



Passive Samplers, an Important Tool for Continuous Monitoring of the COVID-19 Pandemic

Albert Z. Jiang¹ · Fulin Nian² · Han Chen³ · Edward A. McBean¹

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Abstract

The global pandemic caused by COVID-19 has resulted in major costs around the world, costs with dimensions in every aspect, from peoples' daily living to the global economy. As the pandemic progresses, the virus evolves, and more vaccines become available, and the 'battle against the virus' continues. As part of the battle, Wastewater-Based Epidemiology (WBE) technologies are being widely deployed in essential roles for SARS-CoV-2 detection and monitoring. While focusing on demonstrating the advantages of passive samplers as a tool in WBE, this review provides a holistic view of the current WBE applications in monitoring SARS-CoV-2 with the integration of the most up-to-date data. A novel scenario example based on a recent Nanjing (China) outbreak in July 2021 is used to illustrate the potential benefits of using passive samplers to monitor COVID-19 and to facilitate effective control of future major outbreaks. The presented contents and how the application of passive samplers indicates that this technology can be beneficial at different levels, varying from building to community to regional. Countries and regions that have the pandemic well under control or have low positive case occurrences have the potential to significantly benefit from deploying passive samplers as a measure to identify and suppress outbreaks.

Keywords Passive sampler · Wastewater monitoring · COVID-19 · SARS-CoV-2 · Wastewater-based epidemiology · Example scenario

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✉ Albert Z. Jiang
zjiang@uoguelph.ca
Fulin Nian
20211330001@fudan.edu.cn
Han Chen
1004408510@qq.com
Edward A. McBean
emcbean@uoguelph.ca

- ¹ School of Engineering, University of Guelph, 50 Stone Rd. E, Guelph N1G 2W1, Canada
- ² Department of Digestive, Shanghai Pudong Hospital, Fudan University Affiliated Pudong Medical Center, 2800 Gongwei Road, Shanghai 201399, China
- ³ College of Environmental Science and Engineering/Sino-Canada Joint R&D Centre for Water and Environmental Safety, Nankai University, Tianjin 300071, China

Introduction

Since the beginning of December 2019, when the first case of COVID-19 was reported, the global pandemic has been drastically impacting the world in many respects. COVID-19 is a disease caused by the severe acute respiratory syndrome coronavirus (SARS-CoV-2), and the resulting global pandemic has resulted in huge impacts to the global economy and large numbers of confirmed cases and deaths. Meanwhile, medical resources are on the brink of failure and being depleted quickly, as well as not being allocated efficiently due to scattered outbreaks. After two years of the COVID-19 pandemic, toward the end of the year 2021, cumulative worldwide cases reported by the WHO are more than 273 million, with new cases being reported at rates of more than 500,000/day (WHO, 2021). The three countries with highest 7-days newly reported cases are: USA (827,506), United Kingdom (541,872), and France (362,800) (WHO, 2021) (data were last updated on December 21, 2021). As will be used as an example in this paper, Nanjing, China (and henceforth referred as 'City'), experienced an outbreak due to group infection at an airport, with an accumulation of

more than 200 confirmed positive cases within a two-week period in the City (data were last updated on August 17, 2021). One purpose of using Nanjing as a hypothetical scenario example is to demonstrate how passive samplers used in WBE could benefit areas with or without well-established anti-pandemic measures and protocols as the continuous monitoring tool. Further, with massive testing and contact tracing being employed globally as measures to prevent the spread of COVID-19, low- and middle-income countries have severe limits to promote these protocols and including high-income countries, face issues of frequent medical shortages of resources (Donia et al., 2021). In that regard, countries whether they are low-, middle-, or high-income must continue their vigilance in preventing COVID-19 outbreaks and continue to use efficient and cost-effective tools to support their efforts and reinforce their anti-pandemic initiatives.

Given the enormous magnitudes of COVID-19 caseloads, continuing efforts while useful to control the growth of the COVID-19 virus using tools involving clinical testing for SARS-CoV-2 of individuals within their populations via either viral or antibody tests are both onerous and expensive. The challenges of differentiating between non-infected citizens versus pre-symptomatic and/or asymptomatic people represent extra layers of complication as some individuals with the virus are not presenting symptoms. The implications are that clinical testing is somewhat subjective due to limitations of medical resources: people will typically be tested only when they have symptoms, or they are concerned about possible close contact to confirmed cases, or they are required to do so by a regulation. In many cases, this leaves asymptomatic, but infected people not being tested. Even with the most common COVID-19 testing method alone (throat and/or nasal swabbing), patients with positivity could still be missed (Choi et al., 2021). There were also cases of patients who were tested positive after several rounds of negative test results. The quick conclusion is that relying solely on clinical testing has limitations in the task to ‘win the battle against the virus’, when considering the emerging variants and the global view of the number of vaccinated people. As a specific example, during an outbreak in Nanjing (July 2021), in order to de-escalate the situation and control the outbreak, public health authorities implemented multiple rounds of extensive COVID-19 testing to all citizens (translating to a City of nine million people subject to testing). The findings from this enormous effort were that people had negative test results for the SARS-CoV-2 for the first one to three rounds but showed positivity in later tests (NBS, 2021). Therefore, although conventional and popular clinical COVID-19 testing via throat or nose swab are precise, it consumes extensive human labor and medical resources. Having an additional method of monitoring the disease from another perspective and providing near real-time information

has potential to be extremely beneficial and merits urgent implementation.

Individuals whom the SARS-CoV-2 infects can generally be classified into four categories: symptomatic; asymptomatic (infected but no symptoms shown); paucisymptomatic or subclinical (mild symptoms); pre-symptomatic (symptoms become evident after two to 14 days of exposure (UT Health East Texas, 2021)). As identified by the Center for Disease Control and Prevention (CDC) of the USA, common symptoms of those infected by COVID-19 include cough, fatigue, fever, headache, shortness of breath, and sore throat (CDC, 2021; Patel et al., 2021). The gap for clinical testing exists between infected/confirmed cases and asymptomatic people. This gap may also exist where, as examples, there is evidence from the media and university administration indicating some people might remain silent in the hope of avoiding being quarantined. Asymptomatic people are not typically tested, leaving themselves and others, un-informed regarding public danger but, if tested, they typically entail many positive test cases. For example, Snider et al. (2021) showed that children aged 0–9 group had 35.0% asymptomatic cases (of total positive test cases) plus 31.4% cases (of total positive test cases) with only one symptom in Ontario, Canada (Snider et al., 2021). It is also hard for a lay-person to determine whether to take a COVID-19 test when they have a fever, as among those who show up for clinical testing, since ‘fever’ is the most common symptom across all categories (Snider et al., 2021). However, as mentioned above, current clinical testing has an inherent self-selection bias, where generally, the people who show up have symptoms. This self-selection bias results in limited and overstressed medical resources not being utilized efficiently. For example, in Ontario, Canada, there were 219,000 positive cases out of 8.4 million total tests, i.e., only 2.6% (based on the data retrieved as of Jan. 10, 2021). In other words, using Canada as an example, only about 3% of the population has been infected, or ~97% of people have not been infected at that time. Another issue is that as a result of self-selection bias, the 3% SARS-CoV-2 infected patients are utilizing medical resources. As of July 2021, there have been more than 1.4 million positive cases and 26,000 deaths in Canada due to COVID-19. Comparing to motor vehicle accidents in 2019 of Canada, the number of annual deaths was 1,623 (Government of Canada, 2021a).

As the pandemic progresses, an array of vaccines is becoming available, which are now widely promoted by the authorities. Using Canada as an example, 29,165,541 people (76.26% of the population) are fully vaccinated (two doses) as of Dec. 22, 2021 (Government of Canada, 2021b). Worldwide, 45.8% of the population has received at least one dose of the vaccine (Ritchie et al., 2021), all of which indicates that enormous efforts are still required to ‘close the gap’ and form herd immunity. However, with new variants emerging

quickly, the timeline of meeting the herd-immunity threshold is being constantly updated and the endpoint remains unknown (Yadegari et al., 2021). Thus, this leaves major chances for people to become infected, considering vaccination rates continue to be in limited numbers in the current situation. On the positive side, strong evidence has been reported showing vaccines are effective in lowering both the infection and mortality rates (Milman et al., 2021; Netea et al., 2021; Tober-Lau et al., 2021). Although the positive effect of vaccination is undeniable as it reduces the chances of infection and reduce the severity and mortality after the infection (Ashford et al., 2021), even fully vaccinated people are not 100% protected against the virus of COVID-19 as cases are being reported (Apisarnthanarak et al., 2021). Recent studies have revealed that COVID-19 could be more than just a pulmonary disease but also affecting the human Central Nerve System (CNS) (Nagu et al., 2021). For this, and many additional reasons, the need continues to monitor the pandemic to reduce losses in various aspects.

Public Health officials may only respond by implementing more strict policies after the infected cases have reached a predefined epidemic status. For example, one or two positive cases will not result in any major public health actions. However, an epidemiology investigation employing close contact tracing is not easy, and no one can ever definitively know if a scattered infection might evolve into major outbreaks. Therefore, without a means of obtaining a focus in the case of COVID-19, it is very difficult to identify the size of an outbreak based only on current clinical testing. Besides, 'group gatherings' or living in clustered residences such as the situation for university students or long-term care homes, can exponentially increase the risk of an outbreak occurring. Hence, the current situation regarding full understanding of outbreaks has substantial potential to be improved, to alleviate the pressure on the medical system and provide better guidance for Public Health officials.

The current studies support the idea that viral shedding of SARS-CoV-2 continues for periods ranging from 12 to 20 days (Cuffari, 2021). Virus RNAs have been detected in sanitary sewage worldwide via various approaches, where the most widely used approach is RT-qPCR (Aguiar-Oliveira et al., 2020; Cuffari, 2021), making this approach feasible and meaningful. Zhang et al. found a median duration of virus shedding in human feces to be 22 days (Zhang et al., 2020), and more recent research has indicated the viral shedding time was between 20 to 32 days (Fuminari et al., 2021). Patients infected with SARS-CoV-2 have been identified as excreting millions of viral genomes into wastewater daily (Ahmed et al., 2020). Further, 10^4 – 10^8 viral RNAs (with translation) per gram of stool can be detected from asymptomatic individuals after being infected for several weeks. An example reported by Tang et al. (2020) showed that an asymptomatic patient may

excrete stools with virus detected by RT-PCR 17 days after the last exposure to the virus source (Tang et al., 2020). This is dangerous as asymptomatic individuals can thus infect others and release viral genomes for lengthy periods, where studies have illustrated that asymptomatic patients may be as contagious as symptomatic patients (He et al., 2020). In response, there is significant merit to incorporate the feature of SARS-CoV-2 shedding in human feces as part of wastewater-based epidemiology and sewer systems provide a fast 'snapshot' particularly at a community level (e.g., a university residence).

Wastewater-Based Epidemiology.

Given that an individual sanitary sewer and/or wastewater treatment plant (WWTP) receive untreated wastewater from a defined community or region, collecting and analyzing wastewater has the potential to generate near real-time health information about a defined population (Sims & Kasprzyk-Hordern, 2020a). Wastewater-based epidemiology (WBE), the concept based on such knowledge, is crucial to understand that human excreta in wastewater contains chemical and/or biological compounds that can be used as biomarkers to reflect specific populations' health conditions (Sims & Kasprzyk-Hordern, 2020b). Hence, WBE is not a new concept as this idea has been used before in multiple scenarios, including for example, for polio—Israel (Manor et al., 1999) and Brazil (Aguiar-Oliveira et al., 2020) and Salmonella outbreaks—Hawaii (Diemert & Yan, 2020). The concept of WBE has also been used to detect illicit drugs in communities (Benaglia et al., 2020). The methodology using the WBE concept has since been expanded to detect the spread of the virus such as norovirus, sapovirus, rotavirus, and hepatitis A virus. Hence, this concept/methodology is being further extended to the COVID-19 scenario. Intuitively, municipal wastewater treatment plants are hotspots for preliminary sample collection, where results may be identified that can be a very efficient way of detecting upcoming pandemic waves (Colosi et al., 2021). Details below indicate how the concept of WBE is useful for the current global COVID-19 pandemic scenario with a focus on passive samplers, including a scenario based on the recent outbreak in Nanjing, China, to demonstrate the value of passive samplers which could be used as an early warning system and provide guidance to identify outbreaks. It is noted that the difference between WBE or sometimes referred as Wastewater Monitoring (WM) is a measure of passive surveillance (Acosta et al., 2021); alternatively, the use of a passive sampler is a tool within the WBE scope among many others used to collect wastewater samples for further laboratory analyses (Habtewold et al., 2022).

Essential Dimensions of Virus in Feces.

Both symptomatic and asymptomatic persons infected with SARS-CoV-2 for six weeks or more from the time of infection may have their stools contain viral fragments for weeks (Tang et al., 2020). The virus was found to be able to attach on the receptors of ACE2 (Angiotensin-Converting Enzyme 2), an enzyme found in heart, blood, lungs, and intestine (Ghosh et al., 2021). Thus, profiles of ACE in plasma can be used to define the severity of infection from COVID-19 of patients, whereas elevated level of ACE2 can be found in the urine for certain groups of patients (Behl et al., 2020). These characteristics of SARS-CoV-2 have made WBE monitoring tools feasible for the COVID-19 pandemic. Consequently, sewer systems offer near real-time outbreak data depending on the monitoring method because sewers receive human excreta containing viral particles shed by infected persons, and with appropriate wastewater surveillance collection methods (grab or composite sampling), and knowing where and what to sample, such monitoring allows identification of the overall trends of infection. Further, when the clinical testing numbers increased, more people were getting tested, ‘positive signs’ in sewers evidence of COVID-19 infections also increased (Donner et al., 2021). Clearly, the duration of time the sewage contains viral fragments that remain in the sewer system depends on the populations contributing wastewater to the sampling point, where the time of transit of the feces varies between 2 and 24 h. While this number (2–24 h) is for a community, for clustered living such as a student residence, this number would be much shorter. Hence, time poses one of the challenges for monitoring as one of many other factors (e.g., soaps or disinfectants in the wastewater can damage the virus RNA). However, with proper equipment, protocol, and methodology, wastewater monitoring of COVID-19 can serve as an early warning system for detecting new waves of infection outbreaks and/or asymptomatic persons in a community.

Challenges with Wastewater Epidemiology.

The SARS-CoV-2 virus has no DNA, which makes the virus extraction difficult (Hillen et al., 2020). Instead, the SARS-CoV-2 virus has a single-stranded RNA, where one virus has copies of the N1 or N2 fragment. Once the viral RNA gets into a human cell, after it is attached with its spike protein on the receptor protein of the host cell, it enters the ribosome which provides the proteins for the virus replication. The virus is considered ‘alive’ only when it gets into a host cell. It has the genetic information to ‘mis-instruct’ the host cell to perform virus translation and replication. In the host cell, after the infection with the virus, multiple enveloped viruses are produced and eventually are released to infect other cells, and this cycle continues (Kamel et al., 2021).

While it is considered beneficial to use WBE as a tool to serve as an early warning system allowing health authorities’ interventions via wastewater characterization, the virus RNA extraction process is complicated since virus RNA in wastewater may be damaged during the collection, extraction, detection, and subsequent analysis (as well as damage to the RNA occurring during transit in sewers). On the other hand, researchers do not measure the SARS-CoV-2 virus itself but part of the RNA fragments: N1 and/or N2 as the target for analyses. The biological signal of the virus can be amplified through the RT-qPCR (quantitative reverse transcription) process. ‘Noise’ and other potential errors can be corrected/calibrated by normalization using other biomarkers such as pepper mild mottle virus (PMMV), which can be used to account for variations in the sewage due to daily fluctuations and human activities (Aguiar-Oliveira et al., 2020).

As the sample is collected in the extraction tube, usually a few grams of 0.1 mm or 0.7 mm glass beads are added to help the physical lysis; the pre-processed sample is then moved to the sequence of RNA extraction. The general procedure is, in order: adding samples, adding lysis buffer, adding homogenized mixture vortex in the bead mill, and the machine is operated to vibrate and destroy the virus envelope. Such a procedure removes the virus envelope, membrane, protein, and other debris, leaving the target RNA in the solution where it is contained in the supernatant. Consequently, virus genetic material (N1 and N2) can be amplified through the RT-qPCR to be translated into the information about the virus in wastewater in terms of N1 or N2 fragments per liter of wastewater. It is noted that damaging the RNA is always of great concern throughout the entire procedure.

Clearly, WBE can be utilized to comprehensively monitor the spread of an infectious disease in near real-time at the community level by analyzing population-pooled wastewater. While viral shedding varies among individuals and throughout their infection stages, and compounds in the sewer are mostly complex mixtures, considering these variations during the analyses is crucial. The results of the analyses are thus averaged representations that reflect the pandemic status of a community, but the procedure does not identify any specific individual. In the case of congregate living spaces, it is also not possible to use wastewater monitoring to distinguish whether the virus load in the sewer is from newly infected person(s) or previously infected persistent cases (Colosi et al., 2021). Other factors that may interfere with the virus analysis include the unknown persistence of the viral RNA in wastewater under various flow conditions such as dilution (e.g., as occurs due to rainfall/infiltration into sewers) and inputs of wastewater from people without COVID-19 infections. As well, there is variability in the shedding rate of SARS-CoV-2, influenced by a few individuals with high shedding rates or a large population

with a low shedding rate. Furthermore, SARS-CoV-2 is an enveloped virus with poor stability in wastewater, being very susceptible to disinfectants such as commonly found in wastewater, compared to other non-enveloped human enteric viruses such as adenoviruses, rotavirus, norovirus, and hepatitis (Howie et al., 2008). Hence, the longer the virus travels or remains in the wastewater collection system, the greater the virus die-off. Hence, due to the low viral loadings in large volumes of wastewater, effective concentration methods before detection and extraction are needed, to improve detection efficiency. Although most WBE studies have to-date used data from large wastewater treatment plants, timely data from smaller catchments (e.g., university residences where concern is with outbreaks within a student population) also have important merit and are needed for any targeted public health action. This area of application pertains particularly to passive samplers, where this approach is not only more affordable but also can be deployed in small settings such as building or community outlets. Additionally, WBE for COVID-19 pandemic has been proven able to detect viral variants. Precedent studies have demonstrated the capability of identifying the Spike protein G614 variant (Martin et al., 2020), the B.1.1.7 variant (Carcereny et al., 2021), and the B.1.617.2 lineage (Delta variant) (Joshi et al., 2021).

The Passive Sampler—Role of an Alternative Sampling Strategy.

Applying the concept of WBE, many confined areas such as schools, student housing, congested public housing, prisons/correctional facilities, and long-term care facilities can be targeted with diagnostic testing via passive samplers, which can help to focus clinical testing (nasal or throat swabs) by avoiding populations that are likely to be negative and/or be a signal that indicates there is a localized outbreak. This helps identify the need for actual clinical testing and utilizes medical resources more efficiently.

To address this issue, passive samplers provide an important opportunity to sample from fluids such as air and water/wastewater monitoring. Passive samplers are inexpensive (e.g., individual passive samplers themselves are ~\$20 each) and require non-complicated training for a person to deploy and later recover the sampler (Lohmann & Muir, 2010) plus the analytical laboratory for analyses (the chemical costs and time of the lab technician of a passive sample are within the range of CAD\$100 to CAD\$500 per passive sampler, depending upon the laboratory utilized). One example comparable to the potential capability of passive samplers to monitor COVID-19 is the application of POPs (Persistent Organic Pollutants) monitoring in water bodies (Lohmann & Muir, 2010). Hence, passive samplers have the capability to be focused to identify

outbreaks of the virus for a localized facility. Also, reliance upon passive samplers can be implemented at different levels: local community, city, town, or region. For example, the passive sampler technology can be moved upstream or downstream in the sewer network (at any location wherein there is an opportunity to access a sanitary sewer via a man-hole, selected on the basis to isolate/identify the source of the infections).

Alternative sampling methods beyond passive samplers such as autosamplers and grab sampling are also available. However, these alternative sampling measures tend to be more problematic for quick and ad hoc deployment and high-resolution monitoring at smaller scales (Schang et al., 2021). One example is, due to issues of security, autosamplers may be subject to tampering or theft. As well, the absence of a reliable electrical source is an inherent problem for autosamplers. Contrarily, passive samplers are not only inexpensive but also able to avoid many of the issues of other sampling technologies (Hayes et al., 2021). Further, as demonstrated below, passive samplers are easy to assemble and install at nearly any location of interest along a sewer system.

Passive samplers containing sorptive materials such as Q-tips, gauzes, or charged membranes can be contained in a plastic hollow support structure, e.g., a ‘Boat’ (Fig. 1). Inside the support structure, sturdy strings/ropes are used to ensure there are no loose ends and to ensure that deployment and retrieval of the passive sampler can easily occur while minimizing the potential for the sampler becoming ‘stuck/partially blocking’ flow in the sewer. The hollow support structure of the passive sampler can be wrapped with mesh to allow sewage to both enter and leave the ‘boat’ but also prevent too much fouling and clogging (e.g., toilet paper) in the sampler. Once the ‘boat’ containing the adsorptive sample(s) is retrieved from the sewer, the initial processing takes ~8 min, analyzed at that point-in-time, or frozen at -80 °C to analyze at a later time. Although immediate processing is recommended, to store the collected sample, a -80 °C environment is required. It is noted that biological safety cabinet and health & safety protocols are needed for sample processing, following the appropriate safety protocols and PPE guidelines to protect the laboratory technicians. Based on laboratory results, passive samplers have been shown to indicate viral signals in wastewater for up to 48 h, which adds more flexibility for operators to install and collect (Habtewold et al., 2022), and allow for continuous wastewater sampling (Schang et al., 2021).

Applying the WBE concept Hypothetically to an Example Scenario.

As demonstrated above, passive sampler technology has many advantages in monitoring the SARS-CoV-2 in wastewater. This concept may also be useful in areas/regions with

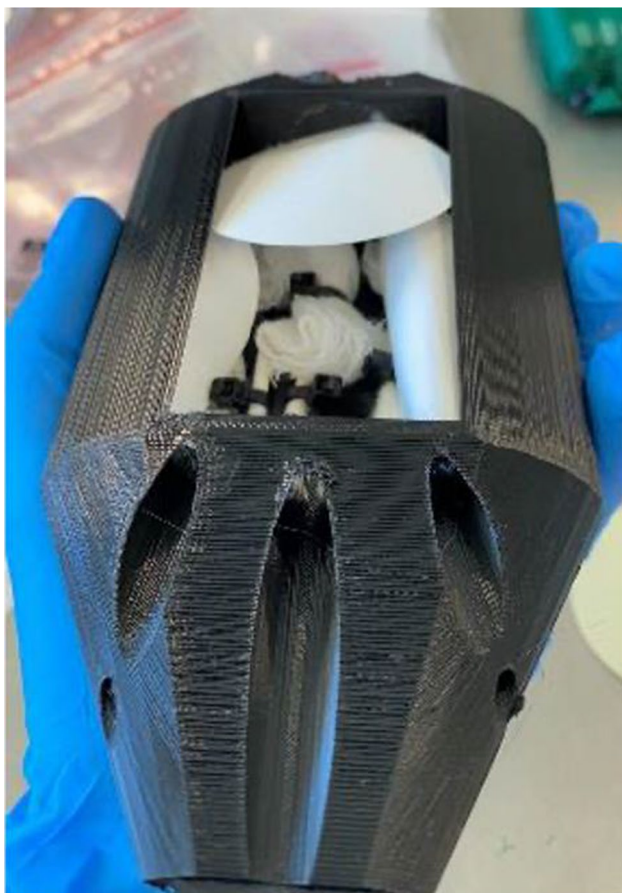


Fig. 1 Field picture of a 'Boat' (obtained from Monash University, Australia)

low COVID-19 occurrence and assist in continuing management of the pandemic, whether it is low- and middle-income countries that cannot afford widespread active sampling or high-income countries that have low infection rates.

Nanjing, a major city near Shanghai and has a population with more than 9 million residents, is located in south-east China. Public health officials of Nanjing reported nine positive cases on July 20, 2021. This event was astonishing from the outset since the COVID-19 pandemic had been well controlled in China throughout the previous year as a result of strict testing measures and substantial quarantine policies. However, this outbreak quickly escalated; by July 23, 2021, Nanjing had a total of 35 identified positive cases. This outbreak started in a group of janitors working at the Nanjing Lukou International Airport (about 40 km away from the city center) and quickly spread into the City due to person-to-person close contacts, where the virus was confirmed to be the Delta variant. In response to the outbreak in the airport and to prevent potential larger outbreaks, public health authorities of Nanjing deployed extensive medical resources and started massive 'all-citizens COVID-19

testing' in the hope of preventing escalations of the outbreak. However, as of August 4, 2021, this outbreak had spread into many cities and provinces within China, where Nanjing alone was identified as having accumulated 223 confirmed positive cases after five rounds of all-citizens COVID-19 testing (NBS, 2021).

The outbreak of COVID-19 in Nanjing, and scattered worldwide, delivers an important message to the public and authorities: the pandemic is not over, and possibly far away from over. Awareness was raised on whether there could be better monitoring methodologies for areas such as Nanjing which had the pandemic well under control and regions with low COVID-19 prevalence. Ideally and practically, passive sampling technology could have played an important role in the screening and prevention processes. Since the outbreak started in a well-defined area—the airport, with regular conventional COVID-19 tests for all employees (the frequency is usually weekly or bi-weekly), wastewater monitoring with passive samplers has important potential, to serve as an early warning system. As indicated herein, an advantage of wastewater monitoring for SARS-CoV-2 is that it can detect virus from asymptomatic people as well as the introduction of the virus to a population sector. And, in terms of using passive samplers, it can be installed on-stream in the sewer or at a sewer outlet, where localized wastewater viral load can be readily available. Although regular clinical COVID-19 testing is more precise in terms of identifying an individual who is positive, it has limits that require extensive resources and is time/labor consuming and provides turnaround of up to 48 to 72 h. Evidence is showing that there were positive cases identified after four rounds of 'all-citizen COVID-19 tests' throughout the City, where patients had negative test results for the first three rounds. As well, passive samplers can be used to show the evidence of onset of an outbreak. Further, since passive sampling can be completed hourly or daily (the frequency is flexible) compared in comparison with conventional clinical COVID-19 tests which are usually done weekly for workers at high-risk to virus exposure, more rapid identification of outbreaks becomes feasible. If a wastewater sampling result suggests there are viral strain(s) found, a quicker and more systematic measure can be implemented to the targeted community. Hence, passive sampling is targeting to monitor a community/area versus clinical testing are individually targeted. To this end, wastewater monitoring using passive samplers can precisely locate the community needing attention quicker than when people show up with symptoms to get a clinical test. This concept is shown graphically in Fig. 2, where the blue-branch is demonstrating the benefits of having regular and frequent wastewater monitoring with passive samplers, completed more frequently than clinical testing, thus allowing more rapid responses from public health

authorities. Nevertheless, the starting block (airport in Fig. 2) is intended to represent any hot zone(s) of infection or communities that require attention.

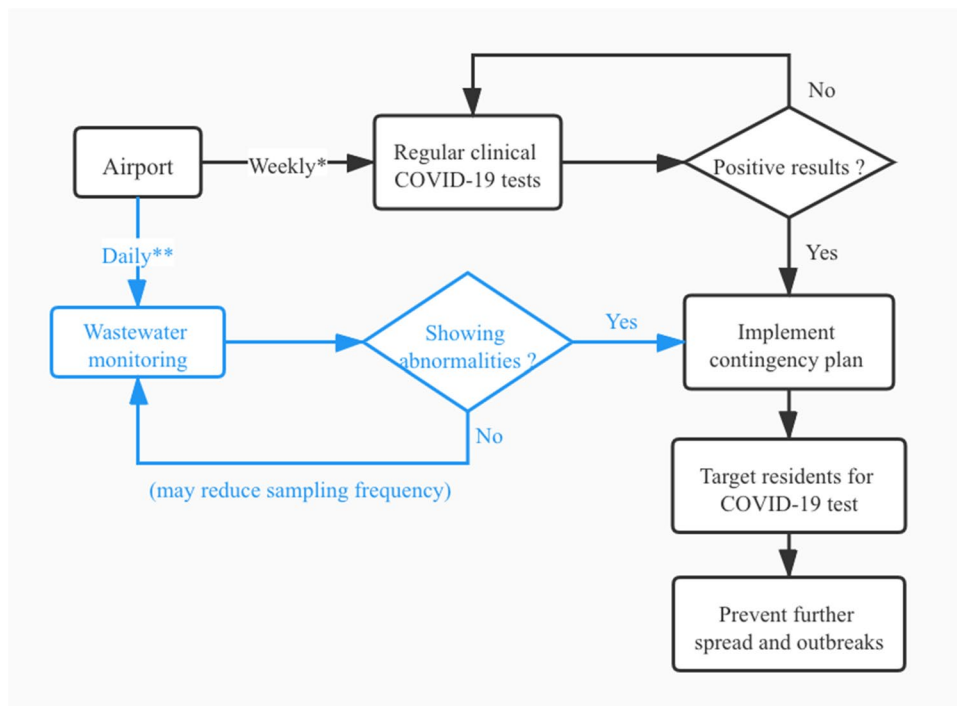
Since wastewater monitoring using passive samplers can be accomplished frequently due to its low cost and minimal training for the operator to deploy and collect the sampler, passive sampler results can provide alerts and valuable early information about whether there are potential positive cases in the community, including variant details. In a well-defined area, as well as a potential hot zone of infection, wastewater monitoring with passive samplers provides more frequent information about the virus concentration and the presence in the community contributing to the wastewater. As soon as the passive sampling shows some abnormalities, attention may be used to allocate medical resources to the community quickly for clinical testing. Hence, wastewater monitoring with passive samplers can be useful in terms of rapid screening and identification of potential outbreaks in a defined community/area. The passive sampler method (or other methods within the scope of WBE) for SARS-CoV-2 is not mutually exclusive with conventional regular COVID-19 tests but can help the latter to be implemented more efficiently and precisely. At various levels from small to large, passive samplers can detect abnormalities and provide early warning, as well as assisting the close contact tracing and epidemiology investigation processes without constant in-person clinical tests. All in all, wastewater monitoring for SARS-CoV-2 with passive samplers serves as a valuable tool for regions with well controlled pandemic situations or regions with low occurrences.

Conclusions

Since viable SARS-CoV-2 sheds its viral RNA in bodily excreta, rapid identification of the presence of the original virus and/or variants, correctly applying the concept of WBE can help monitor variations of the circulating strains and assist in the following of the evolution of the virus genome between regions over time. A major advantage of WBE is that it has the potential to detect the presence of COVID-19 sooner than via random or voluntary clinical testing. This potential can prove very useful in limiting the spread of infection, given that timing is critical for facilitating a head-start on contact tracing.

Wastewater sampling technologies are present in different ways, including autosamplers and passive samplers, both of which are sensitive to detect SARS-CoV-2 in wastewaters. Among the different choices within the WBE scope, passive samplers have the potential for wide use with its attractive feasibility, low costs, ease of deployment at small-scale locations, non-labor-intensive, continuous wastewater sampling, and provide more time/location sensitive information. Wastewater surveillance with passive samplers provides another tool for public health authorities to make early and more informed decisions to prevent and dampen the subsequent waves of the pandemic and future scenarios. The insight learned from the Nanjing outbreak example is transferrable to many other scenarios to help reduce the situations of outbreaks and provide valuable information to public health authorities.

Fig. 2 Flowchart demonstrating the merit of application of passive samplers such as could have been applied in the City outbreak. (*the frequency of testing is usually weekly depending on local guidelines; **the key is to identify situations where the frequency is higher than an alternative method such as the clinical COVID-19 test)



As the pandemic continues globally, with new variants emerging which may become more infectious, (e.g., from the Delta to Omicron variant) providing enough doses from the most effective vaccines remains challenging (Yadegari et al., 2021). Continuing to monitor the pandemic is not only a necessity for public health authorities to understand the infection rate, but also alerts the general public any potential of being infected. Hence, authorities can provide measures to treat infections, and publics can maintain their daily routine while being informed. The University of Guelph has been one of the pilots in using passive sampler to monitor on-campus COVID-19 status, and it has been proven to be useful in alerting relative personnel to stay quarantine and providing a peace of mind for others. This application can be viewed at <https://news.uoguelph.ca/2019-novel-coronavirus-information/u-of-g-covid-19-wastewater-report/>, where the data are updated daily on business days (University of Guelph, 2021). As stated in this review, WBE has been used in many diseases monitoring scenarios, making WBE as an important yet transferrable tool to understand not only the current, but also the future epidemiology.

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Declarations

Ethics approval and consent to participate. Not applicable.

Consent for publication. Not applicable.

Availability of data and materials The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. Public data are also available on the internet.

Competing interests The authors declare they have no competing interests.

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