-treated reclaimed water could be returned to the city's drinking water supply.

One of the biggest concerns about reclaimed water use is whether it carries and spreads pathogens, and until recently the microbiological content of reclaimed water was still largely unknown. Viruses are of particular concern because they include highly stable pathogens that can be resistant to standard wastewater treatment processes, Rosario explained, noting that for practical reasons, current quality control methods do not test the presence of pathogens directly and the spread of viral pathogens through reclaimed water remains a real possibility.

In Tampa - where 55 million gallons of treated wastewater a day is discharged into Tampa Bay - the safety of reclaimed water also has become a large environmental concern.

Rosario was already working on her study with scientists at the Genome Institute of Singapore when the focus on Tampa's use of reclaimed water emerged this summer. But her study could provide some preliminary answers to the first question people ask when they consider reclaimed water: Just what is in there?

"This is a population survey," Rosario explained in a recent interview. "From this study you can look at the different pathogens that are found. For example, you can look at the plant pathogens and find out if this is going to be a problem for agriculture?"

The study - titled "Metagenomic Analysis of Viruses in Reclaimed Water" - describes the novel method for identifying previously unknown viruses that's been developed by Breitbart's lab. Samples containing a host of viruses are processed to extract the virus' DNA. The DNA is

sequenced and then compared to existing databases of known DNA genomes to identify the viruses.

The difficulty for scientists, though, is that with millions of types of viruses in existence, there are still many more viruses that have yet to be identified and mapped. The process used in Breitbart's lab also helps to identify never-before-seen viruses.

Rosario compared samples collected from effluent at a reclaimed water plant; reclaimed water coming from a public sprinkler; reclaimed water used at a plant nursery and drinking water from a plant nursery. She found reclaimed water contained 1,000-fold more virus-like particles than potable water and that reclaimed water may play a role in the dissemination of highly-stable viruses.

The viral community was dominated by viruses that infect bacteria, but viruses related to animal, plant, and insect pathogens were also identified. And while many of those viruses are in themselves not human pathogens, she concluded that further studies are needed to evaluate the impacts of reclaimed water use on human and ecosystem health.

Still, the process produced a laundry list of viruses in the reclaimed water samples that reads like a who's who of the microscopic world.

There are interesting viruses that illustrate - however delicately - that viruses survive the human digestive systems, such as the Banana bunchy top virus, the Beet curly top virus and the Squash leaf curl virus. Those were noted at the point-of-discharge at the treatment plant (effluent) and at the point-of-use in a plant nursery, pointing to the "tough" nature of these plant viruses.

In the samples taken from the effluent and nursery irrigation systems ,viruses related to Rhinovirus - the cause of the human cold - were

present, as well as Enterovirus, which is a large and diverse group of viruses which in some forms can cause human maladies, such as meningitis and foot and mouth disease. Similarities to cow, pig and monkey viruses also were identified in the samples.

Luckily for humans, most viruses pass through their bodies without causing any harm. The value, though, of knowing that viruses exist in treated wastewater is if some day there were to be a viral disease outbreak among people or in the agricultural sector and wastewater is a suspected source of the contamination, scientists now have a baseline list of what viruses are present there.

“I am not sure we answered questions as much as we opened the door for a lot more questions,” Rosario said. “That is the beauty of the technique; you find things you were not looking for.”

And there is no easy answer to getting reclaimed water as clean as people might like, she notes.

“In a perfect world, you could combine different disinfection techniques. Technologically, it’s possible to produce sterile water,” she said. “But in the actual world, it’s not practical because it’s expensive and it can create other problems. For example, if you use chemicals like chlorine to disinfect the water, you still create by-products that could be very harmful to the environment.

“In reality we don’t need sterile water because most microorganisms are not a threat; we just need to be able to eliminate the risk of pathogen transport.”

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