Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
First case of SARS-CoV-2 RNA detection in municipal solid waste leachate from Brazil

Giulliana Mondelli,⁎, Ednei Rodrigues Silva, Ieda Carolina Mantovani Claro, Matheus Ribeiro Augusto, Adriana Feliciano Alves Duran, Aline Diniz Cabral, Lívia de Moraes Bomédiano Camillo, Luísa Helena dos Santos Oliveira, Rodrigo de Freitas Bueno.

HIGHLIGHTS
- This is the first report of SARS-CoV-2 RNA presence in municipal solid waste leachate.
- SARS-CoV-2 RNA concentration was similar to what is already found in wastewater.
- Additional SARS-CoV-2 recovery methods should be evaluated for this different matrix.

GRAPHICAL ABSTRACT

ABSTRACT

This work presents the first case of SARS-CoV-2 RNA detection in leachate collected from a transfer station in the city of São Paulo, Brazil. After calibration of the viral detection method already used for wastewater samples with a pilot leachate sample and virus fragments in laboratory, twelve polyethylene glycol concentrated leachates samples were tested by RT-qPCR. The results confirmed the presence of N1 gene in 9 of the 12 analyzed samples between epidemiological weeks 33 and 38 of the year 2021 (08/15/2021 to 09/19/2021). The occurrence of the N2 gene was only observed in 5 of the 12 samples. The concentration values for N1 and N2 genes varied between 3.1 and 4.6 log_{10}GC·L^{-1}, which are values close to those measured in sanitary wastewater. This method showed to be a promising procedure to verify the presence of viral RNA in municipal solid waste leachate, being especially useful where there is no treatment system and sanitation infrastructure, which makes the conventional wastewater surveillance unfeasible.

Keywords: COVID-19 pandemic, Municipal solid waste management, Leachates, Public health

1. Introduction

The Coronavirus Disease (COVID-19) outbreak in the beginning of 2020 was followed by scientific discoveries involving the virus that causes it, SARS-CoV-2. Several papers and reporters were published with many hypotheses about how the virus is transmitted, transported and how it acts...
in the human body (Bi et al., 2020; Chen et al., 2020; Loder and Husman, 2020; Young et al., 2020). Much of the previous information is now being consolidated in the year 2021, after ongoing vaccination programs and monitoring of transmission processes, new variants, vaccination frequency, number of cases, hospitalizations and deaths (WHO, 2021a, 2021b). The adoption of security measures such as masks and vestments, the overcrowding of hospitals and the numerous lockdowns decreed by different countries and municipalities brought not only a change in the population consumption pattern, but also in the entire system that involves human actions on Earth, generating gases, waste and solid waste.

Worried about the possibility of surface transmission through municipal solid waste (MSW) (Ng et al., 2020; Ren et al., 2020; Gwennzi, 2021), municipalities, agencies, and scientists proposed educational programs about the proper separation of Personal Protection Equipment (PPE) used by common citizens, sorted by what can be recycled or not (Vaverková et al., 2021). Along with the massive participation of households in this process, a 72-h quarantine was also proposed in time to time to decrease the waste amount generated to allow it to be sorted and finally sent to the recycling industries (LIU, 2020; ACRPlus, 2020). Many municipalities had suspended the selective collection or closed the volunteer points to deliver recyclables and, therefore, many sorting plants stopped their operation, decreasing the useful life of sanitary landfills and carrying social, environmental and economical unbalance to the recycling industries and pickers employment (Gwennzi, 2021; Vaverková et al., 2021).

In the beginning of 2021, Lewis (2021) reported that the main SARS-CoV-2 transmission route is respiratory, through individual-to-individual droplets, coughing and speaking, since the aerosols remain in the air for some hours. Although it was already proved that the risk of infection from touching a contaminated surface is low and fomites play a minimal role in SARS-CoV-2 community infection (Harvey et al., 2021), there are other important factors that highlight a demand to investigate SARS-CoV-2 RNA in different environmental matrices to contribute to the monitoring and tracking of the viral presence.

Studies involving the SARS-CoV-2 RNA presence in wastewater systems in Brazil and in other countries (Ahmed et al., 2021; Claro et al., 2021; La Rosa et al., 2020; Medema et al., 2020; Nemudryi et al., 2020; Peccia et al., 2020; Saguti et al., 2021) revealed that the wastewater viral load quantification predicted the clinical peaks 14 weeks early. These studies also demonstrated that later, the results followed the decreased tendency of cases in the same cities and regions. This kind of information was crucial to help the preparation of hospitals and other health services to improve administration and monitoring of the pandemic. Federal University of ABC (UFABC) was a pioneer in this type of detection in Brazil and has been proportionating professional formation and capacitacion in molecular biology technologies to be used in these studies.

UFABC researchers involved in SARS-CoV-2 RNA detection in sanitation systems were encouraged to investigate the RNA presence in the MSW leachate and this present work proposes a sampling methodology used at a transfer station located in São Paulo city (the greater city of the Latin America), considering it an alternative monitoring tool. SARS-CoV-2 RNA presence in the MSW leachate indicates the possibility of virus fragments persistence in different surfaces. No literature references were found until this moment about the use of this environmental matrix as a possible tracking tool for SARS-CoV-2 RNA presence, which makes it a pioneer study.

Although several works around the world have shown the potential of wastewater surveillance, there are major challenges for implementing this methodology, especially in emerging countries with limited financial resources (Daughton, 2020; Foladori et al., 2020; Hamouda et al., 2021; Kitajima et al., 2020; Kumar et al., 2021; Zhu et al., 2021). In Brazil, as in other Latin American countries, several regions do not have wastewater infrastructure (sewage coverage of about 54.1%), which makes the conventional wastewater surveillance unfeasible (Brasil, 2020; Street et al., 2020). Therefore, MSW leachate monitoring emerges as a promising alternative for tracking and monitoring SARS-CoV-2 RNA presence.

2. Material and methods

2.1. Transfer station

The studied waste transfer unit operates with the objective of concentrating loads, reducing vehicle traffic and carbon emissions. In this way, smaller capacity compactor vehicles unload the residues at this unit, which are continuously transferred to larger capacity trucks and, subsequently, disposed in the municipal landfill. The transfer unit, where the leachate samples of the present work were collected, serves the districts of Vila Mariana, Jabaquara, Vila Prudente and Ipiranga in the City of São Paulo (Fig. 1).

2.2. Sampling process and study design

Waste collected in households is packed in plastic bags or, in the case of communities, placed in 1.6 m³ containers. During the trajectory, the residues in the collection trucks can reach a compaction index of 3:1, i.e., the equipment reduces the volume of collected residues up to three times. In this compaction process, some bags are torn and the moisture present in the waste, mainly in the organic fraction representing about 45% of the mass, is somehow drained and it percolates through the mass of enclosed waste in the compaction box.

All MSW interact with humans before they are discarded, but there is also the presence of materials that contain secretions and waste after use, such as tissues, diapers and toilet papers, which may present a greater probability of viral presence. At the transfer station, the compaction box of the collection truck is hydraulically opened, and the waste is pushed into a storage pit. Then, overhead cranes transfer the discharged waste to vehicles with greater capacity, a process that also promotes the wreckage of plastic bags. During the waste collection and transfer processes, the bags are ruined, and the matter is compressed, generating a liquid fraction called leachate, which percolates through the waste deposited in the contained area, going to a drainage channel in the bottom of the pit before proceeding to the authorized effluent treatment process. This channel at the bottom of the pit was the chosen point for the removal of the twelve leachate samples evaluated in this work (Fig. S1). The samples were analyzed for the presence of SARS-CoV-2 RNA titers between epidemiological weeks 33 and 38 of the year 2021 (08/15/2021–09/19/2021).

2.3. Leachate concentration and RNA extraction

The leachate samples were kept refrigerated at 4 °C until the analysis process, for a maximum of 36 h. The samples were processed at the UFABC Environmental Analysis Laboratory, in the same way as the sanitary wastewater samples were processed by Claro et al. (2021). Samples were concentrated by chemical precipitation with Polyethylene Glycol 8000 (PEG 8000), according to Wu et al. (2020). 40 mL of sample homogenized with 4 g of PEG 8000 and 0.9 g of sodium chloride (NaCl) were centrifuged at 8000 × g for 120 min at 4 °C. The pellet generated in this process was resuspended in 0.4 mL of 1 × PBS (pH 7.2), 1 mL of acidic phenol was added to clean the sample and the solution was shaken vigorously. Afterwards, the samples were subjected to centrifugation 12,000 × g for 10 min at 4 °C and after this processing, the aqueous phase was transferred to a microtube with 0.3 mL of lysis buffer. RNA extraction was performed using 80 mL in the input, after eluted into 80 µL of H2O RNAse free, according to the manufacturer guidelines using the PureLink™ Viral RNA/DNA Mini Kit (Thermo Fisher Scientific). To determine the recovery rate of the concentration method, the enveloped bovine respiratory syncytial virus (BRSV – Inforce™ 3, Zoetis, US) was used.

2.4. Viral detection and quantification

The detection and quantification of SARS-CoV-2 RNA were performed according to the Center for Disease Control and Prevention (CDC) protocols, using the 2019-nCoV TaqMan RT-PCR Kit (Norgen, Cat. TM67120).
The RT-qPCR was performed on Bio-Rad CFX96, according to the manufacturer instructions and the RNA samples were analyzed in duplicate with a final volume of 20 μL each, being 10 μL of 2 × One-Step RT-PCR Master Mix, 1.5 μL of Primer&Probe Mix (FAM fluorescence), 3.5 μL of RNase free water and 5 μL of the extracted RNA. The equipment programming consisted of 3 cycles, as shown in Table S1.

N1 and N2 genes were used as genomic targets and for each of them a calibration curve was constructed using the 2019-nCoV_N_Positive Control (Norgen, Cat. PC67102) in 10-fold series dilutions, as shown in Fig. 2.

Both curves presented a linear behavior (N1: \( y = -3.502x + 44,550 \); N2: \( y = -3745x + 46,328 \)). Table 1 presents the calibration curves parameters, considering N1 and N2, as well as the optimal ranges proposed by Bustin (2004), Bustin et al. (2009), and Bivins et al. (2021).

As proposed by Medema et al. (2020) and Wu et al. (2020), all samples with Cycle threshold (Ct) below 40 are considered positive for SARS-CoV-2 RNA, so the limit of detection (LOD) of this method was 3 genomic copies for a Ct value of 39.99 ± 0.05 for N1 gene and 4 genomic copies for a Ct value of 39.99 ± 0.05 for N2 gene. To assess inhibitor influence on the performance of RT-qPCR, a test was performed, where the samples RNA was processed, concentrated and diluted 10 ×. As no significant differences were detected, concentrated samples were used in all analyses. Boxus et al. (2005) and Rajal et al. (2007) established a protocol to assess the
recoverability of the concentration method through RT-qPCR reactions with BRV. This protocol was performed and recovery rates between 0.25 and 5.22% were obtained, presenting a mean of 2.02 ± 1.64.

2.5. Physicochemical analysis

Chemical Oxygen Demand (COD), Ammonium (NH₄⁺-N), pH, Total Phosphorus (TP), Total Suspended Solids (TSS), Fixed Suspended Solids (FSS) and Volatile Suspended Solids (VSS) in the twelve MSW leachate samples were analyzed using the procedures described in the Standard Methods for the Examination of Water and Wastewater (APHA et al., 2017).

3. Results and discussion

3.1. Physicochemical analysis of leachate

Table 2 shows the results of the twelve MSW leachate samples physicochemical analyzes. In this initial characterization, the leachate presented a high organic material concentration, with an average value of 6285 mgO₂L⁻¹ for COD and 1199 mgL⁻¹ for VSS. Furthermore, the resulting pH ranged from 5.2 to 6.0. Despite this complex leachate matrix, the compounds presented in the samples did not negatively interfere in viral RNA concentration and detection methodologies used in this study. This result indicates that diverse sampling concentration and detection methodologies present in the literature, notably for sanitary wastewater, can be adapted to this new matrix.

3.2. SARS-CoV-2 RNA occurrence in MSW leachate

Twelve leachate samples were analyzed for the presence of SARS-CoV-2 RNA titers between epidemiological weeks 33 and 38 of the year 2021 (08/15/2021–09/19/2021). The detected concentrations and the descriptive statistics of the results are presented in Fig. 3. The target regions proposed by the CDC (2020), N1 and N2, were analyzed.

Although a limited number of samples were evaluated, the higher percentage of detection in N1 (75%) indicates the greater sensitivity of this gene region in the analyzed matrix. The coefficient of variation (CV) values also shows that there was less variability and, therefore, greater stability in N1 gene results.

Recent works on detection and quantification of SARS-CoV-2 RNA in sanitary wastewater samples also showed different responses for N1 and N2 target regions (Medema et al., 2020; Randazzo et al., 2020; Wu et al., 2020). Claro et al. (2021), analyzing sanitary wastewater samples from the ABC Region (São Paulo, Brazil), also observed greater sensitivity for N1 gene. The main hypothesis that explains the differences in sensitivity observed between target regions N1 and N2 is that, generally, these regions can respond to inhibitory effects of the analyzed matrix in dissimilar ways and, consequently, generate different results in RT-qPCR (Bustin et al., 2009). Nevertheless, a recent study (Storey et al., 2021) revealed that mutations in the nucleocapsid gene (N) of SARS-CoV-2 can play a role in sensitivity differences observed in RT-qPCR assays. Storey and colleagues compared published primer and probe sequences, including the CDC N1 and N2 target regions and demonstrated that specific variations in the N gene can affect the assay efficiency. These results highlight the necessity of developing more standardized and up to date approaches to monitor SARS-CoV-2 RNA presence.

The mean concentrations of genetic material observed in this work, 4.2 and 4.0 log₁₀GCL⁻¹, were close to the measured concentrations in sanitary wastewater in different studies (Claro et al., 2021; Randazzo et al., 2020; Wu et al., 2020; Wurtzer et al., 2020). As shown in Fig. 4, the temporal variation of SARS-CoV-2 RNA fragments concentration was also evaluated and compared with the clinical data variation (new cases and admissions by COVID-19). Epidemiological data on COVID-19 was obtained from the publicly available repository of the São Paulo State Government (available at https://github.com/seade-R/dados-covid-sp). In Fig. 4, the measured concentrations of N1 and N2 target regions were plotted.

According to previous studies (Claro et al., 2021), the amount of RNA titers present in wastewater can be related to the number of clinical cases observed. As the present work showed that the amount of viral RNA titers present in the leachate is equivalent to what is found in wastewater, the data for the leachate may also suggest a reduction in clinical cases.

However, the sludge monitoring results presented in this study are preliminary and therefore do not allow us to fully assess the applicability of this alternative method for tracking COVID-19 in a community. In addition to a larger dataset, a longer monitoring period will be required so that trends in increasing and decreasing concentrations of viral RNA fragments in sludge can be correlated with clinical data such as moving average of new cases and number of hospitalizations due to Covid-19. A larger dataset will also allow us to perform a more robust statistical analysis of our results.

Although preliminary, the results showed that fragments of the genetic material of SARS-CoV-2 can be detected in the sludge generated from municipal solid waste. Hence, it is possible to affirm that fragments of the virus persist on objects surface and are invariably part of MSW leachate composition. Although we are not evaluating the viability of this virus (and infection potential), the results of this research show the possibility of monitoring and tracking the spread of the disease in a community by the municipal solid waste collection service. Evidently, further studies on the subject should be carried out to prove the potential of monitoring this matrix as an alternative and/or complementary way to SARS-CoV-2 RNA monitoring in sanitary wastewater samples, which is already been carried out in different regions. This alternative detection method becomes useful in regions where there is no wastewater infrastructure, a reality in at least 41% of Central and South America cities (PAHO, 2001) and where there is household waste collection.

Gwenzi (2021) also proposed an alternative methodology for tracking SARS-CoV-2, which involves the detection of viral RNA titers not only in wastewater samples but also in solid waste (papers, metals, fabric, plastics), and raw/untreated and drinking water. The authors draw the attention of the research community, governments, local and international health authorities, and professionals in wastewater-based epidemiology to the development of new low-cost tools for COVID-19 surveillance, especially for

Table 1

| Parameter | N1 | N2 | Optimum range | Reference |
|-----------|----|----|---------------|-----------|
| Slope | −3.309 | −3.118 | −3.10 to −3.58 | Bustin (2004) |
| Y-int. | 41.670 | 41.919 | N1: 36.1 to 42.5 | Bivins et al. (2021) |
| | | | N2: 37.8 to 53.5 | |
| Efficiency (%) | 100.6 | 109.3 | 90 to 110 | Bustin (2004) |
| R² | 0.999 | 0.998 | 0.980 to 1.000 | Bustin (2004) |
| LOD (GC) | 3 | 4 | 3 | Bustin et al. (2009) |

Table 2

| Parameter | COD (mgO₂L⁻¹) | NH₄⁺-N (mgL⁻¹) | pH | TP (mgP·L⁻¹) | TSS (mgL⁻¹) | FSS (mgL⁻¹) | VSS (mgL⁻¹) |
|-----------|---------------|----------------|----|-------------|-------------|-------------|-------------|
| Mean | 6285.0 | 43.0 | 5.5 | 5.1 | 1324.0 | 125.0 | 1199.0 |
| Maximum | 10,274.0 | 56.0 | 6.0 | 9.9 | 2650.0 | 730.0 | 2380.0 |
| Minimum | 1188.0 | 31.0 | 5.2 | 2.2 | 380.0 | 0 | 280.0 |
| SD | 2482.0 | 9.0 | 0.3 | 2.3 | 672.0 | 230.0 | 598.0 |
| CV | 39.5 | 21.5 | 5.4 | 45.1 | 50.7 | 183.9 | 49.9 |
| n= | 12 | | | | | | |

G. Mondelli et al. Science of the Total Environment 824 (2022) 153927

PAHO (2001)
low-income regions. However, data from these new approaches should allow tracking of “hot spots” and temporal “hot moments” of COVID-19, as well as transmission patterns and prevalence in monitored areas.

This is the first report of SARS-CoV-2 RNA fragments in leachate and since there is no reference to compare the results, the methodology presented still needs to be improved to allow a better recovery of the viral genetic material. The leachate matrix is very complex and needs to be well characterized. Hence, other concentration methods should be tested, such as negative membrane and ultracentrifugation. Corpuz et al. (2020) suggest that a leachate appropriate pre-treatment should be conducted to minimize the inhibitors effects, such as an addition of an ultrasonication step and the use of dispersants and enzymes to help break the sludge flocs and release viral material from the leachate aggregated matrix. The detection of SARS-CoV-2 fragments in leachate can help understand the epidemiological situation in collection regions and this pioneer study brings light to other epidemiological studies for other viruses or resistant bacteria present in leachate.

4. Conclusions

The COVID-19 pandemic brought many uncertainties, insecurities and difficulties to MSW management activities in municipalities around the world. In developing countries, as they already lack management and treatment policies, these difficulties are even greater, especially with the speed at which decisions have to be taken to prevent the spread of the virus and the increase in the number of cases, as in the beginning of the pandemic. Furthermore, given the difficulty in analyzing and characterizing MSWs due to their characteristic heterogeneity, the present work proposed a methodology for collecting leachate samples used at a transfer station in the city of São Paulo.

The results obtained by analyzing the polyethylene glycol concentrated leachate by RT-qPCR confirmed the presence of N1 genes in 9 of the 12 samples collected between the epidemiological weeks 08/15/2021 and 09/19/2021. The occurrence of the N2 gene was only observed in 5 of the 12 samples. The concentration values for N1 and N2 genes ranged between 3.1 and 4.6 log_{10} GC·L^{-1}, with higher frequency of N1 gene. These values were close to those found in wastewater in the literature, although they have very different matrices.

It is important to emphasize that, until this day, there is no similar study in the literature that addresses the detection of SARS-CoV-2 RNA virus in the leachate produced by MSW. Thus, more studies are needed for MSW leachate collected in other parts of the MSW management system, such as sorting and incineration plants, in the landfill itself and in the waterproofing, covering and leachate treatment systems.

**Funding**

The authors would like to acknowledge the financial support from the following Brazilian institutions: Federal University of ABC (UFABC) No. 23006.002360/2020-43 and Brazilian National Council of Scientific and
Fig. 4. SARS-CoV-2 RNA viral load (N1 and N2 assays) in leachate samples in relation to COVID-19 clinical data in São Paulo between epidemiological weeks 33 and 38 of the year 2021.

Technological Development (CNPq) in partnership with Ministry of Science, Technology, Innovations and Communications (MCTIC), and Ministry of Health (MS), Secretariat of Science, Technology, Innovation and Strategic Inputs – Decit/SCTIE 07/2020 (Research to cope with COVID-19, its consequences and other severe acute respiratory syndromes – No. 404232/2020-7).

CRediT authorship contribution statement

Giuliana Mondelli: Conceptualization, Writing – review & editing, Supervision, Validation, Funding acquisition. Ednei Rodrigues Silva: Conceptualization, Investigation, Writing – original draft. Ieda Carolina Mantovani Claro: Investigation, Writing – original draft. Matheus Ribeiro Augusto: Writing – review & editing. Adriana Feliciano Alves Duran: Investigation, Writing – review & editing. Aline Diniz Cabral: Writing – review & editing. Lívia de Moraes Bomediano Camillo: Writing – review & editing. Luísa Helena dos Santos Oliveira: Writing – review & editing. Rodrigo de Freitas Bueno: Writing – review & editing, Supervision, Validation, 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.

Acknowledgements

The authors are grateful for the support of the company Ecourbis Ambiental S.A. in collecting municipal solid waste.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.153927.

References

ACRPlus. 2020. Survey on the impact of the COVID-19 on municipal waste management systems [WWW Document]. Association of Cities and Regions for Sustainable Resource Management. https://www.acrplus.org/en/municipal-waste-management-covid-19.
