Water Science & Technology

IWA PUBLISHING

Water Science & Technology Vol 84 No 12, 3508 doi: 10.2166/wst.2021.469

Surveillance of SARS-CoV-2 in extensive monitoring of municipal wastewater: key issues to yield reliable results

F. Cutrupi [a,*], M. Cadonna[b], S. Manara [c] and P. Foladori [a]

[a] Department of Civil, Environmental and Mechanical Engineering (DICAM), University of Trento, via Mesiano, n. 77, 38123 Trento, Italy
[b] ADEP - Agenzia per la Depurazione, Autonomous Province of Trento, via Gilli, n. 3, 38121 Trento, Italy
[c] Department of Cellular Computational and Integrative Biology (CIBIO), University of Trento, via Sommarive, n. 9, 38123 Trento, Italy
*Corresponding author. E-mail: francesca.cutrupi@unitn.it

FC, 0000-0002-3097-5592; SM, 0000-0001-6371-528X; PF, 0000-0002-7483-0416

ABSTRACT

Several studies have detected SARS-CoV-2 in the stool of infected people as in urban wastewater. The quantification of SARS-CoV-2 in wastewater appears today as a powerful tool that can help in wastewater-based epidemiology (WBE). The goal is to improve the prediction of new waves of COVID-19 outbreaks and provide an early warning of the evolution of the infection. In this research, we highlighted some practical and scientific aspects that emerged during an extensive ongoing monitoring campaign carried out on a large number of wastewater treatment plants located in the province of Trento (North Italy) and aimed at the detection of SARS-CoV-2 in raw municipal wastewater. The open issues underline are related to the collection and storage (sampling protocol, storage and heat treatment), to the molecular analysis (enrichment phase), and to the mathematical calculation of SARS-CoV-2 load in wastewater, suitable for WBE (standard curve to obtain the concentration of genomic units and flow rate measurements). This study provides some insights that can help in the implementation of surveillance plans in other regions.

Key words: molecular analysis, monitoring, SARS-CoV-2, wastewater, wastewater-based epidemiology

HIGHLIGHTS

- It is possible to quantify SARS-COV-2 from wastewater.
- Wastewater based epidemiology is a useful tool for predicting new outbreaks.
- Heat treatments make the virus more easily detectable.
- Several storage protocols have been tested.

INTRODUCTION

Since November 2019, the world population has been affected by a pandemic caused by a new virus, the SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) (Coronaviridae Study Group of the International Committee on Taxonomy of Viruses 2020). This virus is a member of the subfamily *Coronavirinae*, genus *Betacoronavirus*, subgenus *Sarbecovirus* (Coronaviridae Study Group of the International Committee on Taxonomy of Viruses 2020) to which also belong the viruses SARS and MERS, which have caused major outbreaks in recent decades. The disease caused by this virus, called COVID-19, mainly affects the respiratory system causing fever, cough, rhinorrhea, dyspnoea, or severe pneumonia (Chen *et al.* 2020; Guan *et al.* 2020). An important interaction is with the gastrointestinal system, which may lead to diarrhoea, vomiting, or abdominal pain (Guan *et al.* 2020; Pan *et al.* 2020) that may be early symptoms of the disease (Huang *et al.* 2020; Lai *et al.* 2020). SARS-CoV-2 can be detected in the stool of positive patients (Wang *et al.* 2020), even if asymptomatic (Park *et al.* 2020), with titres in the range of 10^3–10^7 genomic unit/g faeces (GU/g) depending on the day of sampling (Wölfel *et al.* 2020). The presence of SARS-CoV-2 in faeces leads to finding it in the urban sewers and wastewater treatment plants (WWTPs). When faeces reach the sewerage, the viral titre undergoes a dilution of about 10^3 times due to the discharge of drinking water, rainwater, or infiltrations, and the concentration of SARS-CoV-2 drops to 1–10^3 GU/mL (Foladori *et al.* 2020). The quantification of SARS-CoV-2 in wastewater could give additional information about the spread of the COVID-19 outbreak (Daughton 2020).

This is the topic of the field of wastewater-based epidemiology (WBE), which deals with the use of quantitative measurement of human biomarkers in sewage to evaluate lifestyle, health, and exposure at the community level (https://score-cost.eu/; Sims & Kasprzyk-Hordern 2020). Several nations have moved to develop a monitoring network for their wastewater. For example, in Italy, the severe acute respiratory syndrome (SARI) project was born for the surveillance of SARS-CoV-2 at national level. However, there is still a lack of agreement on an international standard protocol. For monitoring a large number of WWTPs in a territory, sampling, storage, and analysis of samples should be fast and affordable, with moderate efforts in terms of personnel, equipment, and costs, but at the same time ensuring reliable results. The choice of the method is today something closely linked to the skills and availability of each workgroup. For these reasons, the quantification of SARS-CoV-2 in wastewater for WBE applications remains an open issue (Table 1).

In this research we highlighted some practical and scientific aspects that emerged during an extensive ongoing monitoring campaign in August 2020 to January 2021, aimed at quantifying SARS-CoV-2 in 31 WWTPs in the Trento province (North Italy). The monitored WWTPs had daily average flow rates range from 4,000 m^3/d to 40,000 m^3/d and a treatment capacity

Table 1 | Available methods in the literature for the detection of SARS-CoV-2 in wastewater

Ref.	Sampling	Storage	Heat treat.	Enrichment concentration	Extraction
Ahmed *et al.* (2020a)	Automated and grab sampling techniques	Samples were transported on ice to the laboratory and stored at 4 °C		Direct RNA extraction from electronegative membranes or ultrafiltration	For RNA extraction, two kits (RNeasy PowerWater Kit and RNeasy PowerMicrobiome Kit; Qiagen, Germany) were used
La Rosa *et al.* (2020)	24-hour composites collected from the WWTP influent	Immediately stored at −20 °C	30 min treat. at 57 °C	Two-phase (PEG–dextran method) separation	The viral nucleic acids extraction foresaw the use of the NucliSENS miniMAG semi-automated extraction system with magnetic silica carried out following the manufacturer's instructions (Biomerieux, France). Before molecular tests, the extracted nucleic acids were further purified from PCR inhibitors
Medema *et al.* (2020)	24 h flow-dependent composite sample	Samples were transported on ice to the laboratory and stored at 4 °C		The sample was filtered through a Centricon® Plus-70 centrifugal ultrafilter with a cut-off of 10 kDa (Millipore, Amsterdam, the Netherlands)	Method 1: RNeasy PowerMicrobiome Kit (Qiagen, Germany). Method 2: magnetic extraction reagents of the NucliSENS kit (Biomerieux, the Netherlands) in combination with the semi-automated KingFisher mL (Thermo Scientific, The Netherlands) purification system to extract RNA
Randazzo *et al.* (2020)		Collected samples were transferred on ice to the laboratory		Aluminium hydroxide adsorption–precipitation concentration method	Viral RNA was extracted from concentrates using the NucleoSpin RNA virus kit (Macherey-Nagel GmbH & Co., Germany)
Wu *et al.* (2020)	24-h composite samples of raw water		Pasteuriz. at 60 °C for 90 min to inactivate the virus	Concentration with polyethylene glycol 8000 (8% w/v, Millipore Sigma) and centrifugation	

range from 6,000 to 85,000 residents served. This study provides various insights that can be exploited in the implementation of surveillance plans in other regions.

OPEN ISSUES

In the quantification of SARS-CoV-2 in raw wastewater the main areas worthy of further thought are the following: (i) collection and storage of samples, (ii) some steps of the molecular analysis, (iii) mathematical calculation of the viral load in wastewater, suitable for WBE.

Issues related to the collection and storage of wastewater samples

Sampling protocol

The type of sampling may affect the accurate estimation of SARS-CoV-2 content in wastewater, especially for WBE. In general, composite samples are recommended (EPA 2013). They can be formed from: (1) aliquots of wastewater with equal volume taken at equal interval times throughout the day, or (2) aliquots of wastewater with volume proportional to the flow rate, which is often impracticable because this requires a flow measurement device not always available in WWTPs.

To evaluate the fluctuations of SARS-CoV-2 in influent wastewater over the day (24 h), 12 samples collected every 2 hours for 24 hours with an automatic sampler, were analysed. Concentrations, expressed as viral genomic units per litre (GU/L), are shown in Figure 1. Concentration was lower at night while it was higher in the morning, between 9 and 11, due to the human behaviour patterns, e.g. toilet usage, in the early hours, and the corrivation time in the sewerage that causes a delay of approximately 2 hours. Another peak occurred in the afternoon at 15–17. The average concentration was 1.4E + 0.4 GU/L, while the minimum–maximum range was 8.8E + 03–2.9E + 04 GU/L.

Due to these fluctuations, the choice of composite samples is preferable for the WBE (Ahmed *et al.* 2020b). However, grab samples can instead be used to evaluate the peaks of SARS-CoV-2 entering the WWTP (Ahmed *et al.* 2020b), to evaluate its treatment capacity.

Sample storage

Samples should be maintained at low temperature (4 °C) during collection and transport to the laboratory to preserve the viral load and viability (Medema *et al.* 2020). With regards to sample storage, it is worth noting that freeze–thaw cycles can damage the integrity of viral RNA. The optimal temperature and duration of the storage are key factors, but at the moment there is not full international agreement on these aspects. Storage at −80 °C, although recommended by some authors (Medema *et al.* 2020), is not feasible in an extensive monitoring plan with many WWTPs, due to the need to store tens of litres per week. Conversely, the storage at 4 °C or −20 °C appears more feasible. In order to investigate the influence of these two storing temperatures in our monitoring, various aliquots of the same wastewater sample were stored at 4 °C and −20 °C and analysed at 0, 7, 10, 14 and 31 days. As can be seen from the trends in Figure 2, a marked loss of copies occurred in the samples stored at −20 °C; this loss happened especially in the first 10 days of storage, with a reduction of 0.5 log. Conversely, the number of

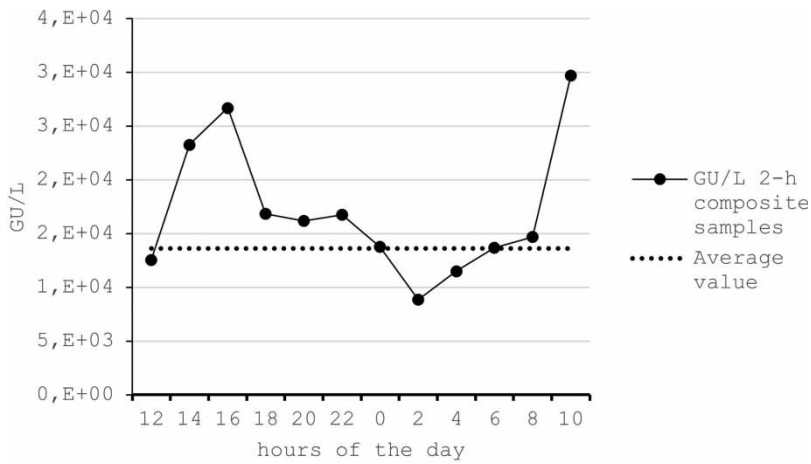

Figure 1 | Profile of SARS-CoV-2 concentration in 2-h composite samples over the day.

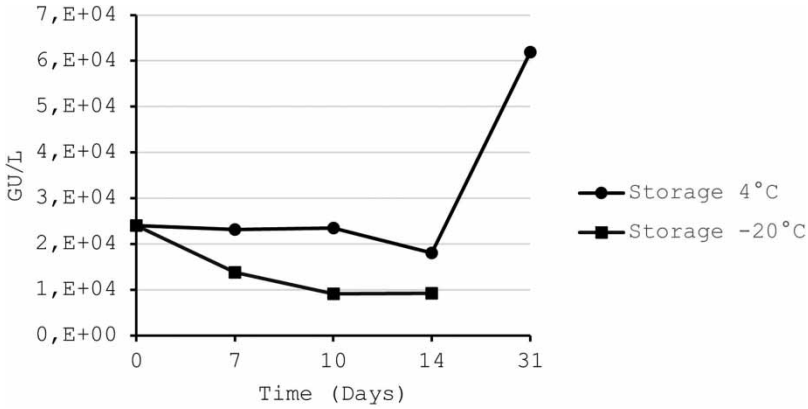

Figure 2 | Trends of SARS-CoV-2 concentration during storage at 4 °C and −20 °C over time.

copies did not decrease significantly when stored at 4 °C for 14 days. This is in agreement with the findings of Medema *et al.* (2020), who indicated that storage at 4 °C is preferable as the viral RNA remains intact for the first 15 days (Medema *et al.* 2020). By contrast, Xiao *et al.* (2020) indicated to keep the samples at 4 °C when the analysis is performed in the following 24 h, otherwise for longer storage (>24 h) the samples should be kept at −20 °C. Therefore, the influence of 4 °C for longer times requires further investigation, especially to find an explanation to the increase phenomena observed.

Pre-treatment

The thermal pre-treatment, aimed at inactivating the virus and making the sample safer for the operators, is another aspect on which a shared agreement has not been reached yet. Some protocols propose pasteurization at 56 °C for 50 min (La Rosa *et al.* 2020), but others advise against it, to avoid a loss of viral RNA integrity, so scarce in wastewater (Weidhaas *et al.* 2020). To investigate the influence of heat treatment on viral RNA detection, four samples from four WWTPs were analysed with and without pasteurization (thermal bath, 56 °C, 30 min). The results are compared in Table 2 in terms of Ct and GU/L. The Ct values in the heat-treated samples were always lower than in the not heat-treated ones. Therefore, a higher concentration of the virus was estimated in the inactivated samples. The heat treatment makes viral RNA more easily detectable. After statistical analysis with the two-way ANOVA test with randomized blocks, the p was significantly lower than the critical p, underlining the important effect of the heat treatment ($p = 0.001$) in our experimental results. The enrichment phase of the analysis protocol used for the pasteurization test was different from that used for the sampling test and for the storage test. The use of these different methods led to a difference in results of about two orders of magnitude. The comparison between these two protocols will be addressed in the following paragraph.

Issues related to the molecular analysis

The molecular analysis of the SARS-CoV-2 virus in wastewater is based on three main steps: (1) enrichment, (2) extraction, and (3) amplification.

Comparison between two methods for enrichment

The very low titre of SARS-CoV-2 in wastewater and the need to recover the total amount of the virus for WBE applications, make the enrichment step very critical. In this research the concentration of SARS-CoV-2 in the wastewater from 31 WWTPs was 3–600

Table 2 | Comparison of Ct and SARS-CoV-2 concentrations in four different wastewater analysed with and without heat treatment at 56 °C for 30 min

	WWTP 1		WWTP 2		WWTP 3		WWTP 4	
	No heat treatment	Heat treatment	No heat treatment	Heat treatment	No heat treatment	Heat treatment	No heat treatment	Heat treatment
Ct	35.96	33.11	36.59	33.44	35.85	32.31	35.6	33.03
GU/L	994	3,201	393	1,829	814	6,952	630	4,105

GU/mL. Without an efficient enrichment virus detection in wastewater is not currently possible. In this research two enrichment methods were compared (Figure 3): (i) biphasic system (PEG–dextran) according to La Rosa *et al.* (2020) and adapted from the WHO procedure for Poliovirus; (ii) centrifugation at 12,000 ***g*** with the addition of PEG, according to Wu *et al.* (2020) except for the first step of 0.2 μm filtration. These methods differ for the time of application: (i) overnight for the biphasic system; (ii) only 2 h for centrifugation. Another difference is the role of solids. A complete elimination of solids is indeed required in the centrifugation method (Wu *et al.* 2020), while in the biphasic method the solids and supernatant are first separated and then combined after the PEG–dextran precipitation (La Rosa *et al.* 2020), according to the scheme in Figure 3. To specifically test for the difference in the two enrichment methods, the same protocol of viral extraction and RT-PCR was used to obtain Ct values.

Analysing samples of different WWTPs with both enrichment methods, a significant difference of results was obtained: Ct were in the range 32.73–38 for the biphasic system and 26.73–36.33 for the centrifugation system. This difference is probably due to the PCR-inhibitory substances (e.g., like humic, and fulvic acids) contained in solids that can prevent the correct amplification of viral RNA, producing false-negative results. The presence of inhibitory substances is another critical factor, beyond the low concentration and the poor integrity of the nucleic acids, in the detection of SARS-CoV-2 RNA in wastewater. This set of limitations is the reason why, unlike for respiratory tract specimens, there is still no standard method for the detection of SARS-CoV-2 in wastewater.

Extraction and amplification

For the extraction and amplification steps, different solutions and combinations exist but there is no evidence of a greater efficacy of one protocol over another. Viral RNA extraction is mainly performed with commercially available kits and serves to isolate RNA and clean it of PCR pollutants and inhibitors (Ahmed *et al.* 2020a; La Rosa *et al.* 2020; Randazzo *et al.* 2020). In this research, the extraction phase consists in (Figure 3): (i) lysis of the virus, (ii) bond between RNA and magnetic silica beads, (iii) washing of impurities with buffers, (iv) final elution. Then the pure RNA is used in the amplification step performed through RT-PCR, also called quantitative PCR (qPCR), and based on the exponential amplification of specific genomic regions of the virus (ORF1ab region in our research). The results of RT-PCR are expressed as Ct (threshold cycle) that is the number of cycles required for the fluorescent signal to cross a threshold. Ct is inversely related to the amount of virus. For example, a low Ct value means that a lower number of amplification cycles are required to surpass the threshold and thus the viral concentration in the sample is higher.

Issues related to the mathematical calculation of SARS-CoV-2 load for WBE

Calculation of GU/L using standard curves

The concentration of SARS-CoV-2 RNA in a sample, expressed as GU/L, can be calculated from the threshold cycle (Ct) values and using a standard curve, obtained with a series of dilutions of a standard with known amounts of viral RNA (e.g., AccuPlex

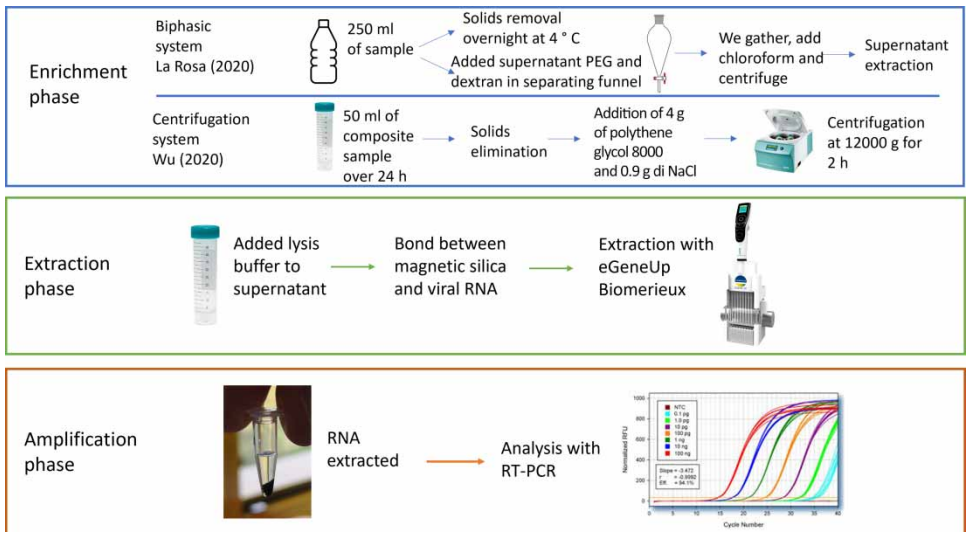

Figure 3 | Scheme of the analytical approach for the detection of SARS-CoV-2 with the two enrichment methods compared in this study. Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/wst.2021.469.

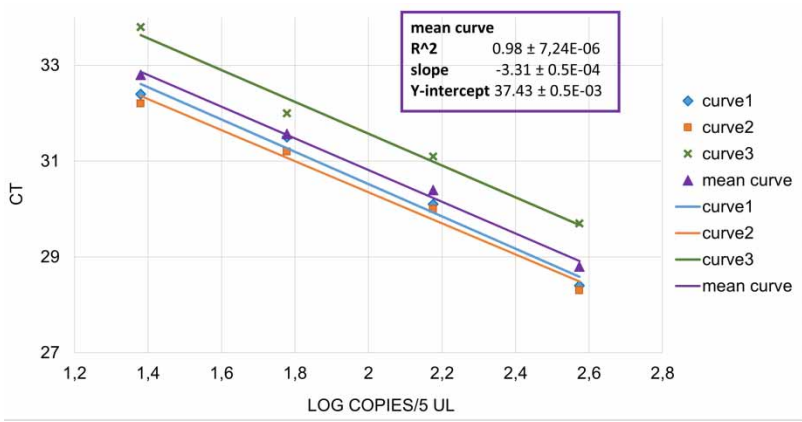

Figure 4 | Examples of daily standard curves for the conversion of Ct into GU/L and mean curve. Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/wst.2021.469.

SARS-CoV-2 Reference Material Kit used in this study). Figure 4 shows some daily PCR standard curves and their average curve: the slope measures the efficiency of the reaction (maximum efficiency corresponds to a slope of –3.32), the correlation coefficient (R2) measures the fitting of the data with the curve and the Y-intercept is the theoretical limit of detection of the reaction. Once the Ct of the sample is known, the concentration of SARS-CoV-2 can be easily calculated. Our extensive monitoring campaign has highlighted the need to run a standard curve on each RT-PCR run in which the samples of interest are analysed, in order to obtain comparable results, because of the efficiency variability between the different runs of qPCR.

Calculation of the SARS-CoV-2 load in wastewater using influent flow rate

Finally, the viral load of SARS-CoV-2 in the influent wastewater can be calculated by multiplying the value of GU/L by the flow rate entering the WWTP during the time interval of sampling. It is worth noting that the real influent flow rate cannot be estimated using the theoretical calculation that is based on the average daily water consumption per capita (L $PE^{-1}d^{-1}$) and the population equivalent (PE), because the real flow rate is almost always affected markedly by industries, stormwater, infiltrations or leakages.

CONCLUSIONS

This monitoring campaign was carried out on 31 WWTPs with more than 100 samples was a robust basis with which to highlight some practical and scientific aspects and advice, such as the use of heat treatment for a better detection of viral RNA, the feasibility of centrifugation to fasten the enrichment or the use of composite samples for WBE. Other aspects still need to be confirmed, such as the influence of different temperatures for the long-term storage of samples, or the role of inhibitory substances present in the wastewater in PCR analysis.

ACKNOWLEDGEMENTS

This research was supported by the Internal Call 2020 ‘Covid 19’, project ‘Surveillance of COVID-19 Pandemic with a Wastewater-Based Epidemiology approach (SCOPE)’, awarded by the University of Trento, Italy. The authors also acknowledge funding from the Italian Ministry of Education, University and Research (MIUR) in the frame of the ‘Departments of Excellence’ grant L. 232/2016.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

REFERENCES

Ahmed, W., Angel, N., Edson, J., Bibby, K., Bivins, A., O’Brien, J. W., Choi, P. M., Kitajimae, M., Simpson, S. L., Li, J., Tscharke, B., Verhagen, R., Smith, W. J. M., Zaugg, J., Dierens, L., Hugenholtz, P., Thomas, K. V. & Mueller, J. F. 2020a 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. *The Science of the Total Environment* **728**, 138764.

Ahmed, W., Bivins, A., Bertsch, P. M., Bibby, K., Gyawali, P., Sherchan, S. P., Simpson, S. L., Thomas, K. V., Verhagen, R., Kitajima, M., Mueller, J. F. & Korajkic, A. 2020b Intraday variability of indicator and pathogenic viruses in 1-h and 24-h composite wastewater samples: implications for wastewater-based epidemiology. *Environmental Research* **193**, 110531.

Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., Qiu, Y., Wang, J., Liu, Y., Wei, Y., Xia, J., Yu, T., Zhang, X. & Zhang, L. 2020 Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *The Lancet* **395** (10223), 507–513.

Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. 2020 The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nature Microbiology* **5** (4), 536–544.

Daughton, C. 2020 The international imperative to rapidly and inexpensively monitor community-wide Covid-19 infection status and trends. *The Science of the Total Environment* **726**, 138149.

EPA. 2013 *Wastewater Sampling*. Number: SESDPROC-306-R3, 28 February 2013.

Foladori, P., Cutrupi, F., Segata, N., Manara, S., Pinto, F., Malpei, F., Bruni, L. & La Rosa, G. 2020 SARS-CoV-2 from faeces to wastewater treatment: what do we know? A review. *The Science of the Total Environment* **743**, 140444.

Guan, W., Ni, Z., Hu, Y., Liang, W., Ou, C., He, J. & Liu, L. 2020 Clinical characteristics of 2019 novel coronavirus infection in China. *MedRxiv*. Available from: https://www.medrxiv.org/content/10.1101/2020.02.06.20020974v1.abstract.

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, F., Gu, X., Cheng, Z., Yu, T., Xia, J., Wei, Y., Wu, W., Xie, X., Yin, W., Li, H., Liu, M., Xiao, Y., Gao, H., Guo, L., Xie, J., Wang, G., Jiang, R., Gao, Z., Jin, Q., Wang, J. & Cao, B. 2020 Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The Lancet* **395**, 497–506.

Lai, C.-C., Ko, W.-C., Lee, P.-I., Jean, S.-S. & Hsueh, P.-R. 2020 Extra-respiratory manifestations of COVID-19. *International Journal of Antimicrobial Agents* **56** (2), 106024.

La Rosa, G., Bonadonna, L., Lucentini, L., Kenmoe, S. & Suffredini, E. 2020 Coronavirus in water environments: occurrence, persistence and concentration methods – A scoping review. *Water Research* **179**, 115899.

Medema, G., Heijnen, L., Elsinga, G., Italiaander, R. & Brouwer, A. 2020 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. *Environmental Science & Technology Letters* **7** (7), 511–516.

Pan, L., Mu, M., Yang, P., Sun, Y., Wang, R., Yan, J., Li, P., Hu, B., Wang, J., Hu, C., Jin, Y., Niu, X., Ping, R., Du, Y., Li, T., Xu, G., Hu, Q. & Tiu, L. 2020 Clinical characteristics of COVID-19 patients with digestive symptoms in Hubei, China: a descriptive, cross-sectional, multicenter study. *The American Journal of Gastroenterology* **115**, 766–773.

Park, S.-K., Lee, C.-W., Park, D.-I., Woo, H.-Y., Cheong, H. S., Shin, H. C., Ahn, K., Kwon, M. -J. & Jooj, E. -J. 2020 Detection of SARS-CoV-2 in fecal samples from patients with asymptomatic and mild COVID-19 in Korea. *Clinical Gastroenterology and Hepatology*. https://doi.org/10.1016/j.cgh.2020.06.005.

Randazzo, W., Truchado, P., Cuevas-Ferrando, E., Simón, P., Allende, A. & Sánchez, G. n.d. *SARS-CoV-2 RNA Titers in Wastewater Anticipated COVID-19 Occurrence in A low Prevalence Area*. https://doi.org/10.1101/2020.04.22.20075200.

Sims, N. & Kasprzyk-Hordern, B. 2020 Future perspectives of wastewater-based epidemiology: monitoring infectious disease spread and resistance to the community level. *Environment International* **139**, 105689.

Wang, W., Xu, Y., Gao, R., Lu, R., Han, K., Wu, G. & Tan, W. 2020 Detection of SARS-CoV-2 in different types of clinical specimens. *JAMA: The Journal of the American Medical Association* **323** (18), 1843–1844.

Weidhaas, J., Aanderud, Z., Keith Roper, D., Van Derslice, J., Gaddis, E. B., Ostermiller, J., Hoffman, K., Jamal, R., Heck, P., Zhang, Y., Torgersen, K., Vander Laan, J. & LaCross, N. 2021 Correlation of SARS-CoV-2 RNA in wastewater with COVID-19 disease burden in sewersheds. *Research Square*. Available from: https://www.researchsquare.com/article/rs-40452/latest.pdf.

Wölfel, R., Corman, V. M., Guggemos, W., Seilmaier, M., Zange, S., Müller, M. A., Niemeyer, D., Jones, T. C., Vollmar, P., Rothe, C., Hoelscher, M., Bleicker, T., Brünink, S., Schneider, J., Ehmann, R., Zwirglmaier, K., Drosten, C. & Wendtner, C. 2020 Virological assessment of hospitalized cases of coronavirus disease 2019. *Nature*. https://doi.org/10.1038/s41586-020-2196-X.

Wu, F., Xiao, A., Zhang, J., Moniz, K., Endo, N., Armas, F., Bonneau, R., Brown, M. A., Bushman, M., Chai, P. R., Duvallet, C., Erickson, T. B., Foppe, K., Ghaeli, N., Gu, X., Hanage, W. P., Huang, K. H., Lee, W. L., Matus, M., McElroy, K. A., Nagler, J., Rhode, S. F., Santillana, M., Tucker, J. A., Wuertz, S., Zhao, S., Thompson, J. & Alm, E. J. 2020 SARS-CoV-2 titers in wastewater foreshadow dynamics and clinical presentation of new COVID-19 cases. *MedRxiv* Available from: https://doi.org/10.1101/2020.06.15.20117747.

Xiao, F., Tang, M., Zheng, X., Liu, Y., Li, X. & Shan, H. 2020 Evidence for gastrointestinal infection of SARS-CoV-2. *Gastroenterology* **158** (6), 1831–1833.e3. https://doi.org/10.1053/j.gastro.2020.02.055.

First received 18 July 2021; accepted in revised form 18 October 2021. Available online 27 October 2021