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Detection of SARS-CoV-2 and Omicron variant RNA in wastewater samples from Manila, Philippines

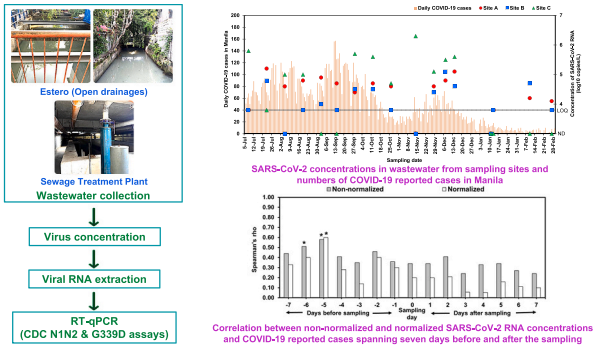
Jessamine Gail M. Inson [a,b], Bikash Malla [c], Divina M. Amalin [a,d], Thaddeus M. Carvajal [a,d], Ma. Luisa D. Enriquez [a], Soichiro Hirai [e], Sunayana Raya [e], Aulia Fajar Rahmani [e], Made Sandhyana Angga [e], Niva Sthapit [c], Sadhana Shrestha [c], Annisa Andarini Ruti [e], Tomoko Takeda [f], Masaaki Kitajima [g], Zeba F. Alam [a,b], Eiji Haramoto [c,*]

[a] Department of Biology, De La Salle University, Manila 1004, Philippines
[b] Environmental Biomonitoring Research Unit, Center for Natural Sciences and Environmental Research, De La Salle University, Manila 1004, Philippines
[c] Interdisciplinary Center for River Basin Environment, University of Yamanashi, 4-3-11 Takeda, Kofu, Yamanashi 400-8511, Japan
[d] Biological Control Research Unit, Center for Natural Sciences and Environmental Research, De La Salle University, Manila 1004, Philippines
[e] Department of Engineering, University of Yamanashi, 4-3-11 Takeda, Kofu, Yamanashi 400-8511, Japan
[f] Department of Earth and Planetary Science, The University of Tokyo, Tokyo 113-0033, Japan
[g] Division of Environmental Engineering, Hokkaido University, Sapporo, Hokkaido 060-8628, Japan

HIGHLIGHTS

- SARS-CoV-2 and Omicron variant finding in wastewater was first reported in Manila.
- SARS-CoV-2 and Omicron were detected in 78 % and 60 % of samples, respectively.
- Omicron variant was detected at all sites mirroring the dominant COVID-19 strain.
- Even when the city had only reported 5 COVID-19 cases, SARS-CoV-2 RNA was detected.

GRAPHICAL ABSTRACT



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ABSTRACT

Manila, a highly urbanized city, is listed as one of the top cities with the highest recorded number of coronavirus disease 2019 (COVID-19) cases in the Philippines. This study aimed to detect and quantify the RNA of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the Omicron variant in 51 wastewater samples collected from three locations in Manila, namely *Estero* de Santa Clara, *Estero* de Pandacan, which are open drainages, and a sewage treatment plant (STP) at De La Salle University–Manila, between July 2022 and February 2023. Using one-step reverse transcription-quantitative polymerase chain reaction, SARS-CoV-2 and Omicron variant RNA were detected in 78 % (40/51; 4.9 ± 0.5 $\log_{10}$ copies/L) and 60 % (24/40; 4.4 ± 0.3 $\log_{10}$

* Corresponding author.
E-mail addresses: jessamine.inson@dlsu.edu.ph (J.G.M. Inson), divina.amalin@dlsu.edu.ph (D.M. Amalin), thaddeus.m.carvajal@dlsu.edu.ph (T.M. Carvajal), ma.luisa.enriquez@dlsu.edu.ph (Ma.L.D. Enriquez), g22tr006@yamanashi.ac.jp (S. Hirai), tomokotakeda@eps.s.u-tokyo.ac.jp (T. Takeda), mkitajima@eng.hokudai.ac.jp (M. Kitajima), zeba.alam@dlsu.edu.ph (Z.F. Alam), eharamoto@yamanashi.ac.jp (E. Haramoto).

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copies/L) of wastewater samples collected from all sampling sites, respectively. SARS-CoV-2 RNA was detected frequently at *Estero* de Santa Clara (88 %, 15/17); its highest concentration was at the STP (6.3 $\log_{10}$ copies/L). The Omicron variant RNA was present in the samples collected (4.4 ± 0.3 $\log_{10}$ copies/L) from all sampling sites, with the highest concentration at the STP (4.9 $\log_{10}$ copies/L). Regardless of normalization, using concentrations of pepper mild mottle virus RNA, SARS-CoV-2 RNA concentrations exhibited the highest positive correlation with COVID-19 reported cases in Manila 5 days after the clinical report. These findings revealed that wastewater-based epidemiology may aid in identifying and monitoring of the presence of pathogens in open drainages and STPs in the Philippines. This paper provides the first documentation on SARS-CoV-2 and the Omicron variant in wastewater from Manila.

1. Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first declared a public health emergency of international concern in January 2020 and categorized as a pandemic in March 2020 by the World Health Organization (WHO) (WHO, 2020). WHO declared an end to the COVID-19 public health emergency on May 5, 2023, and mentioned that this declaration did not imply that the disease was no longer a global threat. Emerging Omicron variant sublineages and the constant risk of new variants could lead to new surges in cases and fatalities (United Nations, 2023).

The Omicron variant, previously a variant of interest and now a variant of concern, is characterized as less virulent; it has a low mortality rate. However, it is also known to be more transmissible and to escape neutralization by antibodies (Brüssow, 2022). More than 50 known mutations in the Omicron variant, most of which occur in the S protein, the target of antibodies, cause vaccine failures (Ou et al., 2022). G339D is one of the specific mutations in the receptor-binding domain of the Omicron variant (Chatterjee et al., 2023; Dhama et al., 2023). Previous studies reported the presence of the Omicron variant in wastewater by detecting G339D and its other specific mutations (Rasmussen et al., 2022; Wilhelm et al., 2022; Vo et al., 2023).

An average of 15 %–83 % of asymptomatic and symptomatic COVID-19 patients have detectable SARS-CoV-2 RNA in feces. Viral shedding is observed even in recovered COVID-19 patients (Barcelo, 2020; Cholankeril et al., 2020; D'Amico et al., 2020; Foladori et al., 2020). The disposal of feces containing SARS-CoV-2 RNA can be through the sewage or the environment in areas with poor sanitation. Open defecation, pit latrines, and septic tanks still exist in lower-resource settings due to the absence of adequate sewage systems. Some communities also discharge their waste into waterways near the neighborhood, contaminating the environment (Adelodun et al., 2020; Elsamadony et al., 2020; Kim et al., 2020). Takeda et al. (2021) stressed the importance of wastewater surveillance in communities facing socio-economic challenges, such as informal settlements, evacuation shelters, and refugee camps, as they may have limited access to clinical testing.

Thus, wastewater-based epidemiology (WBE) can be applied to monitor pathogens (Kitajima et al., 2020; Malla et al., 2022; Shrestha et al., 2023a; Tiwari et al., 2023; Malla et al., 2024) and used to respond to emerging diseases, such as COVID-19 (Kitajima et al., 2020; Betancourt et al., 2021). WBE's advantages include the detection of both asymptomatic and presymptomatic individuals without requiring data collection from individuals (anonymity, protecting privacy, and noninvasive); an indication of viral transmission to implement public health measures; identification of hotspots to perform further surveillance interventions; and providing real-time data. Identifying SARS-CoV-2 in communities through WBE is primarily relevant, especially if there is no comprehensive surveillance data. WBE is cost-effective and provides rapid results in both high and low-resource environments, making it an ideal approach for developing countries (Daughton, 2020; Medema et al., 2020; Takeda et al., 2021).

Identification of the presence of SARS-CoV-2 RNA in the environmental matrix is crucial to the implementation of appropriate safety measures to limit the transmission of viruses. (Aguiar-Oliveira et al., 2020; Michael-Kordatou et al., 2020). SARS-CoV-2 RNA has been successfully detected in wastewater from many countries, such as Australia (Ahmed et al., 2020), Brazil (Barbosa et al., 2022), China (Wang et al., 2020), France (Wurtzer et al., 2020), India (Kumar et al., 2020), Iran (Tanhaei et al., 2021), Israel (Bar Or et al., 2022), Italy (La Rosa et al., 2020; Rimoldi et al., 2020), Japan (Haramoto et al., 2020; Shrestha et al., 2023b), Nepal (Tandukar et al., 2022), the Netherlands (Medema et al., 2020), Spain (Randazzo et al., 2020), Thailand (Sangsanont et al., 2022; Thongpradit et al., 2022), and USA (Nemudryi et al., 2020; Peccia et al., 2020; Sherchan et al., 2020). In the Philippines, Otero et al. (2022) applied WBE to identify SARS-CoV-2 in urban communities in Davao City.

On January 30, 2020, the Department of Health (DOH) confirmed the first case of COVID-19 in the Philippines (DOH, 2020). Challenges exist in the Philippines' healthcare system, leading to delayed COVID-19 surveillance, affecting immediate decision-making to control the outbreak in the country (Haw et al., 2020). In order to compensate for the country's clinical surveillance gaps, it is suggested to integrate environmental detection of SARS-CoV-2 through WBE (Salazar et al., 2022).

Hence, through the WBE approach, this study aimed to detect and quantify SARS-CoV-2 RNA and Omicron variant-specific mutation site (G339D) in raw wastewater from *esteros* (open drainages) and a sewage treatment plant (STP) in Manila. In addition, this study intended to correlate the normalized and non-normalized SARS-CoV-2 RNA concentrations in wastewater with the reported clinical cases in Manila. Thus far, this is the first paper to report SARS-CoV-2 and Omicron variant in wastewater from Manila.

2. Materials and methods

2.1. Description of sampling sites and collection of wastewater samples

The city of Manila, the capital of the Philippines, is located in the National Capital Region of Luzon and the western side of Metro Manila. This densely urbanized city has a total area of 4234 ha (Manila Government City Profile, 2023). The sampling in Manila was carried out at three sites, namely: *Estero* de Santa Clara (site A; 14° 34′ 43″ N; 121° 0′ 49″ E), *Estero* de Pandacan (site B; 14° 34′ 49″ N; 121° 0′ 16″ E), and De La Salle University–Manila STP (site C; 14° 33′ 54″ N; 120° 59′ 37″ E). Only 8 % of the population in the western area of Metro Manila has access to the sewage system (World Bank, 2020). Therefore, open drainages (*esteros*) were selected as sampling sites to represent better the local communities in Manila.

Subsequently, 51 grab samples were collected from the three sites (n = 17 each). Wastewater collection was done twice a month between 7 AM and 11 AM between July 2022 and February 2023. Wastewater samples (1.5 L/site) were collected using sterile polyethylene terephthalate bottles following proper precautions. The collected samples were immediately transported to the laboratory at De La Salle University Science and Technology Research Center for processing.

2.2. Virus concentration, molecular process controls, and RNA extraction

From 1.5 L of wastewater samples collected from each sampling site, a 10-mL aliquot of wastewater sample was transferred to four 15-mL conical centrifuge tubes. The samples were set aside for 1 h to allow sedimentation. The supernatants were removed, and an aliquot of wastewater was transferred to 15-mL centrifuge tubes, followed by centrifugation at 5000 ×*g* for 10 min. After centrifugation, the supernatants were removed and the pellets (800 μL) were transferred to 2 mL-screw cap microtubes. To inactivate pathogens and preserve the integrity of viral RNA in the pellets, an equal volume of DNA/RNA Shield reagent (Zymo Research, Irvine, CA, USA) was added. The pellets with DNA/RNA Shield were stored in a −20 °C freezer before further processing.

To serve as molecular process controls (MPCs), 1.0 μL of a mixture of F-specific RNA coliphage MS2 (ATCC 15597-B1; American Type Culture Collection, Manassas, VA, USA) and *Pseudomonas* bacteriophage Φ6 (NBRC 105899; National Institute of Technology and Evaluation, Tokyo, Japan) was added to 140 μL of a virus concentrate and molecular-grade water (i.e., a noninhibitory control (NIC)), as recommended previously by Haramoto et al. (2018). Using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) in a QIAcube platform (QIAGEN), 60 μL of RNA was obtained from 140 μL of viral concentrate. In this study, the extraction-reverse transcription-quantitative PCR (RT-qPCR) efficiency was calculated only for Φ6 alone, as both Φ6 and SARS-CoV-2 are enveloped viruses. The calculated extraction-RT-qPCR efficiency of the MPC, the concentration ratio of RNA in a sample qPCR tube to that in a NIC tube, was 119.8 ± 25.6 % (n = 51), indicating that there was no substantial viral RNA loss or inhibition in the water samples during RNA extraction and RT-qPCR.

2.3. Quantification of viral genomes by RT-qPCR

Quantification of SARS-CoV-2 and the Omicron variant RNA was conducted using a commercial one-step RT-qPCR kit, SARS-CoV-2 Detection RT-qPCR Kit for Wastewater and Primer/Probe G339D (SARS-CoV-2) (Takara Bio, Kusatsu, Japan), respectively, in a Thermal Cycler Dice Real Time System III (Takara Bio). Furthermore, the SARS-CoV-2 Detection RT-qPCR Kit for Wastewater contained the assays for the detection of SARS-CoV-2, pepper mild mottle virus (PMMoV), and Φ6, wherein Cy5-, FAM-, and HEX-labeled probes are used for the detections of CDC-N1N2, PMMoV, and Φ6 phage, respectively, as previously used by Angga et al. (2022) and Raya et al. (2024). The G339D Primer/Probe (SARS-CoV-2) comprises primers and probes needed to concurrently detect the G339D mutation of the Omicron variant and non-G339D types of SARS-CoV-2 other than the Omicron variant. The probes were labeled with Cy5 for the G339D type and FAM for the non-G339D type. Briefly, 5.0 μL of viral RNA extract was added to a mixture containing 12.5 μL of One-Step RT-qPCR Mix (2×), 2.5 μL of primers and probe of SARS-CoV-2 or PMMoV and Φ6 phage combined in a single tube (10×), or G339D Primer/Probe, and 5.0 μL of RNase-free water. Thermal conditions used for the one-step RT-qPCR involved the following steps: at 25 °C for 10 min, at 52 °C for 5 min, 95 °C for 10 s, and 45 cycles at 95 °C for 5 s, and at 60 °C for 30 s.

To obtain a standard curve, six 10-fold serially diluted (5.0×10^0–5.0×10^5 copies/reaction) Positive Control DNA samples provided in the SARS-CoV-2 Detection RT-qPCR Kit for Wastewater were included in each RT-qPCR run. To quantify the Omicron variant and non-Omicron SARS-CoV-2 RNA, six 10-fold serially diluted samples (5.0×10^0–5.0×10^5 copies/reaction) containing a mixture of positive control RNA of G339D and non-G339D types were prepared from the Positive Control RNA Set (Omicron) (Takara Bio). In each qPCR run, duplicate samples and positive and negative controls were utilized. A sample was considered positive if the threshold cycle value was <40 for the SARS-CoV-2 and Φ6 phage assays, while for the PMMoV assay, the threshold was set at 35. The samples with one-well positive were given a limit of quantification (LOQ) value.

2.4. Statistical analyses

Various statistical analyses were performed using STATA Statistical Software Special Edition 2015 (StataCorp, College Station, Texas, USA). A one-way analysis of variance (ANOVA) was performed to identify the differences in SARS-CoV-2 and PMMoV between sampling sites. The Kruskal–Wallis test was conducted to identify differences in the Omicron variant between sites. Spearman's rank correlation was done to determine the relationship between normalized and non-normalized SARS-CoV-2 concentrations and daily numbers of COVID-19 reported cases in Manila, using a significance level of 0.05 to determine significant differences.

3. Results

3.1. qPCR performance of the assays

Slopes of the standard curves were −3.33 ± 0.01, −3.18 ± 0.03, −3.54, and −3.58 for SARS-CoV-2 (n = 2), PMMoV (n = 2), G339D (n = 1), and non-G339D type assays (n = 1), respectively. The qPCR amplification efficiencies were 99.7 ± 0.5 % (n = 2), 106.5 ± 1.4 % (n = 2), 91.5 %, and 90.3 %; y-intercepts were 40.4 ± 0.0, 40.3 ± 0.1, 40.6, and 40.8; and the correlation coefficient (R^2) values were 1.00 ± 0.00, 1.00 ± 0.00, 0.997, and 0.996 for SARS-CoV-2 (n = 2), PMMoV (n = 2), G339D (n = 1), and non-G339D type assays (n = 1), respectively.

3.2. Detection of SARS-CoV-2 RNA in wastewater samples and number of cases of COVID-19/day in Manila

At all sampling sites, SARS-CoV-2 RNA was detected and quantified by performing one-step RT-qPCR (Table 1). Of 51 wastewater samples collected from the three sites, 40 (78 %) were positive for SARS-CoV-2 RNA, with a mean concentration of 4.9 ± 0.5 $\log_{10}$ copies/L. The SARS-CoV-2 RNA was most frequently quantified in wastewater samples from site A throughout the sampling period, as shown in Fig. 1. SARS-CoV-2 RNA was consistently detected from mid-July to mid-December 2022 at site A, except for samples collected on November 14, 2022. There were no statistically significant differences ($p > 0.05$) in the mean SARS-CoV-2 RNA concentrations across sites.

The data on the daily COVID-19 reported cases in Manila were extracted from the DOH (DOH, 2023). Note that the number of COVID-19-positive individuals tested through RT-PCR is being relayed to DOH by testing laboratories. SARS-CoV-2 RNA was identified in the samples even when the number of reported clinical cases in the city was as few as five. Notably, the number of COVID-19 clinical cases started decreasing in October 2022, as reported by the DOH.

Table 1
The detection and quantification of SARS-CoV-2 RNA in the collected wastewater samples.

Parameters	Site A (n = 17)	Site B (n = 17)	Site C (n = 17)	Total (n = 51)
No. of samples that tested positive in two wells (%)	13 (76)	7 (41)	10 (59)	30 (59)
No. of samples that tested positive in one wells (%)	2 (12)	7 (41)	1 (6)	10 (20)
Concentration (mean ± SD) ($\log_{10}$ copies/L)[a]	4.7 ± 0.3	4.7 ± 0.2	5.4 ± 0.5	4.9 ± 0.5

SD, standard deviation.
[a] Only samples that tested positive in two wells were used during calculation.

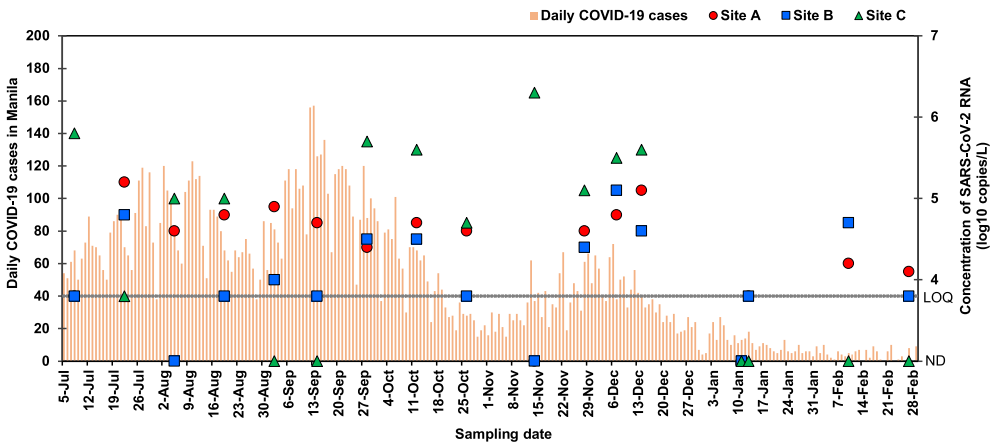

Fig. 1. SARS-CoV-2 RNA concentrations at sampling sites between July 2022 and February 2023. LOQ and ND denote the limits of quantification and those not detected, respectively.

3.3. Detection of the Omicron variant RNA in wastewater samples

G339D, the Omicron variant-specific mutation site, was detected in 24 (60 %) of the 40 samples that tested positive for SARS-CoV-2 RNA, with a mean concentration of 4.4 ± 0.3 $\log_{10}$ copies/L. Table 2 shows the number of positive samples for the Omicron variant in each sampling location. The presence of Omicron variant RNA was confirmed at each sampling site with varying ratios to SARS-CoV-2 RNA (site A, 3:5; site B, 1:2; and site C, 8:11).

Of the 17 samples collected, 15 (88 %) samples from site A were positive for SARS-CoV-2 RNA. The Omicron variant RNA was identified from 9 (60 %) samples that tested positive for SARS-CoV-2 RNA. The highest concentrations of SARS-CoV-2 and the Omicron variant RNA at site A were 5.2 $\log_{10}$ copies/L and 4.7 $\log_{10}$ copies/L, respectively, collected on July 22, 2022.

At site B, SARS-CoV-2 RNA was detected in 14 (82 %) samples, with 7 (50 %) of these samples exhibiting the Omicron variant. The highest RNA concentrations of the SARS-CoV-2 and Omicron variants were 5.1 $\log_{10}$ copies/L and 4.1 $\log_{10}$ copies/L, respectively, collected on December 7, 2022.

SARS-CoV-2 RNA was detected in 11 (64 %) wastewater samples at site C wherein 8 (72 %) of them had the Omicron variant RNA. The highest SARS-CoV-2 RNA concentration at site C was 6.3 $\log_{10}$ copies/L collected on November 14, 2022. The mean concentrations of the Omicron variant RNA in wastewater among sampling sites showed a significant difference, with the highest mean concentration at site C (4.0 ± 0.7 $\log_{10}$ copies/L) (Kruskal–Wallis test; $p < 0.05$).

3.4. Association between SARS-CoV-2 RNA concentration and daily new cases of COVID-19 in wastewater

PMMoV's abundance in wastewater is already well-established (Kitajima et al., 2018; Malla et al., 2019). All 51 (100 %) samples collected from the three sampling sites were positive for PMMoV RNA with a mean concentration of 7.3 ± 0.6 $\log_{10}$ copies/L. The highest concentration of PMMoV RNA (7.5 ± 0.9 $\log_{10}$ copies/L) was identified at site C, followed by site A (7.3 ± 0.2 $\log_{10}$ copies/L) and site B (7.0 ± 0.3 $\log_{10}$ copies/L). No statistically significant differences were observed in the mean PMMoV concentrations among sampling sites (ANOVA; $p > 0.05$).

PMMoV was used to normalize the average concentration of SARS-CoV-2 RNA concentrations measured at three different sites. Subsequently, the association between COVID-19 daily reported cases in Manila that spanned 7 days before and after sampling was examined, considering both normalized and non-normalized SARS-CoV-2 RNA concentrations. Spearman correlation analysis was performed to identify the specific day that exhibited the strongest and most statistically significant positive correlation. As shown in Fig. 2, it was found that both normalized (Spearman's rho = 0.58) and non-normalized SARS-CoV-2 RNA concentrations (Spearman's rho = 0.60) presented the highest positive correlation with COVID-19 daily reported cases 5 days before the sampling (Spearman's rank correlation; $p < 0.05$).

4. Discussion

Large volumes of wastewater in Manila flow into septic tanks or open drainages without undergoing complete treatment due to low sewer line system coverage. Poorly constructed septic tanks and informal housing areas both significantly contribute to wastewater production directly to open canals (Palanca-Tan, 2017; Jalilov, 2018). For instance, *Estero* de Santa Clara and *Estero* de Pandacan, the open drainages in Manila, are heavily polluted due to the direct discharge of domestic waste; consequently, these *esteros* were selected as sampling sites for this study. SARS-CoV-2 and PMMoV RNA were detected in wastewater samples collected from the open drainage sites, revealing that they were substantially contaminated with human fecal matter. SARS-CoV-2 RNA was observed frequently in *Estero* de Santa Clara, attributing to the number of informal settlers found along the open canal. In Davao City, situated

Table 2
The detection and quantification of the Omicron variant and non-Omicron SARS-CoV-2 RNA in the collected wastewater samples.

Parameters	Omicron variant (G339D)				Non-G339D type			
	Site A (n = 15)	Site B (*n* = 14)	Site C (*n* = 11)	Total (*n* = 40)	Site A (*n* = 15)	Site B (n = 14)	Site C (n = 11)	Total (n = 40)
No. of samples that tested positive in two wells (%)	4 (27)	1 (7)	7 (64)	12 (30)	0 (0)	0 (0)	0 (0)	0 (0)
No. of samples that tested positive in one wells (%)	5 (33)	6 (43)	1 (9)	12 (30)	1 (7)	0 (0)	0 (0)	1 (3)
Concentration (mean ± SD) ($\log_{10}$ copies/L)[a]	4.3 ± 0.3	4.1	4.5 ± 0.3	4.4 ± 0.3	NA	NA	NA	NA

SD, standard deviation; NA, not applicable.
[a] Only samples that tested positive in two wells were used during calculation.

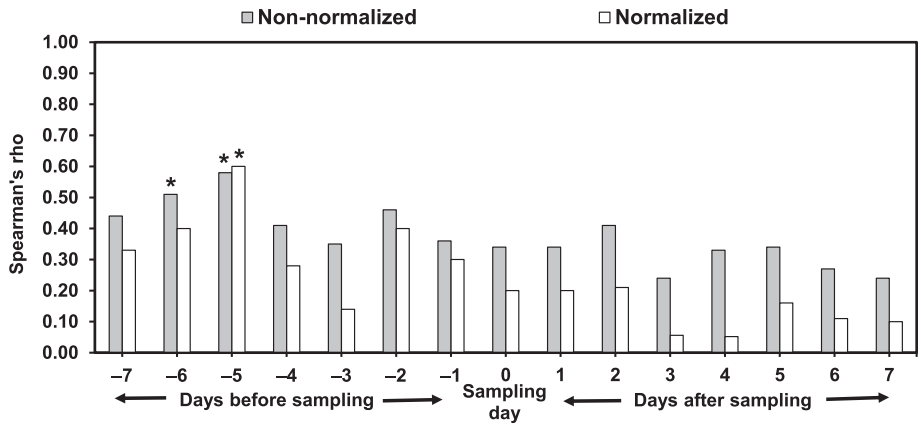

Fig. 2. Spearman rank correlation between normalized and non-normalized SARS-CoV-2 RNA concentrations and COVID-19 daily reported cases spanning 7 days before and after the sampling. An asterisk denotes a significant correlation ($p < 0.05$).

on the island of Mindanao, Otero et al. (2022) performed the Allplex™ 2019-nCoV assay and detected SARS-CoV-2 RNA in wastewater from creeks. The results of this study and Otero et al.'s research accentuate the issue of wastewater management in cities in the Philippines and illustrate the importance of including waterways in epidemiological studies.

Conducting wastewater monitoring for SARS-CoV-2 in buildings at university campuses can persuade testing visitors of the concerned building, facilitate directed response approaches, and provide collated information on the incidence and spread of the virus in the campus (Karthikeyan et al., 2021). Therefore, for this study, the samples were collected from an underground STP at the Henry Sy building of De La Salle University–Manila. The highest concentration of SARS-CoV-2 RNA was observed at this sampling site in November 2022. This result can be associated with increased student and employee access to campus since September 2022; expectedly, daily wastewater production from the building has increased. Similar to the results of the current research, previous studies have also reported the presence of SARS-CoV-2 RNA in wastewater from campus facilities and were able to determine the asymptomatic individuals (Betancourt et al., 2021; Gibas et al., 2021). The results of the present study show that wastewater analysis can be considerably valuable to specific facilities in Manila managing their STPs, such as universities, hospitals, and hotels.

The local government in Manila did not require all citizens to be tested for COVID-19 even though the city is inhabited by 1,846,513 people, as reported in the 2020 census (Philippine Statistics Authority, 2022). Hence, reports from testing laboratories sent to the DOH exclude infected individuals without clinical symptoms and with symptoms who avoid getting tested, stressing the limitations and gaps in the clinical surveillance in the city. Regardless of the decreasing number of infected individuals in Manila from October 2022, SARS-CoV-2 RNA concentrations in wastewater were still detected until the last month of sampling which was February 2023. These results further emphasize that clinical surveillance alone is insufficient to monitor the occurrence of pathogens in Manila. There is limited documentation on SARS-CoV-2 in wastewater from the Philippines, and this study provided the baseline report of SARS-CoV-2 in wastewater from the Capital city. Wastewater surveillance data would be a good combination with Manila's epidemiological data to intensify the city's capacity to monitor various infectious diseases.

The methods performed in this study detected SARS-CoV-2 RNA in wastewater samples from open drainages even when the number of reported daily cases of COVID-19 in the city was as low as 5. Additionally, the presence of SARS-CoV-2 in open drainages is prone to dilution due to meteorological factors and variability in flow rates; remarkably, the methods in this study showed detection efficiency. These results are coherent with the study conducted in Thailand by Thongpradit et al. (2022). The concentrations of SARS-CoV-2 in wastewater from all sampling sites were not significantly different, verifying the processing efficiency and the testing technique sensitivity in this study. These relevant findings underscore the importance of efficient processing methods and sensitive testing techniques for wastewater analysis to provide accurate reports on SARS-CoV-2.

The G339D mutation was detected in SARS-CoV-2 positive wastewater samples, implying that the Omicron variant persisted in the sampling sites. The Omicron variant significantly differs among the samples, denoting the variant diversities in the sampling sites. The results of this study substantiate the biosurveillance report of the DOH (https://doh.gov.ph/covid19-variants), wherein most of the reported clinical cases were caused by the Omicron variant. These findings point out the importance of vigilance and targeted measures to address the unique characteristics of the Omicron variant. The Philippines reported its first two clinical cases of the Omicron variant in December 2021 from incoming travelers. Since then, the transmission of Omicron subvariants in the country has gradually increased, becoming the main variant causing COVID-19 infection. The outcomes of this study suggest the applicability of wastewater analysis in detecting SARS-CoV-2 variants circulating in local communities.

In the present study, the concentration of SARS-CoV-2 RNA was normalized with PMMoV, as suggested in a previous study by Zhan et al. (2022). The reported number of COVID-19-infected persons in Manila 5 days before the wastewater sampling was highly associated with the normalized and non-normalized SARS-CoV-2 RNA concentrations in wastewater from all sampling sites, suggesting that monitoring of SARS-CoV-2 RNA in wastewater can provide insights into disease spread in the community. The findings of this study demonstrate that analysis of wastewater in Manila may aid in observing the fluctuating trends of confirmed clinical cases in the city, further signifying the potential of WBE as an essential indicator of imminent local outbreaks of other infectious diseases in the city.

5. Conclusions

SARS-CoV-2 RNA was successfully detected and quantified through one-step RT-qPCR in wastewater from open drainages and STP in Manila. SARS-CoV-2 RNA detection was possible even when the COVID-19 daily reported cases were as low as five in the city, demonstrating the efficiency and sensitivity of the methods performed. Targeting the G339D mutation determined the presence of the Omicron variant at all sampling locations, highlighting the pertinence of WBE in detecting SARS-CoV-2 variants. There were significant positive correlations between non-normalized and normalized SARS-CoV-2 RNA concentrations and COVID-19 clinical cases reported five days before sampling, denoting that WBE may play a role in identifying and monitoring the trends of clinical cases in the city. Based on these findings, WBE exhibits

the potential to identify and monitor the presence of pathogens in open drainages and STPs in the Philippines. It is recommended to increase the number of samples for collection, include other sampling locations, and target other infectious causative agents for prospective WBE studies in the Philippines.

CRediT authorship contribution statement

Jessamine Gail M. Inson: Writing – original draft, Visualization, Investigation, Formal analysis. **Bikash Malla:** Writing – original draft, Visualization, Investigation, Formal analysis. **Divina M. Amalin:** Resources. **Thaddeus M. Carvajal:** Resources. **Ma. Luisa D. Enriquez:** Resources. **Soichiro Hirai:** Investigation. **Sunayana Raya:** Investigation. **Aulia Fajar Rahmani:** Investigation. **Made Sandhyana Angga:** Investigation. **Niva Sthapit:** Investigation. **Sadhana Shrestha:** Investigation. **Annisa Andarini Ruti:** Investigation. **Tomoko Takeda:** Writing – review & editing, Project administration, Conceptualization. **Masaaki Kitajima:** Writing – review & editing, Methodology, Conceptualization. **Zeba F. Alam:** Writing – review & editing, Supervision, Methodology. **Eiji Haramoto:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

Eiji Haramoto received research funding from Takara Bio Inc. Masaaki Kitajima received research funding from Shionogi & Co., Ltd. and AdvanSentinel, Inc. and patent royalties from Shionogi & Co., Ltd. The other authors declare that they have no conflict of interest.

Data availability

Data will be made available on request.

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Ethics approval

The ethics certificate was provided by the University of Mindanao Ethics Review Committee (UMERC-2022-239).

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