

Detection of SARS-CoV-2 in Environmental Matrices of Rio de Janeiro State

Priscila Santana-Pereira^{1,2}, Lorena da Graça Pedrosa de Macena^{1,2},
Kauê Francisco Corrêa de Souza-Souza^{1,3}, Valéria Teixeira Laneuville²,
Izabel Christina N. P. Paixão¹

¹Laboratory of Molecular Virology and Marine Biotechnology, Department of Cellular and Molecular Biology, Universidade Federal Fluminense-Niterói, Rio de Janeiro, Brazil

²Postgraduate Program in Sciences and Biotechnology, Universidade Federal Fluminense-Niterói, Rio de Janeiro, Brazil

³Laboratory of Immunopharmacology, Department of Biochemistry, Universidade Federal Do Estado Do Rio de Janeiro, Rio de Janeiro, Brazil

Email: ipaixao@vm.uff.br, kauefcorrea@hotmail.com

How to cite this paper: Santana-Pereira, P., Pedrosa de Macena, L.G., Souza-Souza, K.F.C., Laneuville, V.T. and Paixão, I.C.N.P. (2026) Detection of SARS-CoV-2 in Environmental Matrices of Rio de Janeiro State. *Open Journal of Modern Hydrology*, 16, 402-421.

<https://doi.org/10.4236/ojmh.2026.164022>

Received: June 19, 2026

Accepted: October 6, 2026

Published: October 9, 2026

Copyright © 2026 by author(s) and Scientific Research Publishing Inc.

This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).

<http://creativecommons.org/licenses/by/4.0/>



Open Access

Abstract

Since the investigation to discover the possible origin and routes of dissemination of SARS-CoV-2 in 2020 from effluent analyses, countless urban centers have adopted this measure as a research base to understand the factors that would guarantee viral survival and the degree of local human contamination. In this context, the aim of this study was to analyze the presence of SARS-CoV-2 in seven distinct aquatic matrices using RT-qPCR and transmission electron microscopy techniques, as well as to analyze the physico-chemical parameters of aquatic matrices and bacteriological indicators that could influence its survival and propagation. Of the seven aquatic matrices analyzed (coastal seawater, brackish water, freshwater river, drinking water, untreated and treated domestic raw sewage, and untreated hospital raw sewage), six presented detection of SARS-CoV-2 RNA. The brackish water matrix showed a higher frequency of SARS-CoV-2 RNA detection. In addition, an association between SARS-CoV-2 RNA detection and pH was observed. The detection of SARS-CoV-2 RNA was associated with the presence of *E. coli*. However, complementary analyses are needed to identify additional parameters that may interfere with the presence and survival of viruses in aquatic matrices.

Keywords

Aquatic Matrices, Environmental Monitoring, Rio de Janeiro, RT-qPCR, SARS-CoV-2

1. Introduction

Approximately 70% of the planet is composed of water, representing one of the main global concerns regarding availability, use, conservation, and contamination [1] [2]. Therefore, monitoring the quality of water bodies receiving sanitary effluents and water intended for consumption becomes urgent. Despite the urgency, 82% of the world's population still does not benefit from these services [3].

According to the National Water Agency in 2020, the proper identification of a water body occurs through monitoring the quality of surface and groundwater, following the water quality index [4]. The water quality index evaluates parameters such as temperature, pH, dissolved oxygen, total residue, biochemical oxygen demand, thermotolerant coliforms, total nitrogen, total phosphorus, and transparency.

In addition to making water bodies unfit for human consumption, changes in water quality parameters also indicate pathogen proliferation. Studies conducted in several Brazilian states have shown the presence of SARS-CoV-2 in wastewater. In Rio de Janeiro, samples of domestic and hospital wastewater were positive for the presence of SARS-CoV-2 [5]. This can be explained by the unique characteristics of SARS-CoV-2 infection, which is characterized by prolonged shedding and a high viral load in feces [6]. As prolonged shedding of SARS-CoV-2 RNA has been reported in stool samples of both symptomatic and asymptomatic infected individuals [6], sanitary effluent surveillance has become an important tool for monitoring SARS-CoV-2-induced infection in regions with high and low transmission.

Herein, we highlight the importance of wastewater as an environmental monitoring tool. We also evaluated the presence of SARS-CoV-2 in distinct aquatic matrices and measured their physicochemical parameters and bacteriological indicators that may favor viral presence and survival.

2. Materials and Methods

2.1. Study Area and Sampling

Fifteen collection points were preselected in the state of Rio de Janeiro (estuarine beach, lagoon, river, human water supply, domestic sewage, and hospital sewage). Three factors determined these locations: (i) the influence of the discharge of treated or untreated sewage; (ii) proximity to urban agglomerations; and (iii) proximity to pre-existing or field hospitals.

To facilitate sample analysis, we categorized all collection points into seven aquatic matrices: coastal seawater, brackish water, freshwater river, drinking water, treated and untreated domestic raw sewage and raw hospital sewage (Table 1). Which were collected in polypropylene barrels (40 L volume), all inland and coastal seawater samples were collected in polypropylene bottles (1.5 L volume) and processed, concentrated, and stored at -20°C .

The concentration method of coastal, brackish, river, consumption, domestic

and hospital sewage samples was by ultracentrifugation as described by Pina and coworkers [7].

Table 1. Aquatic matrices, locations and methods of collection with their respective abbreviations.

Matrices		Collect Points	Collect Forms
1	Coastal Seawater	Sepetiba	Three collects every seven days (day 1, day 08 and day 15), in July (2020)
		São Bento	
		Dendê	
2	Brackish Water	Jacarepaguá	Three collects every seven days (day 1, day 08 and day 15), in July (2020)
		Rodrigo de Freitas	
3	Freshwater River	Guandu-Mirim	
		Guandu	
		Faria Timbó	
4	Drinking Water	Supply	
5	Domestic Raw Sewage	Entry of Sewage Treatment Plant	
		Complexo Naval de Mocanguê (CNM)	
6	Domestic Treated Sewage	Ilha do Fundão	
		Exit of Sewage Treatment Plant	
7	Hospital Raw Wastewater	Complexo Naval de Mocanguê (CNM)	One monthly collection for three consecutive months, in July, August and September (2020)
		Botafogo Polyclinic	
		Emergency Care Unit of Cabo Frio	
		Municipal Hospital of Cabo Frio	

2.2. Physicochemical Parameters

The physicochemical parameters of the aquatic matrices were determined based on parameters established by the INEA. For the evaluation of these parameters, a conventional thermometer, Secchi disk, and pH strip were used, with data confirmation performed using a benchtop pH meter, conductivity, temperature, and depth probe, and oximeter, as stipulated in CONAMA Resolution 357/2005 [8].

2.3. Bacteriological Analysis

Enterococci sp. are the first-choice marine microbiological indicators according to the United States Environmental Protection Agency and the World Health Organization [9] [10]. To determine the presence of *Enterococci* sp., the commercial kit Colilert and Enterolert, based on defined substrate technology (DST), developed by IDEXX Laboratories, was used. Briefly, a specific water-soluble substrate was added and reacted with the target bacteria in the samples. After bacterial mul-

tiplication in sealed Quant-Tray cards and storage in an incubator, the samples were evaluated by staining and quantified from a table provided by the manufacturer, where concentrations range from 1 to 2×10^3 microorganisms per 100 mL of sample.

For *Escherichia coli*, quantification was performed using the commercial kit Colilert-Quanti-Tray/2000 (IDEXX Laboratories, Inc., Westbrook, ME, USA), and the results were expressed as concentration/100mL. All samples used a dilution of 1:10. Thermotolerant coliforms were quantified using the multiple-tube method described in the Food and Drugs Administration Bacteriologic Analytical Manual [11]. Serial dilutions of 1:10, 1:100, and 1:1000 were performed from 10 mL of each water sample in 90 mL of Butterfield Phosphate Diluent. The substrate was then added to the tube containing the 1:1000 dilution. After homogenization, the suspension was divided into five tubes (20 mL each) and incubated at 35°C for 24 h. Tubes showing turbidity and color change were transferred to *Escherichia coli* soup and incubated at 44.5°C for 24 h to confirm the presence of thermotolerant coliforms [12], which was indicated by the presence of gas in the tube [13].

2.4. Virological Analysis

SARS-CoV-2 was evaluated using three analytical approaches: RT-qPCR (real-time polymerase chain reaction), nucleotide molecular characterization, and transmission electron microscopy. Initially, the samples were processed using different techniques (described in the “Study area and sampling” section), and the final concentrated volume was used for transmission electronic microscopy, nucleic acid extraction, reverse transcription, and RT-qPCR.

2.5. Quantitative Reverse Transcriptase Polymerase Chain Reaction (RT-qPCR)

Viral RNA was extracted using a commercial extraction kit (Allprep PowerViral DNA/RNA kit—Qiagen) on a one-step instrument, as described by the manufacturer.

Briefly, 200 µL of the initial volume (viral concentrated) was used for extraction, followed by the addition of 600 µL of PM1 solution previously incubated for 10 minutes at 55°C and 6 µL of β-mercaptoethanol. The mixture was homogenized for 30 s and incubated for 5 min at room temperature. Next, 150 µL of Interspersed Repetitive Sequence Polymerase Chain Reaction was added to the sample, homogenized, and incubated between 2 and 8°C for 5 min. At the end of the incubation period, the mixture was centrifuged for 1 min at 13,000 x g and the supernatant transferred to a collector tube. A total of 600 µL of PM3 and PM4 solutions were added to the collector tube, homogenized, and centrifuged for 1 min at 13,000 x g. The clarified content was discarded, and the centrifugation process was repeated three more times. Then, 600 µL of PM5 solution was added to the column and centrifuged for 1 min at 13,000 x g, followed by removal of the supernatant, addition of 600 µL of PM4 solution, centrifugation for 1 min at 13,000 x g, removal of the supernatant, and another centrifugation for 2 min at 13,000 x g. After sam-

ple preparation, the column was placed in a new collector tube with the addition of 100 μ L of RNase-free water, incubated for 1 min, and centrifuged again at 13,000 $\times$ g for 1 min. Finally, the column was removed and the liquid containing RNA and DNA was stored at -80°C .

Samples were analysed at the Laboratory of Molecular Virology and Marine Biotechnology (VM/UFF) using the PikoReal™ 96 real-time PCR system (Thermo Scientific™, USA). The SARS-CoV-2 N1 + N2 primer sequences used followed the CDC protocol. Sample cycle Threshold (Ct) values between 16 and 38 were considered positive for SARS-CoV-2 RNA.

Amplification of the S protein gene was performed with six sets of primers targeting the S segment and two sets flanking it, designed based on sequences deposited in GISAID up to September 2020. The SARS-CoV-2 RT-qPCR positives with a Ct value ≤ 16 were selected for confirmation. Selected samples were further analysed by conventional PCR targeting regions of the S gene, followed by 1.2% agarose gel electrophoresis as a complementary molecular analysis. A 100-nucleotide overlap was programmed (**Figure 1**).

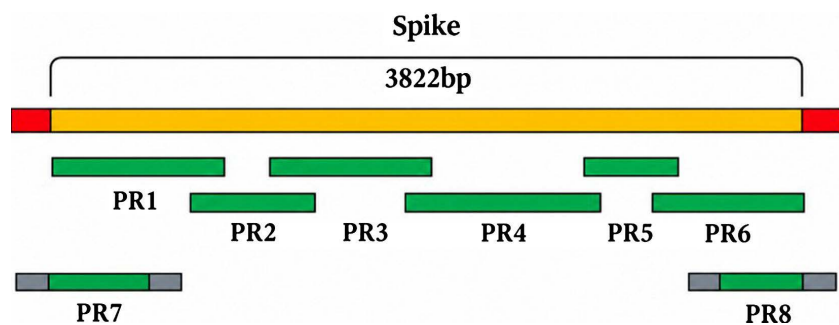


Figure 1. Schematic representation of the fragments of each pair of primers used, where: PR1—primer pair 1, PR2—primer pair 2, PR3—primer pair 3, PR4—primer pair 4, PR5—primer pair 5, PR6—primer pair 6, PR7—primer pair 7 and PR8—primer pair 8.

Table 2 presents the primer sequences used. Standard RT-qPCR was performed using the Superscript III one-step RT-qPCR kit according to the manufacturer's instructions. Amplified fragments were analysed by electrophoresis on a 1.2% agarose gel.

Table 2. Sequence of primer pairs used for identification of protein S, by conventional PCR technique.

Primer S segment*	Sequence (5' -- 3')	Location	Size**
PR1 Sense	GTTTGTTTTCTTGTTTTATT	(21551-21574)	923 pb
Nonsense	ACAGTGAAGGATTTCAACGTACAC	(22450-22474)	
PR2 Sense	CGTGATCTCCCTCAGGGTTTT	(22190-22211)	620 pb
Nonsense	TCAGCAATCTTTCCAGTTTGCC	(22810-22832)	
PR3 Sense	GTAATTAGAGGTGATGAAGTCAGA	(22751-22775)	892 pb
Nonsense	ACATAGTGTAGGCAATGATGGA	(23621-23643)	

Continued

PR4 Sense	CTTGGCGTGTTTATTCTACAG	(23445-23466)	979 pb
Nonsense	GCTTGTGCATTTTGGTTGACC	(24403-24424)	
PR5 Sense	AGACTCACTTTCTTCCACAGCA	(24355-24377)	342 pb
Nonsense	AGATGATAGCCCTTCCACA	(24699-24719)	
PR6 Sense	TTCTGCTAATCTTGCTGCTACT	(24610-24632)	766 pb
Nonsense	GTTTATGTGTAATGTAATTT- GACTCC	(25348-25372)	
PR7 Sense	TAGAGAAAACAACAGAGTT	(21492-21511)	712 pb
Nonsense	TGAGGGAGATCACGCACTAA	(22184-22204)	
PR8 Sense	TTCTGCTAATCTTGCTGCTACT	(24610-24632)	825 pb
Nonsense	CCTTGCTTCAAAGTTACAGTTCCA	(25409-25433)	

*Spike protein segment (S protein)—Primer identification; **Size of the amplified fragment in base pairs (bp).

2.6. Transmission Electron Microscopy

Transmission electron microscopy was performed using 50 µL aliquots of the samples fixed in glutaraldehyde (2.5%) and supplemented with 2% uranyl acetate (final concentration), followed by negative staining.

Copper grids, 400 mesh were coated with formalin and sprayed with carbon for stabilization. Subsequently, the samples were adhered to the grid for 30 sec had their humidity removed with film paper, and the negative contrast was evaluated by adding uranyl acetate (5%), incubating for 30 s, followed by drying with filter paper.

Transmission electronic microscopy images (Hitachi 7800 - 4K) with an acceleration voltage of 100 kV were observed at magnifications of 20,000 to 120,000 x, captured and evaluated using a digital system and the manufacturer's software. The characterization of viral particles follows parameters as morphology, size, and structural uniformity [14].

2.7. Statistical Analysis

Statistical analysis was performed using GraphPad PRISM 8.0.1 software. The Shapiro-Wilk test was used for normality testing, without subsequent analysis of the normal (Gaussian) distribution. Non-parametric analyses were performed and considered significant at $p \leq 0.05$ (*). Qualitative results were presented as detection percentage values, and multinomial linear regression was used to compare physicochemical, bacteriological, and SARS-CoV-2 data. The non-parametric Spearman correlation test was used to evaluate the confidence interval of the correlation coefficient between bacterial and viral detection and physicochemical parameters, being considered significant when $p \leq 0.05$.

3. Results

Five of the nine parameters that determine the water quality index according to

the Water National Agency were analysed: hydrogen ion potential (pH), electrical conductivity (EC), transparency, dissolved oxygen (DO), and temperature (T). Our results showed that pH values for the sampling points ranged from 6.6 in coastal seawater matrices to 8.3 in brackish water matrices, with an overall average of 7.4. Regarding EC, the value ranged from a minimum of 26.7 in brackish water matrices to a maximum of 65.7 in the freshwater river matrix. The transparency showed high variation, with a minimum value of 1.0 for drinking water matrices and a maximum of 87.3 in freshwater river matrices, while DO fluctuate from 0 in freshwater river matrices to 11.7 in coastal seawater matrices. In terms of temperature, three locations presented values that were very divergent, with a minimum of 23.3°C for coastal seawater and ocean seawater matrices, compared with the maximum measurements of 26.8°C recorded for untreated hospital raw sewage (Table 3).

Table 3. Average values and standard deviation of physicochemical parameters per aquatic matrix.

Aquatic matrices and Collect Points (n = 48)	pH	CE	Transparency	OD	T
Costal Seawater					
Sepetiba (n = 3)	6.6 ± 0.5	27.0 ± 2.0	2.3 ± 1.5	5.8 ± 0.6	25.0 ± 0.1
São Bento (n = 3)	7.3 ± 0.3	44.3 ± 1.5	10.0 ± 1.0	8.0 ± 0.5	24.3 ± 0.6
Dendê (n = 3)	7.4 ± 0.5	46.3 ± 1.5	12.7 ± 2.1	11.7 ± 2.1	23.3 ± 0.6
Brackish Water					
Jacarepaguá (n = 3)	7.1 ± 0.1	40.0 ± 1.0	52.9 ± 3.5	0.2 ± 0.1	24.7 ± 0.6
Rodrigo de Freitas (n = 3)	8.3 ± 0.4	26.7 ± 2.1	39.0 ± 1.0	5.1 ± 1.0	23.4 ± 0.7
Freshwater River					
Guandu-Mirim (n = 3)	6.8 ± 0.4	65.7 ± 2.5	87.3 ± 2.5	0.5 ± 0.1	25.3 ± 0.6
Guandu (n = 3)	7.1 ± 0.1	62.0 ± 1.0	41.0 ± 3.6	5.7 ± 1.0	24.9 ± 0.4
Faria Timbó (n = 3)	7.4 ± 0.2	47.3 ± 2.1	38.3 ± 1.5	0.0 ± 0.0	25.7 ± 0.6
Drinking Water					
Supply (n = 3)	7.0 ± 0.5	38.5 ± 27.9	1.0 ± 0.1	9.2 ± 0.8	23.0 ± 1.0
Untreated Domestic Sewage					
Entry of Sewage Treatment Plant CNM (n = 3)	7.3 ± 0.1	51.0 ± 0.6	62.7 ± 1.7	0.1 ± 0.0	26.2 ± 0.3
Ilha do Fundão (n = 3)	7.8 ± 0.0	53.0 ± 0.6	40.4 ± 0.6	0.2 ± 0.1	25.4 ± 0.2
Treated Domestic Sewage					

Continued

Exit of Sewage Treatment Plant CNM (n = 3)	7.5 ± 0.1	62.0 ± 3.0	58.3 ± 2.9	1.2 ± 0.1	25.9 ± 0.1
Hospital Raw Wastewater					
Botafogo Polyclinic (n = 3)	7.7 ± 0.1	64.3 ± 4.0	52.0 ± 1.0	0.1 ± 0.1	26.7 ± 0.5
Emergency Care Unit of Cabo Frio (n = 3)	7.6 ± 0.1	54.3 ± 4.5	59.3 ± 0.6	0.2 ± 0.0	27.0 ± 0.6
Municipal Hospital of Cabo Frio (n = 3)	7.3 ± 0.2	61.7 ± 1.5	55.0 ± 1.0	0.1 ± 0.0	26.7 ± 0.5

Hydrogen ion potential (pH), electrical conductivity (EC), transparency, dissolved oxygen (DO), and temperature (T).

3.1. Bacteriological Analysis

As shown in **Table 4**, the brackish water matrix presented the highest observed concentrations of total coliforms (5.5×10^3 MPN/100mL), thermotolerant coliforms ($2,25 \times 10^3$ MPN/100mL) and *E. coli* (3.15×10^3 MPN/100mL) among the analysed matrices. *Enterococci* ($36,2 \times 10^3$ MPN/100mL) were found in the highest concentration in untreated hospital raw sewage matrices and treated domestic sewage matrices. The lowest concentration of thermotolerant coliforms (1.52×10^3 MPN/100mL) was observed in untreated hospital raw sewage matrices. The freshwater river, treated, and untreated domestic wastewater matrices recorded the lowest average concentrations of thermotolerant coliform (1.10×10^3 MPN/100mL). *E. coli* and *enterococci* showed the lowest concentrations in the matrices of untreated domestic wastewater (1.10×10^3 MPN/100mL) and coastal sea water matrices (13 MPN/100mL), respectively.

Of the 45 samples analysed, contamination indicators were detected in 42 samples, with total coliforms being the most prevalent, followed by *E. coli*, thermotolerant coliforms, and *enterococci* when considering the overall averages. When we classify the aquatic matrices as suitable or unsuitable according with bacteriological parameters established by CONAMA Resolution 274/00 [12], which considers the presence above the permitted level of one bacteriological agent among the four most important (total coliforms, thermotolerant coliforms and *E. coli* and *enterococci*), six of the seven aquatic matrices (coastal seawater, brackish water, freshwater river, untreated domestic raw sewage, treated domestic raw sewage and untreated hospital raw sewage) were classified such as improper.

Table 4. Bacterial indicators of fecal contamination by collection point.

Collect Points (n = 45)	Total coliform	Thermotolerant coliform	<i>E. coli</i>	<i>Enterococci</i>
Coastal Seawater				
Sepetiba (n = 3)	5700	2267 ^{Ct}	1600 ^{Ct}	17

Continued

São Bento (n = 3)	3900	1100 ^{Ct}	1100 ^{Ct}	13
Dendê (n = 3)	1600	1100 ^{Ct}	1100 ^{Ct}	10
Brackish Water				
Jacarepaguá (n = 3)	6867 ^{Ct}	3400 ^{Ct}	1600 ^{Ct}	63
Rodrigo de Freitas (n = 3)	4567	1100 ^{Ct}	4733 ^{Ct}	10
Freshwater River				
Guandu-Mirim (n = 3)	4567	1600 ^{Ct}	2933 ^{Ct}	10
Guandu (n = 3)	3400	1100 ^{Ct}	1467 ^{Ct}	56
Faria Timbó (n = 3)	2267	1100 ^{Ct}	1050 ^{Ct}	16
Drinking Water				
Supply (n = 3)	0	0	0	0
Untreated Domestic Sewage				
Entry of Sewage Treatment Plant CNM (n = 3)	3400	1100 ^{Ct}	1100 ^{Ct}	28
Ilha do Fundão (n = 3)	1100	1100 ^{Ct}	1100 ^{Ct}	17
Treated Domestic Sewage				
Exit of Sewage Treatment Plant CNM (n = 3)	2267	1100 ^{Ct}	1100 ^{Ct}	43
Hospital Raw Wastewater				
Botafoogo Polyclinic (n = 3)	1600	1100 ^{Ct}	1600 ^{Ct}	52
Emergency Care Unit of Cabo Frio (n = 3)	1100	1100 ^{Ct}	1100 ^{Ct}	13
Municipal Hospital of Cabo Frio (n = 3)	1867	897	1600 ^{Ct}	60

^{Ct} indicates the detection of SARS-CoV-2.

3.2. Virological Analysis

The presence of SARS-CoV-2 RNA was also analysed by RT-qPCR. SARS-CoV-2 RNA was detected in samples from six of the seven aquatic matrices analysed, whereas the drinking water sample was negative (**Table 5**).

Table 5. Detection of SARS-CoV-2 RNA by RT-qPCR in each aquatic matrix.

Aquatic Matrices	SARS-CoV-2 RNA detection
Coastal Seawater (n = 9)	2/9 (22%)
Brackish Water (n = 6)	2/6 (33.3%)
Freshwater River (n = 9)	1/9 (11.1%)
Drinking Water (n = 3)	0/3 (0%)
Untreated Domestic Sewage (n = 6)	4/6 (66.7%)
Treated Domestic Sewage (n = 3)	1/3 (33.3%)
Hospital Raw Wastewater (n = 9)	5/9 (55.6%)

Samples with Ct values within the established detection range were considered positive for SARS-CoV-2 RNA. Selected samples were further analysed by conventional PCR targeting regions of the S gene, followed by 1.2% agarose gel electrophoresis as a complementary molecular analysis (Figure 2).

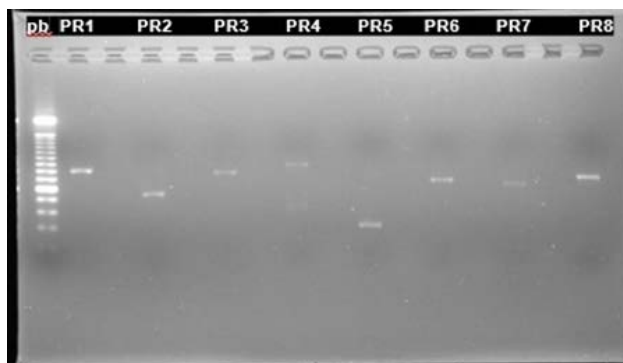


Figure 2. Electrophoresis on 1.2% agarose gel showing amplification of the S gene fragments. The first marker is the molecular weight pattern, with the highest weight bands near 1Kb are observed. The individual bands are the PR1 - PR8 primer pairs, from left to right in interleaved wells (Dendê beach).

3.3. Correlation of the Physicochemical and Bacteriological Parameters of the Matrices in Relation to SARS-CoV-2

When SARS-CoV-2 RNA detection and Ct values were correlated with bacterial concentrations, total coliforms, thermotolerant coliforms, and *E. coli* showed a positive correlation with the SARS-CoV-2 RNA detection. In contrast, *enterococci* showed an inverse correlation with SARS-CoV-2 RNA detection. The correlations between SARS-CoV-2 RNA detection and bacterial and physicochemical parameters were further evaluated. Thus, our results demonstrated a positive association between SARS-CoV-2 RNA detection and the concentrations of total coliforms, thermotolerant coliforms, and *E. coli* (Figure 3).

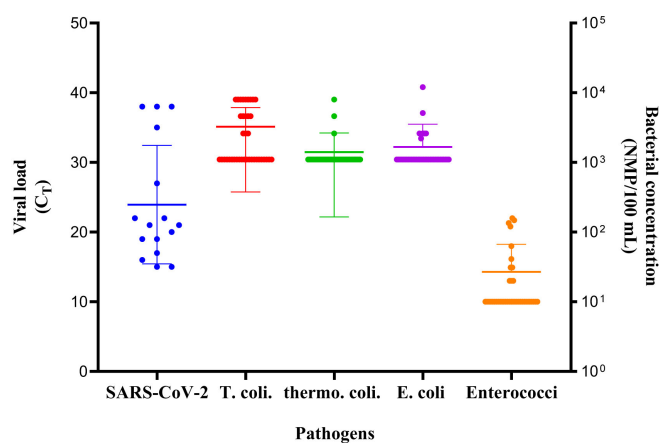


Figure 3. Correlation between bacterial concentration and SARS-CoV-2 RNA detection. (T. Coli.—Total coliforms; Thermo. Coli.—Thermotolerant Coliforms; *E. coli*—*Escherichia coli* and *Enterococci*).

Spearman's correlation (Figure 4) was used to evaluate associations between SARS-CoV-2 RNA detection, bacterial concentration, and physicochemical parameters of aquatic matrices. An association was observed between SARS-CoV-2 RNA detection, pH, and *E. coli*. No similar association was observed with *enterococci*, total coliforms, or thermotolerant coliforms. In addition, it was observed that the bacteria showed weak positive correlations with each other. When we established the correlation between physicochemical parameters and bacterial concentration, we observed that bacterial concentration is more influenced by temperature.

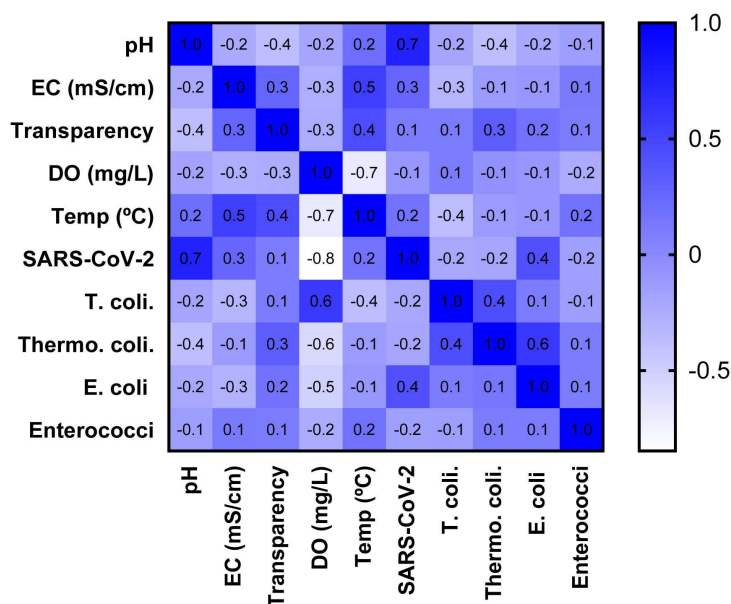


Figure 4. Spearman's correlation to evaluate the relationship between physicochemical and bacteriological parameters and the detection of SARS-CoV-2 RNA. T. Coli.—Total coliforms; Thermo. Coli.—Thermotolerant Coliforms; *E. coli*—*Escherichia coli*; pH—Hydrogen ion potential; EC—electrical conductivity; DO—dissolved oxygen and Temp—Temperature.

3.4. Transmission Electron Microscopy of the Viral Particle and Correlation with Molecular Detection

Electron microscopy results showed that the two particles, approximately 50 nm, are similar in size to enteric viruses (Figure 5(A)). Virus-Like particles (VLPs) of approximately 100 nm, with morphology compatible with coronavirus-like particles, were observed in Figure 5(B). Both Figure 5(A) and Figure 5(B) correspond to the brackish water matrices. VLPs measuring 40 to 60 nm, with different morphologies and dimensions, were also observed in Figure 5(C), while particles of 100nm with morphology similar to SARS-CoV-2 VLP were identified in Figure 5(D), with Figure 5(C) and Figure 5(D) corresponding to the freshwater river matrices. In the untreated sanitary effluent matrix (Figure 5(E)), a structure containing slightly more than 10 particles sizing between 60 and 95 nm, similar to enteropathogens, was found. Figure 5(F) shows a tailed bacteriophage, approximately 50 to 80 nm in size, similar to Siphovirus, identified in the coastal seawater

matrix. In the last two images (**Figure 5(G)**—coastal seawater matrix and **Figure 5(H)**—untreated hospital raw sewage matrix), particles of 100 to 148 nm and 100 to 130 nm, respectively, compatible with the morphology and size of SARS-CoV-2 VLPs, were identified.

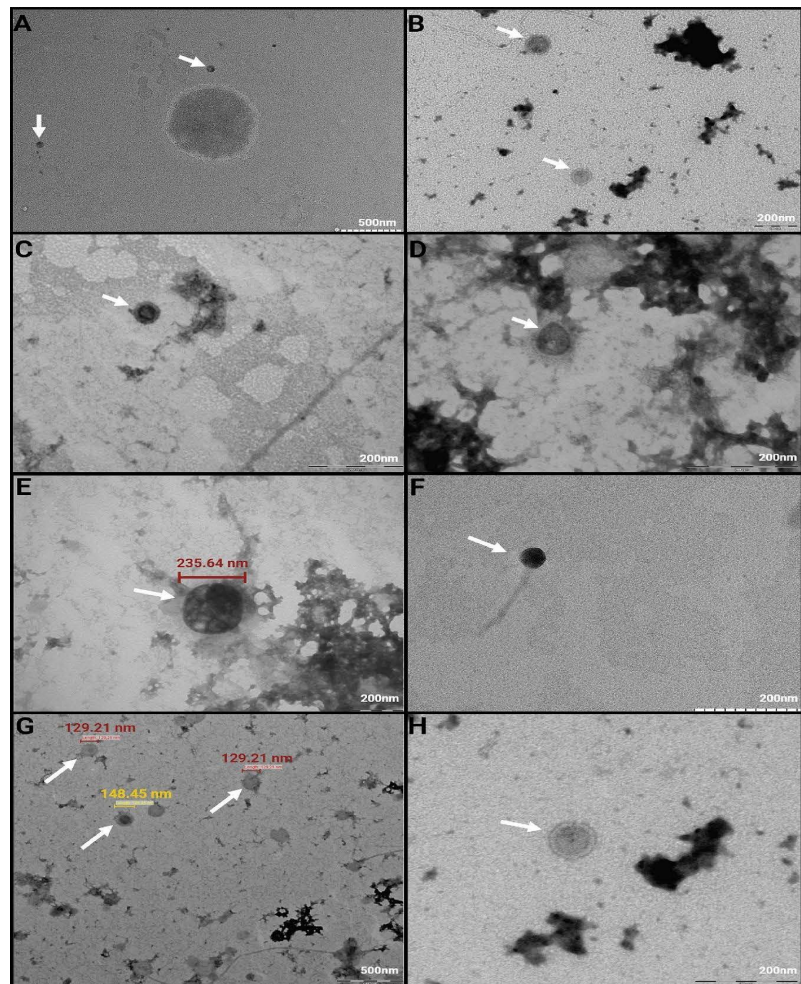


Figure 5. Electron micrograph of environmental matrices. Images A and B—brackish water matrix; C and D—freshwater river matrix; E—untreated domestic sewage matrix; F—coastal seawater matrix; G—coastal seawater matrix; and H—untreated hospital raw sewage matrix.

In addition, **Table 6** shows the overall percentage of samples positive for SARS-CoV-2 VLP by RT-qPCR and transmission electron microscopy (TEM), considering the collection site and grouping by aquatic matrices. For the coastal seawater matrix, two collection sites were positive for SARS-CoV-2 VLP in at least one of the samples, both by RT-qPCR and TEM, suggesting the presence of the virus in 22.2% of the matrix. For the brackish water matrices, one sample from each collection point showed viral presence in both RT-qPCR and TEM, representing an aggregate of 33.3% of SARS-CoV-2 VLP.

The freshwater river matrix was positive in only one sample from the same col-

lection point for both RT-qPCR and TEM techniques, with SARS-CoV-2 VLP being present in only 11.1% of the matrix composition. The drinking water matrix was negative for SARS-CoV-2 VLP in both techniques. In contrast, the untreated domestic sewage matrix was positive in at least two samples from each collection point for both techniques, resulting in viral presence in 66.6% of the matrix. When we analysed the treated domestic sewage matrix, only RT-qPCR showed a positive result for SARS-CoV-2 VLP in at least one sample, whereas transmission electron microscopy showed all negative results. For untreated hospital raw sewage matrices, in both techniques, at least one sample from each collection point was positive, totaling a presence in 55.5% of the matrix.

Table 6. Detection of SARS-CoV-2 by RT-qPCR compared with TEM by collection point and aquatic matrix.

Aquatic Matrices	Collect Points	Presense (+) Absence (-)		Positive samples (%)
		RT-qPCR	MET	
Coastal Seawater (n = 9)	Sepetiba (n = 3)	–	–	0/3 (0%)
	São Bento (n = 3)	+	+	1/3 (33.3%)
	Dendê (n = 3)	+	+	1/3 (33.3%)
Total Positive:		66.6%	66.6%	2/9 (22.2%)
Brackish Water (n = 6)	Jacarepaguá (n = 3)	+	+	1/3 (33.3%)
	Rodrigo de Freitas (n = 3)	+	+	1/3 (33.3%)
Total Positive:		100%	100%	2/6 (33.3%)
Freshwater River (n = 9)	Guandu (n = 3)	–	–	0/3 (0%)
	Guandu - Mirim (n = 3)	–	–	0/3 (0%)
	Faria Timbó (n = 3)	+	+	1/3 (33.3%)
Total Positive:		33.3%	33.3%	1/9 (11.1%)
Drinking Water (n = 3)	Supply (n = 3)	–	–	0/3 (0%)
Total Positive:		0%	0%	0/3 (0%)
Untreated Domestic Sewage (n = 6)	Entry of Sewage Treatment Plant CNM (n = 3)	+	+	2/3 (66.6%)
	Ilha do Fundão (n = 3)	+	+	2/3 (66.6%)
Total Positive:		100%	100%	4/6 (66.7%)
Treated Domestic Sewage (n = 3)	Exit of Sewage Treatment Plant CNM (n = 3)	+	–	1/3 (33.3%)

Continued

Total Positive:		100%	0%	1/3 (33.3%)
Hospital Raw Wastewater (n = 9)	Botafogo Polyclinic (n = 3)	+	+	1/3 (33.3%)
	Emergency Care Unit of Cabo Frio (n = 3)	+	+	2/3 (66.6%)
	Municipal Hospital of Cabo Frio (n = 3)	+	+	2/3 (66.6%)
Total Positive:		100%	100%	5/9 (55.6%)
Overall Positive:		68.75%	62.5%	15/48 (31.25%)

4. Discussion

Water bodies, including marine recreational waters, can contain countless pathogenic organisms originating from untreated sewage, solid waste, and drainage water [15]. According to the Brazilian Institute of Geography and Statistics (2022) 37,5% of the Brazilian population is not supplied by a sewage treatment network, with approximately nine metric tons of effluent discharged untreated daily into the water bodies [16]. In addition, high *E. coli* titres [17] and favouritism of antibiotic-resistant pathogenic strains [18] reinforce the potential risks associated with the domestic use of water in the river catchments and highlight the importance of environmental monitoring in the control and prevention of infections.

Studies have focused their analyses on samples located near treatment stations or in intermediate locations, such as sewage pumping stations [19]-[21]. In 2023, a study on the environmental monitoring of SARS-CoV-2 in the metropolitan area of Porto Alegre, Rio Grande do Sul, Brazil, showed varying levels of viral presence in different sample types, with a direct correlation between environmental viral load and clinical COVID-19 cases, contributing to the understanding of virus persistence and transmission pathways in the environment [22].

In 2021, the presence of SARS-CoV-2 in wastewater in Rio de Janeiro, Brazil, including the discovery of the Mu variant (B.1.621), a strain with mutations (S: E484K, S: N501Y), was reported [23]. Considering the precarious infrastructure of sewage networks and wastewater treatment, we analysed the presence of SARS-CoV-2 in seven different aquatic matrices of Rio de Janeiro state and the association between SARS-CoV-2 RNA detection, *E. coli* and physicochemical parameters.

In this study, seven aquatic matrices were investigated (coastal seawater, brackish water, freshwater river, drinking water, untreated domestic raw sewage, treated domestic raw sewage and untreated hospital raw sewage). SARS-CoV-2 RNA was detected in samples from coastal seawater, brackish water, freshwater river, untreated domestic sewage, treated domestic sewage, and untreated hospital raw sewage, which can be explained by the absence or inefficiency of public policies

that do not prioritize basic sanitation [24]. In contrast, the drinking water did not present contamination by any bacterial group and kept all physicochemical parameters in accordance with CONAMA Resolution 274/00 [12]. The brackish water matrix showed higher concentrations of *E. coli*, total and thermotolerant coliforms, together with a higher frequency of SARS-CoV-2 RNA detection. According to Cavalcante and Abreu (2020), the detection of SARS-CoV-2 RNA is compatible with pollution and is expected owing to the number of confirmed COVID-19 cases near the brackish water matrices [25]. Our data corroborate the findings of Monteiro and coworkers (2022), who suggested that the freshwater introduced by the channels could be a potential source of faecal contamination favoring the growth of bacterial groups [26].

The correlation between bacteriological indicators and SARS-CoV-2 occurred in almost all aquatic matrices, with the excess drinking water matrix. According to Pinon and Vialette (2018), a significant correlation between viruses and bacteria does not occur constantly and may vary depending on the physicochemical parameters of the matrices [27], corroborating our findings.

Parameters such as pH, electrical conductivity, temperature, transparency, and dissolved oxygen were analysed and correlated to the presence of SARS-CoV-2. The pH was maintained within the range of neutral-alkaline (6.6 to 8.3) in all aquatic matrices, showing a strong correlation with SARS-CoV-2, which highlights its importance in maintaining viral viability and persistence in the environment [28]. Additionally, data about the viability of SARS-CoV-2 under a wide range of pH conditions from pH 4 to pH 11 for several days had already been reported [29]. Although temperature interferes directly with the number of ions and salts dissolved in water and influences directly electrical conductivity and inversely dissolved oxygen (CONAMA No. 357/2005 and No. 274/2000) [8] [12], our results did not show a relevant relationship between SARS-CoV-2 and temperature. In contrast, a relationship was found between temperature and *enterococci*, data also found by Adolf and coworkers [30].

Dissolved oxygen and electrical conductivity presented an inverse relationship in brackish water matrices, freshwater rivers, untreated and treated domestic raw sewage, and untreated hospital raw sewage. These data are expected because of the incorporation of materials from the urban environment, which increases the concentrations of phosphorus and ammonia, thereby increasing the electrical conductivity. In addition, phosphorus and ammonia act as bacterial substrates, increasing the rate of cellular respiration, which reduces the concentration of dissolved oxygen [31].

The transparency of the matrices of coastal seawater and drinking water showed the lowest rates, which was expected due to the absence and/or smaller proportions of solid waste and nutrients contributed to maintaining this parameter.

RT-qPCR is the gold standard technique for SARS-CoV-2 diagnosis [32]. Studies in Brazil showed the presence of SARS-CoV-2 in wastewater, rivers, and urban aquatic systems by RT-qPCR [22] [23] [33]. Despite these advances, studies sim-

ultaneously assessing multiple environmental matrices remain limited, highlighting our research, which encompasses the analysis of SARS-CoV-2 by RT-qPCR in seven different interconnected aquatic matrices. The SARS-CoV-2 gene sequence used in this study was aligned with reference prototypes for each variant of concern (VOC) between 2020 and 2021. SARS-CoV-2 was identified by RT-qPCR in at least one sample from the brackish water matrix, coastal seawater, freshwater river, treated and untreated domestic raw sewage, and hospital raw sewage. In contrast, SARS-CoV-2 was not detected in the drinking water matrix. The transmission electron microscopy analysis showed particles morphologically compatible with coronavirus-like particles were observed, and these observations were considered complementary morphological findings. The treated domestic raw sewage matrix showed data different from that obtained by RT-qPCR, which was negative for SARS-CoV-2 RNA. This finding may be explained by the enveloped nature of SARS-CoV-2, which increases its susceptibility to environmental stressors such as oxidizing agents and physicochemical variations [29].

A study conducted by Xiao and coworkers showed that the feces of patients infected with SARS-CoV-2, even 12 days after the onset of symptoms and negative RT-qPCR, presented viral genetic material [34], corroborating the findings of Chen and coworkers (2020) who also showed the presence of SARS-CoV-2 in the feces of patients even 13 days after RT-qPCR was negative [35].

Even if SARS-CoV-2 persists in human feces released in wastewater, the probability of human infection from environmental sources is low [36]; however, theoretically possible, since SARS-CoV-2 can survive in stool for 1 - 2 days but with a 5-log loss of viability [37] and SARS-CoV-2 host receptor was found in the cytoplasm of gastrointestinal epithelial cells of infected patients [38]. Despite the low transmission rate, the analysis of aquatic matrices contributes as a local epidemiological indicator and estimates the extent of pathogen dissemination, favoring the understanding of what public measures should be adopted [32]. Therefore, environmental monitoring combined with efficient public measures has been seen as an effective tool in infection control that could prevent pandemics.

In this study, we demonstrated that environmental and urban factors directly affect the level of contamination of aquatic matrices. We also highlighted the importance of environmental monitoring as a tool to aid in the identification, prevention, and control of future infections.

5. Conclusion

In this study, we evaluated the presence of SARS-CoV-2 VLP and its correlation with bacterial groups and physicochemical parameters in seven different aquatic matrices. We observed that six aquatic matrices presented SARS-CoV-2 RNA detection, with total coliforms, thermotolerant coliforms, *E. coli*, and *enterococci* being presence of SARS-CoV-2 RNA directly associated to *E. coli* and also showing an association between SARS-CoV-2 RNA detection and pH. However, complementary analyses are needed to identify additional parameters that may inter-

ferre with the presence and survival of viruses in aquatic matrices.

Acknowledgements

This work was supported by CAPES (Grant 001), CNPq and FAPERJ. The authors also thank to the Programa de Pós Graduação em Ciências e Biotecnologia da Universidade Federal Fluminense and the Programa de Pós-graduação em Biologia Molecular e Celular da Universidade Federal do Estado do Rio de Janeiro for the financial support.

Data Availability Statement

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

Author Contributions

Priscila Santana Pereira conducted all experiments. Lorena Macena assisted with the statistical analyses. Kauê Souza contributed to manuscript revision. Valéria Teixeira and Izabel Paixão provided supervision.

Conflicts of Interest

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

References

- [1] Detoni, T.L. and Dondoni, P.C. (2008) A Escassez da água: Um olhar global sobre a sustentabilidade e a consciência acadêmica. *Revista Ciências Administrativas*, **14**, 191-204.
- [2] Jacobi, P.R., Empinotti, V.L. and Schmidt, L. (2016) Water Scarcity and Human Rights. *Ambiente & Sociedade*, **19**, Article No. 1. <https://doi.org/10.1590/1809-4422asoceditorialv19i2016>
- [3] Kuiava, V.A., Perin, A.T. and Chielle, E.O. (2019) Hospitalização e taxas de mortalidade por diarreia no Brasil: 2000-2015. *Ciência & Saúde*, **12**, Article 30022. <https://doi.org/10.15448/1983-652x.2019.2.30022>
- [4] Water National Agency (2013) Water Resources Notebook. Current Situation of Water Resources in Brazil. <https://www.gov.br/ana/en/information>
- [5] Prado, T., Fumian, T.M., Mannarino, C.F., Maranhão, A.G., Siqueira, M.M. and Miagostovich, M.P. (2020) Preliminary Results of SARS-CoV-2 Detection in Sewerage System in Niterói Municipality, Rio De Janeiro, Brazil. *Memórias do Instituto Oswaldo Cruz*, **115**, e200196. <https://doi.org/10.1590/0074-02760200196>
- [6] Amirian, E.S. (2020) Potential Fecal Transmission of SARS-CoV-2: Current Evidence and Implications for Public Health. *International Journal of Infectious Diseases*, **95**, 363-370. <https://doi.org/10.1016/j.ijid.2020.04.057>
- [7] Pina, S., Jofre, J., Emerson, S.U., Purcell, R.H. and Girones, R. (1998) Characterization of a Strain of Infectious Hepatitis E Virus Isolated from Sewage in an Area Where Hepatitis E Is Not Endemic. *Applied and Environmental Microbiology*, **64**, 4485-4488. <https://doi.org/10.1128/aem.64.11.4485-4488.1998>

- [8] CONAMA Resolution (2005) Resolution n 357, 18th March 2005. Conselho Nacional do Meio Ambiente.
https://conama.mma.gov.br/?id=450&option=com_sisconama&task=arquivo.download
- [9] United States Environment Protection Agency (2025) Indicators: *Enterococci*.
<https://www.epa.gov/national-aquatic-resource-surveys/indicators-enterococci>
- [10] World Health Organization (2021) Guidelines on Recreational Water Quality, 1: Coastal and Fresh Waters. World Health Organization.
<https://iris.who.int/handle/10665/342625>
- [11] Food and Drugs Administration (2026) Bacteriological Analytical Manual.
<https://www.fda.gov/food/laboratory-methods-food/bacteriological-analytical-manual-bam>
- [12] CONAMA Resolution (2000) Resolution n 274, 29th November 2000. Conselho Nacional do Meio Ambiente.
- [13] Companhia de Tecnologia de Saneamento de São Paulo (2018) Technical Standard L5 202, January 2018. Total Coliforms, Thermotolerant Coliforms and *Escherichia coli*-Determination by the Multiple Tube Technique. CETESB.
<https://www.cetesb.sp.gov.br/dx/api/dam/v1/collections/defd2f83-b83a-4bc3-afba-d3d6f15108b5/items/0465010b-bc07-4671-88c3-49f2927bc473/renditions/b7da71f3-745a-415a-99bd-5c273c596de6?binary=true>
- [14] Weinbauer, M.G. and Höfle, M.G. (1998) Significance of Viral Lysis and Flagellate Grazing as Factors Controlling Bacterioplankton Production in a Eutrophic Lake. *Applied and Environmental Microbiology*, **64**, 431-438.
<https://doi.org/10.1128/aem.64.2.431-438.1998>
- [15] Bosch, A., Guix, S., Sano, D. and Pintó, R.M. (2008) New Tools for the Study and Direct Surveillance of Viral Pathogens in Water. *Current Opinion in Biotechnology*, **19**, 295-301. <https://doi.org/10.1016/j.copbio.2008.04.006>
- [16] Brazilian Institute of Geography and Statistics (2022) Census: Sewerage Reaches 62.5% of the Population, but Regional Inequalities and Inequalities by Color and Race Persist.
<https://static.poder360.com.br/2024/02/censo-2022-caracteristicas-domicilios.pdf>
- [17] Makuwa, S., Tlou, M., Fosso-Kankeu, E. and Green, E. (2020) Evaluation of Fecal Coliform Prevalence and Physicochemical Indicators in the Effluent from a Wastewater Treatment Plant in the North-West Province, South Africa. *International Journal of Environmental Research and Public Health*, **17**, Article 6381.
<https://doi.org/10.3390/ijerph17176381>
- [18] Adefisoye, M.A. and Okoh, A.I. (2016) Identification and Antimicrobial Resistance Prevalence of Pathogenic *Escherichia coli* Strains from Treated Wastewater Effluents in Eastern Cape, South Africa. *MicrobiologyOpen*, **5**, 143-151.
<https://doi.org/10.1002/mbo3.319>
- [19] Bivins, A., Greaves, J., Fischer, R., Yinda, K.C., Ahmed, W., Kitajima, M., *et al.* (2020) Persistence of SARS-CoV-2 in Water and Wastewater. *Environmental Science & Technology Letters*, **7**, 937-942. <https://doi.org/10.1021/acs.estlett.0c00730>
- [20] Wani, H., Menon, S., Desai, D., D'Souza, N., Bhatena, Z., Desai, N., *et al.* (2023) Wastewater-Based Epidemiology of SARS-CoV-2: Assessing Prevalence and Correlation with Clinical Cases. *Food and Environmental Virology*, **15**, 131-143.
<https://doi.org/10.1007/s12560-023-09555-2>
- [21] Bhatia, S., Maswanganye, T.N., Jeje, O., Winston, D., Lamssali, M., Deng, D., *et al.* (2025) Wastewater Speaks: Evaluating Sars-Cov-2 Surveillance, Sampling Methods,

- and Seasonal Infection Trends on a University Campus. *Microorganisms*, **13**, 924. <https://doi.org/10.3390/microorganisms13040924>
- [22] Dutra, L.B., Stein, J.F., da Rocha, B.S., Berger, A., de Souza, B.A., Prandi, B.A., et al. (2024) Environmental Monitoring of SARS-CoV-2 in the Metropolitan Area of Porto Alegre, Rio Grande Do Sul (RS), Brazil. *Environmental Science and Pollution Research*, **31**, 2129-2144. <https://doi.org/10.1007/s11356-023-31081-8>
- [23] Gonçalves-Brito, A.S., Magaldi, M., Farias, B.O., Nascimento, A.P.A., Flores, C., Montenegro, K.S., et al. (2023) Environmental Genomic Surveillance of SARS-CoV-2 in Wastewater in Rio De Janeiro, Brazil. *Journal of Water and Health*, **21**, 653-662. <https://doi.org/10.2166/wh.2023.034>
- [24] Medeiros, M.S., Silva, L.A., Medeiros, Z.M., Souza, R.F., Cruz, J.R., Silva Filho, E.C., et al. (2024) Towards a New Governance Model for Basic Sanitation Management in Amazonas State: A System of Guarantees of Rights-Based Approach. *Saúde em Debate*, **48**, e8724. <https://doi.org/10.1590/2358-289820241438724i>
- [25] Cavalcante, J.R. and Abreu, A.J.L. (2020) COVID-19 no município do Rio de Janeiro: Análise espacial da ocorrência dos primeiros casos e óbitos confirmados. *Epidemiologia e Serviços de Saúde*, **29**, e2020204. <https://doi.org/10.5123/s1679-49742020000300007>
- [26] Sherchan, S., Shahin, S., Alarcon, J., Brosky, H., Potter, C. and Dada, A.C. (2022) Microbial Source Tracking of Fecal Contamination in Stormwater Runoff. *Journal of Water and Health*, **20**, 1271-1283. <https://doi.org/10.2166/wh.2022.286>
- [27] Pinon, A. and Vialette, M. (2018) Survival of Viruses in Water. *Intervirology*, **61**, 214-222. <https://doi.org/10.1159/000484899>
- [28] Zare Jeddi, M., Hopf, N.B., Louro, H., Viegas, S., Galea, K.S., Pasanen-Kase, R., et al. (2022) Developing Human Biomonitoring as a 21st Century Toolbox within the European Exposure Science Strategy 2020-2030. *Environment International*, **168**, Article 107476. <https://doi.org/10.1016/j.envint.2022.107476>
- [29] Chan, K., Sridhar, S., Zhang, R.R., Chu, H., Fung, A.Y., Chan, G., et al. (2020) Factors Affecting Stability and Infectivity of SARS-CoV-2. *Journal of Hospital Infection*, **106**, 226-231. <https://doi.org/10.1016/j.jhin.2020.07.009>
- [30] Adolf, J.E., Weisburg, J., Hanna, K. and Lohnes, V. (2022) Enterococcus Exceedances Related to Environmental Variability at New Jersey Ocean Beaches. *Environmental Monitoring and Assessment*, **195**, Article No. 250. <https://doi.org/10.1007/s10661-022-10788-0>
- [31] Toledo, L.G.D. and Nicolella, G. (2002) Índice de qualidade de água em microbacia sob uso agrícola e urbano. *Scientia Agricola*, **59**, 181-186. <https://doi.org/10.1590/s0103-90162002000100026>
- [32] Jin, Y., Cai, L., Cheng, Z., Cheng, H., Deng, T., Fan, Y., et al. (2020) A Rapid Advice Guideline for the Diagnosis and Treatment of 2019 Novel Coronavirus (2019-Ncov) Infected Pneumonia (Standard Version). *Military Medical Research*, **7**, 4-26. <https://doi.org/10.1186/s40779-020-0233-6>
- [33] Sangsanont, J., Rattanakul, S., Kongprajug, A., Chyerochana, N., Sresung, M., Sripokratana, N., et al. (2022) SARS-CoV-2 RNA Surveillance in Large to Small Centralized Wastewater Treatment Plants Preceding the Third COVID-19 Resurgence in Bangkok, Thailand. *Science of The Total Environment*, **809**, Article 151169. <https://doi.org/10.1016/j.scitotenv.2021.151169>
- [34] Xiao, K., Zhai, J., Feng, Y., Zhou, N., Zhang, X., Zou, J., et al. (2020) Isolation of SARS-CoV-2-Related Coronavirus from Malayan Pangolins. *Nature*, **583**, 286-289. <https://doi.org/10.1038/s41586-020-2313-x>

- [35] Chen, Y., Chen, L., Deng, Q., Zhang, G., Wu, K., Ni, L., *et al.* (2020) The Presence of SARS-CoV-2 RNA in the Feces of COVID-19 Patients. *Journal of Medical Virology*, **92**, 833-840. <https://doi.org/10.1002/jmv.25825>
- [36] Cahill, N. and Morris, D. (2020) Recreational Waters—A Potential Transmission Route for SARS-CoV-2 to Humans? *Science of The Total Environment*, **740**, Article 140122. <https://doi.org/10.1016/j.scitotenv.2020.140122>
- [37] World Health Organization (2020) First Data on Stability and Resistance of SARS Coronavirus Compiled by Members of WHO Laboratory Network. https://www.who.int/csr/sars/survival_2003_05_04/en/
- [38] Hindson, J. (2020) COVID-19: Faecal-Oral Transmission? *Nature Reviews Gastroenterology & Hepatology*, **17**, 259. <https://doi.org/10.1038/s41575-020-0295-7>