“pySewage”: a hybrid approach to predict the number of SARS-CoV-2-infected people from wastewater in Brazil

Adriano Roberto Vieira de Sousa1 · Lívia do Carmo Silva1 · Juliana Santana de Curcio1 · Hugo Delleon da Silva1,2 · Carlos Eduardo Anunciação3 · Silvia Maria Salem Izacc1 · Flavio Olimpio Sanches Neto4 · Elisângela de Paula Silveira Lacerda1

Received: 27 December 2021 / Accepted: 30 April 2022 / Published online: 6 May 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

Abstract
It is well known that the new coronavirus pandemic has global environmental, public health, and economic implications. In this sense, this study aims to monitor SARS-CoV-2 in the largest wastewater treatment plant of Goiânia, which processes wastewater from more than 700,000 inhabitants, and to correlate the molecular and clinical data collected. Influent and effluent samples were collected at Dr. Helio de Seixo Britto’s wastewater treatment plant from January to August 2021. Viral concentration was performed with polyethylene glycol before viral RNA extraction. Real-time qPCR (N1 and N2 gene assays) was performed to detect and quantify the viral RNA present in the samples. The results showed that 43.63% of the samples were positive. There is no significant difference between the detection of primers N1 (mean 3.23 log10 genome copies/L, std 0.23) and N2 (mean 2.95 log10 genome copies/L, std 0.29); also, there is no significant difference between the detection of influent and effluent samples. Our molecular data revealed a positive correlation with clinical data, and infection prevalence was higher than clinical data. In addition, we developed a user-friendly web application to predict the number of infected people based on the detection of viral load present in wastewater samples and may be applied as a public policy strategy for monitoring ongoing outbreaks.

Keywords COVID-19 · Web application · Coronavirus · SARS-CoV-2 · Wastewater-based epidemiology · Surveillance

Introduction
In December 2019, a cluster of patients with pneumonia symptoms was observed in Wuhan, Hubei, China. After further investigation, the outbreak was identified as coronavirus disease (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Dong et al. 2020; Graham Carlos et al. 2020). The rapid global spread of SARS-CoV-2 led the World Health Organization to classify COVID-19 as a pandemic in March 2020 (World Health Organization 2020). The current COVID-19 outbreak has become a serious threat to human health around the world (Liu et al. 2020).

In the early days of the pandemic, nonpharmaceutical measures (NPIs) such as isolation through quarantine, social distancing, wearing face masks, and travel restrictions were implemented in many countries to contain the peak of COVID-19 infection (Coccia 2020a; Bo et al. 2021). These measures have had a positive impact on the natural environment, as many sources of pollution have been reduced or eliminated (Elsaid et al. 2021), and transmission of COVID-19 within the local population has also decreased (Flaxman et al. 2020; Huang et al. 2021). However, some NPIs, such as prolonged lockdown, could have negative impacts on many aspects of life, including psychological, social, and economic consequences (Brooks et al. 2020; Coccia 2021a, b).
On the other hand, the transmission dynamics and diffusion of SARS-CoV-2 appear to be a complex relationship between climate, environment, and social aspects (Coccia 2020a, b, 2021a). Cities with high levels of air pollution combined with low production of renewable energy may have accelerated the spread of COVID-19, especially in people with concomitant diseases such as heart disease, chronic obstructive pulmonary disease, and lung cancer (Amoatey et al. 2020; Coccia 2020a, b, 2021a, e). Gross domestic product (GDP) per capita, health expenditures, population mobility, and social interactions are also factors that can contribute to the spread and mortality of COVID-19 (Bontempi et al. 2020; Coccia 2021d).

SARS-CoV-2 is readily transmitted from human-to-human through contact with aerosols, droplets, and fomites from infected individuals (He et al. 2020; Meyerowitz et al. 2021). However, active viral replication in the gut and detection of SARS in intestinal samples have been reported, even when some patients did not have any gastrointestinal symptoms (Zuo et al. 2021). Released SARS-CoV-2 viral particles are rapidly inactivated in gastrointestinal fluid (Larsen and Wigginton 2020). There is evidence of cell tropism of SARS-CoV-2 in multiple organs, not only in the lung but also in the small intestine, pancreas, blood vessels, and other tissues (Liu et al. 2021). Moreover, SARS-CoV-2 was detected in stool samples over a longer time, namely after 5 weeks, whereas it was undetectable in respiratory swab samples (Wu et al. 2020b), indicating possible contamination via the fecal–oral route (Heller et al. 2020; Guo et al. 2021).

Hence, epidemiological monitoring should be prioritized by surveillance services in the context of the pandemic, as it allows a better knowledge of the current epidemiological scenario (Peccia et al. 2020). Concentrations of SARS-CoV-2 RNA in wastewater samples can be correlated with reported COVID-19 cases and predict the outcomes of community clinical trials, sometimes 6 to 14 days before the onset of symptoms (Peccia et al. 2020; Cervantes-Avilés et al. 2021; Kumar et al. 2021a; Barua et al. 2022). One strategy that has been used is mass testing, but this strategy is costly and not feasible for low-income countries, even with existing adaptations such as the use of sample pools (Brault et al. 2021). Thus, cost-effective alternatives that can help with epidemiological monitoring and community surveillance are needed.

Wastewater-based epidemiology (WEB) is a population-wide potential tool for monitoring the chemical or microbiological profile of a community. This methodology allows assembly inferences about infected asymptomatic and symptomatic individuals by assessing the viral load in wastewater in a given population (Bivins et al. 2020; Hewitt et al. 2022). Also, it allows the collection of data from people who lack access to health care and provides health authorities with real-time monitoring of outbreaks (Larsen and Wigginton 2020).

Furthermore, this approach has been supported by several studies on COVID-19 epidemiological control; Ahmed et al. (2021) estimated the average number of infected individuals in Australia to range from 171 to 1090, which was consistent with clinical observations. In Brazil, Claro et al. (2021) observed for 44 weeks at five different sites. The prevalence of infection ranged from 0.05 to 0.38%, showing a positive correlation with clinical observations. Other studies detected SARS-CoV-2 viral RNA in wastewater in India (Arora et al. 2020), Germany (Westhaus et al. 2021), the USA (Wu et al. 2021; Barua et al. 2022), Croatia (Brnić et al. 2022), and the United Arab Emirates (Hasan et al. 2021).

Since the beginning of the pandemic, an avalanche of molecular data has been produced worldwide, making the development of data science and bioinformatics protocols essential (Mercatelli et al. 2021b). Once new data is daily produced, computational tools may accelerate the monitoring of infected people around the world (Hufsky et al. 2021), although several web tools have been developed for analysis regarding SARS-CoV-2 (Mercatelli et al. 2021a).

Bioinformatics tools play an important role in monitoring SARS-CoV-2 and provide non-computational users with the possibility to analyze data and advanced knowledge related to COVID-19 (Hufsky et al. 2021). This is probably part of the reason that although several studies have been conducted to monitor SARS-CoV-2, and estimate the number of infected people from wastewater samples, they are mostly used by a small group of researchers (Sanches-Neto et al. 2021). Therefore, in addition to studies of SARS-CoV-2 monitoring by experimental techniques such as RT-qPCR and genomic sequencing, it is extremely important to develop web applications to automate the SARS-CoV-2 detection combined with COVID-19 monitoring (Pérez-Cataluña et al. 2022). Moreover, the use of our web application can provide early prediction in wastewater, potentially identifying new variants for recurrent outbreaks (Peterson et al. 2022; Wurtzer et al. 2022).

Nevertheless, to the best of our knowledge, there are no web applications regarding the monitoring of the number of people infected with SARS-CoV-2 from the viral load detected in wastewater. Thus, the focus of this work aimed to monitor the SARS-CoV-2 viral load in wastewater samples in a large community in Brazil-Midwest combined with the development of a user-friendly web application to predict the number of infected people.

Materials and methods

Sample and data

A total of 55 wastewater samples were collected from January to August 2021 (30 weeks) at Dr Hélio Seixo
de Britto’s wastewater treatment plant (WWTP) located in Goiânia, Goiás, Brazil (latitude $-16.6799$, longitude $-49.25516^\circ 40' 48''$ South, $49^\circ 15' 18''$ West). A total of 200 mL of influent and effluent samples was collected between 2 and 3 pm (BRT – Brazilian time zone, –03:00 UTC) in sterile bottles using the grab sample method, transported on wet ice to the testing laboratory within 15 min, and frozen at $-80^\circ$ C until the samples were processed for virus concentration. The WWTP serves about 57.51% of the population of Goiania (over 700,000 people) and its treatment consists of chemically enhanced primary treatment with a maximum capacity for sewage treatment of 2300L/s $^{-1}$ T (Fig. 1).

**Virus concentration**

Vir
al concentration was performed according to Fongaro et al. (2021), and 50 mL of influent and effluent were processed. The pH of the sample was adjusted to 3.5 using HCl 1 M (Sigma-Aldrich, MO, USA). Samples were then shaken at 4°C for 30 min and centrifuged at 2474 g at 4°C for 30 min. The supernatant was discarded and the pellet was resuspended with 25 mL of glycine–NaOH (0.25 M) buffer, pH 9.5, shaken for 30 min at 4°C, and centrifuged at 2474 g at 4°C for 60 min. The supernatant was homogenized with 4 mL polyethylene glycol 24% PEG 6000 (Sigma-Aldrich, MO, USA) by shaking for 120 min at 4°C and then centrifuged for 180 min at 2474 g at 4°C. The supernatant was discarded and the pellet formed was eluted with 1 mL of phosphate-buffered saline (PBS 1x—137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, and 1.8 mM KH2PO4, pH 7.0). Samples were stored at $-80^\circ$ C until RNA extraction. To evaluate the viral concentration protocol, samples were spiked with a double-stranded DNA control cloned into a plasmid containing the complete nucleocapsid gene sequence of the 2019-nCoV virus containing the two regions analyzed with the 2019-nCoV RUO N1 and N2 kit (IDT, Integrated and Technologies).

**Viral RNA extraction**

SARS-CoV-2 RNA was extracted from 100 μL of the samples using the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (Thermo Fisher Scientific, MA, USA) according to the manufacturer’s protocol. The extracted nucleic acid was stored at $-80^\circ$ C until additional downstream analysis.

**RT-qPCR analysis**

The different regions of the SARS-CoV-2 nucleocapsid gene were detected by RT-qPCR using two primers pairs—N1 and N2 (IDT, IA, USA) and according to the recommendations of the Centers for Disease Control and Prevention protocols (https://www.fda.gov/media/134922/download) and GoTaq® Probe 1-Step RT-qPCR System (Promega, WI, USA). The total reaction volume was 20 μL (10 μL GoTaq Probe qPCR Master Mix, 0.4 μL GoScript RT Mix for 1-Step RT-qPCR, 3.6 μL of phosphate-buffered saline (PBS 1x—137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, and 1.8 mM KH2PO4, pH 7.0). Samples were stored at $-80^\circ$ C until RNA extraction. To evaluate the viral concentration protocol, samples were spiked with a double-stranded DNA control cloned into a plasmid containing the complete nucleocapsid gene sequence of the 2019-nCoV virus containing the two regions analyzed with the 2019-nCoV RUO N1 and N2 kit (IDT, Integrated and Technologies).

**Viral RNA extraction**

SARS-CoV-2 RNA was extracted from 100 μL of the samples using the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (Thermo Fisher Scientific, MA, USA) according to the manufacturer’s protocol. The extracted nucleic acid was stored at $-80^\circ$ C until additional downstream analysis.

**RT-qPCR analysis**

The different regions of the SARS-CoV-2 nucleocapsid gene were detected by RT-qPCR using two primers pairs—N1 and N2 (IDT, IA, USA) and according to the recommendations of the Centers for Disease Control and Prevention protocols (https://www.fda.gov/media/134922/download) and GoTaq® Probe 1-Step RT-qPCR System (Promega, WI, USA). The total reaction volume was 20 μL (10 μL GoTaq Probe qPCR Master Mix, 0.4 μL Go Script RT Mix for 1-Step RT-qPCR, 3.6 μL
nuclease-free water, 1.0 primer/probe, and 5.0 μL of RNA). Nuclease-Free Water for Molecular Biology (Sigma-Aldrich) was used as a negative control. The RT-qPCR was performed using the AriaMX Real-Time PCR System (Agilent, CA, USA). The amplification program consisted of one cycle at 45 °C for 15 min for reverse transcription, one cycle at 95 °C for 2 min for denaturation of reverse transcriptase and activation of DNA polymerase, followed by 45 cycles at 95 °C for 15 s, and 60 °C for 1 min for denaturation and amplification. We compared the amplification curves in the RT-qPCR reactions with both the commercial 2019-nCoV_N_Positive Control and the biological positive control using AriaMx software. All samples that presented Cq values lower than 40 were considered positive for SARS-CoV-2. This value was determined by the construction of a standard curve. We used a \( \log_{10} \) dilution series ranging from \( 10^5 \) to 1 copy/reaction. The straight equation generated through the standard curve was used to determine the number of viral genetic material present in samples. All the reactions were performed in triplicate and nuclease-free water was used as a negative control. Viral load (viral gene copies/L of wastewater) was determined by Eq. (1):

\[
\text{Viral Load} = \frac{\text{viral genomic copies} \times \mu L \text{ eluted RNA} \times 40 \mu L \text{ (total volume of eluted RNA)}}{50 \text{ mL (initial volume of concentrated wastewater sample)} \times 20}
\]

### Data analysis and procedures

As reported by Ahmed et al. (2021), the number of infected people (NIP) was estimated as follows Eq. (2):

\[
NIP = \frac{VGC \times FR}{\alpha \times \beta \times \gamma}
\]

where VGC is viral RNA genomic copies (per liter) of N1 or N2 gene targets detected in wastewater samples; FR is the flow rate of the wastewater treatment plant; \( \alpha \) is the viral load shedding in the stool; \( \beta \) is the estimated daily production of stool per capita; and \( \gamma \) is the percentage of infected people shedding viral RNA in the stool. To calculate the flow rate (FR) of the wastewater treatment plant, data were collected and analyzed from health agencies, such as inflow rate, maximum daily inflow rate, and the mean inflow rate, to determine the average flow rate/day/L/s. Moreover, due to the wide variation in parameters such as viral load shedding rate (\( \alpha \)), daily stool mass production per capita (\( \beta \)), and percentage of infected individuals shedding SARS-CoV-2 viral RNA in the stool (\( \gamma \)), it is difficult to interpret the infection prediction results. Thus, a Monte Carlo simulation was performed in Eq. 2 using the Python programming language. The viral load shedding rate in the stool (\( \alpha \)) was modeled as a uniform distribution with a range of 0.8 to 7.5 \( \log_{10} \) gc∙g\(^{-1}\)∙feces\(^{-1}\) (Ahmed et al. 2021). Daily stool mass production per capita (\( \beta \)) was modeled as a normal distribution with a range of 75 to 520 g/capita/day in low-income countries, as reported by Rose et al. (2015). The percentage
of infected people shedding SARS-CoV-2 viral RNA in the stool ($\gamma$) was modeled with uniform distribution with a range of 30–90% (Ahmed et al. 2021).

COVID-19 surveillance data, such as the number of new confirmed cases, number of intensive care unit hospitalizations (ICU), and number of deaths, were obtained from the Goiânia Health Agencies database (available at https://saude.goiania.go.gov.br/goiania-contra-o-coronavirus/informe-epidemiologico-covid-19/) to compare clinical data and SARS-CoV-2 viral load detected in wastewater. According to the Goiânia Health Agencies database, the prevalence of confirmed cases is 289.69 cases per 100 thousand inhabitants.

### Results and discussion

#### SARS-CoV-2 RNA detection in wastewater samples

In this study, the presence of SARS-CoV-2 RNA in sewage was analyzed using probes directed to the regions of SARS-CoV-2 N (N1 and N2 gene assays), and the quantification

| EPI week | Year-month | Sample type | $\log_{10}$ RNA concentration GC/L | NIP | Prevalence (%) |
|---|---|---|---|---|---|
| 1 | 2021-Jan | Effluent | 2.81 | N.D | 2490 | 0.35 |
| 2 | 2021-Jan | Influent/effluent | 3.25 | 2.47 | 7995 | 1.14 |
| 3 | 2021-Jan | Influent/effluent | 3.21 | 3.19 | 12,256 | 1.75 |
| 4 | 2021-Jan | Influent/effluent | N.D | N.D | - | - |
| 5 | 2021-Jan | N.A | N.A | N.A | N.A | N.A |
| 6 | 2021-Feb | Effluent | 3.35 | N.D | 8746 | 1.25 |
| 7 | 2021-Feb | Influent/effluent | N.D | N.D | - | - |
| 8 | 2021-Feb | Influent/effluent | 3.30 | 2.86 | 10,509 | 1.50 |
| 9 | 2021-Feb | Influent/effluent | 3.46 | 2.69 | 13,145 | 1.87 |
| 10 | 2021-March | N.A | N.A | N.A | N.A | N.A |
| 11 | 2021-March | Influent/effluent | 3.11 | 2.69 | 6862 | 0.98 |
| 12 | 2021-March | Influent/effluent | N.D | N.D | - | - |
| 13 | 2021-March | Influent | N.D | 3.56 | 14,146 | 2.01 |
| 14 | 2021-April | Influent/effluent | N.D | N.D | - | - |
| 15 | 2021-April | Influent/effluent | N.D | N.D | - | - |
| 16 | 2021-April | Influent | N.D | 3.41 | 10,015 | 1.43 |
| 17 | 2021-April | Effluent | 3.14 | N.D | 5296 | 0.75 |
| 18 | 2021-May | Influent | 3.30 | N.D | 7675 | 1.09 |
| 19 | 2021-May | Influent/effluent | N.D | N.D | - | - |
| 20 | 2021-May | Influent/effluent | N.D | N.D | - | - |
| 21 | 2021-May | Effluent | N.D | 3.50 | 12,368 | 1.76 |
| 22 | 2021-May | N.A | N.A | N.A | N.A | N.A |
| 23 | 2021-June | Effluent | N.D | 2.92 | 3250 | 0.46 |
| 24 | 2021-June | Influent/effluent | 3.37 | 3.43 | 30,147 | 4.29 |
| 25 | 2021-June | Influent | 3.72 | N.D | 20,418 | 2.91 |
| 26 | 2021-June | Influent/effluent | N.D | N.D | - | - |
| 27 | 2021-July | Influent | 3.29 | 3.17 | 13,307 | 1.90 |
| 28 | 2021-July | Influent/effluent | N.D | N.D | - | - |
| 29 | 2021-July | Influent | N.D | 2.72 | 2051 | 0.29 |
| 30 | 2021-July | Influent/effluent | N.D | N.D | - | - |
| 31 | 2021-Aug | Influent/effluent | N.D | N.D | - | - |
| 32 | 2021-Aug | Effluent | 3.24 | N.D | 6753 | 0.96 |
| 33 | 2021-Aug | Influent/effluent | N.D | N.D | - | - |

*EPI*, epidemiological; *GC/L*, genomic copies per liters; *NIP*, number of infected people; *N.D*, not detected; *N.A*, data not available
cycle (Cq) values obtained in RT-qPCR were considered positive below 40. A total of 55 samples (30 weeks) were analyzed, among them, 24 (43.6%) showed positive for the occurrence of RNA of SARS-CoV-2. N1 gene assay was detected in 43.3% of the samples (13/30 weeks), while N2 gene assay was detected in 40% (12/30 weeks). The percentage of positive samples here is similar to other studies. Prado et al. (2020, 2021) and Ahmed et al. (2021) presented 41.6% and 48.4% of positivity in their samples, respectively. We found a viral load ranging from 2.73 to 3.73 log10 for N1 gene assay, and 2.69 to 5.47 log10 for N2 gene assay. Wastewater epidemiology is a useful public health tool to understand the dissemination of COVID-19 in a community from SARS-CoV-2 RNA quantification (Orive et al. 2020).

Table 1 shows some different results regarding the RNA concentration. This detection difference is consistent with what is reported in the literature (Guerrero-Latorre et al. 2020; Randazzo et al. 2020; Ahmed et al. 2021; Claro et al. 2021), and it is suggested to be related to the analytical sensitivity of gene assays (Randazzo et al. 2020). Additionally, PCR assays can be affected by potential inhibitors present in sewage (Kitajima et al. 2020), especially those WWTP that receive clandestine sewage. The wastewater-based epidemiology could vary depending on laboratory resources, as there is no gold standard methodology leading to differences in viral load in the sample’s positivity (Sherchan et al. 2021), suggesting that a widely standardized methodology would be required (Kaya et al. 2022).

Kumar et al. (2021a, b) evaluated the efficacy of three gene assays: N-genes (nucleocapsid), S-genes (spike glycoprotein), and ORF-1ab genes (polyprotein) before and after the treatment. N genes showed to be more stable after the treatment when compared to S genes and ORF 1ab genes. It is due to these genes being protected in SARS-CoV-2 structures, and they are common genes among the family Coronaviridae (Kumar et al. 2021b).

There is no significant difference between N1 and N2 gene assays in both influent (raw sewage) and final effluent (treated sewage) sites (considering p-value < 0.05). The occurrence of positive samples in treated wastewater suggests that the treatment employed by the WWTP is not able to completely remove the virus as reported by Randazzo et al. (2020). Furthermore, discriminating between infectious and non-infectious viral particles in treated wastewater using RT-qPCR technics is promising for further outbreaks (Monteiro et al. 2022).
Correlation between the prevalence of reported cases of COVID-19 and predicted numbers of infected

The number of infected people was estimated from wastewater data. Although there are limitations to the data, studies have suggested that wastewater parameters should be performed via Monte Carlo (Ahmed et al. 2021; Claro et al. 2021; Hasan et al. 2021). The estimation of the possible number of infected people was performed via Monte Carlos simulation with 50,000 random samples of each parameter of Eq. 1. According to Eq. 1, the number of infected people by SARS-CoV-2 in this study ranged from 2490 to 30,147 people, with a minimum and maximum prevalence of 0.35, and 4.29%, respectively (Table 2). Ahmed et al. (2021) found a range of 122–1090 infected people. Wu et al. (2020a, b) have found a prevalence ranging from 0.1 to 5%, higher than the clinical cases (0.026), in MA, USA. Claro et al. (2021) have found a prevalence in the ABC region (point 4—São Paulo state) ranging from 0.1 to 4.39. There are some factors to be considered in estimating the prevalence of infected people. First, there is no consensus in the scientific community about the timeframe of SARS-CoV-2 shedding in the stool; there are no standardized protocols for wastewater-based epidemiology, especially in viral concentration step; and little is known about the loss of viral particles in sewage and experimental procedures (Wu et al. 2020a). Although surveillance in wastewater is an important tool for monitoring pathogens that are circulating in the communities, further studies are needed to construct robust models of prevalence. Additionally, it is important to note that the accuracy and significance of the estimate performed depend on several parameters due to the large uncertainties and variability associated with the relevant SARS-CoV-2 data.
On the main page of the web application, the user can choose between two options: (i) Home and (ii) Simulation. If “Home” is chosen, a description of the pySewage application will be displayed. If the option “Simulation” is chosen, a page will be displayed for the user to estimate the prevalence of infection using Monte Carlo simulation due to the uncertainty of the variables (see Eq. 2). On the Simulation interface, the user can choose either “automatic or manual simulation.” For both cases, the user must add genomic copies per liter of sewage (excel format), and wastewater flow rate input (liters per second per day). However, the user can change some variables such as α, β, and γ (see Eq. 2) when manual mode is selected.

**Conclusion**

This study detected SARS-CoV-2 in the biggest wastewater treatment plant in a large city in the Midwest region of Brazil from January to August 2021. SARS-CoV-2 was detected in 43.63% of the samples. A correlation was observed between the clinical data and the viral load detected in this study. The main limitations of this work were the lack of viral concentration standardization in the scientific literature and the manifold factors associated with the uncertainty of the prevalence prediction variables. However, the viral concentration was performed with polyethylene glycol and extracted with commercial RNA kits. Moreover, to estimate the number of infected people, we performed a Monte Carlo simulation with 95% confidence intervals in the simulated variables to deal with uncertainty. To disseminate our results, we developed a user-friendly web application for the automatic prediction of the number of infected people. Therefore, the use of the web application combined with the WBE methodology for the detection of SARS-CoV-2 becomes an essential tool for epidemiological management and may be applied as a public policy strategy for monitoring ongoing outbreaks. Several factors regarding the viral pathogens outbreaks are related to complex interactions between humans and the environment. Additionally, the environmental climate changes affect not only human chronic diseases but also infectious diseases. Thus, to further disseminate the use of our results, the future perspectives of this investigation should include arboviruses monitoring, such as DENV, CHIKV, and ZIKV, once tropical countries are endemic to these viruses.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s11356-022-20609-z.

**Acknowledgements** We are grateful to Dr. Gislaine Fongaro for the experimental support of the viral concentration protocol and SANEAGO wastewater treatment plant for the collaboration.
Author contribution Adrian Roberto Vieira de Sousa contributed to conceptualization, methodology, validation, formal analysis, writing—original draft, review and editing, investigation, and data curation. Lívia do Carmo Silva contributed to methodology, investigation, data curation, and writing—review and editing. Juliana Santana de Curiico contributed to methodology, investigation, data curation, and writing—review and editing. Hugo Delleon contributed to conceptualization, methodology, investigation, writing—review and editing, and funding acquisition. Carlos Eduardo Anunciação contributed to conceptualization, methodology, and investigation. Sílvia Maria Salem Izacc contributed to methodology. Flavio Olimpio Sanches Neto contributed to formal analysis, data curation, and writing—review and editing. Elisângela de Paula Silveira Lacerda contributed to conceptualization, methodology, validation, formal analysis, writing—original draft, writing—review and editing, investigation, data curation, supervision, project administration, and funding acquisition.

Funding This work was performed at Universidade Federal de Goiás supported by CAPES, JBS-Swift Foods Company – PI04742.2020, and by Academic Cooperation Program in National Defense (PROCAD) – 88887.387750/2019–00.

Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

References

Ahmed W, Tscharke B, Bertsch PM et al (2021) SARS-CoV-2 RNA monitoring in wastewater as a potential early warning system for COVID-19 transmission in the community: a temporal case study. Sci Total Environ 761:144216

Amoatey P, Sicard P, De Marco A, Khaniabadi YO (2020) Long-term exposure to ambient PM2.5 and impacts on health in Rome. Italy Clin Epidemiol Glob Heal 8:531–535

Arora S, Nag A, Sethi J et al (2020) Sewage surveillance for the presence of SARS-CoV-2 genome as a useful wastewater based epidemiology (WBE) tracking tool in India. Water Sci Technol 82:2823–2836

Barua VB, Juel MAI, Blackwood AD et al (2022) Tracking the temporal variation of COVID-19 surges through wastewater-based epidemiology during the peak of the pandemic: a six-month long study in Charlotte. North Carolina Sci Total Environ 814:152503

Bivins A, North D, Ahmad A et al (2020) Wastewater-based epidemiology: global collaborative to maximize contributions in the fight against COVID-19. Environ Sci Technol 54:7754–7757

Bo Y, Guo C, Lin C et al (2021) Effectiveness of non-pharmaceutical interventions on COVID-19 transmission in 190 countries from 23 January to 13 April 2020. Int J Infect Dis 102:247–253

Bontempi E, Vergalli S, Squazzoni F (2020) Understanding COVID-19 diffusion requires an interdisciplinary, multi-dimensional approach. Environ Res 188:109814

Brault V, Mallein, Rupprechter JF (2021) Group testing as a strategy for COVID-19 epidemiological monitoring and community surveillance. PLoS Comput Biol 17:1–25

Briñi D, Lojkic I, Škoko I et al (2022) SARS-CoV-2 circulation in Croatian wastewaters and the absence of SARS-CoV-2 in bivalve molluscan shellfish. Environ Res 207:112638

Brooks SK, Webster RK, Smith LE et al (2020) The psychological impact of quarantine and how to reduce it: rapid review of the evidence. Lancet 395:912–920

Cervantes-Aviliés P, Moreno-Andrade I, Carrillo-Reyes J (2021) Approaches applied to detect SARS-CoV-2 in wastewater and perspectives post-COVID-19. J Water Process Eng 40:10

Cevik M, Tate M, Lloyd O et al (2021) SARS-CoV-2, SARS-CoV, and MERS-CoV viral load dynamics, duration of viral shedding, and infectiousness: a systematic review and meta-analysis. The Lancet Microbe 2:e13–e22

Claro ICM, Cabral AD, Augusto MR et al (2021) Long-term monitoring of SARS-COV-2 RNA in wastewater in Brazil: A more responsive and economical approach. Water Res 203:117534

Coccia M (2020) How (Un)sustainable environments are related to the diffusion of covid-19: the relation between coronavirus disease 2019, air pollution, wind resource and energy. Sustain 12:1–12

Coccia M (2020) Factors determining the diffusion of COVID-19 and suggested strategy to prevent future accelerated viral infectivity similar to COVID. Sci Total Environ 729:138474

Coccia M (2021) Pandemic prevention: lessons from COVID-19. Encyclopedia 1:433–444

Coccia M (2021) The relation between length of lockdown, numbers of infected people and deaths of Covid-19, and economic growth of countries: lessons learned to cope with future pandemics similar to Covid-19 and to constrain the deterioration of economic system. Sci Total Environ 775:145801

Coccia M (2021) Effects of the spread of COVID-19 on public health of polluted cities: results of the first wave for explaining the déjà vu in the second wave of COVID-19 pandemic and epidemics of future vital agents. Environ Sci Pollut Res 28:19147–19154

Coccia M (2021) High health expenditures and low exposure of population to air pollution as critical factors that can reduce fatality rate in COVID-19 pandemic crisis: a global analysis. Environ Res 199:111339

Coccia M (2022) Preparedness of countries to face COVID-19 pandemic crisis: strategic positioning and factors supporting effective strategies of prevention of pandemic threats. Environ Res 203:111678

Dong E, Du H, Gardner L (2020) An interactive web-based dashboard to track COVID-19 in real time. Lancet Infect Dis 20:533–534

Elsaid K, Olabi V, Sayed ET et al (2021) Effects of COVID-19 on the environment: an overview on air, water, wastewater, and solid waste. J Environ Manage 292:112694

Flaxman S, Mishra S, Gandy A et al (2020) Estimating the effects of non-pharmaceutical interventions on COVID-19 in Europe. Nature 584:257–261

Fongaro G, Hermes P, Sobral D et al (2021) The presence of SARS-CoV-2 RNA in human sewage in Santa Catarina, Brazil, November 2019. Sci Total Environ 778:146198

Graham Carlos W, Dela Cruz CS, Cao B et al (2020) Novel Wuhan coronavirus. Am J Respir Crit Care Med 201:P7–P8

Guerrero-Latorre L, Ballesteros I, Villacrés-Granda I et al (2020) An approach to detect SARS-CoV-2 in wastewater: implications in low sanitation countries. Sci Total Environ 775:145801

Hasan SW, Ibrahim Y, Daou M et al (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
He X, Lao EHY, Wu P et al (2020) Temporal dynamics in viral shedding and transmissibility of COVID-19. Nat Med 26:672–675
Heller L, Mota CR, Greco DB (2020) COVID-19 faecal-oral transmission: are we asking the right questions? Sci Total Environ 729:138919
Hewitt J, Trowsdale S, Armstrong BA et al (2022) Sensitivity of wastewater-based epidemiology for detection of SARS-CoV-2 RNA in a low prevalence setting. Water Res 211:118032
Huang X, Shao X, Xing L et al (2021) The impact of lockdown timing on COVID-19 transmission across US counties. E ClinicalMedicine 38:101035
Hufsky F, Lamkiewicz K, Almeida A et al (2021) Computational strategies to combat COVID-19: useful tools to accelerate SARS-CoV-2 and coronavirus research. Brief Bioinform 22:642–663
Kaya D, Niemeier D, Ahmed W, Kjellerup BV (2022) Evaluation of multiple analytical methods for SARS-CoV-2 surveillance in wastewater samples. Sci Total Environ 808:152033
Kitajima M, Ahmed W, Bibby K et al (2020) SARS-CoV-2 in wastewater State of the knowledge and research needs. Sci Total Environ 739:139076
Kumar M, Joshi M, Patel AK, Joshi CG (2021) 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
Kumar M, Kuroda K, Joshi M et al (2021) First comparison of conventional activated sludge versus root-zone treatment for SARS-CoV-2 RNA removal from wastewaters: statistical and temporal significance. Chem Eng J 425:130635
Larsen DA, Wigginton KR (2020) Tracking COVID-19 with wastewater. Nat Biotechnol 38:1151–1153
Levine-tiefenbrun M, Yelin I, Katz R et al (2021) Initial report of decreased SARS-CoV-2 viral load after inoculation with the BNT162b2 vaccine. Nat Med 27:790–792
Li L, Mazurowski L, Dewan A et al (2022) Longitudinal monitoring of SARS-CoV-2 in wastewater using viral genetic markers and the estimation of unconﬁrmed COVID-19 cases. Sci Total Environ 817:152958
Liu J, Zheng X, Tong Q et al (2020) Overlapping and discrete aspects of the pathology and pathogenesis of the emerging human pathogenic coronaviruses SARS-CoV, MERS-CoV, and 2019-nCoV. J Virol 94:371–404
Liu J, Li Y, Liu Q et al (2021) SARS-CoV-2 cell tropism and multicellular infection. Cell Discov 7:2–5
Mercatelli D, Holding AN, Giorgi FM (2021) Web tools to ﬁght pandemics: the COVID-19 experience. Brief Bioinform 22:690–700
Mercatelli D, Triboli L, Fornasari E et al (2021) Coronapp: a web application to annotate and monitor SARS-CoV-2 mutations. J Med Virol 93:3238–3245
Meyerowitz EA, Richterman A, Gandhi RT, Sax PE (2021) Transmission of SARS-CoV-2: a review of viral, host, and environmental factors. Ann Intern Med 174:69–79
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
Monteiro S, Rente D, Cunha MV et al (2022) Discrimination and surveillance of infectious severe acute respiratory syndrome coronavirus 2 in wastewater using cell culture and RT-qPCR. Sci Total Environ 815:152914
Mota CR, Bressani-Ribeiro T, Araújo JC et al (2021) Assessing spatial distribution of COVID-19 prevalence in Brazil using decentralised sewage monitoring. Water Res 202:117388
Orive G, Lertxundi U, Barcelo D (2020) Early SARS-CoV-2 outbreak detection by sewage-based epidemiology. Sci Total Environ 732:139298
Peccia J, Zulli A, Brackney DE et al (2020) Measurement of SARS-CoV-2 RNA in wastewater tracks community infection dynamics. Nat Biotechnol 38:1164–1167
Pérez-Cataluña A, Chiner-Oms A, Cuevas-Ferrando E et al (2022) Spatial and temporal distribution of SARS-CoV-2 diversity circulating in wastewater. Water Res 211:118007
Peterson SW, Lidder R, Daigle J et al (2022) RT-qPCR detection of SARS-CoV-2 mutations S 69–70 del, S N501Y and N D3L associated with variants of concern in Canadian wastewater samples. Sci Total Environ 810:151283
Prado T, Fumian TM, Mannarino CF et al (2020) Preliminary results of SARS-CoV-2 detection in sewerage system in niterói municipality, Rio de Janeiro, Brazil. Mem Inst Oswaldo Cruz 115:1–3
Prado T, Fumian TM, Mannarino CF et al (2021) Wastewater-based epidemiology as a useful tool to track SARS-CoV-2 and support public health policies at municipal level in Brazil. Water Res 191:116810
Randazzo W, Truchado P, Cuevas-Ferrando E et al (2020) SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area. Water Res 181:115942
Rose C, Parker A, Jefferson B, Cartmell E (2015) The characterization of feaces and urine: a review of the literature to inform advanced treatment technology. Crit Rev Environ Sci Technol 45:1827–1879
Sanchez-Neto FO, Dias-Silva JR, KengQueiroz Junior LH, Carvalho-Silva VH (2021) “pySIRC”: machine learning combined with molecular fingerprints to predict the reaction rate constant of the radical-based oxidation processes of aqueous organic contaminants. Environ Sci Technol 55:12437–12448
Sherchan SP, Shahin S, Ward LM et al (2020) First detection of SARS-CoV-2 RNA in wastewater in North America: a study in Louisiana, USA. Sci Total Environ 743:140621
Sherchan SP, Shahin S, Patel J et al (2021) Occurrence of SARS-CoV-2 RNA in six municipal wastewater treatment plants at the early stage of COVID-19 pandemic in the United States. Pathogens 10:798
Westhaus S, Weber F-A, Schiwy S et al (2021) Detection of SARS-CoV-2 in raw and treated wastewater in Germany – suitability for COVID-19 surveillance and potential transmission risks. Sci Total Environ 751:1–12
World Health Organization (2020) WHO Director-General’s opening remarks at the media briefing on COVID-19 - 11 March 2020. WHO Dir. Gen. speeches 4.
Wu F, Zhang J, Xiao A et al (2020) SARS-CoV-2 titers in wastewater are higher than expected from clinically conﬁrmed cases. mSystems 5:1–9
Wu Y, Guo C, Tang L et al (2020) Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. Lancet Gastroenterol Hepatol 5:1–9
Wu F, Xiao A, Zhang J et al (2021) Wastewater surveillance of SARS-CoV-2 across 40 US states from February to June 2020. Water Res 211:117400
Wurtzer S, Waldman P, Levert M et al (2022) SARS-CoV-2 genome quantification in wastewaters at regional and city scale allows precise monitoring of the whole outbreaks dynamics and variants spreading in the population. Sci Total Environ 810:152213
Zuo T, Liu Q, Zhang F et al (2021) Depicting SARS-CoV-2 faecal viral activity in association with gut microbiota composition in patients with COVID-19. Gut 70:276–284

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