NEWS FEATURE

Interested in gauging a population's health? Look to sewage

Researchers are mining the stuff we excrete to get a window on drug use, antibiotic resistance, and the overall health of populations.

John Carey, *Science Writer*

When the Canadian government wanted to assess the impact of legalizing marijuana on its citizens' drug use, it turned to a rich, though often unappreciated, source of data—sewage.

All of the drugs people take journey through the body, and significant amounts end up in the toilet. So since March of last year, researchers have been filling bottles once a month with sewage flowing into wastewater treatment plants in the Canadian cities of Vancouver, Montreal, Edmonton, Toronto, and Halifax. They send the samples to the lab of Viviane Yargeau, chair of McGill University's chemical engineering department. There, Yargeau and her team measure the levels of 18 different drugs or their metabolites, including cannabis and cocaine. "It's a quick way to have a snapshot of what people are consuming," she says.

Once the results are analyzed, that monthly snapshot will enable the government to see how drug use changed after marijuana became legal in October 2018. The results might even help tease out the relative proportions of legal and illegal drug use by comparing sewage levels to sales data. In sampling the output from 8.5 million Canadians every month, the study is the largest effort ever to mine wastewater's plentiful data. "We had to buy a couple more freezers," says Yargeau.

But that work represents only a small part of a surge of interest in analyzing sewage. The aims are generally twofold: gleaning fresh insights into human

The sewage that ends up in treatment plants such as this one may hold important clues about the health of populations. But such plants may also be hotspots for the spread of antibiotic-resistance genes. Image credit: Shutterstock/Daniel Jedzura.

behavior, biology, and health; and opening a new front in the battle to understand and combat antibiotic resistance. In 2013, health authorities in Israel successfully quashed a potential polio outbreak by stepping up vaccinations after detecting the virus in wastewater before anyone developed symptoms. In March 2018, city workers in Cary, NC, began lowering robots into manholes to get real-time insights into the extent and geography of opioid addiction. And elsewhere in the United States, researchers have been able to distinguish cities with healthier populations from those with more obese individuals by identifying microbes in wastewater. "We showed pretty clearly that sewage did accurately represent human gut communities," says microbiologist Ryan Newton at the University of Wisconsin-Milwaukee.

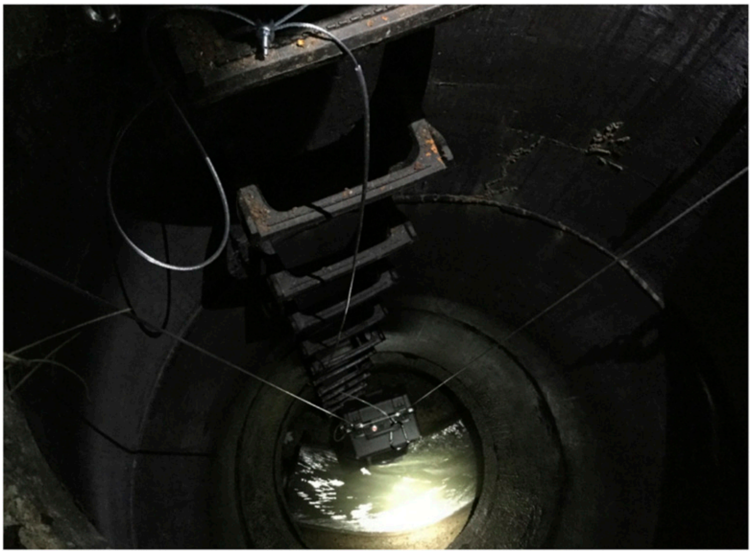
On the antibiotic-resistance front, samples taken from cities all over the world show that resistant bacteria—and resistance genes—survive and persist in sewage, raising fears that wastewater could be fueling the increase of resistance. "It's only logical," says Amy Pruden, a civil and environmental engineer at Virginia Tech in Blacksburg. "Everything we are flushing down the drain is important—it's where the antibiotics and pathogens all end up."

The CDC is now funding studies to understand the size of the threat. "We want to know the levels of antibiotic-resistance genes going into wastewater treatment plants and how effectively they are being removed," explains microbiologist and epidemiologist Amy Kirby, a Senior Service Fellow in CDC's Waterborne Disease Prevention Branch and manager of the effort.

Community-Wide Urinalysis

For environmental chemist Christian Daughton, the growing attention on sewage is long overdue. Daughton kick-started the notion of mining sewage for health indicators and population data back in the 1990s while at the US Environmental Protection Agency's (EPA's) National Exposure Research Laboratory. At the time, the EPA focused on so-called priority water pollutants such as pesticides, solvents, and heavy metals, recalls Daughton. "I was frustrated that no one had stopped to consider that the chemicals humans use in everyday activities, such as drugs, make their way to sewage, and then to the environment," he explains. His landmark 1999 article (1) "alerted the scientific world that we need to be paying more attention to drug residues in the environment," he says. At first, he concentrated on pharmaceuticals. For a 2001 book (2), however, Daughton also described how monitoring for illegal substances and their metabolites could offer a new window into drug use.

European countries quickly took notice. In the mid-2000s, a group in Italy tested the hypothesis that sewage was an effective monitoring tool. The researchers showed that levels of illicit drugs in wastewater roughly match data from surveys of drug use. More recently, a consortium of academic laboratories called SCORE (Sewage Analysis CORe group Europe) has been analyzing wastewater from 56 cities in 19 European countries. "They use it to not just figure out consumption rates, but also as an early warning of new drugs that have entered the market," explains Daughton. "It's essentially a way to do a community-wide urinalysis."



To more easily sample sewage from multiple locations before it reaches a treatment plant, MIT scientists designed sophisticated robots that can be lowered into manholes dotted throughout a community. Image credit: Biobot Analytics.

Recent SCORE results show that cocaine use is much higher in cities in Belgium, The Netherlands, Spain, and the United Kingdom than in eastern European cities, for instance, and that cocaine and MDMA (also known as ecstasy) levels jump during weekends in most cities (3), providing key data for European Union drug reports (4). A next step, researchers hope, will be using the data to help guide policy decisions. "One of the strengths of the methodology is quickly assessing if an intervention has an effect on substance abuse," explains Alexander van Nuijs, a pharmaceutical scientist at the University of Antwerp in Belgium.

In Canada, McGill's Yargeau was also paying attention. Intrigued by the Italian study, she began in 2008 to look in wastewater for a wide range of drugs and chemicals that people ingest or use, such as painkillers, caffeine, and silver nanoparticles from wound dressings and sanitary products. "The biggest surprise is that every time we thought about something we could look for, we always could find it," she says. Impressed, Statistics Canada, the government's statistics agency, enlisted Yargeau's laboratory to help monitor the effects of marijuana legalization.

Sewage-Sampling Robots

The United States was slower to read the tales in sewage. In the mid-2000s, the US White House Office of National Drug Control Policy (ONDCP) was struggling to get a handle on substance abuse by using surveys and estimates of cocaine and heroin production in countries such as Colombia and Afghanistan. Then, ONDCP officials heard about Daughton's "crazy idea to look for the metabolite of cocaine, benzoylecgonine, in wastewater," recalls David Murray, ONDCP's chief scientist at the time. The idea offered what Murray calls "the holy grail" of drug policy—an objective measure of consumption. He started a pilot in 2006, measuring benzoylecgonine in the wastewater of dozens of cities. "It was a good idea that might have produced benefits," says Murray, now a senior fellow at the Hudson Institute and codirector of the Center for Substance Abuse Policy Research. But before there were any firm results, the project was quashed amid misguided privacy concerns. Such sewage research languished in the United States for several years.

No longer. In 2013, for example, MIT biological engineer Eric Alm and architect and engineer Carlo Ratti, who directs the Senseable City Lab in MIT's urban studies and planning department, figured that sewage could help cities make smarter decisions by spotting the precise locations of disease outbreaks or other changes in health. "Wastewater is very rich source of information that's not being mined in the way other data streams are mined," explains Alm.

In most sewage surveillance efforts, like Yargeau's Canadian drug study, researchers collect samples of the wastewater flowing into treatment plants, which aggregates what's happening across an entire city. But pinpointing where a new case of typhoid or a new illegal drug might pop up requires sampling in several or many locations. So does spotting substances that degrade before they reach the treatment plant.

"In the next 5 to 10 years, I can't imagine that any major city will not incorporate sewage data into their decision-making."

—Eric Alm

That's why the MIT scientists, in an interdisciplinary project dubbed "Underworlds," designed sophisticated sewage-sampling robots that can be lowered into manholes dotted throughout a community. The robots filter out solids and bacteria. Then they pump the water through cartridges that act like a chromatography column to capture small molecules, such as illicit drugs and their metabolites, before expelling the water back to the sewer. The robots can continuously sample up to a day's worth of sewage at one-tenth of the cost of the standard method of bottling samples of unfiltered wastewater, explains Mariana Matus, who helped develop the robot as an MIT graduate student.

The team decided to target opioids, although it wasn't easy. "Many of the metabolites break down quickly, so the sampling has to be done within four hours after being flushed into the sewers," explains Matus. To determine where to probe wastewater in Cambridge, MA, she and her colleagues mapped the sewage system, including the rate of flow. They calculated that the sampling spots needed to be within four hours of sewage travel from every toilet flush. And to guarantee that it would be impossible to trace a drug or virus back to a specific house or apartment building, they needed each sampling manhole to represent at least 4,000 people. Cambridge is a dense city, so the MIT team is able to probe the entire city with just three manholes or identify neighborhood trends with seven manholes.

Believing that the technology could help numerous cities improve public health, Matus and colleague Newsha Ghaeli cofounded Biobot Analytics to commercialize the approach. In March 2018, they won a competition earning them the opportunity to present their idea at the US Conference of Mayors in June.

Even before then, the company had started its first pilot project in Cary. After a 70% increase in opioid overdoses in 2017 over the previous year, the city formed an opioid task force and was looking for answers about where and why drug use was increasing—and how to fight the growing problem. City officials found articles describing the MIT Underworlds project and contacted Matus. The Biobot approach was new and especially attractive compared with the existing efforts in Europe because it could spot trends within individual sections of a city, explains Mike Bajorek, then Cary's deputy city manager. The city was anxious to give it a try. "If we have the opportunity to make a difference, why wouldn't we want to do it?" says Bajorek.

Biobot first modeled Cary's sewage system, showing that most of the city could be monitored from 10 manhole locations. Then, starting in March, city workers collected samples for 1 day per month for 12 weeks at each site and analyzed them for metabolites of morphine, codeine, oxycodone, heroin, fentanyl, and other drugs, including the overdose-reversal drug naloxone. The first results have been "pretty impressive," says Bajorek. The sampling found metabolites of both opioids and naloxone in every location, "even areas where we had no recorded instances of overdoses," he says. That indicates that private citizens, not just first responders, are using naloxone to rescue people from overdoses. And overall, "the information enabled us to successfully start an extensive conversation about drug misuses and abuse, and what can be done about it," says Bajorek, who retired from his city post in early 2019.

Meanwhile, Biobot's team has worked in South Korea and the Middle East and has talked with about 100 more municipalities in the United States about new efforts, with projects expected to begin in several cities in late spring of 2019. "I now know what sewage smells like across three continents," quips cofounder Ghaeli.

But perhaps just as promising as monitoring drugs will be assessing the health of populations. "Our work on illicit drugs has been a springboard," says Malcolm Reid, a research manager in environmental chemistry at the Norwegian Institute for Water Research, who works with SCORE. "But I think the greatest value of this technique will be gained in other fields," he says, such as public and environmental health.

In one case, researchers measured bacteria from human guts to learn about the health of the people flushing their toilets. Collecting wastewater from 71 cities across the United States, Wisconsin's Newton found that the sewage of some cities, such as Steamboat Springs, CO, and Palo Alto, CA, had the signature microbiomes of thinner people, with higher amounts of *Faecalibacterium* species and lower levels of *Bacteroides* species. In contrast, cities such as Milwaukee and municipalities in Alabama had the opposite bacterial proportions—and a higher percentage of obese people (5). The results suggest that sewage could be used to see other measures of health, once studies of human gut microbes identify relevant biomarkers, Newton says.

Antibiotic-Resistance Hotspots

More urgent, though, is a different sort of public health concern—discerning whether sewage is fueling the spread of antibiotic resistance.

Public health officials have known for decades that overprescribing antibiotics and administering them widely to livestock have led to an alarming increase in the number and type of drug-resistant bacteria. But even after the US Food and Drug Administration banned the use of a common class of antibiotics used to treat people, fluoroquinolones, in poultry production in 2005, levels of bacteria resistant to those drugs didn't decline, says the CDC's Kirby. That and other evidence have "shown us that we are missing a link in the resistance story," she says.

That missing link is the environment, with wastewater being a key factor. When people take antibiotics, a full 30 to 90% of the active chemical substance passes through the body intact. It's excreted in the urine and is therefore flushed into the teeming communities of bacteria in wastewater. Moreover, sewage treatment plants, says Kirby, have all three conditions necessary to stimulate gene transfer—lots of biofilms, where bacteria like to swap genes; high environmental stresses, such as rapid changes in temperature and pH that are known to cause bacteria to exchange genes as a strategy for surviving the stresses; and plenty of ruptured bacteria, which release intact genes. As a result, "we think sewage treatment plants will be hotspots where resistance genes move around," says Kirby.

In fact, antibiotic-resistant bacteria (and resistance genes) are popping up just about everywhere scientists look. In one of the first studies, Pruden measured resistance genes in the Poudre River using quantitative PCR when she was a professor at Colorado State University in the mid-2000s. Levels of the genes soared in two places: downstream of livestock operations and downstream from water treatment facilities (6). "We were pretty surprised how striking the relationship was," Pruden says.

Since then, researchers have similarly found genes in sewage that convey resistance to antibiotics such as tetracyclines, sulfonamides, and fluoroquinolones—and in the outflow from treatment plants—in cities all around the world, from Romania and Peru to China and India. And in early 2018, environmental engineer Barth Smets of the Technical University of Denmark was able to show that bacteria in activated sludge easily shared plasmids containing antibiotic-resistance genes (7). "The evidence is growing that antibiotic resistance is being maintained in the environment, and that just controlling the use of antibiotics in the clinic or in agriculture won't be enough," says Kirby.

How much of a threat does antibiotic resistance in wastewater pose? The question is becoming even more urgent as water-limited areas of the United States and the world increasingly reuse treated wastewater for irrigation, for recharging aquifers, or even for drinking supplies. There are no good answers yet, but the CDC aims to find out as part of the US National Action Plan for Combating Antibiotic-Resistant Bacteria, an effort launched in 2015 by presidential executive order (8). The agency is funding research by Virginia Tech's Pruden and others to measure levels of resistant bacteria and resistance genes flowing into treatment plants—and how effectively the facilities remove those bugs and genes. Those treatment processes could then be enhanced to keep resistance from leaving the facility. "We can remove pretty much any pathogen and bacteria if we want to spend enough money," says Pruden. The challenge is finding the most cost-effective methods. "The basic aim is to put up multiple barriers from the toilet to the environment or to the use of water for our homes and for recreation," she says.

Such potentially insightful work suggests sewage sleuthing is poised for rapid growth. "In the next 5 to 10 years, I can't imagine that any major city will not incorporate sewage data into their decision-making," says MIT's Alm. "All the chemistry and biology is there in the wastewater."

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