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Long-term monitoring of SARS-CoV-2 RNA in sewage samples from specific public places and STPs to track COVID-19 spread and identify potential hotspots

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ABSTRACT

Coronavirus pandemic started in March 2020 and since then has caused millions of deaths worldwide. Water-based epidemiology (WBE) can be used as an epidemiological surveillance tool to track SARS-CoV-2 dissemination and provide warning of COVID-19 outbreaks. Considering that there are public places that could be potential hotspots of infected people that may reflect the local epidemiological situation, the presence of SARS-CoV-2 RNA was analyzed by RT-qPCR for approximately 16 months in sewage samples from 5 public places located in the metropolitan area of Belo Horizonte, MG, Brazil: the sewage treatment plant of Confins International Airport (AIR), the main interstate bus terminal (BUS), an upscale shopping centre (SHC1), a popular shopping centre (SHC2) and a university institute (UNI). The results were compared to those of the influent sewage of the two main sewage treatment plants of Belo Horizonte, MG, Brazil: the sewage treatment plant of Confins International Airport (AIR), the main interstate bus terminal (BUS), an upscale shopping centre (SHC1), a popular shopping centre (SHC2) and a university institute (UNI). The results were compared to those of the influent sewage of the two main sewage treatment plants of Belo Horizonte (STP1 and STP2). Viral monitoring in the STPs proved to be an useful regional surveillance tool, reflecting the trends of COVID-19 cases. However, the viral concentrations in the samples from the selected public places were generally much lower than those of the municipal STPs, which may be due to the behaviour of the non-infected or asymptomatic people, who are likely to visit these places relatively more than the symptomatic infected ones.

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1. Introduction

The SARS-CoV-2 virus, also known as the new coronavirus, was first detected in the city of Wuhan, China, through laboratory analysis of patients with symptoms of severe respiratory disease (Huang et al., 2020). A member of the Coronaviridae family, it is an enveloped virus composed of a RNA strand. Being highly transmissible, the associated disease (COVID-19) quickly spread to several countries. On 11 March 2020, the World Health Organization (WHO) declared the COVID-19 pandemic. Several countries were severely affected by the new coronavirus, regardless of their wealth status.

Initially SARS-CoV-2 was found to be transmitted by air and droplets (Yao et al., 2020). Then, other transmission routes were considered, such as contact with surfaces, food, insects and sewage (Esilami and Jalili, 2020; Xiao et al., 2020). Based on evidence of the presence of other coronaviruses in stool, such as SARS-CoV (causative of acute and severe respiratory syndrome) and MERS-CoV (causative of Middle Eastern respiratory syndrome), it was found that SARS-CoV-2 infects the intestine, and its RNA was found in the feces of infected people (Wu et al., 2020). Urine as well as respiratory fluids and saliva have also been found to contribute to the virus load in sewage, but in smaller amounts than feces (Crank et al., 2021). However, despite the detection of relatively high concentrations of viral genetic material, the replication and infectivity of the SARS-CoV-2 virus from feces and sewage is limited (Wölfel et al., 2020; Elsamadony et al., 2021; Rimoldi et al., 2020; Westhaus et al., 2021), and the risks of waterborne infection appear to be low (Rimoldi et al., 2020).

The analysis of the genetic material of the SARS-CoV-2 virus in domestic sewage makes it possible to infer on the dissemination of COVID-19. Considering the high costs and lack of resources, among other challenging factors for mass testing of the population, wastewater-based epidemiology (WBE) appears as a complementary surveillance tool to track viral dissemination. Nationwide WBE studies for SARS-CoV-2 monitoring have already been in place. In the US, the Centers for Disease Control and Prevention (CDC) launched the National Wastewater Surveillance System (NWSS) to coordinate the tracking of the presence of SARS-CoV-2 in wastewater samples collected across the country. It works in close partnership with health departments responsible for decision-making to combat the COVID-19 pandemic (CDC, 2022). In Europe, sewage-based SARS-CoV-2 surveillance programs have been created in several countries. The European Commission (EC) provided a guide for the elaboration of SARS-CoV-2 sewage monitoring plans seeking a unified approach (EC, 2021). Among the European initiatives are: the Dutch Sewage Surveillance, which started on a small scale when the SARS-CoV-2 was first detected in sewage from the Netherlands (Medema et al., 2020), and currently monitors more than 300 sewage treatment plants in its national territory (RIVM, 2022); and the nationwide surveillance in Turkey, which started as a study monitoring WWTWs located in 81 cities from May to June 2020, and since September 2020 has been routinely monitoring the sewage from 22 cities and regularly indicating COVID-19 spread on a map (Kocamemi et al., 2020). WBE studies have proven to be a useful tool to provide early warnings of emerging COVID-19 cases and to monitor cases that had not been tested, including the asymptomatic ones (Bonanno Ferrari et al., 2020) and might estimate the number of infections in the community as demonstrated in Australia by Ahmed et al. (2020a). For instance, Medema et al. (2020) detected SARS-CoV-2 RNA in sewage 1 week before the first confirmed case reported in the Netherlands. Similarly, Randazzo et al. (2020) detected the virus in sewage before the first COVID-19 cases were declared by local authorities in the Region of Murcia (Spain). In Southeastern Virginia, USA, Gonzalez et al. (2020), detected SARS-CoV-2 RNA in sewage from at least one WWTP on all samples dates (during the 21-week study), even during the first week of sampling when there were only two clinical detections in the region. In Massachusetts, USA, Wu et al. (2022) also detected the virus in sewage when there were only two confirmed cases, and they observed that viral titer trends in wastewater preceded 4–10 days earlier than in clinical data. In Louisiana, USA, Sherchan et al. (2020) detected the virus RNA in influent samples when the total confirmed cases were 6173 and 308 in parishes served by WWTPs A and B, respectively. In India, Kumar et al. (2021) reported that the percentage change in virus genome concentration level on a particular date was found in the lead of 1–2 weeks of time with respect to the confirmed cases registered based on clinical tests on a temporal scale. In addition, it is a very cost-effective tool when sampling sewage from the community of a city detecting both symptomatic and asymptomatic cases for a low-cost value (Manuel et al., 2022).

WBE can monitor even people who do not have access to health care (CDC, 2022), thus giving a better picture of the epidemic situation in a region. In places where clinical testing is limited and/or directed for symptomatic people, such as Brazil, India, Canada, and others, this is a great tool to help health authorities to adopt and intensify measures to mitigate COVID-19 spread.

In Brazil, the rate of COVID-19 testing has been relatively low (<300,000 tests/ 1 M pop) (Worldometers, 2022a). Thus, WBE applying decentralized sewage monitoring could play an important role in Brazil as demonstrated by Prado et al. (2021), Chernicharo et al. (2020) and Mota et al. (2021). Nevertheless, the monitoring of several sampling points is time and money consuming, and in a scarce resources scenario this could pose a challenge in the long term. Therefore, it is crucial to identify strategic places that can serve as hotspots to be monitored continually even after the ongoing pandemic.

In this context, this work presents the results of long-term monitoring of SARS-CoV-2 RNA concentrations in sewage samples from strategic public places in the city of Belo Horizonte and its metropolitan area, in the state of Minas Gerais, Brazil. These places comprise two shopping centres, a university institute, the main bus station of Belo Horizonte and the International Airport of Confins. The data obtained from these locations were compared to those of the influent samples from the two main sewage treatment plants of Belo Horizonte city (STP Arrudas and STP Onça). In addition, we sought to understand whether monitoring the presence of the SARS-CoV-2 RNA in sewage from the public places can be used as an epidemiological surveillance tool to detect infected people who visit or work in these locations, and possibly report local outbreaks.

2. Material and methods

2.1. Description of the places

Belo Horizonte is a large city located in the state of Minas Gerais, Brazil, with a population around 2.7 million people and with a metropolitan area of 6 million people (IBGE, 2020). To monitor SARS-CoV-2 RNA concentration in sewage, five public places in the city and metropolitan area of Belo Horizonte considered strategic from the epidemiological point of view of the dissemination of COVID-19 were selected. SARS-CoV-2 RNA concentrations in the sewage samples from these places were compared to those of the influent sewage of the two main sewage treatment plants (STPs) of Belo Horizonte. Both STPs serve mainly the population of Belo Horizonte, but also part of the city of Contagem. Table 1 shows the approximate...
daily number of passersby and the monitoring period for each place investigated. Fig. 1 shows the geographic location of the monitored locations.

Although these places used to be crowded, the number of visitors or workers sharply reduced, due to the pandemic restrictions, during most of the monitoring period. Almost all non-essential activities were suspended in Belo Horizonte from 11 to 31 January 2021 and from 06 March to 22 April 2021. In the other periods the restrictions were variable (PBH, 2022a).

### Table 1

| Name         | Location Description                                                                 | Average daily number of passersby | Monitoring period       |
|--------------|---------------------------------------------------------------------------------------|-----------------------------------|-------------------------|
| AIR          | Sewage Treatment Plant of Confins International Airport                               | 22,513\(^a\)                      | 09/30/2020-01/25/2022   |
| BUS          | Interstate and intermunicipal bus station Governor Israel Pinheiro                    | 11,651\(^b\)                      | 09/30/2020-01/25/2022   |
| SHC1         | Shopping centre Diamond Mall                                                         | 12,000\(^c\)                      | 02/24/2021-01/25/2022   |
| SHC2         | Shopping centre Oiapoque                                                              | 23,000\(^d\)                      | 12/09/2020-01/25/2022   |
| UNI          | The Institute of Biological Science of the Federal University of Minas Gerais (UFMG)  | 500\(^e\)                         | 12/02/2020-01/25/2022   |
| STP1         | STP Arrudas                                                                           | 1.160,488\(^d\)                   | 09/30/2020-01/25/2022   |
| STP2         | STP Onça                                                                              | 1.084,779\(^d\)                   | 09/20/2020-01/25/2022   |

\(a\) Number of employees (equivalent to 5124 people) and passengers boarding, landing and in connections, as informed by the Confins International Airport (CNF) administrative sector.

\(b\) Number of embarking and disembarking passengers and employees of the bus station, the latter being equivalent to approximately 220 people, as informed by the administrative sector of the bus station.

\(c\) Approximate number of people who attended the malls daily during the monitoring period as reported by the respective administrative sectors.

\(d\) Population served by the municipal STPs.

Fig. 1. Geographic locations of the monitored places. In the left, Belo Horizonte city in Brazil, in the right, the monitored places in the metropolitan area of Belo Horizonte.
2.2. Composite samples were collected once a week, every Tuesday. For all monitoring points except the STPs 1 and 2, sampling was performed from 8:30 am to 12:30 pm using refrigerated autosamplers (keeping the samples at 4 °C). The automatic sampling device was set as described by Mota et al. (2021), i.e., one portion (approximate 400 mL) was taken every 10 min, resulting in 10 L after 4 h sampling. As for the monitoring points at the entrance of STPs, 24 h-composite samples were collected. Following that, the samples were perfectly homogenized and then transferred to two 1 L bottles. One bottle was taken to the microbiology laboratory in the Department of Sanitary and Environmental Engineering (DESA) of the Federal University of Minas Gerais to quantify the SARS-CoV-2 RNA (Section 2.3). The second bottle was taken to the laboratory of the state sanitation company (COPASA) for the determination of chemical oxygen demand (COD) and E. coli, according to APHA (Baird and Bridgewater, 2017). Both bottles were kept on ice during transport.

2.2. Sample collection

2.3. Molecular analyses

Viruses concentration was determined by the adsorption-extraction method using electrogenetic membranes (0.45 μm-HAWP04700-Millipore) as previously described (Symonds et al., 2014; Ahmed et al., 2015). MgCl₂ (2.5 M) was added to the sewage samples (1 mL MgCl₂ for 100 mL sample) and the pH was adjusted to 3.0 to 3.5 using acetic acid (1 M) (Symonds et al., 2014). Then, the samples were filtered using the electrogenetic membrane (30 mL to 100 mL). After the filtration, the membranes were folded and kept in 2 mL tubes containing 600 μL of PM1 solution (provided in the DNA/RNA extraction kit), glass beads and 6 μL of beta-mercaptoethanol, and stored at −80 °C until RNA extraction. RNA was extracted using a commercial kit (AllPrep PowerViral DNA/RNA, Qiagen®, Hildem, Germany), according to instructions provided by the manufacturer. A bead beater was used in the first step of RNA extraction to remove viral particles from the membrane. Following the extraction, RNA was resuspended in 100 μL of ultrapure water (free of RNases) and stored at −80 °C.

The detection of SARS-CoV-2 was performed using available RT-qPCR assays using different primer-probe sets recommended by the CDC (2019), targeting the nucleocapsid protein (N) gene-region N1 (Lu et al., 2019). All RT-qPCR amplifications were performed in 20 μL reaction mixtures using iTaq™ Universal probes One Step reaction mix and reverse transcriptase. The RT-qPCR assays were performed in triplicate using an Applied Biosystems 7500 Real Time PCR System. Plasmid containing the full sequence of the SARS-CoV-2 nucleocapsid (N) gene (Integrated DNA Technologies (Coralville, IA, USA) was used to generate the standard curves and as positive control. Standard dilutions ranged from 1 × 10⁵ to 1 GC μL⁻¹, 5 × 10⁴ to 5 GC μL⁻¹ and 2.5 × 10³ to 2.5 GC μL⁻¹ (see Table S1 for RT-qPCR standard curves summary data). For each RT-qPCR run, a series of three positive (2019-nCoV Positive control) and negative controls (No Template Control- NTC with sterile, nuclease-free water used to check for contamination during specimen extraction and/or plate set-up) were included. Optional controls (SARS-CoV and MERS-CoV) were also used to prove specificity and sensitivity of the reactions for the chosen primers/probes. Both optional controls must be negative for the N1 region. In order to check PCR inhibition, internal control HsRPP 30 was used, as recommended by the CDC 2020 protocol (CDC, 2019). Whenever the sample did not amplify with the internal control (negative for RNase P), the sample was diluted 10 × and 100 × to check for inhibition and confirm that the result was negative. Data analysis was performed following the recommendations of Bustin et al. (2009), such as standard deviation (SD <0.5) in replicate analysis and slope values \( \mu = -3.3 \pm 10\% \) (100%), Y-intercept between 33 and 37 and R² ≥ 0.99. The same threshold was used in the data analysis of all runs.

The limit of detection (LOD) was defined as the number of copies corresponding with 95% probability of amplification (Bustin et al., 2009). The calculation for the LOD was carried out following the exponential model established by Verbyla et al. (2016), considering the qPCR standard curves generated (Table S1). In the present study the calculated LOD was 3.6 copies.mL⁻¹. In-sewer decay of the RT-qPCR signal for SARS-CoV-2 RNA was assumed negligible based on Mota et al. (2021).

2.4. COVID-19 data

The number of suspected cases (including cases of flu and severe acute respiratory syndrome), confirmed cases of COVID-19 and vaccination rate were obtained from the epidemiological and assistance bulletins of Belo Horizonte health secretariat available at the municipal administration website (PBH, 2022b).

3. Results and discussion

3.1. Detection and quantification of SARS-CoV-2 virus in sewage samples

Table 2 shows the summary of the viral concentrations detected in the sewage samples. The overall statistics are divided in two monitoring periods: (i) Period I: from September 30, 2020, up to July 13th, 2021, and (ii) Period II: when the proportion of vaccinated people increased considerably (>60% of people >12 years old with at least 1 dose of vaccine), from July 20th, 2021, up to January 25, 2022 (Fig. 2). The increase in the vaccination rate was accompanied by the reduction of COVID-19 cases and reduced viral concentrations in sewage samples up to the end of 2021. With the emergence of the Omicron (B.1.1.529) variant, with the first infections in Belo Horizonte being detected in December 2021 (PBH, 2021), the number of COVID-19 cases raised abruptly in January 2022, despite the relatively high vaccination rate (above 90% of the target population). In fact, the effectiveness of most of the vaccines approved so far, including those that have been being applied in Brazil (CoronaVac, Oxford-AstraZeneca, Pfizer-BioNTech and Janssen), is reduced against the Omicron variant compared to the ancestral lineage and other widely disseminated variants (Liu et al., 2021a; Lu et al., 2021; Minka and Minka, 2022; Xie et al., 2022).

The samples from the municipal STPs (STP Arrudas and STP Onça) presented the highest viral concentrations, having 100% positivity rate (Table 2). On the other hand, the viral concentrations in the samples from the other selected locations were usually low, many of them below the LOD, which was 3.6 copies.mL⁻¹. The positivity rate up to July 13th, 2021 was relatively high, ranging from 53% in the BUS to 91% in the AIR. Regardless the concentration, the detection of the virus in the sewers indicates the circulation of infected people (symptomatic and asymptomatic) in the monitored places. In the study of Medema et al. (2020), signs of viral RNA amplification in sewage were observed when the prevalence of COVID-19 was ≤1.0 case in 100,000 people. Hong et al. (2021), in their study in hospitals, observed that a minimum number of infected people were required for the detection of the virus in the sewage, which ranged between 253 and 409 positive cases per 10,000 people. Therefore, the rate of infected people that is enough to the viral detection varies to a great extent. Using a mathematical model, Hart and Halden (2020) concluded that it is theoretically possible to detect the genetic material of SARS-CoV-2 in sewage if there is 1.0 infected per 100 to 2,000,000 people, depending on local conditions, such as temperature, in-sewer travel time, and water consumption per inhabitant.

Except the STPs, AIR samples presented the highest frequency of positivity and the highest SARS-CoV-2 median concentrations. Nevertheless, the fact that a considerable percentage of samples tested positive from the bus terminal, shopping malls and university campus, indicates that the monitoring of the sewage from these places can reveal the presence of infected people who have visited or worked in these places. Consequently, providing this information to the administrative agents would be useful to promote clinical testing and contact tracing. Ahmed et al. (2020b) tested sewage samples from three commercial aircraft and from a cruise ship. Although the concentrations were close to the LOD (which in this case was 1 copy.mL⁻¹), the positive samples indicated the presence of infected passengers, prioritizing
testing and contact tracing. Similarly, Albastaki et al. (2021) investigated the wastewater samples from 198 commercial aircrafts arriving at Dubai Airport, giving a positive result percentage of 13.6%. The authors proposed to use this data to eventually ban the flights coming from specific locations when the respective flight samples were positive.

In the second period of monitoring, the positivity rate reduced for the selected public places frequency (Table 2). The viral concentrations in sewage lowered when COVID-19 new cases decreased from mid-July up to end of December 2021. Besides, such concentrations raised amid the new wave of COVID-19 due to the emergence of the Omicron variant, as shown in the next section. These results demonstrate how the WBE can be a reliable and useful tool to reflect the epidemiological situation.

3.2. Temporal analysis of SARS-CoV-2 in the public places and STPs

In the temporal analysis, AIR samples showed the greatest correspondence with the municipal STPs’ samples, generally following the same trends of increasing and decreasing concentrations (Fig. 3), even though the AIR samples come from the airport and aircrafts, thus not being exclusive of the Belo Horizonte metropolitan area. Also considering the positivity rate and viral concentrations results, it is inferred that, among the selected places, the Confi nis International Airport (AIR) has the greatest potential to be used as a permanent monitoring point (functioning as a sentinel surveillance point for infectious disease, particularly COVID-19). Although less representative than the municipal STPs, AIR monitoring can provide an indication of the regional epidemiological situation and eventually of the national one, since this airport is an important hub for flights connecting the different regions of the country and its STP also treats sewage from the airplanes. However, when the Omicron variant became predominant in the beginning of 2022, the viral concentration in the AIR samples did not increase as in the STPs samples. Possibly the increased viral load of this variant leads to a shorter time of incubation and early symptom onset (Jansen et al., 2021), which likely contribute to prevent in some extent the infected people to travel.

![Fig. 2. Follow-up COVID-19 cases and vaccination rate (at least 1 dose) in the population > 12 years old in the city of Belo Horizonte.](image-url)
Although the daily number of passersby in the bus station was relatively high in the monitoring period (Table 1), the frequency of positivity and SARS-CoV-2 concentrations were quite low, excepting by the pick in the early January 2022 (Fig. 3). It is important to note that sampling was carried out in the morning period, between 8:30 am and 12:30 pm. Then, the samples might not be representative of the overall population using the bus station premises. Sampling during the busiest periods (around 8 am and 7 pm) could be more suitable for on-site monitoring. As in BUS, it is noteworthy that viral concentrations in SHC1, SHC2 and UNI were usually very low. The number of passersby was reduced due to the restrictions imposed by the pandemic, then the sewage contribution from these places could have been too low to be representative of the municipal epidemiological situation. This may explain the heterogeneous viral concentrations over the time, especially in the UNI, where the number of passersby was the lowest (Table 1). In this location the real number of people using the toilets might have been even lower which might explain the variability of viral concentration in the sewage samples from this place and other non-residential locations.

Chemical oxygen demand (COD) and E. coli analyses indicated the presence of organic matter and fecal material, respectively, in the samples from BUS, SHC1 and SHC2 in similar or even higher values than those of influent sewage of the municipal STPs (Fig. S1). Since E. coli usually correlates well with the presence of feces (Tallon et al., 2005), these analyses indicate that the proportion of fecal contribution in the sewage from these locations was comparable to the municipal domestic sewage. Therefore, the lower viral concentrations in the sewage of the selected locations in relation to the influent sewage of the STPs are likely to be associated with the health profile of the people who visit these places rather than with the occasional non-domestic contributions to the sewage. One hypothesis is that symptomatic infected people visit these locations less frequently than uninfected and asymptomatic people. This is less evident in the airport likely due to the fact that air travels are usually planned in advance, hence, it is expected that the infected people, including the symptomatic, are more willing to keep their plans regarding air travels than regarding bus travels and going shopping and working at university.

In the studies by Prado et al. (2021) and Mota et al. (2021) the concentrations of the genetic material of SARS-CoV-2 in sewage were usually similar between samples from sewer networks and from the STPs serving those areas. Thus, decentralized monitoring of sewage from different neighborhoods and areas of the city seems to be a more appropriate surveillance tool at a municipal scale. In the study of Tandukar et al. (2022), concentrations of SARS-CoV-2 in sewage were similar between samples from WWTP, sewer lines, hospitals and urban river water. Their results indicate that sampling of river water can be carried out in polluted urban rivers to implement WBE in some developing countries where insufficient sewage treatment is carried out before discharge into rivers. As also observed by Guerrero-Latorre et al. (2020), in river water samples in Quito (Ecuador). On the other hand, the viral monitoring of sewage from specific public places can reveal possible infections among people who visit or work in these locations, allowing public authorities to target groups of people and increase the testing capacity, possibly anticipating local outbreaks.

### 3.3. Relationship between SARS-CoV-2 in sewage and COVID-19 cases

Fig. 4 shows the SARS-CoV-2 genetic material concentrations in the sewage samples and the number of suspected and confirmed cases of COVID-19 in Belo Horizonte city. During the monitoring period, the evolution of cases followed the national trend, as seen at Worldometer website (Worldometers, 2022b) and at the Brazilian Coronavirus Panel website (MS, 2022).

Since the beginning of monitoring up to December 2021, before the emergence of Omicron variant, peaks of viral concentrations in AIR and especially in STP1 and STP2 were observed in certain periods, such as December 2020 and March 2021, which preceded by a couple of weeks the peaks of suspected and confirmed COVID-19 cases in the city (as shown by the dashed circles and arrows indicating the waves in viral concentrations in the sewage (blue or purple circles) and COVID-19 cases (red circles) in Fig. 4a and b). Many other studies found that STP’s influent monitoring can provide early warning for SARS-CoV-2 infections (Peccia et al., 2020; Nemudry et al., 2020; Agrawal et al., 2021; Claro et al., 2021; Kumar et al., 2021; Galani et al., 2022; Tandukar et al., 2022). However, Tandukar et al. (2022) observed moderate correlation between the number of weekly COVID-19 cases and SARS-CoV-2 RNA concentration in WWTPs. In the case of their study, this fact is related to the sampling strategy adopt. They performed grab sampling and did not collect weekly sampling since lockdowns were imposed in the Kathmandu Valley (Nepal). In the present study, in which weekly sewage samples were collected, the anticipation of the increase of viral concentrations in sewage in relation to the new confirmed COVID-19 cases was not observed in the end of monitoring, in January 2022, when the number of cases raised concurrently with the increase
Fig. 4. SARS-CoV-2 in sewage and number of suspected and confirmed cases of COVID-19 in Belo Horizonte over the monitoring period. STP1: influent of Arrudas STP. STP2: influent of Onça STP. AIR: influent of BH-Confiins International Airport STP. BUS: sewage from Interstate and intermunicipal bus station Governador Israel Pinheiro. SHC1: sewage from the shopping centre Diamond Mall. SHC2: sewage from the shopping centre Oiapoque. UNI: sewage from the Institute of Biological Sciences of the Federal University of Minas Gerais (UFMG). *At UNI, the University’s superintendence reported 2 cases on 12/04/20, a local outbreak of about 6 cases between 05/15/21 and 05/27/21, 1 case on 07/12/21, 1 case on 07/22/21 and 6 cases between 01/04/22 and 01/12/22. In Figures a and b, the dashed circles in blue or purple indicate the viral concentration waves and the arrows point to the following waves of COVID-19 cases (in red dashed circles).
in sewage viral concentration (as can be seen in Fig. 4a, b, c and f). This may be linked to the shorter incubation time before symptom onset of Omicron variant compared to the other variants (Jansen et al., 2021). Nevertheless the peak in sewage viral concentration observed for the STP1 and STP2 (Fig. 4a) was reached one week before (01/18/2022) the end of January in which the number of cases were still very high.

Viral concentrations in samples from the other locations, however, were very variable and had no relation with the number of COVID-19 cases reported in the city. Similarly, Zdenkova et al. (2021) found a weak correlation between SARS-CoV-2 RNA concentration in samples from locations dominated by activities other than regular living and COVID-19 cases, as opposed to what they observed for samples from some residential areas. However, for all monitoring points in the present study, except the UNI, it is notable that viral concentrations were generally very low or null between August and December 2021, in accordance with the reduction trend of COVID-19 cases in the city (Fig. 2).

The peaks in viral concentrations in the shopping centres samples on 03/23/21 and 04/20/21 (SHC1) and on 01/12/21 and 05/11/21 (SHC2) occurred in periods when all non-essential activities were suspended (except on 05/11/21). Therefore, the increase in concentrations probably occurred due to the presence of infected people among the employees (working on internal and delivery services). In the case of the University institute, its superintendence reported the notifications of local COVID-19 cases. The reported local outbreaks of approximately 6 cases between
05/15/21 and 05/27/21 and 6 cases between 01/04/22 and 01/12/22 coincided with the detection of the SARS-CoV-2 in the sewage on 05/18/21 and its increased concentration on 05/25/21 and on 01/11/22 (Fig. 4f).

Scott et al. (2021) found some correspondence between SARS-CoV-2 concentrations in the sewage of university dormitories and the number of COVID-19 cases in the university campus. However, there was no official report of COVID-19 cases in the UNI in early January, mid September and early November 2021, when sewage viral concentrations increased. Sewage viral monitoring especially in places frequented by the same groups of people (e.g. university institutes, gyms, clubs) can aid decision-making in disease prevention and control. Such strategy was also adopted by Betancourt et al. (2021), that monitored the SARS-CoV-2 in the sewage of a university dormitory. When SARS-CoV-2 concentrations were above the LOD, clinical testing of residents was performed, ultimately resulting in the identification and isolation of the infected students. Similarly, Liu et al. (2021b) observed that the positivity rate for SARS-CoV-2 in sewage was proportional to the number of cases in student residence halls and positive samples led to subsequent diagnostic testing of building residents. Thus, the monitoring of specific places can be used as a surveillance tool to detect infected people, helping in the local epidemiological control, directing testing and contact tracing among employees and regular visitors. In the case of cities with low sewage treatment coverage, sampling urban river waters impacted by untreated sewage discharges might be considered as an optional strategy (as demonstrated by Guerrero-Latorre et al., 2020 in Ecuador and Tandukar et al., 2022 in Nepal).

4. Conclusions

Sewage monitoring of the municipal STPs showed to be the most appropriated WBE surveillance to track COVID-19 dynamics in the city of Belo Horizonte. On the other hand, monitoring the sewage from specific places can be used as a surveillance tool to detect infected people locally, directing testing and contact tracing among employees and regular visitors. Among the public places monitored, the sewage samples from the Airport showed a greater potential to be selected as a hot spot for SARS-CoV-2 dissemination. As the COVID-19 symptoms become milder and circulation restrictions diminish, it is likely that the passersby would be more representative of the general population, as observed in the last month of monitoring with the emergence of Omicron variant cases, enhancing the possibilities of WBE surveillance in public spots. Sewage sampling duration could also be increased from 4 to 6 or 8 h in public places whenever possible and feasible. Finally, it is important to add that hot spots could be an alternative for cities without extensive sewage treatment coverage.

Data curation, Writing original draft. AmandA TeodorO: Molecular analyses. Cíntia Leal: Molecular analyses. Deborah Leroy: Molecular analyses. Camila Madeira: Writing – review. Elayne C. Machado: Molecular analyses. Marcela F. Dias: Molecular analyses, Writing – review. Cassandra C. Souza: Molecular analyses. Gabriela Coelho: Molecular analyses. Thiago Bressani: Sample acquisition, Physical-chemical analyses, Writing – review. Gabriel T. Freitas: Sample acquisition. Physical-chemical analyses. Thiago Morandi: Data curation. Alyne Duarte: Data curation, Writing – review. Carlos Perdigão: Conceptualization, Writing – review. Flávio Tröger: Conceptualization, Writing – review. Sérgio Ayirmonaes: Conceptualization, Writing – review. Marília Carvalho de Melo: Conceptualization, Funding acquisition. Filipe Laguardia: Sample acquisition, Writing – review. Marcus Tulius Reis: Conceptualization, Sample acquisition. César Mota: Conceptualization, Funding acquisition. Carlos A. L. Chernicharo: Conceptualization, Writing – review, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.155959.

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