scientific reports

OPEN

Measuring SARS-CoV-2 RNA in Bangkok wastewater treatment plants and estimating infected population after fully opening the country in 2023, Thailand

Thanchira Saita[1,2], Bussarakam Thitanuwat[1], Nattamon Niyomdecha[3], Jarunee Prasertsopon[4], Hatairat Lerdsamran[4], Pilaipan Puthavathana[4] & Pirom Noisumdaeng[1,2]✉

Wastewater-based epidemiology (WBE) has been employed for monitoring the presence of SARS-CoV-2 infected population. Herein, the study aims to apply the WBE for surveillance and monitoring SARS-CoV-2 in Bangkok, where the highest official covid-19 cases reported in Thailand, during the fully opening for international tourists in early 2023. A total of 200 wastewater samples (100 influent and 100 effluent samples) were collected from 10 wastewater treatment plants (WWTPs) during January–May 2023. SARS-CoV-2 RNA was detected by real time qRT-PCR with accounting for 51% (102/200). Of these, 88% (88/100) and 14% (14/100) were detected in influent and effluent samples, respectively. The SARS-CoV-2 RNA concentration was detected in ranged of 4.76×10^2–1.48×10^5 copies/L. The amount of SARS-CoV-2 RNA has increased approximately 4 times from the lag phase (January–March) to the log phase (April–May). Spearman's correlation coefficient revealed that correlation between estimated infected population and weekly reported cases was statistically significant (p-value = 0.017). SARS-CoV-2 RNA in influent had a statistically significant relationship with weekly reported cases ($r = 0.481$, p-value < 0.001). Lag time analysis revealed early warning 1–3 weeks before rising covid-19 cases observed. GIS was applied for spatial-temporal analysis at the province level, suggesting real time dashboard should be further developed.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent for covid-19, emerged in December 2019 in Wuhan, China[1,2]. The virus belongs to the *Coronaviridae* family which contains a positive-sense single-stranded RNA genome of approximately 30,000 nucleotides in length[3]. It has rapidly spread worldwide to become a global health crisis. As of 04 August 2024, WHO reported the cumulative numbers of 775,867,547 infected cases, and significant numbers of fatalities around 7,057,145 deaths[4,5]. Significantly, multiple SARS-CoV-2 variants (VOCs and VOIs), such as Alpha, Beta, Gamma, Delta, and Omicron variants, have emerged over time posing additional challenges due to their increased transmissibility and potential for immune evasion[6]. The primary mode of viral transmission is respiratory droplets and airborne transmission[2,7]; nevertheless, the virus can be replicated in the gastrointestinal tract and excreted through the patient's feces and urine[8], leading to contamination of wastewater and the environment[9].

SARS-CoV-2 infections have caused varying degrees of clinical manifestations from asymptomatic to severe complications, mainly respiratory disease involvement[10]. However, a significant prevalence of patients ranging from 26.7 to 90% was reported for gastrointestinal tract symptoms, 6.7–40% for diarrhea, and 20–50% for related symptoms, i.e., anorexia, nausea, vomiting, and abdominal pain[11]. The virus was detected in respiratory secretions including saliva, sputum, nasal secretions, and also non-respiratory specimens including serum, feces and urine in infected persons[8,12]. Interestingly, the SARS-CoV-2 RNA shedding can be seen as high as 10^2–10^{10}

[1]Faculty of Public Health, Thammasat University, Pathum Thani 12121, Thailand. [2]Thammasat University Research Unit in Modern Microbiology and Public Health Genomics, Thammasat University, Pathum Thani 12121, Thailand. [3]Department of Medical Technology, Faculty of Allied Health Sciences, Thammasat University, Pathum Thani 12121, Thailand. [4]Faculty of Medical Technology, Mahidol University, Nakhon Pathom 73170, Thailand. ✉email: pirom.n@fph.tu.ac.th

RNA copies/g of feces[13,14], 10^2–10^5 RNA copies/ml in urine[15], and 10^5 to 10^{11} RNA copies/ml found in saliva and sputum[16], and serum viral load was found 10^1 to 10^5 RNA copies/ml[17]. The viral shedding in respiratory and gastrointestinal specimens significantly contributed to the presence of the virus in wastewater. Several studies have demonstrated that SARS-CoV-2 RNA can remain stable for extended periods in various environments such as surfaces or carriers of particulate matter in air[18], including wastewater[19]. SARS-CoV-2 RNA has stability in wastewater for 2 days at 20 °C and > 14 days at 4 °C[20].

Wastewater-based epidemiology (WBE) has emerged as a complementary approach to traditional testing methods for monitoring the prevalence and spread of SARS-CoV-2 in a particular area[9,21,22]. It also serves as an alternative method for monitoring the presence of the virus in the community and an indicator of active infections. Over 72 countries on seven continents (i.e., Australia, New Zealand, India, Japan, Spain, USA, South Africa, Canada Brazil, and Argentina) applied WBE as a surveillance and monitoring tool to provide early warning of outbreaks[13,19,23–31]. Thailand has also been significantly affected by multiple SARS-CoV-2 variants introduced to the country[32–34] and has also applied WBE to detect and investigate the dynamics of SARS-CoV-2 outbreaks[34–36]. Several previous studies reported the WBE-based monitoring and surveillance in urban areas such as Bangkok and metropolitan areas[33–35,37], and also in the rural areas in the southern and northern provinces of Thailand[33,35,36]. The results of monitoring served as early warning before 14–20 days[35]. Previous reports showed a correlation between the SARS-CoV-2 RNA concentration presented in wastewater and reported cases[37]. In addition, SARS-CoV-2 variants were found and identified in wastewater, reflected in clinical specimens from patients at the same time[33,34]. All the results of those studies demonstrated that WBE can be applied in Thailand as an efficient tool for monitoring SARS-CoV-2 new variants or other emerging infectious diseases. Nevertheless, those studies were performed during SARS-CoV-2 outbreaks and lacked information on the estimated infected population and spatio-temporal area-based analysis.

Bangkok is the capital and most populous city of Thailand. It is the central hub of traveling, governance, education, transportation, finance, banking, commerce, communication, and the prosperity of the country. Bangkok is the 14th world largest city[38] by population with more than 8.3 million identified by both registered and non-register populations[39,40], and it is the 1st best city in 2024 for tourist destinations with an average of more than 25 million travelers per year[41]. It has also been affected by SARS-CoV-2 outbreaks, resulting in the highest numbers of covid-19 patients officially reported (20.8%, (997,059/4,793,505) at the national level as of 30th August 2024[42]. Therefore, the study aims to apply WBE for surveillance and monitoring covid-19 in Bangkok after fully opening the country. The influent and effluent wastewater were collected from large-scale WWTPs which collected and treated wastewater generated from the inner city, and small-scale WWTPs which collected and treated wastewater from areas under supervision of the National Housing Authority with residents over 15,000 people. In addition, this study overcame the previous limitations by demonstrating the estimated infected population from the prediction model and used the Geographic Information System (GIS) for spatio-temporal area-based analysis.

Materials and methods

Biosafety approval

The biosafety issue of the study was approved by the Institutional Biosafety Committee (TU-IBC), Thammasat University with the certificate of approval number 084/2565. All experiments were conducted under enhanced biosafety laboratory level 2 (BSL2+) at the Faculty of Public Health, Thammasat University.

SARS-CoV-2 RNA for validation

Purified RNA of three SARS-CoV-2 strains, i.e., hCoV-19/Thailand/MUMT-3/2020 (Wuhan-like strain), hCoV-19/Thailand/MUMT-53/2021 (Delta variant), and hCoV-19/Thailand/MUMT-11/2022 (Omicron variant) were used as positive controls for validation in real time quantitative reverse transcription polymerase chain reaction (real time qRT-PCR) system.

Study area

Bangkok, the capital of Thailand, has eight large-scale wastewater treatment plants (WWTPs) responsible for 216 km^2 covering 22 districts and twelve small-scale WWTPs that collect wastewater from the National Housing Authority (Supplementary Table S1). These WWTPs have a total capacity of approximately 1,136,800 cubic meters per day. An estimated 48% of the wastewater generated in Bangkok was collected and treated in WWTPs, and the remaining 52% of wastewater was discharged directly to the canal. In the study, we selected 10 representative WWTPs, i.e., six large-scale WWTPs responsible for the inner city (including Si Phraya, Chong Nonsi, Rattanakosin, Din Daeng, Bang Sue, and Chatuchak), and four small-scale WWTPs (including Thung Song Hong 1, Huai Khwang, Khlong Chan and Romklao) which are responsible for collecting and treatment of wastewater at area of registered-population over 15,000 people (Fig. 1; Table 1).

Wastewater collection, concentration, and total nucleic acid extraction

Approximately 1.5 Liters (L) of influent (untreated wastewater) and effluent (treated wastewater) samples from WWTPs were collected by a 24-h composite sampling technique using an autosampler or manual composite sampling in the sterile polyethylene bottle container. The sampling events were conducted between January to May 2023 by continuous sampling intervals every 14 days. Therefore, a total of 200 composite wastewater samples, including 100 influent and 100 effluent samples were obtained. Wastewater samples were contained in triple packaging, preserved at 4 °C, and transported to the laboratory within 8 h.

Wastewater samples were pasteurized at 60 °C for 30 min and mixed by inversion to generate homogeneously. The 40 ml of wastewater was concentrated and extracted for total nucleic acid by using Wizard® Enviro Total

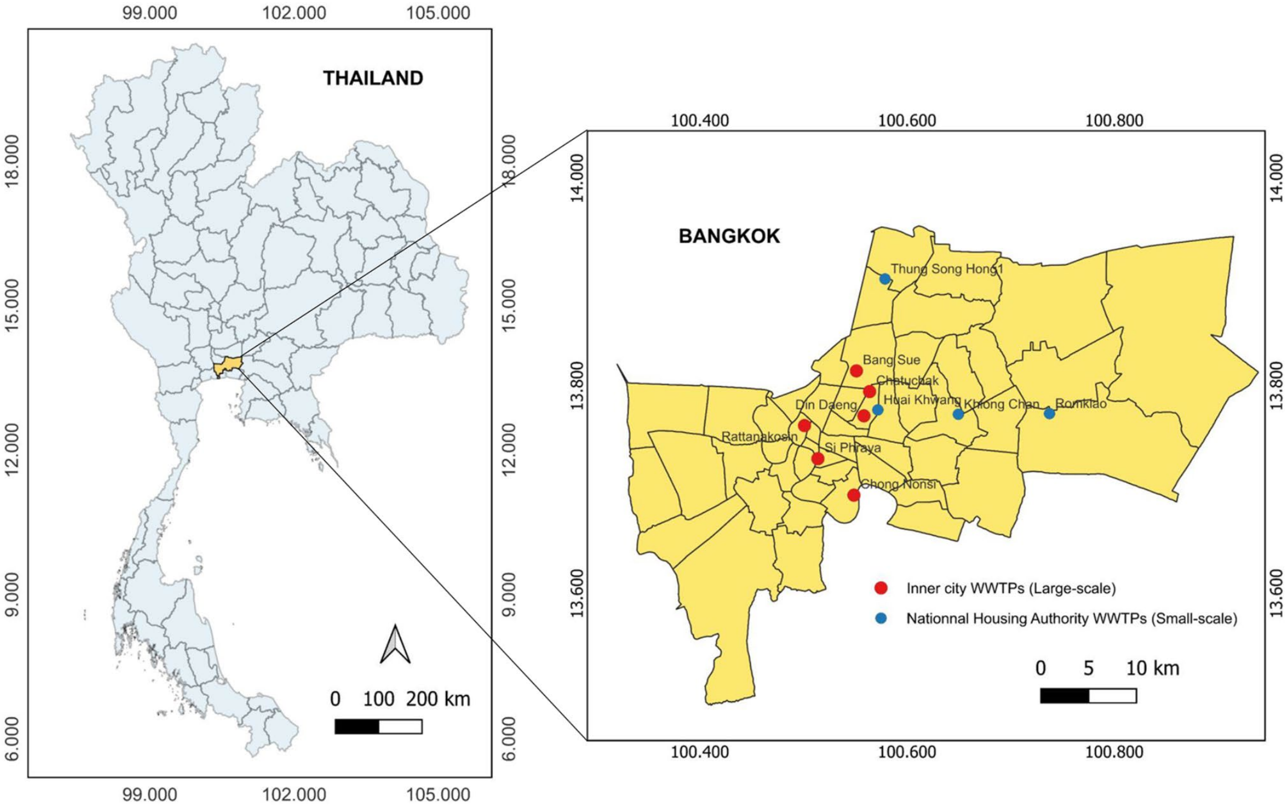

Fig. 1. Study sites of Bangkok WWTPs including six large-scale WWTPs located in the inner city and four small-scales WWTPs which collected wastewater from the National Housing Authority.

Nucleic Acid kit (catalog number: A2991) (Promega, Madison, WI) as following the manufacturer's instructions. The extracted total nucleic acid with a final volume of 40 µl was stored at -70 °C until further analysis.

Detection and measurement of SARS-CoV-2 RNA using real time qRT-PCR

Real time qRT-PCR assay was performed using GoTaq® Enviro Wastewater SARS-CoV-2 system (catalog numbers: AM2100 and AM2110) (Promega, Madison, WI) as following manufacturer's instruction. This system included primer/probe sets prescribed by the U.S. Center for Disease Control (CDC), and designed for one-step real time qRT-PCR. The system comprised an internal amplification control (IAC) and primers for detecting Pepper Mild Mottle Virus (PMMoV), an RNA virus commonly found in wastewater. The 10 µl reaction mixture for SARS-CoV-2 detection comprised 5 µl of 2X GoTaq® Enviro Master Mix, 0.2 µl of GoScript™ Enzyme Mix, 0.5 µl of 20X Primer/Probe/IAC Mix, 0.3 µl of nuclease-free water and 4 µl of extracted nucleic acid. The real time qRT-PCR was performed on a CFX96 Touch Real Time PCR Detection System (Bio-Rad) with the standard cycling condition including reverse transcription (45 °C for 15 min) for 1 cycle, RT inactivation/ GoTaq® activation (95 °C for 2 min) for 1 cycle, denaturation (95 °C for 15 s) and annealing/extension (62 °C for 60 s) for 40 cycles. The results were analyzed using Bio-Rad CFX Maestro software 2.3 (https://www.bio-rad.com/en-in/sku/12013758-cfx-maestro-software-2-3-for-windows-pc?ID=12013758).

For preparing standard dilution for SARS-CoV-2 and PMMoV RNAs, five-serially concentration dilutions (1.6×10^5, 1.6×10^4, 1.6×10^3, 1.6×10^2, 1.6×10^1 copies/reaction) were prepared as followed manufacturer's instruction. The standard curve with the average slope and R squared (R^2 value) was determined (Supplementary Fig. S1). The quantitatively amount of SARS-CoV-2 viral genomes (copies/L) based on N1 detection were calculated as Eq. (1):

$$\text{Viral genome (copies/L)} = \frac{\text{Copies in real time qRT} - \text{PCR} \times 1000}{\text{Volume of nucleic acid extract used in real time qRT} - \text{PCR (ml)} \times \text{Concentration factor}} \quad (1)$$

Where:

$$\text{Concentration factor} = \frac{\text{Wastewater sample volume used (ml)}}{\text{The volume of nucleic acid extracted (ml)}} \quad (2)$$

Categories	WWTPs (Site code)	Flowrate (m^3/ day)	Serve population	Sampling point (rate of detection)	SARS-CoV-2 RNA concentration (copies/L) in wastewater samples collected on									
					24-Jan-23	7-Feb-23	21-Feb-23	7-Mar-23	21-Mar-23	4-Apr-23	18-Apr-23	2-May-23	16-May-23	30-May-23
Inner city of Bangkok	Din Daeng (L1)	350,000	1,080,000	Influent (100%)	6.48×10^{3}	8.24×10^{3}	3.71×10^{3}	1.20×10^{4}	1.43×10^{4}	3.57×10^{4}	4.62×10^{4}	3.57×10^{4}	2.66×10^{3}	2.85×10^{4}
				Effluent (0%)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Chong Nonsi (L2)	200,000	580,000	Influent (80%)	ND	1.42×10^{3}	7.22×10^{3}	ND	2.85×10^{4}	9.23×10^{3}	4.65×10^{4}	2.43×10^{4}	5.18×10^{4}	6.56×10^{3}
				Effluent (40%)	ND	6.27×10^{2}	ND	ND	ND	1.21×10^{3}	4.76×10^{2}	1.93×10^{3}	ND	ND
	Chatuchak (L3)	200,000	360,000	Influent (100%)	8.49×10^{3}	2.72×10^{3}	8.24×10^{3}	7.22×10^{3}	7.18×10^{3}	9.12×10^{3}	1.79×10^{4}	4.05×10^{4}	1.21×10^{4}	9.86×10^{3}
				Effluent (0%)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Bang Sue (L4)	120,000	223,990	Influent (80%)	7.01×10^{3}	3.76×10^{3}	7.90×10^{3}	8.85×10^{3}	4.21×10^{3}	6.92×10^{3}	3.38×10^{4}	3.02×10^{4}	7.31×10^{3}	4.10×10^{4}
				Effluent (0%)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Si Phraya (L5)	30,000	120,000	Influent (90%)	2.41×10^{3}	1.79×10^{3}	ND	2.31×10^{4}	4.20×10^{4}	6.72×10^{3}	7.78×10^{4}	2.60×10^{4}	2.33×10^{3}	1.51×10^{3}
				Effluent (30%)	ND	ND	ND	ND	1.36×10^{3}	ND	ND	4.34×10^{3}	ND	7.73×10^{2}
	Rattanakosin (L6)	40,000	70,000	Influent (70%)	8.77×10^{2}	ND	3.74×10^{3}	ND	1.23×10^{4}	7.26×10^{3}	4.22×10^{4}	3.49×10^{4}	ND	3.37×10^{3}
				Effluent (20%)	ND	ND	ND	ND	ND	ND	1.16×10^{3}	1.10×10^{3}	ND	ND
National housing authority	Khlong Chan (S1)	6500	36,000	Influent (90%)	6.64×10^{3}	2.55×10^{3}	5.51×10^{3}	ND	4.24×10^{3}	8.29×10^{3}	2.86×10^{4}	2.15×10^{4}	4.95×10^{3}	3.53×10^{4}
				Effluent (20%)	ND	ND	ND	ND	1.46×10^{3}	ND	ND	1.99×10^{3}	ND	ND
	Romklao (S2)	3800	19,000	Influent (100%)	9.04×10^{2}	2.48×10^{4}	9.74×10^{3}	3.08×10^{3}	2.22×10^{3}	1.03×10^{3}	2.25×10^{4}	1.26×10^{4}	3.98×10^{4}	9.26×10^{4}
				Effluent (10%)	ND	ND	ND	ND	1.46×10^{3}	ND	ND	ND	ND	ND
	Huai Khwang (S3)	2400	16,800	Influent (80%)	2.21×10^{4}	7.09×10^{3}	ND	1.48×10^{3}	ND	6.82×10^{4}	1.86×10^{4}	9.60×10^{4}	1.48×10^{5}	7.16×10^{4}
				Effluent (20%)	ND	ND	ND	ND	ND	1.28×10^{3}	ND	ND	5.70×10^{2}	ND
	Thung Song Hong 1 (S4)	3000	15,000	Influent (70%)	ND	1.29×10^{3}	1.11×10^{3}	ND	2.44×10^{3}	7.80×10^{3}	2.54×10^{4}	1.75×10^{4}	1.05×10^{5}	ND
				Effluent (0%)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table 1. Characteristics of WWTP study sites and SARS-CoV-2 RNA detected by real time qRT-PCR during January - May 2023. *ND* not detected, *L* large-scale WWTPs, *S* small-scale WWTPs.

Covid-19 epidemiological data and Estimation of SARS-CoV-2 infected population

The new officially reported covid-19 cases in Bangkok were retrieved from the Department of Disease Control, Ministry of Public Health (publicly accessible from https://ddc.moph.go.th/covid19-dashboard/) and provided by the Institute for Urban Disease Control and Prevention.

The estimation of the SARS-CoV-2 infected population was determined by using the formula previously reported by Pillay et al.[13], Ahmed et al.[19], and Saththasivam et al.[43]. However, we modified the Eq. (3) to fit in our study area as:

$$\text{Estimated Infected population} = \frac{\left(\text{RNA concentration}\left(\frac{\text{copies}}{\text{L}}\right)\right) \times \left(\text{Flowrate}\left(\frac{\text{L}}{\text{day}}\right)\right)}{\left(\text{RNA rate}\left(\frac{\text{copies}}{\text{gram}}\right)\text{ of excretion}\right) \times \left(\text{Excretion rate}\left(\frac{\text{gram}}{\text{person}} \cdot \text{day}\right)\right)} \quad (3)$$

Where the viable gram of feces per person per day was used Bangkok's excretion rate was 113.96 g/person/day (data from the Information Department of Health[44]). The viral RNA load was used at a constant value of 3.634×10^6 copies/gram as previously reported by Zheng et al.[45]. For determining the validity of the adjusted prediction model, our study categorized WWTPs as small- and large-scale based on their treatment capacity and type of services area. Next, we divided peaks in clinically reported covid-19 cases into two phases: (1) lag phase is the initial period of covid-19 cases before reaching high level of peaks (2) log phase is the peak period that the number of cases is rapidly increasing. These two phases (lag and log) were also statistical tested using one sample t-test for accurately divided confirmation. For each type of WWTP (small and large) and each duration phase (lag and log), the difference between the estimated infected population and the reported infected cases was calculated. The result of difference represented the underreporting of covid-19 cases. A 95% confidence interval was determined to provide a range of values in which the true underreporting is likely to fall with 95% probability.

Lag time correlation analysis for early warning

Lag time correlation analysis was carried out based on three data sets, including SARS-CoV-2 RNA concentration in wastewater, weekly covid-19 reported cases, and estimated infected populations obtained in the study. The analysis was divided into two categories: (1) lag time correlation analysis between SARS-CoV-2 RNA concentration and 1-week average reported cases, and (2) lag time correlation analysis between estimated infected population and 1-week average reported cases. For preparing the data set of each sampling time, the data of reported cases was prepared as a 1-week average, with weeks 0 to 6 representing the sampling week, then the final data were obtained as lag 0 to lag 6. For the calculation of lag 0–6, it is calculated from the reported cases of the sampling week plus the case reports of the following week, and then the average is taken as shown in the Supplementary Figs. S2A and S2B. After that, the data set as shown in the Supplementary Table S2 were obtained for input and analyzing lag time correlation analysis by using the Spearman's correlation coefficient in the SPSS program version 28 (SPSS Inc., USA). The results of lag time correlation were expressed in r (rho, $\boldsymbol{\rho}$) and *p*-value. The result was a significant interpretation under consideration of p-value < 0.05.

Spatio-temporal analysis by geographic information system (GIS)

The spatial relationship between the SARS-CoV-2 RNA presented in wastewater and the numbers of weekly reported cases of individual areas was analyzed using QGIS software version 3.32.2 (https://www.qgis.org/project/overview/). The location, latitude, and longitude were retrieved from Google Maps. ShapeFile as database information for GIS generated by 50 districts in Bangkok was retrieved and downloaded from Bangkok GIS, Bangkok GIS Center (http://www.bangkokgis.com/modules.php?m=download_shapefile). The new officially reported covid-19 cases, SARS-CoV-2 viral concentration, and estimated infected population were used as values for spatio-temporal categories.

Results

Situation and epidemiological data of covid-19 in Thailand

Thailand has opened the country for international tourism since 01 October 2022, and welcome back for Chinese tourists which is the key market returns on 09 January 2023. However, the number of confirmed covid-19 cases has increased in November 2022 and declined overtime between December 2022 to April 2023. Nevertheless, rapidly increasing officially reported cases were investigated after the Songkran festival (Thailand's new year). Overall, over 33,554 national cases were reported between 01 January and 30 September 2023. During this time, Bangkok is the area that reported the highest cases with approximately 20,343 accounting for 60.6% compared to the national level (Fig. 2).

Qualitative detection of SARS-CoV-2 RNA in wastewater from Bangkok WWTPs

Firstly, we determined the detection efficiency of primer/probe sets (N1, N2 and E) in real time qRT-PCR assay by using purified RNA of known SARS-CoV-2 strains, i.e., hCoV-19/Thailand/MUMT-3/2020 (Wuhan-like strain), hCoV-19/Thailand/MUMT-53/2021 (Delta variant), and hCoV-19/Thailand/MUMT-11/2022 (Omicron variant). The real time qRT-PCR Cq targeting SARS-CoV-2 N1 was 12.51, 12.27, and 12.61 for Wuhan-like strain, Delta, and Omicron variants, respectively. The Cq value targeting the N2 region was 13.83, 12.59, and 12.69 for Wuhan-like strain, Delta, and Omicron variants, respectively; meanwhile, the Cq value targeting the E region was 13.45, 12.19, and 33.60 for Wuhan-like strain, Delta, and Omicron variants, respectively (Supplementary Table S3 and Supplementary Fig. S3). This result demonstrated that the N1 primer/probe set showed the highest detection covering all of SARS-CoV-2 variants; therefore, we selected N1 primer/probe set in the study.

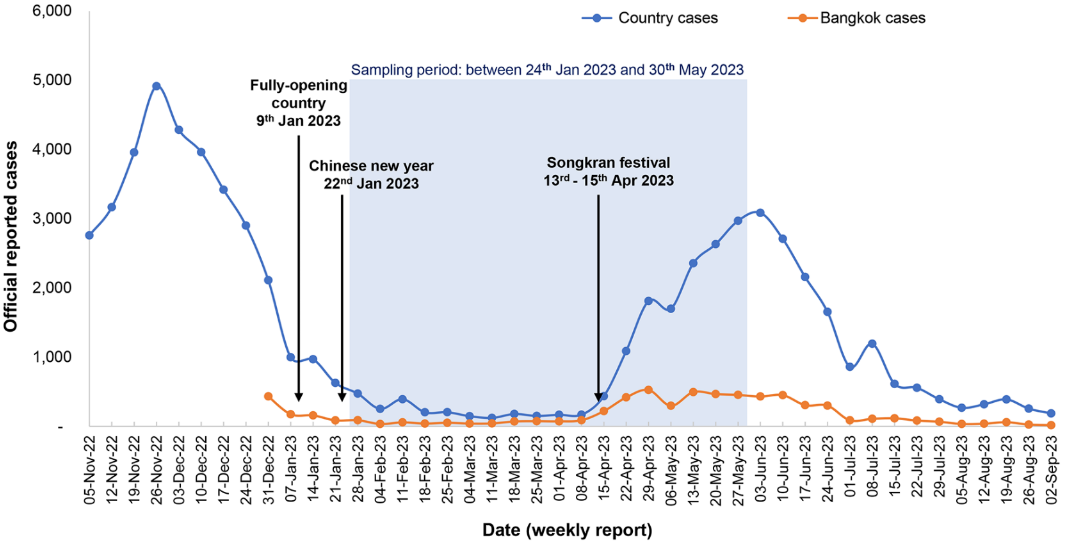

Fig. 2. Sampling duration and weekly reported SARS-CoV-2 infected cases in Thailand and Bangkok between November 2022 and September 2023.

A total of 200 wastewater samples (100 influent and 100 effluent samples) were collected between 23 January and 30 May 2023 by every 2 weeks across 10 WWTPs located in Bangkok. Overall, SARS-CoV-2 RNA was detectable in 51.0% (102/200) (Table 1). The higher positive detection rate was noted in influent samples showing 88% (88/100) with mean ± SD Cq values of 34.74 ± 2.07 and 26.05 ± 2.30 for SARS-CoV-2 N1 gene and PMMoV, respectively. Meanwhile, only 14% (14/100) was found in effluent samples. The mean ± SD Cq values of the SARS-CoV-2 N1 gene and PMMoV detected in effluent samples were 38.38 ± 0.92 and 28.35 ± 2.49, respectively. However, the detection rate was varied by locations of WWTPs by showing a range of 70–100% in influent samples and 0–40% in effluent samples. The mean ± SD of Cq values by time collection and WWTPs were shown in Supplementary Tables S4 and S5.

Quantitative measurement of SARS-CoV-2 RNA in wastewater from Bangkok WWTPs

The quantitative measurement of SARS-CoV-2 RNA presented in wastewater was assessed based on measuring N gene target detection. The geometric mean concentrations (copies/L) of SARS-CoV-2 RNA and PMMoV in the influent samples were $2.10 \times 10^4 \pm 2.62 \times 10^4$ (ranged of 7.73×10^2–1.48×10^5; $n = 88$) and $7.61 \times 10^6 \pm 6.96 \times 10^6$ (ranged of 4.32×10^3–2.76×10^7; $n = 100$), respectively. Meanwhile, the geometric mean concentrations (copies/L) of SARS-CoV-2 RNA and PMMoV in the effluent samples were $1.46 \times 10^3 \pm 9.10 \times 10^2$ (ranged of 4.76×10^2–4.34×10^3; $n = 14$) and $2.48 \times 10^6 \pm 3.20 \times 10^6$ (ranged of 1.12×10^4–1.71×10^7; $n = 100$), respectively. Based on large and small WWTPs, the ranges of SARS-CoV-2 RNA concentrations in wastewaters collected from the inner city of Bangkok (L1–L6) and the National Housing Authority WWTPs (S1-S4) were $1.72 \times 10^4 \pm 1.68 \times 10^4$ (ranged of 7.73×10^2–7.78×10^4; $n = 54$) and $2.71 \times 10^4 \pm 3.56 \times 10^4$ (ranged of 9.04×10^2–1.48×10^5; $n = 34$), respectively (Table 1, Supplementary Tables S4 and S5). The viral concentration time series was investigated and found low viral RNA concentration was detected from January to March 2023 with an average of $8.22 \times 10^3 \pm 8.59 \times 10^3$ copies/L; nevertheless, it was found to rapidly increase starting from April to May 2023 by showing approximately 4 times of higher concentration ($3.17 \times 10^4 \pm 3.07 \times 10^4$ copies/L) (Fig. 3A).

Correlation between SARS-CoV-2 RNA in wastewater and covid-19 reported cases

Spearman's correlation coefficient analysis demonstrated that SARS-CoV-2 RNA concentration detected in the influent samples showed a significant correlation with weekly covid-19 reported cases ($r = 0.481$, p-value < 0.001); while non-significant correlation (p-value = 0.405) was observed between SARS-CoV-2 RNA concentration detected in effluent samples and number of covid-19 reported cases (Supplementary Table S6).

Estimation of the infected population

Spearman's correlation coefficient analysis demonstrated a significant correlation between estimated infected population and reported cases (p-value = 0.017). To ensure a reliable range of potential values associated with the estimated infected populations, our study calculated 95% confidence intervals based on four different groups (small-scale WWTP, large-scale WWTP, lag phase and log phase). The prediction model revealed that the small-scale WWTPs had overestimated infected cases between 12 and 68 cases and between 175 and 443 cases than real world reported cases during the lag (January-March) and log phases (April-May), respectively. For large-scale WWTPs, the estimated infected cases had overestimated than real word reported cases between 1,635 and 4,427 cases, and between 5,656 and 14,085 cases, during the lag phase and log phase, respectively (Supplementary Table S7). A comparison of estimated infected population and reported cases by plotting a graph shows a similar trend as shown in Fig. 3B. During the late March and April surge, all estimated infected cases in WWTPs reached their highest peak simultaneously. The illustration of the correlation between estimated infected cases and reported

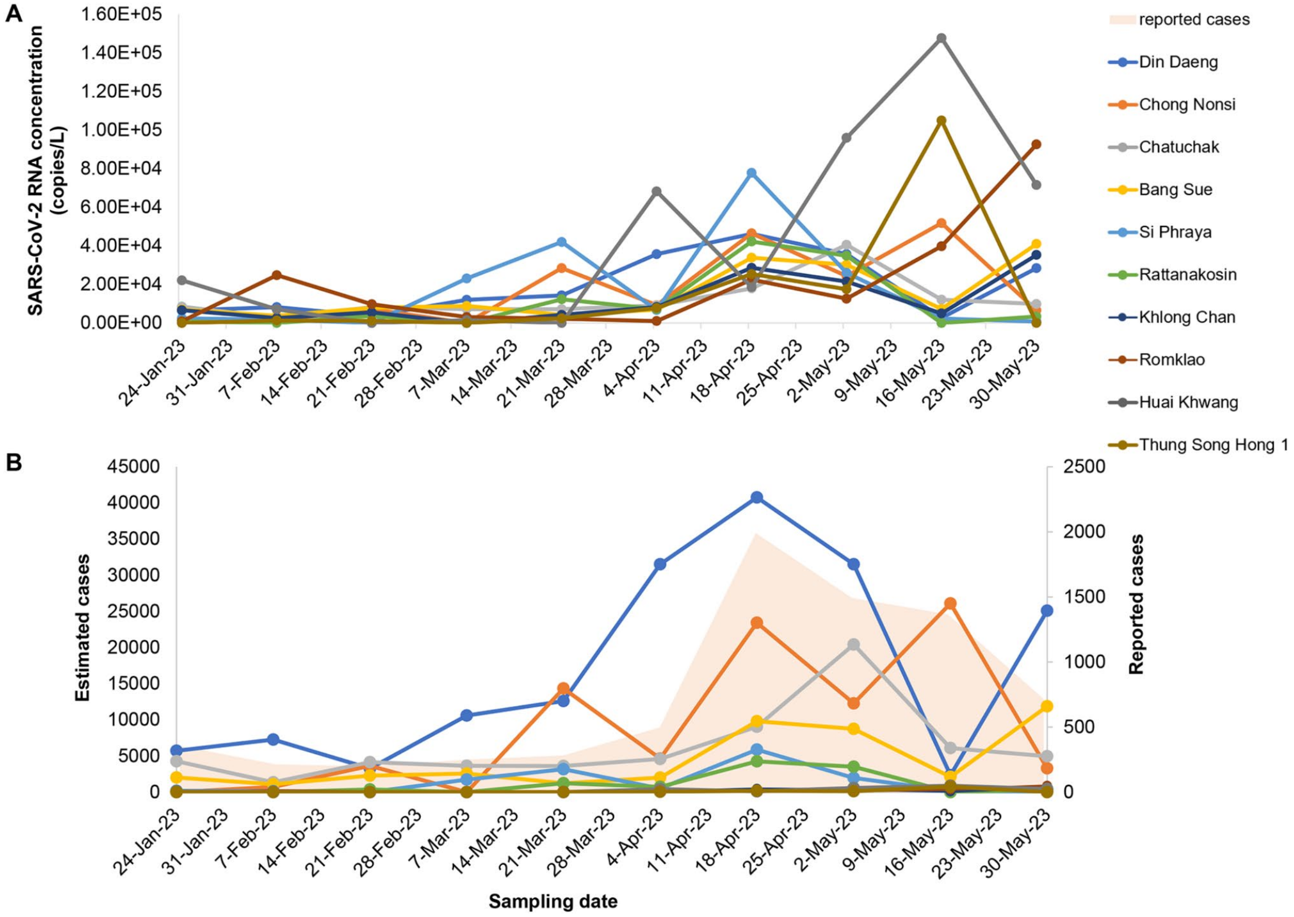

Fig. 3. SARS-CoV-2 RNA concentration in wastewater from 10 Bangkok WWTPs (**A**) plotting graph of officially reported cases (line graph) and estimated infected cases (shaded area under the curve) (**B**).

cases at individual WWTPs within service areas demonstrated consistent peak and trending patterns for both large and small-scale WWTPs (Fig. 4 and Supplementary Table S8).

Lag time correlation analysis for early warning

Lag time correlation analysis was conducted to assess the potential of wastewater-based epidemiology (WBE) as an early warning system for covid-19 outbreaks in Bangkok by comparing SARS-CoV-2 RNA concentrations in wastewater to officially reported cases. During the post-pandemic period in Bangkok, lag time correlation analysis revealed a strong correlation ($\rho = 0.620$) between estimated infected cases and reported cases, with a lag time of 1–3 weeks (Table 2). However, the correlation between SARS-CoV-2 RNA concentration and reported cases was weaker ($\rho = 0.430$) with a similar lag time (Supplementary Table S9).

GIS application for SARS-CoV-2 surveillance

Large scales of WWTPs (L1–L6) serving the inner city of Bangkok were applied for GIS by using data on viral RNA concentration (copies/L), estimated infected population, and weekly reported cases from 50 districts of Bangkok. The viral RNA concentrations and estimated infected population were able to be early detected on 07 February 2023, in the inner city of Bangkok, before a reported case on 21 February 2023 (2 weeks). Nevertheless, some of the spatial analysis demonstrated varying patterns due to the movement dynamics of the population (Fig. 5).

Discussion

Between 2020 and 2022, Thailand encountered multiple SARS-CoV-2 variants causing a total of five waves of covid-19, e.g., S-clade for the 1st wave, GH-clade for the 2nd wave, Alpha variant for the 3rd wave, Delta variant for the 4th wave and finally Omicron variant for the 5th wave[46]. Bangkok, the capital city of Thailand, was greatly affected with the highest number of infected cases reported[34]. It is an area where tourists come in first, allowing new variants and outbreaks before the viruses spread to other provinces. After declining of covid-19 reported cases in early 2023, this allows to fully-open the country for welcoming foreign tourists, including Chinese tourists, on January 9, 2023. However, there are still concerns about a new wave of outbreaks that may occur after the country opening, Chinese New Year, and Songkran Festival.

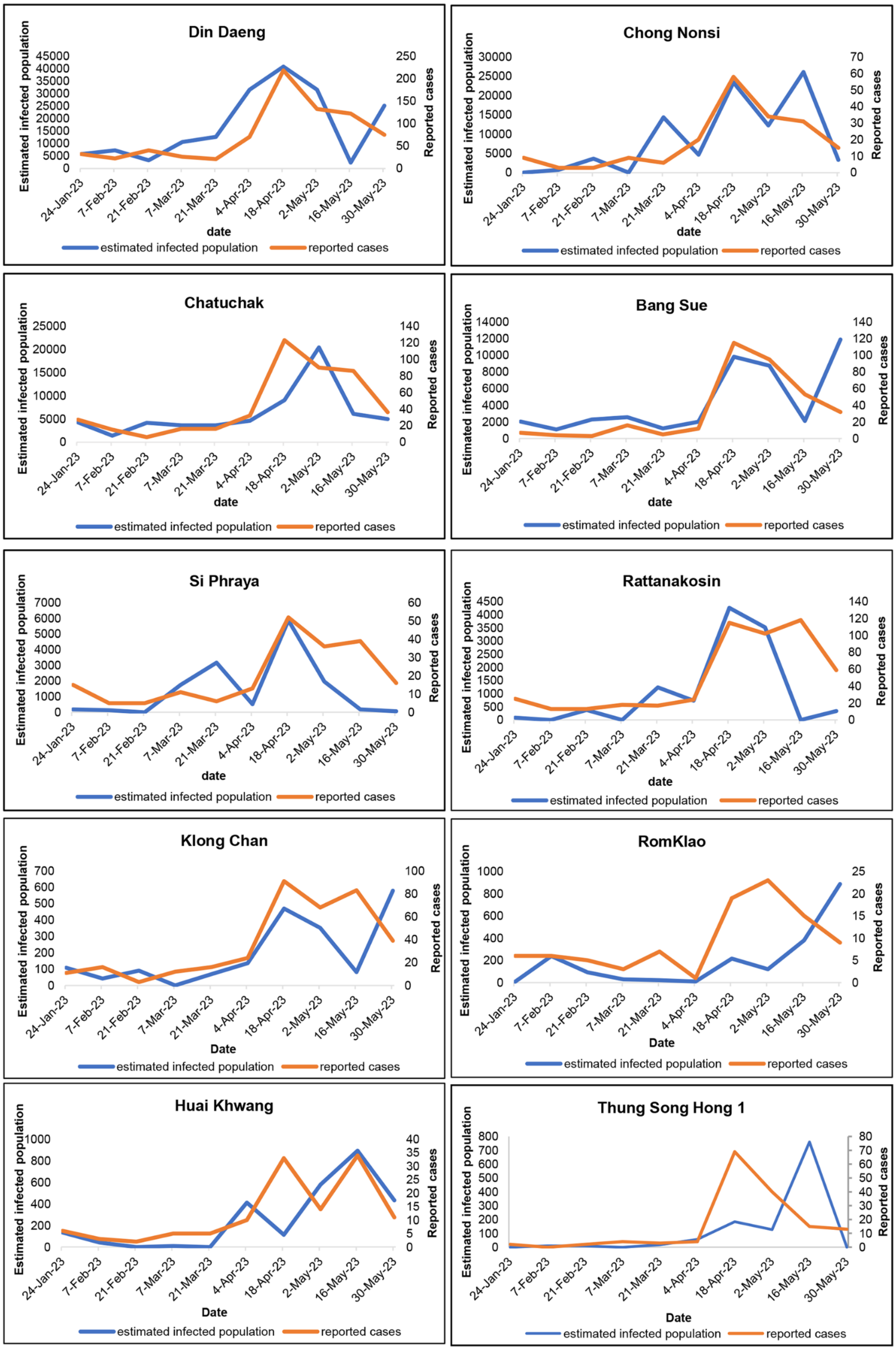

Fig. 4. Correlation between officially reported cases and estimated infected cases in individual Bangkok WWTPs.

Wastewater-based epidemiology (WBE) has been widely used as a tool for evaluating monitoring, surveillance, estimating infected populations, and outbreak warning prediction in several countries covering seven continents[13,19,23,24,26–29,47,48]. Therefore, this research has applied WBE for studying in Bangkok after fully opening the country. The premise of WBE is that people infected with SARS-CoV-2 can shed the virus in their respiratory secretions and gastrointestinal tract, such as feces, into wastewater treatment systems. The SARS-CoV-2 RNA could be detected in the wastewater, it would indicate the presence of infected persons in that

WWTPs	Spearman's correlation at different lag time (0–6 weeks)						
	0	1	2	3	4	5	6
L1	$\rho = 0.456$	$\rho = 0.608$	**$\rho = 0.675$**	$\rho = 0.608$	$\rho = 0.462$	$\rho = 0.359$	$\rho = 0.316$
	p-value = 0.185	p-value = 0.062	**p-value = 0.032***	p-value = 0.062	p-value = 0.179	p-value = 0.309	p-value = 0.374
L2	$\rho = 0.620$	**$\rho = 0.705$**	$\rho = 0.48$	$\rho = 0.602$	$\rho = 0.748$	$\rho = 0.389$	$\rho = 0.170$
	p-value = 0.056	**p-value = 0.023***	p-value = 0.160	p-value = 0.066	p-value = 0.013	p-value = 0.266	p-value = 0.638
L3	**$\rho = 0.754$**	**$\rho = 0.758$**	**$\rho = 0.697$**	$\rho = 0.418$	$\rho = 0.236$	$\rho = 0.03$	$\rho = -0.285$
	p-value = 0.012*	**p-value = 0.011***	**p-value = 0.025***	p-value = 0.229	p-value = 0.511	p-value = 0.934	p-value = 0.425
L4	**$\rho = 0.739$**	$\rho = 0.607$	**$\rho = 0.748$**	$\rho = 0.103$	$\rho = 0.116$	$\rho = 0.073$	$\rho = 0.043$
	p-value = 0.018*	p-value = 0.062	**p-value = 0.016***	p-value = 0.776	p-value = 0.751	p-value = 0.841	p-value = 0.907
L5	$\rho = 0.345$	$\rho = 0.383$	$\rho = 0.486$	**$\rho = 0.821$**	**$\rho = 0.754$**	$\rho = 0.624$	$\rho = 0.596$
	p-value = 0.328	p-value = 0.275	p-value = 0.154	**p-value = 0.004***	**p-value = 0.012***	p-value = 0.054	p-value = 0.069
L6	$\rho = 0.362$	$\rho = 0.572$	$\rho = 0.546$	**$\rho = 0.681$**	$\rho = 0.607$	$\rho = 0.46$	$\rho = 0.468$
	p-value = 0.304	p-value = 0.084	p-value = 0.102	**p-value = 0.03***	p-value = 0.063	p-value = 0.181	p-value = 0.173
S1	**$\rho = 0.644$**	$\rho = 0.600$	**$\rho = 0.693$**	$\rho = 0.249$	$\rho = 0.109$	$\rho = -0.285$	$\rho = -0.249$
	p-value = 0.044*	p-value = 0.067	**p-value = 0.026***	p-value = 0.487	p-value = 0.763	p-value = 0.425	p-value = 0.487
S2	**$\rho = 0.685$**	$\rho = 0.421$	$\rho = 0.267$	$\rho = 0.140$	$\rho = -0.012$	$\rho = -0.111$	$\rho = -0.213$
	p-value = 0.029*	p-value = 0.226	p-value = 0.455	p-value = 0.699	p-value = 0.973	p-value = 0.761	p-value = 0.554
S3	$\rho = 0.559$	$\rho = 0.383$	$\rho = 0.377$	$\rho = 0.122$	$\rho = -0.061$	$\rho = -0.298$	$\rho = -0.571$
	p-value = 0.093	p-value = 0.275	p-value = 0.283	p-value = 0.738	p-value = 0.868	p-value = 0.403	p-value = 0.084
S4	$\rho = 0.568$	**$\rho = 0.656$**	$\rho = 0.617$	$\rho = 0.571$	$\rho = 0.580$	$\rho = 0.239$	$\rho = 0.185$
	p-value = 0.087	**p-value = 0.039***	p-value = 0.057	p-value = 0.085	p-value = 0.079	p-value = 0.506	p-value = 0.610
Overall	**$\rho = 0.620$**	**$\rho = 0.605$**	**$\rho = 0.594$**	**$\rho = 0.549$**	**$\rho = 0.519$**	**$\rho = 0.429$**	**$\rho = 0.378$**
	p-value < 0.001*	**p-value < 0.001***	**p-value < 0.001***	**p -value < 0.001***	**p-value < 0.001***	**p-value < 0.001***	**p-value < 0.001***

Table 2. Lag time correlation analysis between estimated infected cases and 1-week average reported cases at different lag time (0–6 weeks). Significant values are in (bold).

area[29,49]. Several gene targets, i.e., N, S, E, and ORF1ab regions have been used for qualitative and quantitative detecting SARS-CoV-2 RNA in wastewater by using real time qRT-PCR, ddPCR, and next generation sequencing, and varying in termed of water concentration method, extraction, and different wastewater characteristics was noted[33–37]. Nevertheless, the N target region was widely reported for measuring RNA since its conservation across multiple SARS-CoV-2 variants[50]. Our study demonstrated the superior detection using N1 primer/probe set for detecting three variants, (hCoV-19/Thailand/MUMT-3/2020 [Wuhan-like strain], hCoV-19/Thailand/MUMT-53/2021 [Delta variant, and hCoV-19/Thailand/MUMT-11/2022 [Omicron variant]), used as the test viruses for real time qRT-PCR validation.

The current study was conducted on 10 representative WWTPs (six large-scale and four small-scale), covering 132.03 km^2 for a registered population of approximately 2,520,700 people[51]. SARS-CoV-2 RNA was detected in 51% (102/200) wastewater samples, 88% in influent and 14% effluent samples, demonstrating a high concentration of SARS-CoV-2 RNA present in influent and RNA degradation (decay) over time[52,53]. The range of SARS-CoV-2 RNA was between 4.76×10^2 and 1.48×10^5 copies/L which corresponded to other previous reported[13,35,37,54]. Most previous studies collected untreated or raw wastewater and found a high SARS-CoV-2 RNA positive rate with a concentration of 10^1 to 10^6 copies/L[10,22]. There are a few studies that could detect SARS-CoV-2 RNA in effluent wastewater collected from WWTPs in Paris and Spain[27,55]. Corresponding to our study, we detected SARS-CoV-2 RNA in the effluent in ranges of 4.76×10^2 to 4.34×10^3 copies/L. Nevertheless, the RNA concentration detected in the effluent, treated wastewater, showed a reduction of 1.5–260 times compared to the RNA concentration detected in the influent. Even though our study could detect SARS-CoV-2 RNA in effluent samples, implying that the virus was potentially released to the environment. Several investigators demonstrated that SARS-CoV-2 RNA could be detected in the secondary and tertiary treated wastewater; but the infectious SARS-CoV-2 particle could not be isolated, demonstrating no viable viruses presented in effluent[14,56,57].

Estimated infected populations have been calculated using modified estimated models reported from previous studies[13,19,43]. The estimated infected population corresponded to the weekly reported cases by showing a significant correlation ($r = 0.351$, p-value < 0.001). However, the estimated infected population from our study was higher than the reported cases, reflecting the real covid-19 infected cases were underestimated[19,42]. The estimation model depended on two variables: (1) the feces or urine rate per person per day, and (2) the RNA rate per gram of feces of Thais. However, the current sewage disposal system in Thailand could not effectively separate human feces and urine resulting no data available on feces or urine rates per person for Thais. Therefore, our study used a total excretion rate reported from the Department of Health for calculation[44]. In addition, for the amount of RNA shedding per gram in excretion of Thai people, our study applied the average SARS-CoV-2 RNA of 3.634×10^6 $\text{copies/g}_{\text{feces}}$ found in Asian persons as reported by Saththasivam et al.[43] and Zheng et al.[45], which studied in Qatar and China, respectively. Furthermore, our study used an average daily flowrate annually reported from the Drainage and Sewerage Department in the estimation model. However, the use of the average

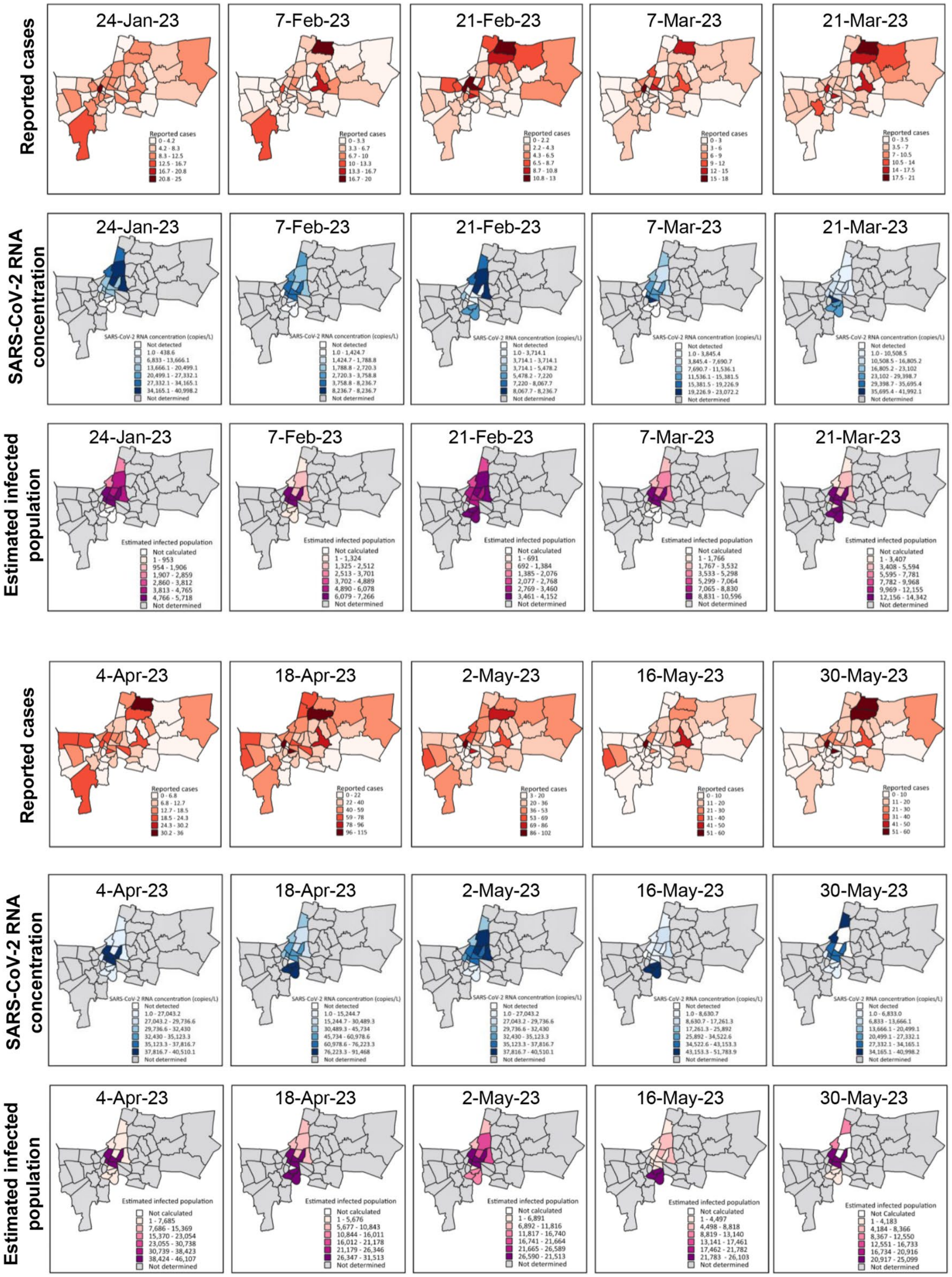

Fig. 5. GIS application for six large-scales of WWTPs (L1 to L6) serving the inner city of Bangkok based on data of viral RNA concentration (copies/L), estimated infected population, and reported cases.

daily flowrate obtained in a study period (January–May) might be more accurate than the annual average daily flowrate, particularly in regions such as Thailand where there is a wet and dry period of the year. Nevertheless, the flowrate could also vary from one week to another depending on the number of tourists that are present in the city.

Several studies reported the viral RNA concentration in wastewater has been related to reported cases[21,24,27,29,31]. Similarly, this study found that the amount of viral RNA in the influent significantly correlated with weekly reported cases ($r = 0.481$, p-value < 0.001). Two large-scale WWTPs, Chatuchak and Bang Sue, showed a strong correlation ($r = 0.964$, p-value < 0.001) and $r = 0.842$, p-value = 0.002), respectively; while, a Huai Kwang small-scale WWTP showed a moderate correlation ($r = 0.675$, p-value = 0.032), implying that size of WWTPs was not a factor effected the correlation analysis. However, Sangsanont J. et al.[37], studied the WWTPs in Bangkok and found no correlation with viral RNA concentration and reported cases in the serve area. This was probably different by sampling, concentration, and extraction methods. According to the correlation between viral RNA concentration and reported cases in the service area, WWTPs are related on both small and large scales, which means that the scale of WWTPs is not a factor in the result.

In addition, our detected SARS-CoV-2 RNA in effluent samples did not show the correlation to the reported cases. Unfortunately, we did not have information on the wastewater characteristics such as BOD, COD, pH, Temperature, Total suspended solid (TSS), Total Kjeldahl nitrogen (TKN), etc., in influent and effluent samples. However, we know that most of Bangkok WWTPs (90%) used the activated sludge (AS) system for treating wastewater resulting in sludge formation presented in the treatment process. Previous reports revealed that the majority of SARS-CoV-2 RNA (82.5–92.5%) was found in sludge[58], postulated that it is the main factor causing no correlation between SARS-CoV-2 RNA in effluent and covid-19 reported cases. For further investigation, WWTP treatment efficiency should be studied in mass balance of SARS-CoV-2 RNA in all treatment processes to determine the efficiency of wastewater treatment systems.

The WBE can be used as an early warning 2–14 days before an outbreak by using lag time correlation analysis in several countries[9,24]. Early warning of 4–10 days before an outbreak was reported from Massachusetts, USA[31]. The signal early warning 2 days before new covid-19 cases and 4 days before hospitalizations were reported in Canada[29]. Early warning was issued about 12–16 days before new cases were discovered in Spain, allowing governance to prepare resources and plan for mitigation outbreaks[27]. Likewise, in Thailand, Wannigama et al.[35] studied WBE with reporting early warning of 20 days for rural areas and 14 days for urban areas, before new cases were reported. The study of Sangsanont S. et al.[37] found correlation time to be as 22–24 days early warning in Bangkok. Both of the studies were conducted during the third wave SARS-CoV-2 outbreak in Thailand. The variable used for estimation is reported cases, some studies use the daily reported cases[21,24,29], and some studies use accumulative cases by weekly report[26,30,31]. The estimated infected population in the post-pandemic period in Bangkok was calculated in our study. The official covid-19 cases were reported by week; therefore, this study used the 1-week average reported cases at different lag times (0–6 weeks). The lag time correlation was considered to be 6 weeks because SARS-CoV-2 can be shed in feces for more than 20 days[31], and a previous study in Bangkok considered 40 days at a different lag time[37]. Consequently, a recent study found that a higher and stronger correlation was seen in the lag time correlation analysis between estimated infected cases and the 1-week average reported cases compared to viral RNA concentration. Although the estimated infected population had a higher correlation than viral RNA concentration, the overall lag time correlation in Bangkok was found to be significant time from a day to a week with a moderate correlation (p-value < 0.001).

Previous studies demonstrated that spatial analysis using GIS could be used to monitor the density and movement of infected populations, identify clusters, and evaluate efficiency after using prevention and control strategies[59]. Around 72 countries use the data from WBE to create GIS and dashboards for announcements to citizens among those countries[60]. The GIS has been used to control the covid-19 outbreaks, and the web-GIS system has provided beneficial information to the citizens, including the location of the detected patients, high-risk locations, and information about the available medical facilities[61]. This study first applied GIS for SARS-CoV-2 surveillance based on WBE in Bangkok. As spatial analysis, early detection was detected on mapping during the study period. Nevertheless, some of the spatial analysis demonstrated varying patterns due to the movement dynamics of a population. Bangkok is the most popular city for tourist destination, and a highest covid-19 cases was reported. In 2023, approximately 27 million cumulative tourists come to Thailand as reported by the Economics Tourism and Sport Division, Thailand. It is noted that the new SARS-CoV-2 variants might be potentially imported to the country. Nowadays, covid-19 cases have been frequently reported in many crowned places especially in the inner district of Bangkok. The real time dashboard of wastewater-based SARS-CoV-2 surveillance should be developed for monitoring new variants and supporting epidemiological data to government agencies and healthcare sectors for outbreak preparedness. Our study first demonstrated that the WBE combining with GIS might be a powerful tool implemented for disease surveillance systems in Bangkok, Thailand.

Conclusion

WBE was successfully applied in Bangkok, Thailand, and can be a complemented tool for surveillance and predicting covid-19 during the post-pandemic period. Spearman's correlation coefficient showed that SARS-CoV-2 RNA and the estimated infected population significantly correlated with covid-19 officially reported cases. Lag time analysis demonstrated the early warning prediction around 1–3 weeks before large-scale outbreaks. SARS-CoV-2 RNA should be routinely monitored in both large-scale and small-scale WWTPs across the countries for disease surveillance at the community level. In addition, GIS application has been applied for spatio-temporal area-based analysis for further creating a dashboard or hotspot map to support government initiatives, and policy decision-making for prevention and control program implementations.

Data availability

The raw data are available in the Supplementary files.

Received: 31 August 2024; Accepted: 18 March 2025

References

1. Prem, K. et al. The effect of control strategies to reduce social mixing on outcomes of the COVID-19 epidemic in Wuhan, China: A modelling study. *Lancet Public. Health* **5**, e261-e270 (2020).
2. Tantrakarnapa, K., Bhopdhornangkul, B. & Nakhaapakorn, K. Influencing factors of COVID-19 spreading: A case study of Thailand. *J. Public. Health: Theory Pract.* **30**, 1–7 (2020).
3. Naqvi, A. A. T. et al. Insights into SARS-CoV-2 genome, structure, evolution, pathogenesis and therapies: Structural genomics approach. *Biochim. Et Biophys. Acta Mol. Basis Dis.* **1866**, 165878 (2020).
4. WHO. COVID-19 deaths | WHO COVID-19 dashboard. *datadot* (2024). https://data.who.int/dashboards/covid19/deaths
5. WHO. WHO COVID-19 dashboard. *datadot* (2024). https://data.who.int/dashboards/covid19/cases?n=c
6. Rubio-Casillas, A., Redwan, E. M. & Uversky, V. N. SARS-CoV-2: A master of immune evasion. *Biomedicines* **10**, 1339 (2022).
7. Handiso, T. B., Jifar, M. S. & Nuriye Hagisso, S. Coronavirus's (SARS-CoV-2) airborne transmission. *SAGE Open. Med.* **10**, 1–5 (2022).
8. Kim, J. M. et al. Detection and isolation of SARS-CoV-2 in serum, urine, and stool specimens of COVID-19 patients from the Republic of Korea. *Osong Public. Health Res. Perspect.* **11**, 112–117 (2020).
9. Medema, G., Heijnen, L., Elsinga, G., Italiaander, R. & Brouwer, A. Presence of SARS-Coronavirus-2 RNA in sewage and correlation with reported COVID-19 prevalence in the early stage of the epidemic in the Netherlands. *Environ. Sci. Technol. Lett.* **7**, 511–516 (2020).
10. Chen, Y. et al. The presence of SARS-CoV-2 RNA in the feces of COVID-19 patients. *J. Med. Virol.* **92**, 833–840 (2020).
11. Aumpan, N., Nunanan, P. & Vilaichone, R. Gastrointestinal manifestation as clinical predictor of severe COVID -19: A retrospective experience and literature review of COVID -19 in association of Southeast Asian nations (ASEAN). *JGH Open.* **4**, 1096–1101 (2020).
12. Mohammadi, A., Esmaeilzadeh, E., Li, Y., Bosch, R. J. & Li, J. Z. SARS-CoV-2 detection in different respiratory sites: A systematic review and meta-analysis. *EBioMedicine* **59**, 102903 (2020).
13. Pillay, L. et al. Monitoring changes in COVID-19 infection using wastewater-based epidemiology: A South African perspective. *Sci. Total Environ.* **786**, 147273 (2021).
14. Atoui, A., Cordevant, C., Chesnot, T. & Gassilloud, B. SARS-CoV-2 in the environment: Contamination routes, detection methods, persistence and removal in wastewater treatment plants. *Sci. Total Environ.* **881**, 163453 (2023).
15. Jones, D. L. et al. Shedding of SARS-CoV-2 in feces and urine and its potential role in person-to-person transmission and the environment-based spread of COVID-19. *Sci. Total Environ.* **749**, 141364 (2020).
16. Savela, E. S. et al. Quantitative SARS-CoV-2 viral-load curves in paired saliva and nasal swabs inform appropriate respiratory sampling site and analytical test sensitivity required for earliest viral detection. *MedRxiv* https://doi.org/10.1101/2021.04.02.21254771 (2021). 2021.04.02.21254771.
17. Piqué, B. et al. SARS-CoV-2 serum viral load and prognostic markers proposal for COVID-19 pneumonia in low-dose radiation therapy treated patients. *J. Clin. Med.* **12**, 798 (2023).
18. Anand, U. et al. A review of the presence of SARS-CoV-2 RNA in wastewater and airborne particulates and its use for virus spreading surveillance. *Environ. Res.* **196**, 110929 (2021).
19. Ahmed, W. et al. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: A proof of concept for the wastewater surveillance of COVID-19 in the community. *Sci. Total Environ.* **728**, 138764 (2020).
20. Wang, X. W. et al. Excretion and detection of SARS coronavirus and its nucleic acid from digestive system. *World J. Gastroenterol.* **11**, 4390 (2005).
21. Peccia, J. et al. Measurement of SARS-CoV-2 RNA in wastewater tracks community infection dynamics. *Nat. Biotechnol.* **38**, 1164–1167 (2020).
22. Wani, H. et al. Wastewater-Based epidemiology of SARS-CoV-2: Assessing prevalence and correlation with clinical cases. *Food Environ. Virol.* **15**, 131–143 (2023).
23. Hewitt, J. et al. Sensitivity of wastewater-based epidemiology for detection of SARS-CoV-2 RNA in a low prevalence setting. *Water Res.* **211**, 118032 (2022).
24. Kumar, M., Joshi, M., Patel, A. K. & Joshi, C. G. Unravelling the early warning capability of wastewater surveillance for COVID-19: A temporal study on SARS-CoV-2 RNA detection and need for the escalation. *Environ. Res.* **196**, 110946 (2021).
25. Haramoto, E., Malla, B., Thakali, O. & Kitajima, M. First environmental surveillance for the presence of SARS-CoV-2 RNA in wastewater and river water in Japan. *Sci. Total Environ.* **737**, 140405 (2020).
26. Lastra, A. et al. SARS-CoV-2 detection in wastewater as an early warning indicator for COVID-19 pandemic. Madrid region case study. *Environ. Res.* **203**, 111852 (2022).
27. Randazzo, W. et al. SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area. *Water Res.* **181**, 115942 (2020).
28. Li, X. et al. Wastewater-based epidemiology predicts COVID-19-induced weekly new hospital admissions in over 150 USA counties. *Nat. Commun.* **14**, 4548 (2023).
29. D'Aoust, P. M. et al. Catching a resurgence: Increase in SARS-CoV-2 viral RNA identified in wastewater 48 h before COVID-19 clinical tests and 96 h before hospitalizations. *Sci. Total Environ.* **770**, 145319 (2021).
30. Rusiñol, M. et al. Monitoring waves of the COVID-19 pandemic: Inferences from WWTPs of different sizes. *Sci. Total Environ.* **787**, 147463–147463 (2021).
31. Wu, F. et al. SARS-CoV-2 RNA concentrations in wastewater foreshadow dynamics and clinical presentation of new COVID-19 cases. *Sci. Total Environ.* **805**, 150121 (2022).
32. Kumthip, K., Khamrin, P., Yodmeeklin, A., Ushijima, H. & Maneekarn, N. Molecular detection and characterization of SARS-CoV-2 in wastewater in Thailand during 2020–2022. *J. Infect. Public Health* **16**, 1884–1890 (2023).
33. Sangsanont, J. et al. Wastewater monitoring in tourist cities as potential Sentinel sites for near real-time dynamics of imported SARS-CoV-2 variants. *Sci. Total Environ.* **860**, 160317 (2023).
34. Tangwangvivat, R. et al. SARS-CoV-2 variants detection strategies in wastewater samples collected in the Bangkok metropolitan region. *Viruses* **15**, 876 (2023).
35. Wannigama, D. L. et al. COVID-19 monitoring with sparse sampling of sewered and non-sewered wastewater in urban and rural communities. *iScience* **26**, 107019 (2023).
36. Thongpradit, S. et al. A Simple method to detect SARS-CoV-2 in wastewater at low virus concentration. *J. Environ. Publ. Health* **2022**, 867626 (2022).
37. Sangsanont, J. et al. SARS-CoV-2 RNA surveillance in large to small centralized wastewater treatment plants preceding the third COVID-19 resurgence in Bangkok, Thailand. *Sci. Total Environ.* **809**, 151169 (2022).
38. Johnston, M. List of the world's largest cities by population | Agglomeration, Conglomeration, Metropolitan Area, Estimate, & Facts | Britannica. *britannica.* (2024). https://www.britannica.com/topic/list-of-the-worlds-largest-cities-by-population
39. Thailand National Statistical Office. Summary of important results of the latent population in Thailand in 2023. (2023). https://www.nso.go.th/nsoweb/storage/survey_detail/2024/20240418144915_50809.pdf
40. The Bureau of Registration Administration. Top 10 provinces with the largest population (all) 2021–2023. *Dopa.go.th* (2024). https://stat.bora.dopa.go.th/StatMIS/#/ReportTopTen/1
41. Ministry of Tourism and Sports. Domestic Tourism Statistics (Classify by region and province 2023). *Ministry of Tourism and Sports* (2023). https://www.mots.go.th/news/category/705#

42. DDC. DDC COVID-19 Interactive Dashboard | 1-dash-tiles-w. ddc.moph.go.th.https://ddc.moph.go.th/covid19-dashboard/ (2022).
43. Saththasivam, J. et al. COVID-19 (SARS-CoV-2) outbreak monitoring using wastewater-based epidemiology in Qatar. *Sci. Total Environ.* **774**, 145608 (2021).
44. Department of Health. *Driving Waste Management According to the Resolution of the Meeting to Discuss Guidelines for Creating a Waste Disposal System*. (2022). https://www.dla.go.th/upload/document/type2/2022/7/27748_2_1658475061855.pdf
45. Zheng, S. et al. Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January–March 2020: Retrospective cohort study. *BMJ* **2020**, m1443 (2020).
46. Aiewsakun, P. et al. Spatiotemporal evolution of SARS-CoV-2 in the Bangkok metropolitan region, Thailand, 2020–2022: Implications for future outbreak preparedness. *Microb. Genomics* **9**, 001170 (2023).
47. de Bueno, F. Wastewater-based epidemiology: A Brazilian SARS-COV-2 surveillance experience. *J. Environ. Chem. Eng.* **10**, 108298 (2022).
48. Giraud-Billoud, M. et al. Monitoring of SARS-CoV-2 RNA in wastewater as an epidemiological surveillance tool in Mendoza, Argentina. *Sci. Total Environ.* **796**, 148887 (2021).
49. Betancourt, W. Q. et al. COVID-19 containment on a college campus via wastewater-based epidemiology, targeted clinical testing and an intervention. *Sci. Total Environ.* **779**, 146408 (2021).
50. Song, W. et al. The role of SARS-CoV-2 N protein in diagnosis and vaccination in the context of emerging variants: present status and prospects. *Front. Microbiol.* **14**, 1217567 (2023).
51. DDS. Anual report 2021. *Water Quality Management Office*. https://drive.google.com/file/d/1GZTy_kVdugwdkb0XfHQoCcs2qiKwweyG/view (2021).
52. Kumar, M. et al. Decay of SARS-CoV-2 RNA along the wastewater treatment outfitted with upflow anaerobic sludge blanket (UASB) system evaluated through two sample concentration techniques. *Sci. Total Environ.* **754**, 142329 (2021).
53. Yang, S. et al. Persistence of SARS-CoV-2 RNA in wastewater after the end of the COVID-19 epidemics. *J. Hazard. Mater.* **429**, 128358 (2022).
54. Hata, A., Hara-Yamamura, H., Meuchi, Y., Imai, S. & Honda, R. Detection of SARS-CoV-2 in wastewater in Japan during a COVID-19 outbreak. *Sci. Total Environ.* **758**, 143578 (2020).
55. Wurtzer, S. et al. Evaluation of lockdown effect on SARS-CoV-2 dynamics through viral genome quantification in wastewater, greater Paris, France, 5 March to 23 April 2020. *Eurosurveillance* **25**, 2000776 (2020).
56. Giacobbo, A., Rodrigues, M. A. S., Ferreira, Z., Bernardes, J., de Pinho & A. M. & M. N. A critical review on SARS-CoV-2 infectivity in water and wastewater. What do we know? *Sci. Total Environ.* **774**, 145721 (2021).
57. Kallem, P., Hegab, H. M., Alsafar, H., Hasan, S. W. & Banat, F. SARS-CoV-2 detection and inactivation in water and wastewater: review on analytical methods, limitations and future research recommendations. *Emerg. Microb. Infect.* **12**, 2222850 (2023).
58. Li, B., Di, D. Y. W., Saingam, P., Jeon, M. K. & Yan, T. Fine-scale temporal dynamics of SARS-CoV-2 RNA abundance in wastewater during A COVID-19 lockdown. *Water Res.* **197**, 117093 (2021).
59. Raju, K., Lavanya, R., Manikandan, S. & Srilekha, K. Application of GIS in COVID – 19 monitoring and surveillance. *Int. J. Res. Appl. Sci. Eng. Technol.* **8**, 1435–1440 (2020).
60. UC Merced. COVIDPoops19 Summary of Global SARS-CoV-2 Wastewater Monitoring. *Arcgis* https://www.arcgis.com/apps/dashboards/c778145ea5bb4daeb58d31afee389082
61. Rezael, M., Nouri, A. A., Park, G. S. & Kim, D. H. Application of geographic information system in monitoring and detecting the COVID-19 outbreak. *Iran. J. Public. Health* **49**, 114–116 (2020).

Acknowledgements

The authors thank the Drainage and Sewerage Department, Bangkok Metropolitan Administration for kindly support the WWTP study sites. This research is supported by Thailand Science Research and Innovation (TSRI) Fundamental Fund, fiscal year 2023.

Author contributions

P.N. conceptualized the project, designed and performed the study, analyzed the data and wrote the manuscript. T.S. performed the study, analyzed the data and wrote the manuscript. B.T. provide the samples and analyzed the data. N.N., J.P., H.L, and P.P., provided the reagents and viruses in the study. All authors have reviewed the manuscripts.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-94938-7.

Correspondence and requests for materials should be addressed to P.N.