



## Wastewater - A Tool to Better Understand and Manage Infectious Disease

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#### Abstract

In light of the COVID-19 pandemic around the globe, scientists have been paying extended attention to the virus transmission through wastewater collection and treatment. Pathogens could enter urban sewage systems from human waste, animal farming, and hospital effluents. It has become crucial to assess the potential exposure and transmission of viruses like SARS-CoV-2 through wastewater systems. Successful strategies to control the spread of this pathogen rely on effective surveillance. As viruses have the potential to adsorb on surfaces with certain pH, the interaction between SARS-CoV-2 and solid particles implies their behaviour in sewage sludge and their concentration by water treatment. In the study, we investigate the current methodologies used to monitor and analyze the statistics of SARS-CoV-2 in wastewater. To understand the dynamics of SARS-CoV-2 outbreaks, risk analysis and modelling in the research are significant.

# Keywords

COVID-19, wastewater surveillance, treatment methods

# Introduction

For the past three years, the world has been facing health threats from SARS-CoV-2, which have resulted in a global pandemic. Starting with the outbreak in December 2019 reported in China, the SARS-CoV-2 spread rapidly, with almost 600 million confirmed cases and more than 6 million deaths by 26th August 2022 [4]. With the emergence of the pathogen, researchers around the world have been delving into the field of effective control strategies for SARS-CoV-2. Although it was difficult to halt the rapid spread of the highly contagious SARS-CoV-2, various methods of surveillance have been developed to monitor the spread of the pathogen. Due to the diverse variants of the virus, monitoring individual symptoms and receiving screening in a clinical environment have been challenging at such a large population scale. It is also impossible to activate testing for the entire population due to resource and economic constraints. Therefore, there is an urgent demand to develop effective epidemiological models that effectively assess the prevalence of SARS-CoV-2 at a community level. Research has been done to comprehend the spread of the virus to provide more information that can mitigate outbreaks in a community.

Wastewater Surveillance System is one method to assess the impact of SARS-CoV-2 in a community. Although the main route of virus transmission was through inhalation by interaction with people, there is a valuable potential to discover the role of wastewater as a potential source of epidemiological data. Wastewater analysis could provide an overview of the prevalence of SARS-CoV-2 in populations. Wastewater, in terms of this review, is defined as all the sewage generated from human activities and industries. Coming from various sources, the wastewater will enter the sewerage system and be conveyed to treatment plants. Subsequently, the water will undergo a series of treatments and be discharged into the environment. Wastewater-based epidemiology (WBE) monitoring can guide the protection of public health and epidemiological surveillance. In the past, this technology has been a tool in mitigating infectious diseases like the Global Polio Eradication Initiative [9]. Considering the current epidemic situation created by SARS-CoV-2, appropriate measures need to be taken to continue closely monitoring the prevalence of the pathogen. Taking into the consideration of more future variants of SARS-CoV-2 and more waves, a wastewater surveillance system would be an effective method to trace and track the spread of SARS-CoV-2 in the population.





Research has been conducted to testify to the persistence of viruses in the aquatic environment. It has been proven that the genetic material RNA of SARS-CoV-2 has been discovered in the feces of patients, but currently, no data proved that SARS-CoV-2 in wastewater is a possible transmission method of the virus [11]. As an enveloped virus, SARS-CoV-2 rapidly inactivates without a host [7]. Consequently, tracking periodic changes in viral concentrations in aquatic environments is a feasible method to determine the presence of the virus, the scale of related outbreaks in the population, and its effect on public health. Gaining more attention from researchers, domestic wastewater surveillance systems became an important way to monitor the development of SARS-CoV-2 in a population. With a focus on SARS-CoV-2, this literature review provides a detailed assessment of the effectiveness and recent usage of wastewater surveillance systems in Ontario to determine public health, along with a future portrait of disease surveillance systems.

### Analysis

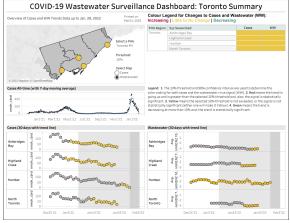
Wastewater testing has been and continues to be a useful tool for the surveillance of SARS-CoV-2 and its variants. Wastewater testing has helped guide the decisions of public health officials regarding SARS-CoV-2 and supplement public health officials on types of diseases virally reaching local communities (Manuel et al., 2021). Ottawa Public Health used wastewater testing to support the identification of new COVID-19 surges with early notification to public health and the general population through the use of social media, news conferences, and public reporting. All thirty-four of Ontario's public health units have also signed onto Ontario's Wastewater Surveillance Initiative, where the province, researchers, and public health units work together in testing wastewater samples (Chandler, 2022). At the time of August 26, 2021, five Ontario municipalities publicly reported wastewater test results and three other municipalities included the wastewater results in their regular public briefings (Manuel et al., 2021).

The process of wastewater testing is efficient as it is capable of providing data quicker than the data from collecting and testing individual samples. It can take up to fourteen days for symptoms of COVID-19 to show up, with some cases showing little to no symptoms. Wastewater testing can identify positive cases before those fourteen days and can identify cases where individuals do not experience any symptoms [12]. There is also little to no difference in stool shedding based on the severity of the virus. In Northern Italy, the first SARS-CoV-2 positive wastewater samples were found weeks before the first imported COVID-19 cases were reported. After positive wastewater tests in Yellowknife, Northwest Territories in December of 2020 recent travellers were encouraged to be tested and clinical testing identified five cases of COVID-19 in recent travellers who were at the end of their self-isolation period. Wastewater testing was implemented at residential housing for approximately five thousand students at the University of Arizona in August of 2020 [12]. When SARS-CoV-2 RNA was detected in one of the dormitory's wastewater samples further clinical testing of all building residents found 2 positive asymptomatic cases who were then isolated which resulted in no further transmission. Without the use of wastewater testing these cases might have been identified later and could have potentially infected others. Wastewater testing is also able to sample a wide population or populations which are underserved and vulnerable as well as access settings where testing is limited [12]. As of August 26, 2021, wastewater testing has been performed in seventy-seven upstream locations in Ontario, this includes nineteen university campus locations, five retirement homes, four longterm care homes, three correctional facilities, three shelters, and two hospitals, and community-level testing in various Indigenous communities [12].

Wastewater testing has been used alongside reported cases in the surveillance of SARS-CoV-2 due to wastewater testing's ability to identify if the overall virus is increasing or decreasing in a community. The City of Toronto's report of COVID-19 surveillance from February 1, 2022, showed that surveillance based on cases reported within the last thirty days and wastewater surveillance within the last thirty days both showed that the Ashbridges Bay, Highland Creek, Humber, and North Toronto region all had trends that were similar to the previous month (Figure 1) [3].

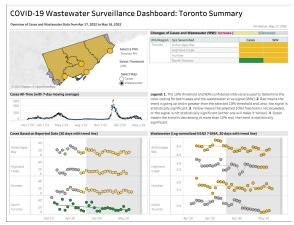






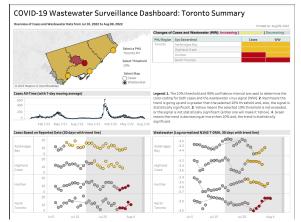
February 1, 2022 COVID-19 Wastewater Surveillance Dashboard: Toronto Summary (Figure 1).

The City of Toronto's report of COVID-19 surveillance from May 17, 2022, showed that based on both reported cases and wastewater surveillance the Ashbridges Bay, Highland Creek, and Humber region had trends that were similar to the previous month and the only variation between cases reported and wastewater surveillance data was for the North Toronto region where, with cases reported, showed that trends were going down while, with wastewater surveillance, showed that trends were similar to the month before (Figure 2) [3].



May 17, 2022 COVID-19 Surveillance Dashboard: Toronto Summary (Figure 2).

The City of Toronto's report of COVID-19 surveillance from August 9, 2022, showed that based on both reported cases and wastewater surveillance the Ashbridges Bay, Highland Creek, and North Toronto regions had similar results with the data (Figure 3) [3].



August 9, 2022 COVID-19 Surveillance Dashboard: Toronto Summary (Figure 3)

The Humber region had variations between cases reported and wastewater surveillance where, with cases reported, showed that trends were increasing from the previous month, whereas with wastewater surveillance showed trends were similar to the month before. The data from cases reported and the data from wastewater surveillance are generally similar, however, there are variations within the reports [3].

# Discussion

Mark Servos, a research advisor with the COVID-19 Wastewater Coalition and a biology professor for the university of Waterloo stated, "Now that the clinical testing is overwhelmed, wastewater is this reliable tool that is continuing to run in parallel that we can now use [2]." Public health officials watch for sustained increasing levels of the virus in wastewater and use this data to provide insight into public health decisions and an impactful response to COVID-19; however, this data transcends the singular impact of influencing public health actions. Bringing us to our question: "How are wastewater data and wastewater systems tools to help manage and understand COVID-19 in Ontario?"

#### **Collaborative Tool**

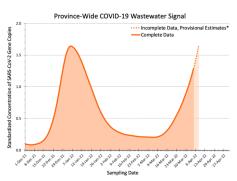
Wastewater Surveillance Systems have been re-established with a means to be a stabilizing tool for COVID-19. However, this data encourages further collaboration with other coronavirus regulating methods; it works in tandem with other methods in efforts to accurately trace COVID-19. Dr. Lawrence





Goodridge, a team leader for Ontario's wastewater surveillance initiative reported to CTV News Toronto, "I like to say that wastewater testing was never meant to replace clinical testing," he said. "It's supposed to be complimentary. It's supposed to be another data point [5]." Wastewater surveillance data should not be an independent action solely affecting public health actions; as it excludes several factors such as, social and behavioural factors.





Ontario's COVID-19 Science Advisory Table (Figure 4) https://toronto.ctvnews.ca/what-is-wastewater-actuallytelling-us-about-covid-19-in-ontario-1.5859978

Wastewater testing is another tracking point, it is a complementary strategy alongside clinical testing. Both are effective, and one can not replace the other. Wastewater data accurately determines virus concentrations on a population level, disregarding the calculation of the number of individuals sick with COVID-19. Advantageously, variants of COVID-19 can be identified, recognizing dominants in populations. For example, in January, Delta viral infections, including multiple other sub-variants, were the most concentrated. These sub-variants originated in different regions of the world, so wastewater data gives an indication where people have travelled: situations like these have an influence on public health decisions. According to Figure 4, concentrations of COVID-19 signals in wastewater decreased in February into early March, before taking on a huge spike. It is speculated to have been caused by alterations in public messaging and the lifting of mask mandate in Ontario, giving feedback on future public health actions, including the occupancy of hospitalizations [6]. Wastewater surveillance has been immensely helpful exposing COVID-19 trends in collaboration with other testing methods; however it's capacities do not end here. This long-established

method is multi-purposed and wastewater surveillance will benefit future issues.

#### Acts As a Multi-Purposed Method

Wastewater surveillance systems are tools that are only a step in the COVID-19 identification process; nonetheless, this deep-rooted process has been used to investigate past issues, and will be beneficial in scrutinizing future diseases. Generally, wastewater is collected to expose food borne pathogens, such as Listeria and E. coli, and monitor pollutants, for example mercury: this is demonstrating that wastewater surveillance systems are not restricted to only producing COVID-19 data. Furthermore, wastewater testing has also been used to detect illicit drug use and viruses such as poliovirus in populations. Surveillance systems of poliovirus continue to persist, but the focus was more relevant during it's peak (1960s).

#### Past use of WWSS: Poliomyelitis virus

In the 1960s, scientists tracked wastewater contaminants, their primary concern was directed towards the poliomyelitis virus, as it was a viral debilitating and life-threatening disease. Testing sewage for poliovirus RNA was 4-5 times more sensitive in detecting outbreaks than monitoring communities for an increase in cases of acute flaccid paralysis. This sensitive detection allows entire communities to be continuously monitored with attentive care. Whenever poliovirus was identified in wastewater, mop-up vaccination campaigns was one of the many strategies that prevented new cases of paralysis. Meaning, when limited poliovirus cases settled, door-to-door immunization ("mopping up") took place in high-risk districts where the virus was known or suspected to be circulating [10].

# Similarities Between Past Diseases with COVID-19

Poliomyelitis contaminates mainly by the fecal bacteria-oral route, but also through aerosols. Similarly, COVID-19 propagates through respiratory droplets; nonetheless, SAR-CoV-2 RNA can still be detected in human stool samples. SARS coronavirus (SARS-CoV-1 RNA) epidemic of 2003 is often compared with COVID-19, a disease caused by SARS-CoV-2. Both causative pathogens are positive-sense RNA





viruses, they belong to the family of Coronaviridae, severe respiratory diseases. The family of Coronaviridae share the common aspect that SARS and COVID-19 infect lung alveolar epithelial cells, penetrating the cells through receptor-mediated endocytosis via the angiotensin-converting enzyme II (ACE2), it's entry receptor [1]. The rapid, unprecedented development of the COVID-19 pandemic requires comparisons with previous epidemics, analysis of infection trends and, based off of observations, findings of the most effective prevention measures, as was done in the past for similar cases.

#### **Future Advancement**

Although, in the present, this surveillance has the main responsibility of detecting COVID-19, it has the potential to help prevent future outbreaks of diseases. However, room for improvement remains. More research needs to be directed towards the conversion of viral RNA concentrations in wastewater to disease prevalence in a community. This is because the biological variability in viral RNA is difficult for reliable estimation. The variability is caused by changes in sewer systems, across communities, mainly due to size, configuration, and influence of stormwater and industrial waste [10]. Nonetheless, longitudinal trends of SARS-CoV-2 RNA levels in wastewater continue to support traditional surveillance methods to understand communal trends in transmission.

#### **Building of Future Method**

Perceiving present strategies can impact scientists' next steps. Scientists collect daily wastewater samples from sludge, the solids that settle during the first processes of municipal wastewater treatment. They directly separate nucleic acids from small volumes of mixed sludge samples and undergo reverse transcription quantitative PCR, with a purpose to quantify the multiple gene targets of SARs-CoV-2. The RNA was detected in all collected samples, with concentrations ranging from 1.7 x 103 to 4.6 x 105 virus RNA copies per mL of primary sludge [10]. It is evident that coronaviruses have an attraction for wastewater solids. These observations propose future surveillance methods for scientists. For example, sludge monitoring may contribute greater sensitivity when compared with wastewater influent monitoring. At the same time, scientists must consider the variables, such as diverse transmission dynamics, diverse geographic areas and different sludge collection systems at wastewater treatment plants.

#### **Complexity of Limiting Factors**

Mark Servos, a research advisor with the COVID-19 Wastewater Coalition of Canada, likens building wastewater testing capacity in Ontario to "building an airplane while trying to fly it." He communicates that creating mechanisms to share information, equipment, and expertise requires time and energy [2]. The Ontario Science Table's report, published in August of 2021, states: "There is a need for improved quality assurance between analytical methods, as well as consistency in data interpretation within local context [2]." There is still a lot more to apprehend about wastewater testing. As other factors change in the population, we learn more about wastewater testing, including the proportion of the population vaccinated and the circulating virus strains.

Wastewater testing can provide trend data that complements other surveillance data to help public health decisions and actions. However, there are limiting factors in these systems which affect collected data on COVID-19 rates.

#### Limitation: Disconnection Between Systems

Limitations include the inability to test water systems that are not connected to a wastewater system. Wastewater testing includes entire populations serviced by the sample wastewater infrastructure. In order to accurately illustrate COVID-19 trends in communities, all citizens need to be involved. However, community-level wastewater surveillance at a treatment plant will not capture homes on a septic system. There are approximately 1.2 million septic systems in Ontario and this has a detrimental affect on the accuracy of wastewater data. Additionally, wastewater surveillance systems will not often observe facilities using decentralized systems: such as, prisons, universities, or hospitals. Geographically, infection trends within the community interacts with their sewer shed. Sewer sheds with largely transient populations, for example, with high tourism, may provide less stable signals, and a factor that should be recognized when collecting data for a specific region [14]. Overall, one main aspect that causes wastewater data instability is the disconnection be-

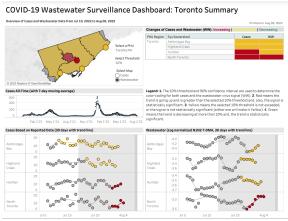




tween Ontario's population and different wastewater systems with wastewater data collection.

#### Limitation: Weak COVID-19 Strands

A major limitation is wastewater surveillance systems' inability to identify weak COVID-19 signals. The detection of SARS-CoV-2 in wastewater is influenced by the sensitivity of the test being used, and the amount of SARS-CoV-2 being shed by the community. If SARS-CoV-2 is not detected in wastewater, either there is no SARS-CoV-2 in the sampled community or the concentration of virus in wastewater is below the level the test has the ability to detect.



Statistical trends drawn from Toronto's COVID-19 wastewater surveillance systems. (Figure 5) https://www.toronto.ca/wp-content/uploads/2022/04/9501-

2022-04-19d-Toronto-PHU.pdf

Referencing Figure 5, from July 10, 2022 to August 08, 2022, reported cases started to rise in Humber and North Toronto, Ashbridges Bay and Highland Creek had little to no change in cases. According to wastewater data, it predicted an increase in North Toronto cases. During this time period, the mask mandate had already been lifted during March of 2022. The increase in cases only definitively changed late July, conveying that the North Toronto region is the more easily influenced; however the effects of public health actions have a delayed impact on Ontario; proving why early warning signals from wastewater surveillance systems, are effective. Also, the wastewater data did not capture the increasing rate of COVID-19 in Humber. Based on the cases reported, there was now a sudden spike, whereas wastewater signals captured the trend as gradual and low. This shows to have had a weak influence on

wastewater surveillance levels, and it was not dramatic enough to cause disruptance in COVID-19 wastewater rates. More research is yet to be done in order to find out the minimum number of infected individuals for the tests to detect a viral RNA signal in the wastewater.

#### **Several Other Limiting Factors**

The COVID-19 pandemic has resulted in rapid expansion of wastewater testing; however, multiple factors influence the quantity of viral material in wastewater and thus, the quantity of reported RNA measured in a given testing site and between testing sites. The overall amount of RNA excreted in stools vary depending on age, fecal excretion patterns, and other characteristics. In terms of limitations, there are variations in the transit time and quantity of RNA that remains intact between an individual's stool and the sample collection point. These variations are due to a number of factors which include the variation in the size and design of sewer shed, and the fragility of the viral RNA [12].

#### Limitation: Environmental Persistence

Environmental persistence refers to the length of time a pathogen, such as the SARS CoV-2, is able to survive outside the human body: the longer it survives, the more likely it is to cause infection. For the most part, investigations on the fate of viruses in the aquatic environment have focused on non-enveloped enteric viruses, given that these viruses are characterized by high resistance under a variety of environmental conditions [11]. The persistence of viruses can be affected both by the type of environment, and by the physical and chemical properties of the environment. COVID-19 is capable of airborne transmission, meaning the virus is viable in the air from several minutes to hours. Research has also shown that humidity levels influence the infectivity of COVID-19. For example, in 50% humidity there is an instant loss of infectivity in 50-60% of the virus. Whereas, 90% humidity sustained the virus' infectiousness for 2 minutes before degenerating [18].

#### Benefits

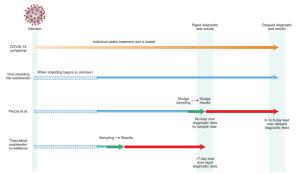
Nonetheless, wastewater surveillance data can help state, tribal, local, and territorial health departments approach the COVID-19 pandemic. Beneficially, wastewater surveillance lacks biases existing in tra-





ditional epidemiological indicators used to determine where disease transmissions are occurring, increasing, or decreasing. Also, these surveillance systems are a cost-effective approach to survey transmission dynamics of entire communities. For example, wastewater data is collected for communities where consecutive COVID-19 clinical testing is under-utilized or unavailable.

Wastewater surveillance systems are also a signal on the emergence or reemergence of infections within a region before case reporting takes place. Wastewater testing has been shown to identify SARS-CoV-2 RNA before laboratory-based clinical confirmation of COVID-19. In Northern Italy, the first SARS-CoV-2 positive wastewater samples, which were found weeks before the first imported COVID-19 cases, were reported. Studies from the Netherlands show a correlation between the concentration of viral RNA copies in wastewater and the number of active COVID-19 cases reported in six cities of March 2020. Canadian reports of SARS-CoV-2 detection through wastewater testing, occurred before case detection through clinical testing in Yellowknife, Northwest Territories, December of 2020 [12]. These are international second-hand instances of wastewater surveillance systems having the ability to provide early detection signals in comparison to COVID-19 case reports.



Observed and theoretical time delays between infection and detection of SARS-CoV-2 transmission in wastewater and the health system (Figure 6). <u>https://</u> www.nature.com/articles/s41587-020-0690-1

Overall, wastewater testing for SARS-CoV-2 is a rapidly developing surveillance approach. Evidence of the effectiveness of wastewater testing in informing public health action is accumulating. There is an increasing number of case reports where wastewater testing has been involved in inaugurating public health policies. There are also fewer resources and clinical staff for wastewater testing, compared to clinical testing. It is important to consider low-resource settings, as it lacks numerous staff wages, and less costly equipment is acquired. As COVID-19 becomes endemic, wastewater testing will be useful for the ongoing surveillance of low-risk populations. The science should constantly be evolving and finding room for improvement. All the while limitations are being pushed and acknowledged, advanced sources of data, such as wastewater surveillance systems, are encouraging safe practices in communities and promoting all-encompassing awareness.

# Conclusion

Wastewater testing has been used to detect past diseases such as polio and SARS and is currently used for the surveillance of SARS-CoV-2. While the diseases were all different from each other, they all could be detected within stool samples [10]. Wastewater testing does come with its weaknesses; due to the way that the samples are collected, samples can end up being diluted and samples can vary [10]. This causes the data that comes from wastewater testing to be inaccurate due to the variations of the samples. In Toronto, wastewater testing data has been compared with data from cases reported to represent the trends of SARS-CoV-2 in the city, though the data from wastewater testing and cases reported are frequently similar, there are inconsistencies between the two [3].

In Ontario, data that is collected from wastewater testing has been used by public health officials to guide future actions and all thirty-four of Ontario's public health units have signed onto Ontario's Wastewater Surveillance Initiative [2]. Wastewater testing has been able to provide testing for those in high-risk residential settings and those in vulnerable communities or communities that lack access to clinical testing. In Ontario, wastewater testing is performed in seventy-seven upstream locations which include nineteen university campus locations, five retirement homes, four long-term care facilities, three correctional facilities, three shelters, two hospitals, as well as various Indigenous communities [12].

Wastewater testing is also more efficient compared to clinical testing. Given that it takes up to fourteen days to show symptoms and some cases are asymptomatic or mildly symptomatic, cases can be identi-





fied later or not identified at all by clinical testing, with wastewater testing positive cases can be picked up before those fourteen days even if those cases are showing little to no symptoms [12]. In Yellowknife, Northwest Territories, after positive wastewater tests recent travellers were encouraged to get clinical tests and from that five cases were able to be identified [12].

Wastewater testing has shown to be a useful tool, given that its process is efficient and can identify cases that show little to no symptoms and can provide testing for those in communities that are in high-risk or vulnerable settings, not only in the context of SARS-CoV-2 but also in the context of past virus outbreaks [10]. However, due to the variations that come from samples which leads to inaccuracy in data, as shown in the reports from the City of Toronto, and that we cannot currently directly link viral RNA in wastewater to the prevalence of SARS-CoV-2 in communities, wastewater testing is a tool that should be used alongside other methods of surveillance of SARS-CoV-2 and not as a replacement for clinical testing [3]. This way wastewater testing is still able to be utilized and, due to the fact that it is being used with other methods, the weaknesses of wastewater testing are not as large of an issue.

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