Review

Lead time of early warning by wastewater surveillance for COVID-19: Geographical variations and impacting factors

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ABSTRACT

The global data on the temporal tracking of the COVID-19 through wastewater surveillance needs to be comparatively evaluated to generate a proper and precise understanding of the robustness, advantages, and sensitivity of the wastewater-based epidemiological (WBE) approach. We reviewed the current state of knowledge based on several scientific articles pertaining to temporal variations in COVID-19 cases captured via viral RNA predictions in wastewater. This paper primarily focuses on analyzing the WBE-based temporal variation reported globally to check if the reported early warning lead-time generated through environmental surveillance is pragmatic or latent. We have compiled the geographical variations reported as lead time in various WBE reports to strike a precise correlation between COVID-19 cases and genome copies detected through wastewater surveillance, with respect to the sampling dates, separately for WASH and non-WASH countries. We highlighted sampling methods, climatic and weather conditions that significantly affected the concentration of viral SARS-CoV-2 RNA detected in wastewater, and thus the lead time reported from the various climatic zones with diverse WASH situations were different. Our major findings are: i) WBE reports around the world are not comparable, especially in terms of gene copies detected, lag-time gained between monitored RNA peak and outbreak/peak of reported case, as well as per capita RNA concentrations; ii) Varying sanitation facility and climatic conditions that impact virus degradation rate are two major interfering features limiting the comparability of WBE results, and iii) WBE is better applicable to WASH countries having well-connected sewerage system.

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1. Introduction

While the pandemic has entered its third-year span and the world is dealing with several mutants of SARS-CoV-2 viruses, the extent of the COVID-19 infection is not fully explored due to insufficient testing in several countries struggling with the economic slowdown. The health facilities are lagging behind the desired level of coverage for the clinical diagnosis of the infected patients in both developing and developed countries [1]. In the past, Wastewater Based Epidemiology (WBE) has proven to be a promising tool for several pathogens surveillance and has also been implemented to track the infection during the COVID-19 pandemic [2,3]. WBE provides a soft signal of virus transmission at a community scale [4], as well as tracks the presence of emerging contaminants in the ambient environment, including drug abuse and antibiotic use [5,6]. Although wastewater surveillance at community-scale wastewater treatment plants (WWTPs) has been widely recognized to track infectious diseases [5,6] yet until its effective use in the recent COVID-19 pandemic monitoring, WBE had a meagre presence worldwide, as a handful of researchers were obtaining first-hand data and funding to such research was still confined to few expertise or organizations.

WBE provides a non-invasive, near-real-time analysis of SARS-CoV-2 signal within the area served by the sewer network. WBE consists of several steps: (i) representative composite or grab raw wastewater samples are collected; (ii) the wastewater is analyzed for the genomic components of SARS-CoV-2, and the concentration is measured; (iii) the concentrations of the SARS-CoV-2 genome copies are multiplied by the daily flow rates of sewage to obtain the daily viral loads; (iv) the sewer line exhibiting a positive signal is traced back to the contributing communities. Thus, WBE approach assists in the pre-detection of SARS-CoV-2 infected cases by a week or two weeks at city scale [7]. The procedure of detecting the RNA of viral pathogens can monitor the health risk of approximately one-third of the entire globe with access to around one hundred thousand WWTPs worldwide [8,9]. The WBE method gives an initial measure of disease spread at the community level prior to clinical symptoms; however, it does not precisely estimate the number of infected citizens at present and/or in near future. In addition, it does not essentially consider the effect of seasonal variations, temperature, time, and type of sampling. Overall, prevalence, population density, sanitation conditions, sampling frequency, air temperature variations, and testing coverage are important considerations that have not yet received enough attention.

The early warning is the single most widely accepted outcome of WBE; however, there is a load of speculation around the extractable lead-time through WBE. WBE performance relies on multiple factors, including sampling and analytical procedures, climatic variability, WWTP features and population dynamics rather than SARS-CoV-2 RNA concentrations (C_{RNA}) in wastewater samples alone [10]. Due to varying catchment characteristics and uncertainties associated with the data handling, including reliability and the availability of epidemiological data, the lead-time reported globally are likely to have many variations and may not be constant universally [11,12]. Thus, for the actual realization of the importance of WBE studies during COVID-19 or any future infectious disease outbreak, it is highly recommended to have comprehensive comparability among the global record of the environmental epidemiology data, especially its virtue of providing the lead-time and frequency to achieve better accuracy. The impact of the socio-economic and demographic conditions on the ratio of infections captured by the clinical testing further necessitates future studies.

SARS-CoV-2 concentrations in wastewater and sludge is linked with the COVID-19 infection [58], however, lead time variation across studies has not been normalized yet to arrive at a consistent deduction. Here we review and majorly collate lead-time variation in the WBE reports and further attempt to correlate COVID-19 cases and genome copies obtained from sewage surveillance, mainly focusing on the countries with and without proper sewerage collection systems.

Wastewater viral genome copies (C_{RNA}) and COVID-19 incidence in countries with and without proper water, sanitation, and health (WASH) conditions were emphasized while assessing several factors mentioned above that may influence the spatial and temporal trend in the lead time.

Recognizing the urgency to normalize lead-time reported in many studies, this article mainly: a) emphasizes the temporal variation and early warning period reported worldwide to establish a global perspective, b) critically analyzes and sheds light on any latent causes that govern the variation in reported genome data in environmental matrices, c) discusses alternative WBE strategies and future perspectives. Primarily we compared the global data on the temporal WBE tracking of the COVID-19 to generate an understanding of the robustness of the WBE approach for predicting pragmatic early warning lead time. As the sampling technique was summarized as a category variable, its impact on the correlation coefficients between wastewater viral genome copies (C_{RNA}) and COVID-19 prevalence are also assessed through point-biserial correlation using R software package. This shows that sewage-based surveillance i.e., WBE is better applicable to WASH countries having well-connected sewerage systems, and the lead-time around the world is not consistent owing to their varying climatic conditions.

2. Materials and methods

2.1. Quality control and quality assurance

PRISMA guidelines were used for a systematic literature search around global data on the temporal WBE on October 10, 2021 [13]. Databases such as Web of Science core collection, Scopus, and PubMed were searched using the term "SARS-CoV-2 AND wastewater AND prevalence AND temporal variation". After eliminating duplicates using the EndNote Reference Manager software, a total of 102 unique papers were identified. Titles and abstracts of the retained articles were screened and assessed for eligibility using two basic criteria: i) must be a peer-reviewed article and in the English language; ii) must have had reported data of both lead-time/early prediction and clinically confirmed COVID-19 prevalence. Manuscripts that passed the initial screening were further evaluated by full-text reading, and finally, 36 articles were included in this study. If data were available in graphs, then GetData Graph Digitizer was used to extract the number corresponding prevalence or lead-time. Despite various information sources available, we trusted for impacting factors, including sampling methods, climatic and weather conditions [4,14–17]. The impacts of various variables related to change in geographical locations on the correlation coefficients between C_{RNA} and COVID-19 prevalence were further assessed through Pearson correlation analysis which could potentially play an important role in lead time variation.

3. Human viral shedding

SARS-CoV-2 RNA can be shedded in feces, urine, blood, saliva, and sputum from COVID-19 patients [18–22]. The human excretion of SARS-CoV-2 via different shedding sources varies on the magnitude, probability, and time distribution. However, a recent *meta*-analysis found urine is not a major shedding source due to the very low shedding probability in patients, i.e., 0.026 (95% CI 6.3 × 10^-4^- 0.10 [19]. In contrast, feces and sputum were identified as major viral shedding sources in wastewater [23,24]. It was also reported that the oral cavity is an important SARS-CoV-2 infection site and saliva is thus a critical shedding source [25]. Another study also confirmed that viral loads in saliva were comparable to those in sputum and throat swabs, with an analogous detection rate for respiratory tract samples [26].

A significant difference also exists among the timing of viral shedding, swab results, blood results and the appearance of the first symptom. Based on clinical data about fecal shedding, a *meta*-analysis estimated that fecal shedding peaked at 0.34 day after the symptom onset and the peak concentration was about 10^3 times higher than the

median concentration over the whole shedding period [27]. In contrast, another study reported that the sputum shedding peaked at about 7 days after the symptom onset, with the concentration 10 times higher than the average over the shedding period [28]. The viral load in saliva was high since symptom onset (median 4.02 log10 [copies/mL]), peaked within 5 days (median 4.27 log10 [copies/mL]), and gradually declined one week after symptom onset.

Based on the research published by Zhurakivska et al. (2020), it was further found that most patients had a positive pharyngeal swab result for the first ten days of illness [29]. After this period, the percentage of patients whose pharyngeal swab result was negative exceeded the positive ones around day 12 of the disease. In the case of blood samples, viral RNA was not initially detected in most patients. In < 15% of patients, the virus in blood was registered during the 2nd week; however, sputum mucus carried the viral RNA during the entire disease period. Toward the end of the first week, viral RNA was found in the blood of approximately 40% of patients. However, the urine of COVID-19 patients was almost always negative for the presence of the virus.

In another study, He et al. (2020) studied the difference in the viral shedding loads. The group observed the start and peak of infectiousness in around three days and two days, respectively, before the onset of symptoms [30]. After the onset of symptoms, viral loads were found to be decreased, i.e., after eight days, and the infectious virus could not be detected. Wölfel et al. (2020) proposed a median of 20 days for the virus prevalence after symptoms onset [31]. Linking it to the shedding rate, Miura et al. (2021) studied the viral shedding in feces using the shedding dynamic model [27]. The shedding rate obtained from the model stood at 3.4 log copies/g feces, and the duration was found to be 26 days at the Ct value of 40. A previous *meta*-analysis also reported that the mean shedding magnitude was $10^{4.52\pm0.13}$ gene copies/g feces, and the mean shedding probability was 0.54 ± 0.09 [19]. The viral load in saliva varied from about 10^4 to 10^8 copies per mL during the first week of symptoms [26]. Other shedding sources like sputum may be a potentially major contributor to the viral load in wastewater [23,24]. Thus, it is evident that viral shedding is one of the major factors which is commonly used in the WBE to convert the load or of SARS-CoV-2 RNA in wastewater by the population. In addition, SARS-COV-2 RNA concentration is primarily influenced by factors such as gender, age, and pathological conditions of patients. Usually, patients experience prolonged shedding ranging 4 to 10 days after the onset of first symptoms and after they are clinically negative, which is normally not considered in WBE studies [7]. Thus, such aspects should be taken into account while performing WBE.

4. Virus in wastewater and epidemiology

4.1. The lead time of WBE for early warning

The virus and/or its genetic materials enter WWTPs through the sewerage network following shedding. In Ottawa, Canada, D'Aoust et al. (2021) reported that viral SARS-CoV-2 signal increased by > 400% in wastewater just 48 h after a > 300% increase in reported cases [32]. In Utah, another study over a 9 weeks duration reported a positive correlation between community outbreak and increased SARS-CoV-2 RNA [33]. Similarly, Trottier et al. (2020) studied the temporal variation in viral RNA concentration in wastewater for two months post lockdown in France [34]. However, the virus was already circulating in the area but with a very low incidence rate, and an increase in SARS-CoV-2 RNA in wastewater was seen 2–3 weeks before the increase in COVID-19 cases. Although no correlation was noticed between the factors mentioned above, the author suggested a few hypothetical factors that impact the post-lockdown rise, such as migration from clusters and underestimation of prevalence rates. From these studies, it is evident that systematic sewage surveillance, i.e., WBE, can decipher the health status of the community by analyzing lead time much earlier before the clinical results.

In Table 1, important observations and WBE lead time is shown for worldwide cases varying from 5 to 14 days [35–40]. D'Aoust et al. (2021) observed a rapid increase in the signal 48–96 h prior to the new COVID-19 cases [32]. However, it is interesting to notice that the 2 weeks lead time observed by Kumar et al. (2020), could provide sufficient time for a proper management plan [7]. In another study, the WBE data reported was 6 to 8 days ahead of patients being clinically tested positive when compared with different parameters [41]. Here, the biomarker testing was entirely dependent on primary sludge rather than wastewater, which is proven to have a more diverse range of viral strains [41]. The study suggested having a same-day sampling and analysis of sludge leads us to have better information about the infection rate in case the normal clinical testing is delayed due to some reason. Another reason to sample and analyze sludge over wastewater is the affinity of this enveloped virus towards wastewater solids [42].

A study also suggested that upstream sampling of the sewerage manholes is preferred to downstream as it could provide more accurate knowledge of infected individuals in the neighborhood of 2000 to 5000 people [43]. Another study conducted in Virginia reported SARS-CoV-2 concentration in the range of 10 to 10,000 copies per 100 mL based on the 21 weeks of sampling [6]. The results aligned with the results obtained from Australia and Turkey but differed from Spain and France. The reason for the difference is assumed to be COVID prevalence, concentration efficacy, and PCR test variations [44]. However, analyzing wastewater from sewer manholes is the way forward.

4.2. Effects of temperature, seasonal variations, and sampling time of WBE

Most WBE studies that report SARS-CoV-2 RNA in wastewater do not include temperature as a co-factor. A computational model study was carried out to examine the feasibility of WBE while considering the effect of temperature, in-sewer travel time, and water usage per individual to quantify infection rates at the global and local scales [8]. Hart and Halden (2020) [15] simulated the SARS-CoV-2 infection at a community scale and implemented WBE to detect one infected case per hundred to 2 million non-infected people. The temperature has one of the significant effects on the biomarkers, which would otherwise have degraded at a rate proportional to the system's residence time [8].

The equations (1) and (2) estimate the concentration of biomarkers up to a particular amount of time. Also, the population normalization for predicting the actual virus prevalence in the community served was taken care of by equation (3) [6].

$$R_2 = R_1 \times Q_{10}^{\frac{(T_2 - T_1)}{10^{o}C}} \tag{1}$$

$$t_{\frac{1}{2},2} = t_{\frac{1}{2},1} \times \frac{\ln(2)}{\ln(2) \times Q_{10}^{\frac{(T_2 - T_1)}{10^{o}C}}} \tag{2}$$

$$L_{WWTP} = \frac{C_{WWTP} \times V \times (conversion factor 'L' and 'MG')}{Population} \tag{3}$$

Here, R_2 and R_1 are final and initial decay rates, $t_{\frac{1}{2},2}$ is the adjusted half-life (as per spatial and seasonal variation), $t_{\frac{1}{2},1}$ is the initial half-life, Q_{10} is temperature-dependent factor ranging between 2 and 3, L_{WWTP} is normalized loading of SARS-CoV-2 as per population, C_{WWTP} is the concentration of a particular assay in 100 mL, V is the amount of wastewater sampled.

In winter months, the viral titer was available to detect up to 100 h after shedding, which was reduced to 20 h in summer months. These temperature-related variations were comparatively less in locations with relatively lower water temperatures, as the viral RNA was preserved for a longer duration [8]. The variation in soil, water, and air temperatures affect the heat transfer rate between the wastewater sampled and the nearby environmental factors, changing the

Table 1
Lead time and significant observations of global WBE studies.

City	Duration of study	Genes/Assay Focused	Gene concentration	Lead time	Important Observation	References
Gandhinagar, India	August-September 2020	S gene, N gene and ORF 1ab gene	897.5 to 924.5 copies/L	14 days	Hike of 75% in genome concentration resulted in 11% increment of confirmed cases.	[7]
Ishikawa and Toyama, Japan	April-June 2020	N2, N3 and N (2019-nCoV)	12,000 to 35,000 copies/L	-NA-	Detection frequency exceeded when cases were > 10 per 100,000 people.	[65]
Connecticut, USA	March-June 2020	N gene	1,700,000 to 460,000,000 copies/L	6–8 days	Sludge results were not a positive indicator compared to + ve test results. Data are susceptible and prone to factors like handling.	[38]
Netherland	February-March 2020	N1 – N3 and E	790,000 to 2,200,000 copies/L	6 days	Correlation of SARS-CoV-2 RNA and reported COVID-19 prevalence	[36]
Paris, France	March-April 2020	E	50,000 to 3,000,000 units/L	8 days	Decrease in genome units with a decrease in COVID cases	[40]
Queensland, Australia	March-April 2020	N	120 copies/L	–	Strong correlation between no. of infection and $\log_{10}$SARS-CoV-2 copies in stool	[35]
Montana, US	March-June 2020	–	30,000 copies/L	5–8 days	RNA concentration in wastewater correlates COVID-19 epidemiology	[37]
Milano and Monza, Italy	April 2020	N, E, ORF 1ab	–	–	SARS-CoV-2 infectious nature is null and is zero in effluents	[39]
Milan and Rome	February-April 2020	ORF 1ab	–	–	Virus detection despite low infections is due to inclusion of mild and asymptomatic cases.	[66]
Ottawa, Canada	June-August 2020	N1 and N2	–	6–14 days (wastewater) 1–5 days (primary sludge)	WW viral signal increases 48 h and 96 h prior to increase in new COVID-19 cases and admission to hospitals	[32]

degradation of RNAs over the year, thus reducing the efficiency of the WBE approach further. The errors in interpreting the effect of temperature will result in variation in lead time prediction. The study also reported the temperature effect on ambient half-lives of biomarkers by 27 to 7010 % [8]. Overall, temperatures lead to variations of C_{RNA}, but these variations would be similar within the months of same season and as the lead-time is shorter than one month, may be temperature would not affect lead time in given city or region. However, the same will not be true in different geographical regions or say in the countries of different hemispheres.

Fig. 3 shows the reported genome copies worldwide divided among tropical, sub-tropical and temperate zones. Based on the geographical distribution (Fig. 3), it is found that the virus flourished in winter and did not lose its contagious nature beyond the preferred temperature range. Atmospheric heat is the primary cause of apparent seasonal variation in COVID-19. Thus, Paraskevis et al. (2021) suggested measuring UV-A and UV-B distribution and correlating with the seasonality of COVID-19 [45]. Other factors such as humidity and cloud cover could be considered while reporting seasonal variation in virus concentration [45]. A recent study included air temperature and humidity in the WBE modelling to estimate the prevalence better [19]. Therefore, a model that incorporates all these factors can help policymakers control any future outbreak waves of the disease.

The sampling time is another critical factor affecting RNA detection in wastewater. There is a massive difference in the quantity of viral genome detected in the initial viral load per day, decreasing drastically with time. This, when measured over the year, shows a sharp decrease from July to September (Table 2) but remains more consistent for the colder months, i.e. November to January [15]. This variation could have originated due to the infection shift over different seasons. However, the onset of the summer has underestimated the SARS-CoV-2 RNA level in

wastewater in countries located in the northern hemisphere. Thus, failure to consider the time and temperature variation has also affected decoding health metrics. The temporal trends in the detected concentration of RNA genomic copies and infected cases worldwide are summarized in Table 3. Further, Fig. 4 clearly highlights the variation in cases with respect to genome copies on the prior, during, and after sampling day.

Although decay of SARS-CoV-2 in wastewater follows the first-order degradation kinetics (decay constant: k), most of the studies reported SARS-CoV-2 RNA concentration in influent wastewater samples without considering travel time (t) of SARS-CoV-2 RNA in sewers, or more specifically, hydraulic retention time (HRT) of sewers which is significantly correlated to the catchment size of WWTPs. More specifically, the HRT varies from several minutes and 6–10 h in small- and large-scale catchments, respectively. The uncertainty caused due to SARS-CoV-2 RNA decay (U_D) in wastewater while traveling in the sewerage system may be calculated through equation (4) [19].

$$U_D = kt\sqrt{U_k^2 + U_t^2} \qquad (4)$$

where U_D, U_k, and U_t are the uncertainties of D, k and t, respectively. The U_D is calculated to vary between 5 and 10% when the relative standard deviation (RSD) of k for SARS-CoV-2 RNA in wastewater varies from 10 to 15 % and temperature ranges from 15 °C to 37 °C [19].

4.3. Other challenges of WBE application

Six main factors play a critical role in WBE studies [26]: (a) shedding profile of infected individuals; b) recovery efficiency and detection limit of the instruments; c) dilution factor and sampling strategy; d) in-sewer time and degradation; e) turnaround time for sample treatment and quantification and lastly f) data interpretation and reporting (Fig. 1). Although WBE is critical in monitoring pathogens and community disease tracking, it comes with several challenges, such as the complexity of wastewater matrix, estimation of infected population size [9] and the stability and concentrations of biomarkers [46,47]. Another aspect, which may have crawled in WBE, is the variation in SARS-CoV-2 RNA in stool shedding [6]. A viral shedding model study by Miura et al. (2021) observed the difference in estimation factor by nearly 17.2 when a difference of one week was noticed between symptoms onset and

Table 2
Reduction in the viral genome in summer months (Hart and Halden, 2020b).

Initial Load 100 Million Viral Genomes per day	Time (h)	Maximum Reduction (July-September) (%) (±5%)
	1	10%
	2	40%
	5	70%
	10	90%

Table 3
Trends in Temporal Cases and RNA copies worldwide.

Countries	Copies/L	Duration of Study	Cases on	Cases prior	Cases after	References
Australia	120	27th March 2020	328	537	145	[35]
	19	1st April 2020	285			
Bangladesh	39,380	10th August 2020	2907	2654	2174	
	30,180	26th August 2020	2519			
Brazil	25,110	15th April 2020	3348	1644	48,590	[60]
	794,320	25th August 2020	46,959			
Germany	2,000	15th July 2020	486	390	1584	[61]
	3,000,000	15th August 2020	704			
Japan	365,000	17th March 2020	28	57	373	[62]
	355,000	7th July 2020	206			
Netherland	30,000	5th March 2020	44	N.D.	884	[36]
	650,000	25th March 2020	852			
France	50,000	5th March 2020	138	N.D.	5497	[40]
	3,000,000	9th April 2020	4286			
Italy	1,300	28th February 2021	234	147	588	[64]
Spain	3,162,277	14th April 2020	2208	3208	1122	[63]
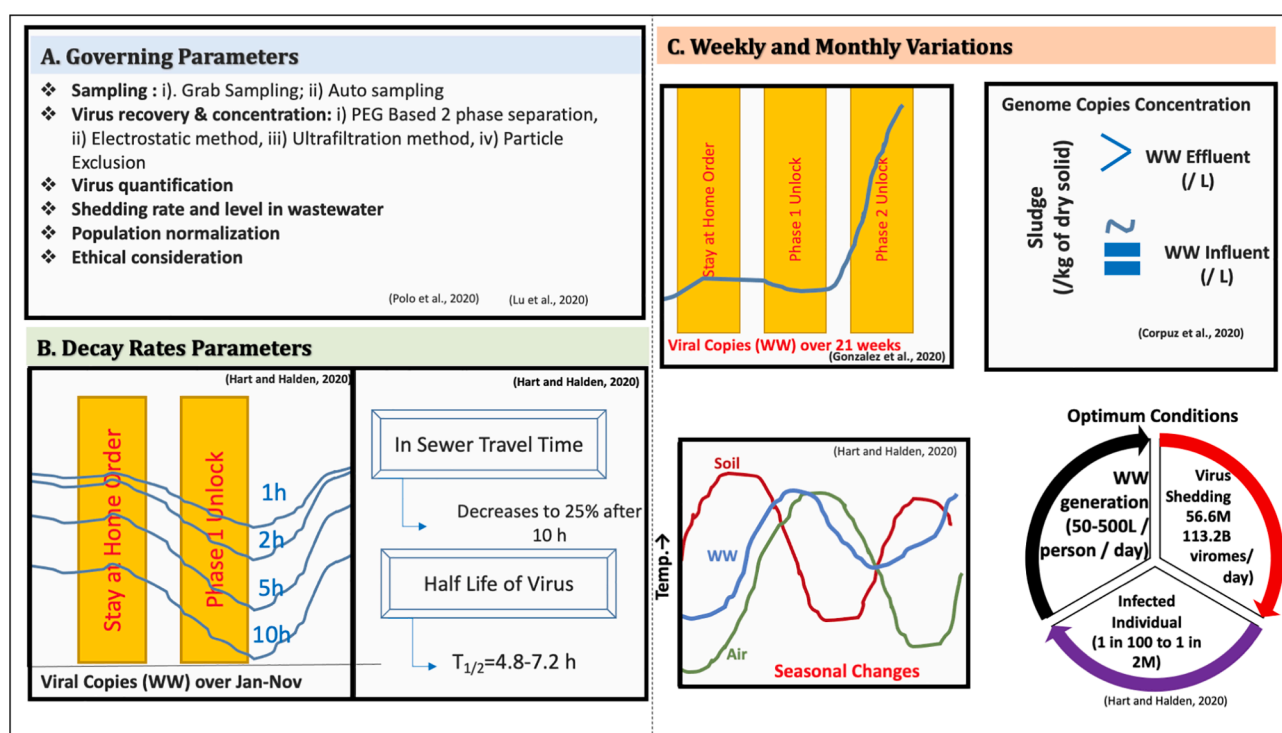
Fig. 1. Criticalities of wastewater-based epidemiology a) effects of WBE parameters and b) temporal variations of SARS-CoV-2 RNA concentrations.

wastewater sampling [27]. This result points to one of the major variations explained by Weidhaas et al. (2021) that could affect WBE studies worldwide [33]. Weidhaas et al. (2021) discussed the importance of wastewater inflow volume, infiltration rate, viral load decay, and sewer shed characteristics while performing correlation analysis between WBE studies and SARS-CoV-2 cases [33].

Fig. 1 shows the different areas of WBE: a) various parameters affecting the WBE approach, b) weekly and monthly variations. However, Fig. 2 explicitly summarises the challenges mentioned above in a graphical manner. Broadly these challenges are grouped into four categories: (i) complex wastewater matrix related difficulties such as PCR inhibitors present in wastewater, variation in the extraction efficiency and the effect of wastewater dilution; (ii) SARS-CoV-2 RNA or biomarker related uncertainties such as biomarker specificity, and concentration range; (iii) uncertainties associated with sample collection (composite vs grab), virus concentration and quantification; (iv) variability in population size such as the influx of tourists, and population normalization. However, it is possible to implement WBE globally by addressing all

these issues, and the cost of conducting WBE is almost negligible than clinical testing, as highlighted in Fig. 2.

One of the limitations of WBE has been its application in the area without drainage network catchment. Recently, Kumar et al. (2022) have recommended that surface waters receiving direct sewage or effluent discharge possess vital clues on possible COVID-19 transmission, possible outbreak, and prevalent conditions and thus may be included in the COVID-19 surveillance [48]. Through a study carried out in Guwahati, a city without any wastewater treatment plant, it was shown that regular monitoring of lake, and river, can surely be helpful in at least predicting the upcoming wave, if any. In a study carried out in Bangladesh by Ahmed et al. (2021), it was illustrated that SARS-CoV-2 surveillance is possible through monitoring of drains and the collected data can be used to predict city health in general, with a caution of interreference owing to dilution, accumulation processes along the way. However, it will remain a fact that well connected-sewer network will provide better results [49].
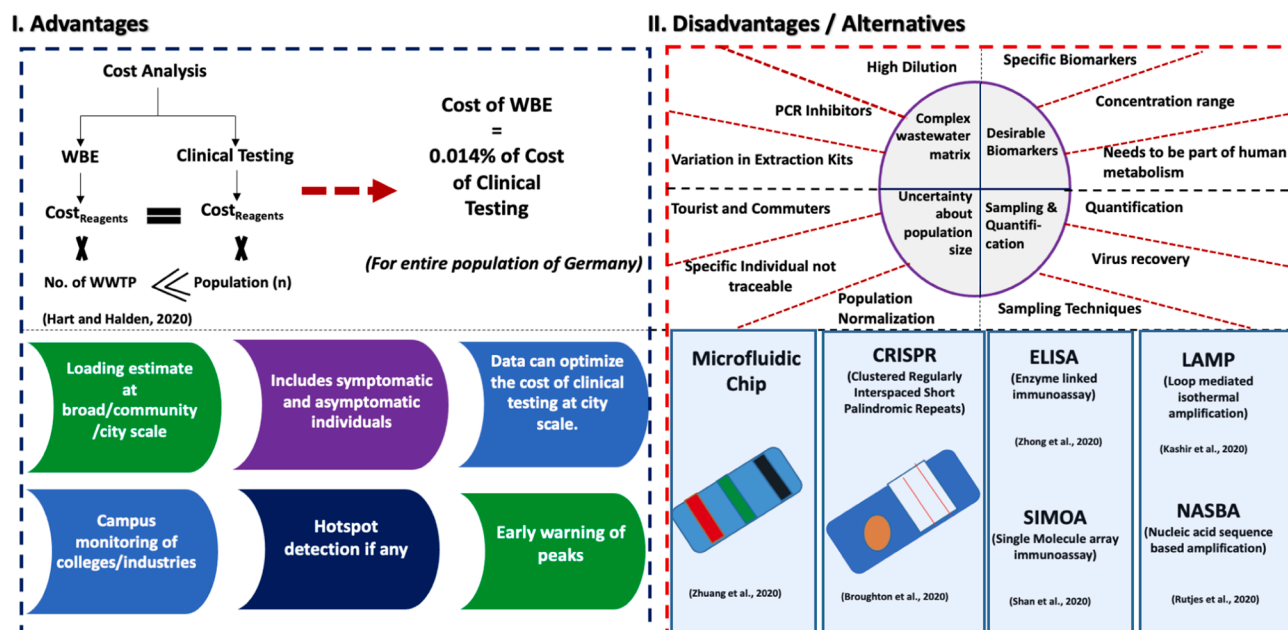
Fig. 2. Advantages, disadvantages and alternatives of WBE considerations. While cost of the WBE along with some explicit uses of the techniques are in favour of WBE relevance in the future; process optimization and method sensitivity will remain a challenge. Several future alternatives being among quick methods can further enhance the WBE result-delivery.

Fig. 3. Effect of temperate and temporal zones on viral RNA quantification in wastewater.

5. Implications of lockdown and subsequent COVID-19 waves on WBE

Pons et al. (2020) studied the 3 months lockdown effect on wastewater quality in two urban areas of France with 0.25 M and 0.3 M populations, respectively [50]. The study also considered the commuters traveling into and out of the city. The number of outward and inward daily commuters was extracted from national census databases related to the population and their commuting habits. Wastewater quality parameters such as chemical oxygen demand (COD), biochemical oxygen demand (BOD), total nitrogen (N) and phosphorus (P), and total dissolved solids (TDS) were also monitored to establish correlation among wastewater quality, quantity, number of infected individuals and virus concentration. The amount of ammonium load generated remained constant due to the use of toilets before leaving for work and after returning from work [50]. Subsequently, the decrease in COD concentration was related to the increase in greywater volume. These little to no variation in the water quality during the lockdown period conforms to the WBE technique and validate its efficiency.

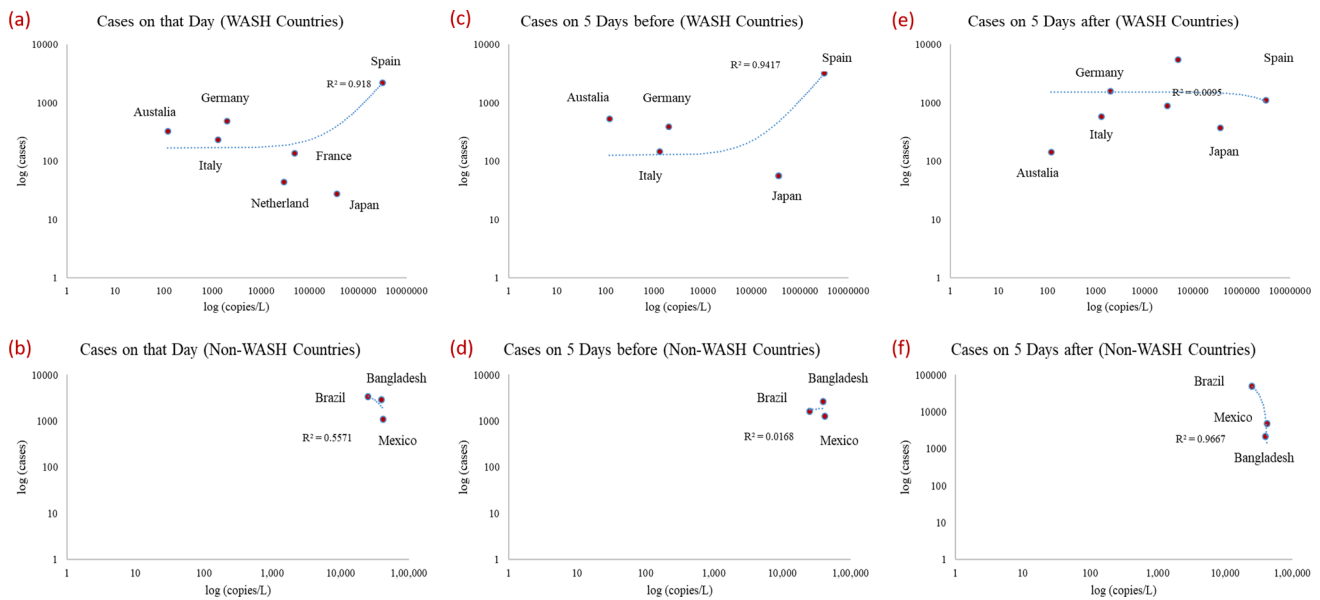WBE has been proven to be beneficial in the nations experiencing

Fig. 4. Correlation between cases and genome copies in two types of countries with respect to sampling Day.

subsequent waves of COVID-19 infections. WWTPs in Spain were monitored during the two waves experienced by the country. In both scenarios, the daily load of SARS-CoV-2 gene copies strongly correlated with the number of cases with a lead time of 7 days [51]. Monitoring small WWTPs was more challenging than larger ones due to lower gene copies loads. Another study in Barcelona, Spain, suggested that the WBE can anticipate future outbreaks, the mitigation measure impacts and the number of infected individuals, including symptomatic and asymptomatic cases [52]. Kumar et al. (2021) also found the potential of WBE surveillance in predicting the fluctuation by a lead of 1–2 weeks in cases, which could help provide an early warning [53]. However, the virus has

been adapting, and new emerging variants might change the observed lead time for other variants (Fig. 5).

The impactful 2nd COVID-19 wave has infected millions and claimed thousands of lives just in April 2020. Our study here has corroborated all other studies, suggesting possible reasons for the massive outbreak. 1) In an Indian study conducted on the SARS-CoV-2 antibodies prevalent, 7 out of 100 people showed the antibodies prevalence indicating that antibodies range between 3 and 6 months in most of the population [54]. 2) The new variants B.1.617 (Indian-Variant), B.1.1.7 (UK-Variant) and B.1.351 (South-Africa Variant) were detected as VOCs (Variant of Concern). B.1.617 mutated variant is known to have higher
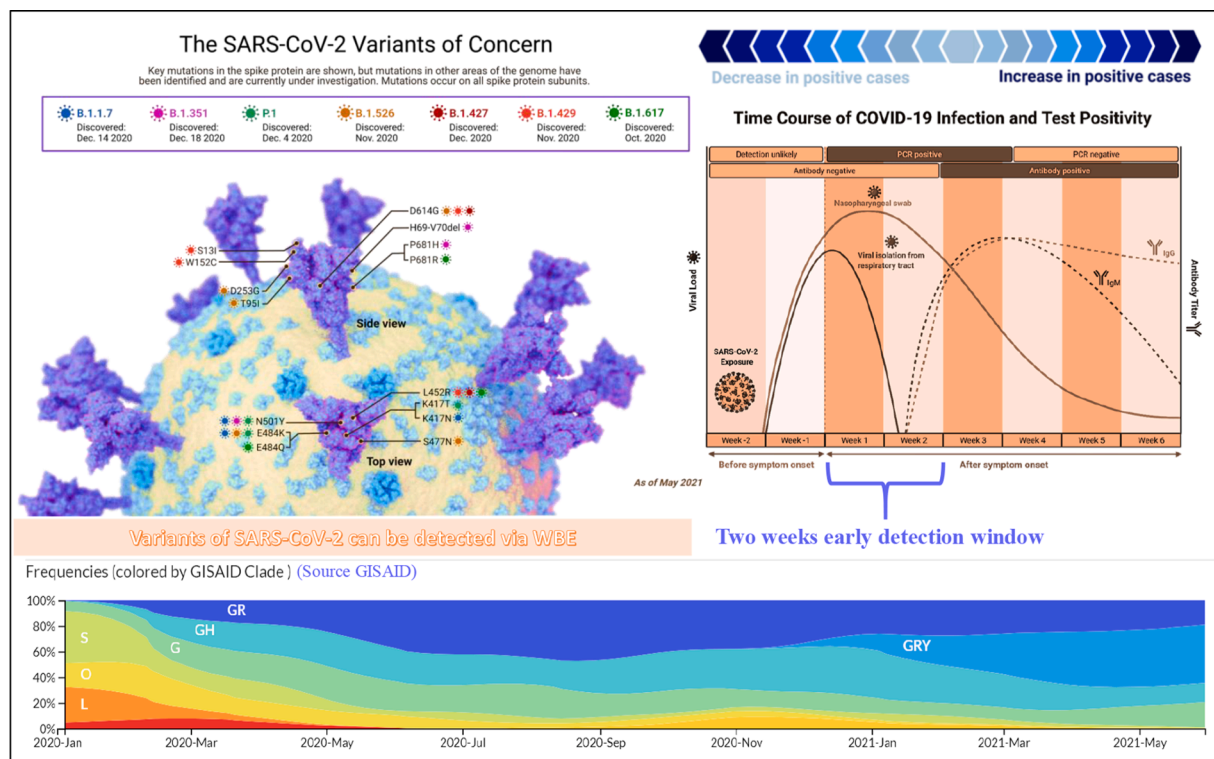

Fig. 5. The modifications in variants of SARS-CoV-2 and time-course of variants of SARS-CoV-2 detected through WBE studies.

pathogenicity and transmissibility [55,56] and was part of most samples tested in Maharashtra, India [57]. 3) Apart from the contagious double mutant variant, the other reasons included the population's negligent behavior and relaxations from lockdown [58]. 4) The fourth reason the article suggests is that the airborne transmissions of the mutated variant are applicable over a larger area and for a longer duration. WBE must have pace with the emerging variants and continue to improve strategies to be more distinctive in predicting trends before the actual rise in infection.

6. Considerations

Among the approaches to curtail the COVID-19 pandemic, including cost, time and labor-intensive clinical testing, WBE would be a highly cost-effective alternative during this unprecedented time. It is more effective for economically fragile countries than mass testing of individuals. The nationwide WBE approach can be more successful in accurately predicting the number of infected people in each catchment when consistent sampling and analytical procedures are adopted. Thus, some of the future outlooks include:

- 24-hour flow proportional composite sampling should be adopted to account for the diurnal variation in the SARS-CoV-2 genome concentration.
- The sampling of manholes located upstream of the sewerage network is recommended over downstream for more accurate and immediate information of infected individuals in a catchment.
- Wherever possible same-day sampling and analysis of sludge may be conducted to have better information on the infection rate in case the regular clinical testing is impossible.
- While collecting samples at the WWTP inlet, uncertainties associated with the SARS-CoV-2 RNA decay and sewer HRT must be considered
- Adopting an efficient SARS-CoV-2 RNA extraction protocol and normalization strategy such as PMMoV-normalized SARS-CoV-2 concentrations may be incorporated while reporting WBE results.
- Appropriate SARS-CoV-2 surrogate marker may be used as a quality assurance/quality control (QA/QC) criterion while reporting the virus concentrations in wastewater samples which can minimize the analytical uncertainties
- The temperature correction factor may be incorporated to correct the diurnal and seasonal variations in the SARS-CoV-2 genome concentration.
- Keeping track of tourists or commuters entering and leaving a catchment and taking them into account while reporting WBE data are also recommended.
- With the growing WBE data available, we further recommend artificial intelligence and machine learning-based simulation studies for a precise understanding of the impacts of various governing features of WBE results before using it as a precise predictor for any upcoming outbreak wave.

7. Conclusions

The COVID-19 pandemic has proved that efficient environmental monitoring through WBE for infectious disease is critically important for source apportionment, early warning, and mega to micro-level management of the pandemic/endemic. Due to the significant amount of data on WBE for various climatic conditions, we reviewed the influence of geographical location/climatic condition on the extent of lead-time feasible for COVID-19 over the last two years. Although we found that WBE provides non-invasive, near-real-time monitoring of COVID-19 infections through analyzing SARS-CoV-2 in wastewater from the area served by sewerage network, it is not practical in communities lacking well-connected sewer networks. As the number of genome copies detected per liter of wastewater was higher in temperate countries, the study cautions the (rough) comparison of WBE data without a thorough

consideration of population dynamics, sanitary features, geographical locations, previous COVID-19 cases, background positivity rates, and climatic conditions (season, dilution, sampling time, humidity, and temperatures). Thus, the normalization of WBE data while acknowledging wide variation in the climatic, sanitation, environmental, and catchment characteristics is a way forward to predict the lead-time accurately.

CRediT authorship contribution statement

Manish Kumar: Supervision, Conceptualization, Visualization, Writing – review & editing, Project administration. **Guangming Jiang:** Writing – original draft and editing, Revision. **Alok Kumar Thakur:** Writing – original draft and editing, Revision. **Shreya Chatterjee:** Writing – original draft and editing, Revision. **Tanushree Bhattacharya:** Writing and editing, Revision. **Sanjeeb Mohapatra:** Writing – original draft and editing, Revision. **Tushara Chaminda:** Writing and editing, Revision. **Vinay Kumar Tyagi:** Writing and editing, Revision. **Meththika Vithanage:** Writing and editing, Revision. **Prosun Bhattacharya:** Writing and editing, Revision. **Long D. Nghiem:** Writing and editing, Revision. **Dibyendu Sarkar:** Writing and editing, Revision. **Christian Sonne:** Writing and editing, Revision. **Jürgen Mahlknecht:** Writing and editing, Revision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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