Municipal wastewater viral pollution in Saudi Arabia: effect of hot climate on COVID-19 disease spreading

Hadil M. Alahdal¹ · Fuad Ameen² · Sami AlYahya³ · Hana Sonbol¹ · Anas Khan^{4,5} · Yousef Alsofayan⁵ · Ahmed Alahmari⁵

Received: 28 April 2021 / Accepted: 7 June 2021 / Published online: 17 June 2021

© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2021

Abstract

The viral RNA of SARS-Coronavirus-2 is known to be contaminating municipal wastewater. We aimed to assess if COVID-19 disease is spreading through wastewater. We studied the amount of viral RNA in raw sewage and the efficiency of the sewage treatment to remove the virus. Sewage water was collected before and after the activated sludge process three times during summer 2020 from three different sewage treatment plants. The sewage treatment was efficient in removing SARS-CoV-2 viral RNA. Each sewage treatment plant gathered wastewater from one hospital, of which COVID-19 admissions were used to describe the level of disease occurrence in the area. The presence of SARS-CoV-2 viral RNA-specific target genes (N1, N2, and E) was confirmed using RT-qPCR analysis. However, hospital admission did not correlate significantly with viral RNA. Moreover, viral RNA loads were relatively low, suggesting that sewage might preserve viral RNA in a hot climate only for a short time.

Keywords Coronavirus · Health effects · Viral contamination · Sewage treatment

Introduction

The contamination of wastewaters with viruses causing diseases is an emerging threat that the global climate change seems to worsen (El-Sayed and Kamel 2020). The new coronavirus disease COVID-19 causing the global pandemic is

needing great efforts to be controlled. It has also been speculated that the SARS-CoV-2 virus will circulate over the world like seasonal influenza (Li et al. 2020). Therefore, it seems that we will need preventing actions for a long time in the future. The disease symptoms appear 4 to 14 days after exposure; meanwhile, the person can further spread the virus. Viruses usually spread through the air, but they can spread through wastewater as well. The virus can be transmitted from sewage through the workers that are exposed to the virus (Elsamadony et al. 2021). This transmission pathway was recommended to be studied in a recent review (Bogler et al. 2020).

Wastewater surveillance has been shown to be helpful in assessing the disease spreading (Mohapatra et al. 2020; Polo et al. 2020; Thompson et al. 2020; Zhu et al. 2021). Disease incidence has correlated with viral RNA, which can be analyzed using the molecular method of quantitative real-time PCR (RT-qPCR). Viral RNA has been observed to correlate strongly positively with local hospital admission; a correlation coefficient (r) has been higher than 0.9 (Medema et al. 2020; Peccia et al. 2020). The efforts to utilize wastewater surveillance to control the disease has already been made throughout the world, for instance, in China (Bar-Or et al. 2020),

Hadil M. Alahdal and Fuad Ameen contributed equally to this work.

Responsible Editor: Lotfi Aleya

✉ Fuad Ameen
fuadameen@ksu.edu.sa

¹ Department of Biology, Faculty of Science, Princess Nourah Bint Abdulrahman University, Riyadh, Saudi Arabia

² Department of Botany & Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

³ National Center for Biotechnology, King Abdulaziz City for Science & Technology, Riyadh, Saudi Arabia

⁴ Department of Emergency Medicine, College of Medicine, King Saud University, Riyadh, Saudi Arabia

⁵ Global Center for Mass Gatherings Medicine, Ministry of Health, P.O. Box 11461, Riyadh, Saudi Arabia

Australia (Ahmed et al. 2020), Netherlands (Medema et al. 2020), Japan (Haramoto et al. 2020), Turkey (Kocamemi et al. 2020), and France (Wurtzer et al. 2020). Several reviews have already been published (Saawarn and Hait 2020; Hasan et al. 2021; Ihsanullah et al. 2021; Zhou et al. 2021). The wastewater approach has scarcely been studied in Arabic countries. We found only one study in UAE (Hasan et al. 2021). It is vital to study sewage and sewage treatments locally because the environmental conditions, most of all temperature, are known to affect the survival of viruses (Aboubakr et al. 2020). The survival of SARS-CoV-2 virus in sewage is still not well known. Coronavirus causing an epidemic in 2003 SARS-CoV was found in sewage for 2 days at 20 °C and for 14 days at 4 °C (La Rosa et al. 2020). Recently, high temperatures have been shown to inhibit the spreading of COVID-19 disease (Guo et al. 2021; Notari 2021). It can be assumed that the high temperature (around 50 °C) prevailing in the Arabian Gulf countries during summer is destroying the virus more rapidly than cool or moderate temperatures.

Our aim was to assess how SARS-CoV-2 viral RNA persists in sewage systems in a hot climate. High temperatures prevailing in Saudi Arabia may destroy the virus, and thus, the spreading of the virus may not be significant. We sampled untreated wastewater from three sewage treatment plants three times and compared the results to reported disease cases in the hospitals nearby. We also sampled treated sewage to analyze the efficiency of the treatment procedure to remove viral RNA.

Materials and methods

Waste water sample collection

Three replicated untreated and treated (cleaned) wastewater samples (2 L) were collected to sterilized glass bottles from three sewage treatment plants situated in Riyadh city three times monthly in 15th June, 17th July, and 16th August 2020. The sewage plants were DQ sewage water treatment facility (SW1), KFSHRC water treatment plant (SW2), and NWC strategic water systems (SW3) (Fig. 1). The samples were collected after activated sludge treatment before the disinfection step. The temperatures of sewage were 35 °C, 38 °C, and 40 °C $\pm$ 2 °C in June, July, and August, respectively. The samples were immediately cooled at 4 °C, and the analyses were carried out within 48 h.

RNA extraction

The wastewater was concentrated using the flocculation method using 3.0% w/v beef extract solution (Sigma-Aldrich, St. Louis, USA), pH 9.5 in 0.05 M glycine buffer. Each sewage

water (SW1, SW2, and SW3) sample (500 mL) was acidified (Michael-Kordatou et al. 2020) at pH 3.5 $\pm$ 0.1 and 10 mL of the beef extract was agglomerated by the addition of 0.1 M HCl at pH 3.0 followed by stirring for 10 h. The stirred suspension was centrifuged at 10,000 $\times$ g for 30 min at 4 °C. The pellet was dissolved in 8 mL of phosphate buffered saline (PSB) and the RNA was extracted using NucliSENS® miniMAG® system (BioMerieux, Marcy l'Etoile, France) according to the manufacturer's instructions.

RT-qPCR analysis

RT-qPCR was carried out using Allplex COVID 19 RT-PCR kit (Seegene Inc) specific for SARS-CoV-2. RNA was amplified using R-Biopharm AG RIDA®CYCLER system with initial activation (2 min) at 25 °C, reverse transcription at 50 °C for 15 min, initial denaturation at 95 °C for 2 min, annealing with 45 cycles at 95 °C for 10 s, and extension at 55 °C for 15 s. The fluorescence analysis was carried out using Light Cycler 480 (Roche Molecular Diagnostics, Mannheim, Germany). The length of the PCR product was analyzed with high-resolution automatic electrophoresis (Agilent, Santa Clara, USA). Viral load was determined spectrophotometrically (277 nm) as described by Rostislav et al. (2021). Cycle threshold value (Ct), which is inversely related to the viral load, was reported to assess the SARS-CoV-2 viral load as recommended by Tom and Mina (2020). A rise of three in the Ct value indicates a tenfold reduction in viral load, and Ct < 29 indicates strong, Ct 30–37 moderate, and Ct 38–40 weak or negative load (Tom and Mina, 2020).

Disease incidence

The epidemic was at a relatively high level in Saudi Arabia during the study period. The 14-day notification rate per 100,000 population varied between 57 and 160 during the study period (ECDC 2020). In Riyadh, the disease incidence was higher; the 14-day notification rate per 100,000 population was 100 in May, 650 in June, 633 in July, and 413 in August (MOH 2020).

Patients analyzed to have the COVID-19 disease in hospitals near the sewage treatment plants were used to assess disease incidence. The data from three hospitals were used. Each sewage treatment plant collected the wastewater of one hospital. The hospitals were King Khalid University Hospital; King Faisal Specialist Hospital & Research Center; and King Salman Bin Abdulaziz Hospital corresponding SW1, SW2, and SW3 sewage treatment plants (Fig. 1). The hospital admission data were divided into 2 weeks period starting 1 month before the first sewage sampling and ending 2 weeks after the last sewage sampling.

Fig. 1 Map of the sewage treatment plants in Riyadh city

Statistical analysis

Spearman rank correlation between the Ct value and the disease cases during 2 weeks after the sampling was calculated. Paired t-test was used to analyze the difference between untreated and treated sewage. $P < 0.05$ was considered significant.

Results

The levels of viral RNA load in untreated sewage differed between the treatment plants SW1 having the highest load. Inversely related to viral load, the Ct values varied between 14 and 22 for all three target genes (N1, N2, E) during the 3 months. For all genes, lower Ct values (14–16), indicating higher viral load, were observed in June and August than in July (Ct = 21) (Fig. 2a). In SW2 and SW3, the Ct values were

at a slightly higher level in general (Fig. 2b, c). In SW2, the June samples were the highest, over 30, indicating only moderate viral load. In SW3, the months did not differ much. The three treatment plants showed different trends during the sampling SW1 and SW2 differing from SW3 where the Ct values were almost equal each month.

A total of 2374 (SW3), 3499 (SW1), and 5761 (SW2) disease cases were reported in the hospitals. In May, almost no cases were reported and the rapid spreading of the disease started in June when the first samples were taken (Table 1). Disease cases and target gene Ct values did not correlate (Fig. 3). Spearman rank correlation between Ct values and disease cases (data of all hospitals combined, $n = 9$) were not significant, and the correlation coefficients (R_s) were 0.42, 0.37, and 0.42 for N1, N2, and E, respectively.

The sewage treatment procedures removed viral RNA efficiently in all three treatment plants. The means of the viral loads of the 3 months varied between 70 and 85 copies mL^{-1} before the sewage treatment (Fig. 4). Viral load was significantly (paired t-test, $p < 0.05$) lower in treated sewage than in untreated sewage. Viral load varied between 3 and 10 copies mL^{-1} in treated sewage.

Discussion

Wastewater surveillance has been shown to give an early indication about the starting epidemic of COVID-19 disease (Medema et al. 2020; Randazzo et al. 2020b). Disease incidence and SARS-CoV-2 viral RNA load have been shown to correlate strongly positively already many times, as reviewed by Mandal et al. (2020). A predictive regression model has been published (Vallejo et al. 2020). A high correlation ($r > 0.9$) between viral RNA and hospital admission was observed in the USA (Peccia et al. 2020). Lower correlations have also been reported, for instance, $r = 0.4$ – 0.5 in Japan (Hata et al.

Fig. 2 Disease cases reported during 2 weeks after sewage sampling and Ct values (note the reverse order) of RT-qPCR analysis of SARS-CoV-2 RNA of untreated sewage for three genes (N1, N2, and E) (mean, SD, $n = 3$). (a) SW1, (b) SW2, and (c) SW3 refer to sewage sampled from the sewage treatment plants three times during 2020

Table 1 COVID-19 disease cases in three hospitals near the sewage treatment plants during 15th May–31st August 2020 in Riyadh

Time period	Sewage treatment plants		
	SW1	SW2	SW3
15th May–31st May	24	1	86
1st June–15th June	1109	1399	634
16th June–30th June	896	938	559
1st July–17th July	705	1212	532
18th July–31st July	344	1067	281
1st August–16th August	221	738	160
17th August–31st August	200	406	122
Total	3499	5761	2374

Fig. 3 Scatter plot of the disease cases reported during 2 weeks after sewage sampling and Ct values of RT-qPCR analysis of SARS-CoV-2 RNA of untreated sewage for genes N1 (blue square), N2 (red circle), and E (green triangle)

2021). In some studies, no correlation was found (Trottier et al. 2020; Wu et al. 2020). In our study, no significant correlation was observed between the disease cases reported in hospitals during 2 weeks after the sampling of sewage and the Ct values of the three genes. One explanation is that the strength of the correlation depends on the variation in the data set, which was low in our case. Moreover, we had only nine observations that we sampled during 3 months from three hospitals when the disease incidence was relatively high, the 14-day notification rate per 100,000 population varying between 57 and 160 during the study period (ECDC 2020). Unfortunately, we were not able to sample in April when disease incidence was very low (<10). In general, the monitoring of viral load has been assessed as a reliable tool to give an early warning about the spreading of the disease (Polo et al.

2020; Thompson et al. 2020). However, wastewater surveillance might not be sensitive enough to detect small changes in the epidemic at the high level of disease incidence, as was the case in our study.

One aspect to be taken into consideration in our surveillance results is the hot temperature that prevailed in Riyadh during the sampling period. Viral RNA is known to be destroyed in high temperatures. Average temperatures in Riyadh were 42 °C, 43 °C, and 43 °C in June, July, and August, respectively. The maximum temperature reached 45 °C during July and August. Despite the high temperatures, disease incidence was high in Riyadh. However, the viral loads seemed to be at a relatively low level in sewage compared to previously reported values. Our RNA loads of N1, N2, and E genes varied between 70 and 85 copies per mL. In previous reports from sewage, when the prevalence of COVID-19 disease increased, the RNA load increased from 2.6–30 gene copies to 790–2 200 gene copies per mL (N1–N3 genes) (Medema et al. 2020). Elsewhere, a maximum of 3000 copies per mL has been reported in untreated sewage (Wurtzer et al. 2020). In the feces of COVID-19-positive people, the viral RNA load has been 10^3 – 10^8 gene copies per mL (Foladori et al. 2020). It may be that the high temperature of sewage (35–40 °C) had destroyed viruses in Riyadh. A study from a neighboring country UAE in the Arabian Peninsula reported viral load from 0.75 to 340 gene copies per mL and 0.3 to 2.9 from untreated wastewaters (Hasan et al. 2021). These values are relatively low and support our suggestion that the hot climate might have destroyed viral RNA. However, comparing viral loads with previous studies is difficult because of the slightly different procedures used in viral RNA analyses. A notable observation was that viral RNA

Fig. 4 Viral load of SARS-CoV-2 RNA in untreated and treated sewage (mean, SD, $n = 9$) from three sewage treatment plants (SW1, SW2, SW3) sampled three times during 2020

seemed to be concentrated in activated sludge as compared to influent (Carrillo-Reyes et al. 2021). This indicates the need to standardize the procedures, which has been recognized in several studies already (Michael-Kordatou et al. 2020). The summary interpretation of our results in Riyadh is as follows. Disease incidence was high during the hot summer 2020 and the viral RNA was largely destroyed by the high temperature of sewage. However, due to relatively small number of samples in our study and small changes in disease incidence during the study period, the results must be interpreted with caution.

The risk of disease spreading from wastewater has been assessed to be low (Rimoldi et al. 2020; Dada and Gyawali 2021). In contrast, many authors suggest that wastewater may be a relevant transmission route for the disease (Amoah et al. 2020; Bogler et al. 2020; Gormley et al. 2020). Viral RNA has been found from fecal samples up to 5 weeks and viable up to 2 days in urine samples, as reviewed by Langone et al. (2020).

Several studies have shown that enteric viruses are destroyed in different wastewater treatment procedures (Kumar et al. 2021). However, SARS-CoV-2 RNA has been studied scarcely from this point of view. Moreover, viral loads and treatment procedures have often been reported insufficiently in published studies. An unidentified treatment removed SARS-CoV-2 RNA from sewage in Italy (Rimoldi et al. 2020). The RNA was removed from sewage by thermal hydrolysis and anaerobic digestion (Balboa et al. 2021) as well as by conventional wastewater treatments followed by a tertiary disinfection step with peracetic acid or high-intensity UV lamps in Spain (Randazzo et al. 2020a). Free chlorine is known to be an especially efficient disinfectant (Wang et al. 2005; Tran et al. 2020) (Tran et al. 2020). In India, up-flow anaerobic sludge blanket technology reduced SARS-CoV-2 RNA remarkably: inlet sewage had a Ct value of 30, whereas in the final effluent, the values were zero (Kumar et al. 2021). The published studies show that common sewage treatments were mostly able to remove viral RNA. However, it is possible that some viruses might be left after the treatment. Metagenomic data revealed high diversity of respiratory viruses, including coronaviruses, to be present in anaerobic digester effluent in the USA (Bibby and Peccia 2013). We found one article where SARS-CoV-2 RNA presence was confirmed with RT-qPCR method in treated sewage. After an unidentified wastewater process in Paris, viral RNA was found at a concentration up to 0.11 copies per mL (Wurtzer et al. 2020). The sewage treatment had reduced viral load to a hundredth part. In our study, while the primary sewage had the RNA load of 70–85 copies per mL, the treated sewage had 3–10 copies per mL. The removal of viral RNA was assessed as efficient but treated sewage still contained some SARS-CoV-2 RNA.

Coronaviruses are generally thought to survive viable for a few days depending on the environment (Kampf et al. 2020). However, some studies have found that coronaviruses might

survive in wastewater for a week (Casanova and Weaver 2015). When SARS-CoV was inoculated into sewage, it remained infectious for 2 days at 20 °C (Masaaki et al., 2020). A recent review concluded that coronaviruses would survive a maximum of 3 days at 20 °C in wastewater (Amoah et al. 2020). Wastewater composition (organic matter), temperature, and pH are among the most important factors controlling the survival of viruses in wastewater (Medema et al. 2020). Because it is known that higher temperatures destroy viruses, our observation is notable. Although we interpret that most viruses had been destroyed by the high temperature, a part of SARS-CoV-2 RNA survived in wastewater at 35–40 °C. However, the viability of the virus has not been studied previously or in our study, which should be the next step in our research.

Several potential treatment technologies to remove SARS-CoV-2 RNA were presented in two recent reviews (Bhatt et al. 2020; Lesimple et al. 2020). For instance, membrane bioreactors have been suggested to remove viral RNA efficiently (Naddeo and Liu 2020). We showed that the activated sludge process without any disinfection step removed SARS-CoV-2 RNA efficiently. The removal was also reported from Mexico recently (Carrillo-Reyes et al. 2021). However, in our Riyadh case, the high temperature possibly had a remarkable effect, and more studies about the efficiency in different environments are needed.

Conclusion

The persistence of SARS-CoV-2 viral RNA in untreated sewage was confirmed in a hot climate in Saudi Arabia. However, the high temperature prevailing in Riyadh seemed to destroy viruses relatively rapidly. The sewage treatment procedures used mostly destroyed the viral RNA, and the spread of the disease through wastewater and sewage treatment plant workers is assessed as minimal. The wastewater surveillance has now been suggested as a routine approach to help in controlling COVID-19, and it seems to work in low-prevalence areas (Black et al. 2021; Rooney et al. 2021; Tiwari et al. 2021). However, in a hot climate, the efficiency of wastewater surveillance is not proved, and we can only make tentative conclusions. The hot climate, such as in Saudi Arabia, may affect the epidemiological factors of SARS-CoV-2 virus, and the effect of climate should be studied further.

Acknowledgements The authors extend their appreciation to the researcher A. Almansob for his support during practical work.

Availability of data and material All data related to this manuscript is incorporated in the manuscript only.

Code availability Not applicable.

Author contribution HA: designed the experiments. FA: drafted the manuscript. SA: contributed to and finalized the draft. HS: analyzed the results. AK: providing the official data. YA: providing the official data. AA: providing the official data. All authors read and approved the final manuscript.

Funding This research project was funded by the Deanship of Scientific Research, Princess Nourah bint Abdulrahman University, through the Targeted Research Program, Grant No (PNU-DRI-Targeted-20-028).

Declarations

Ethics approval Ethical approval was obtained from the Institutional Review Boards Committee of Princess Nourah bint Abdulrahman University (20-0452).

Consent to participate All authors have participated and approved the final version of the manuscript.

Consent to publish All authors have approved the final version of the manuscript and have given their consent for publication.

Conflict of interest The authors declare no competing interests.

References

- Aoubakr HA, Sharafeldin TA, Goyal SM (2020) Stability of SARS-CoV-2 and other coronaviruses in the environment and on common touch surfaces and the influence of climatic conditions: a review. *Transbound Emerg Dis* 68(2):296–312
- Ahmed W, Angel N, Edson J, Bibby K, Bivins A, O'Brien JW, Choi PM, Kitajima M, Simpson SL, Li J, Tschärke B, Verhagen R, Smith WJM, Zaugg J, Dierens L, Hugenholtz P, Thomas KV, Mueller JF (2020) 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
- Amoah ID, Kumari S, Bux F (2020) Coronaviruses in wastewater processes: source, fate and potential risks. *Environ Int* 143:105962
- Balboa S, Mauricio-Iglesias M, Rodríguez S, et al. (2021) The fate of SARS-CoV-2 in WWTPS points out the sludge line as a suitable spot for detection of COVID-19. *Sci Total Environ* 145268
- Bar-Or IB, Yaniv K, Shagan M, et al. (2020) Regressing SARS-CoV-2 sewage measurements onto COVID-19 burden in the population: a proof-of-concept for quantitative environmental surveillance. *MedRxiv*
- Bhatt A, Arora P, Prajapati SK (2020) Occurrence, fates and potential treatment approaches for removal of viruses from wastewater: a review with emphasis on SARS-CoV-2. *J Environ Chem Eng* 104429
- Bibby K, Peccia J (2013) Identification of viral pathogen diversity in sewage sludge by metagenome analysis. *Environ Sci Technol* 47:1945–1951
- Black J, Aung P, Nolan M, et al. (2021) Epidemiological evaluation of sewage surveillance as a tool to detect the presence of COVID-19 cases in a low case load setting. *Sci Total Environ* 147469
- Bogler A, Packman A, Furman A, et al. (2020) Rethinking wastewater risks and monitoring in light of the COVID-19 pandemic. *Nat Sustain* 1–10
- Carrillo-Reyes J, Barragán-Trinidad M, Buitrón G (2021) Surveillance of SARS-CoV-2 in sewage and wastewater treatment plants in Mexico. *J Water Process Eng* 40:101815
- Casanova LM, Weaver SR (2015) Evaluation of eluents for the recovery of an enveloped virus from hands by whole-hand sampling. *J Appl Microbiol* 118:1210–1216
- Dada AC, Gyawali P (2021) Quantitative microbial risk assessment (QMRA) of occupational exposure to SARS-CoV-2 in wastewater treatment plants. *Sci Total Environ* 763:142989
- ECDC (2020) European Centre for Disease Prevention and Control. <https://www.ecdc.europa.eu/en/publications-data/data-national-14-day-notification-rate-covid-19>
- Elsamadony M, Fujii M, Miura T, Watanabe T (2021) Possible transmission of viruses from contaminated human feces and sewage: implications for SARS-CoV-2. *Sci Total Environ* 755:142575
- El-Sayed A, Kamel M (2020) Climatic changes and their role in emergence and re-emergence of diseases. *Environ Sci Pollut Res* 27:22336–22352
- 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. *Sci Total Environ* 743:140444
- Gormley M, Aspray TJ, Kelly DA (2020) COVID-19: mitigating transmission via wastewater plumbing systems. *Lancet Glob Health* 8:e643
- Guo C, Bo Y, Lin C, Li HB, Zeng Y, Zhang Y, Hossain MS, Chan JWM, Yeung DW, Kwok KO, Wong SYS, Lau AKH, Lao XQ (2021) Meteorological factors and COVID-19 incidence in 190 countries: an observational study. *Sci Total Environ* 757:143783
- Haramoto E, Malla B, Thakali O, Kitajima M (2020) First environmental surveillance for the presence of SARS-CoV-2 RNA in wastewater and river water in Japan. *Sci Total Environ* 737:140405
- Hasan SW, Ibrahim Y, Daou M, Kannout H, Jan N, Lopes A, Alsafar H, Yousef AF (2021) Detection and quantification of SARS-CoV-2 RNA in wastewater and treated effluents: Surveillance of COVID-19 epidemic in the United Arab Emirates. *Sci Total Environ* 764:142929
- Hata A, Hara-Yamamura H, Meuchi Y, Imai S, Honda R (2021) Detection of SARS-CoV-2 in wastewater in Japan during a COVID-19 outbreak. *Sci Total Environ* 758:143578
- Ihsanullah I, Bilal M, Naushad M (2021) Coronavirus 2 (SARS-CoV-2) in water environments: current status, challenges and research opportunities. *J Water Process Eng* 39:101735
- Kampf G, Todt D, Pfaender S, Steinmann E (2020) Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. *J Hosp Infect* 104:246–251
- Kocameci BA, Kurt H, Sait A, et al. (2020) SARS-CoV-2 detection in Istanbul wastewater treatment plant sludges. *medRxiv*
- Kumar M, Kuroda K, Patel AK, Patel N, Bhattacharya P, Joshi M, Joshi CG (2021) 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
- La Rosa G, Bonadonna L, Lucentini L, et al. (2020) Coronavirus in water environments: occurrence, persistence and concentration methods—a scoping review. *Water Res* 115899
- Langone M, Petta L, Cellamare CM, et al. (2020) SARS-CoV-2 in water services: presence and impacts. *Environ Pollut* 115806
- Lesimple A, Jasim SY, Johnson DJ, Hilal N (2020) The role of wastewater treatment plants as tools for SARS-CoV-2 early detection and removal. *J Water Process Eng* 101544
- Li Y, Wang X, Nair H (2020) Global seasonality of human seasonal coronaviruses: a clue for post-pandemic circulating season of SARS-CoV-2 virus? *J Infect Dis*
- Mandal P, Gupta AK, Dubey BK (2020) A review on presence, survival, disinfection/removal methods of coronavirus in wastewater and progress of wastewater-based epidemiology. *J Environ Chem Eng* 104317
- 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. *Environ Sci Technol Lett* 7:511–516
- Michael-Kordatou I, Karaolia P, Fatta-Kassinos D (2020) Sewage analysis as a tool for the COVID-19 pandemic response and management: the urgent need for optimised protocols for SARS-CoV-2 detection and quantification. *J Environ Chem Eng* 8:104306
- MOH (2020) Ministry of Health, Kingdom of Saudi Arabia. <https://www.moh.gov.sa/en/Pages/default.aspx>
- Mohapatra S, Menon NG, Mohapatra G, et al. (2020) The novel SARS-CoV-2 pandemic: possible environmental transmission, detection, persistence and fate during wastewater and water treatment. *Sci Total Environ* 142746
- Naddeo V, Liu H (2020) Editorial Perspectives: 2019 novel coronavirus (SARS-CoV-2): what is its fate in urban water cycle and how can the water research community respond? *Environ Sci Water Res Technol* 6:1213–1216
- Notari A (2021) Temperature dependence of COVID-19 transmission. *Sci Total Environ* 763:144390
- Peccia J, Zulli A, Brackney DE, et al. (2020) SARS-CoV-2 RNA concentrations in primary municipal sewage sludge as a leading indicator of COVID-19 outbreak dynamics. *MedRxiv*
- Polo D, Quintela-Baluja M, Corbishley A, Jones DL, Singer AC, Graham DW, Romalde JL (2020) Making waves: wastewater-based epidemiology for COVID-19—approaches and challenges for surveillance and prediction. *Water Res* 186:116404
- Randazzo W, Cuevas-Ferrando E, Sanjuán R, Domingo-Calap P, Sánchez G (2020a) Metropolitan wastewater analysis for COVID-19 epidemiological surveillance. *Int J Hyg Environ Health* 230:113621
- Randazzo W, Truchado P, Cuevas-Ferrando E, Simón P, Allende A, Sánchez G (2020b) SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area. *Water Res* 181:115942
- Rimoldi SG, Stefani F, Gigantiello A, et al. (2020) Presence and vitality of SARS-CoV-2 virus in wastewaters and rivers. *medRxiv* 2020.05.01.20086009
- Rooney CM, Moura IB, Wilcox MH (2021) Tracking COVID-19 via sewage. *Curr Opin Gastroenterol* 37:4–8
- Saawarn B, Hait S (2020) Occurrence, fate and removal of SARS-CoV-2 in wastewater: current knowledge and future perspectives. *J Environ Chem Eng* 104870
- Thompson JR, Nanchaiah YV, Gu X, Lee WL, Rajal VB, Haines MB, Girones R, Ng LC, Alm EJ, Wuertz S (2020) Making waves: wastewater surveillance of SARS-CoV-2 for population-based health management. *Water Res* 184:116181
- Tiwari SB, Gahlot P, Tyagi VK, Zhang L, Zhou Y, Kazmi AA, Kumar M (2021) Surveillance of Wastewater for Early Epidemic Prediction (SWEEP): environmental and health security perspectives in the post COVID-19 Anthropocene. *Environ Res* 195:110831
- Tran HN, Le GT, Nguyen DT, et al. (2020) SARS-CoV-2 coronavirus in water and wastewater: a critical review about presence and concern. *Environ Res* 110265
- Trottier J, Darques R, Mouheb NA et al (2020) Post-lockdown detection of SARS-CoV-2 RNA in the wastewater of Montpellier, France. *One Heal* 10:100157
- Vallejo JA, Rumbo-Feal S, Conde-Pérez K, et al. (2020) Highly predictive regression model of active cases of COVID-19 in a population by screening wastewater viral load. *MedRxiv*
- Wang X-W, Li J-S, Guo T-K, Zhen B, Kong QX, Yi B, Li Z, Song N, Jin M, Xiao WJ, Zhu XM, Gu CQ, Yin J, Wei W, Yao W, Liu C, Li JF, Ou GR, Wang MN, Fang TY, Wang GJ, Qiu YH, Wu HH, Chao FH, Li JW (2005) Concentration and detection of SARS coronavirus in sewage from Xiao Tang Shan Hospital and the 309th Hospital. *J Virol Methods* 128:156–161
- Wu F, Zhang J, Xiao A, et al. (2020) SARS-CoV-2 titers in wastewater are higher than expected from clinically confirmed cases. *Msystems* 5
- Wurtzer S, Marechal V, Mouchel J-M, et al. (2020) Evaluation of lockdown impact on SARS-CoV-2 dynamics through viral genome quantification in Paris wastewaters. *MedRxiv*
- Zhou NA, Tharpe C, Meschke JS, Ferguson C (2021) Survey of rapid development of environmental surveillance methods for SARS-CoV-2 detection in wastewater. *Sci Total Environ* 144852
- Zhu Y, Oishi W, Maruo C, et al. (2021) Early warning of COVID-19 via wastewater-based epidemiology: potential and bottlenecks. *Sci Total Environ* 145124

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.