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.
Spectre of SARS-CoV-2 RNA in the ambient urban waters of Ahmedabad and Guwahati: A tale of two Indian cities

Manish Kumar a,*, Vaibhav Srivastava b, Payal Mazumder c, Jyoti Prakash Deka d, Shilangi Gupta b, Ritusmita Goswami e, Pravin K. Mutiyar f, Shyamnarayan Dave g, Chandan Mahanta h, A.L. Ramanathan i, Madhvi Joshi j

a Sustainability Cluster, School of Engineering, University of Petroleum & Energy Studies, Dehradun, Uttrakhand, 248007, India
b Discipline of Earth Science, Indian Institute of Technology Gandhinagar, Gujarat, 382 355, India
c Centre for the Environment, Indian Institute of Technology Guwahati, Assam, 781039, India
d Discipline of Environmental Sciences, Gauhati Commerce College, Guwahati, Assam, 781021, India
e Centre for Ecology, Environment and Sustainable Development, Tata Institute of Social Sciences, Guwahati, Assam, 781013, India
f National Mission for Clean Ganga, Department of Water Resources, Ministry of Jal Shakti, Govt. of India, New Delhi, India
g UNICEF Gujarat State Office, Sector- 20, Gandhinagar, Gujarat, 382021, India
h Department of Civil Engineering, Indian Institute of Technology Guwahati, Assam, 781039, India
i School of Environmental Sciences, Jawaharlal Nehru University, New Delhi, 110067, India
j Gujarat Biotechnology Research Centre (GBRC), Sector- 11, Gandhinagar, Gujarat, 382 011, India

ARTICLE INFO

Keywords:
COVID-19
Urban water
Wastewater
Surveillance
SARS-CoV-2 RNA

ABSTRACT

COVID-19 positive patients can egest live SARS-CoV-2 virus and viral genome fragments through faecal matter and urine, raising concerns about viral transmission through the faecal-oral route and/or contaminated aerosolized water. These concerns are amplified in many low- and middle-income countries, where raw sewage is often discharged into surface waterways and open defecation is common. Nonetheless, there has been no evidence of COVID-19 transmission via ambient urban water, and the virus viability in such aquatic matrices is believed to be minimal and not a matter of concern. In this manuscript, we attempt to discern the presence of SARS-CoV-2 genetic material (ORF-1ab, N and S genes) in the urban waterways (lakes, rivers, and drains) of the two Indian cities viz., Ahmedabad (AMD), in western India with 9 wastewater treatment plants (WWTPs) and Guwahati (GHY), in the north-east of the country with no such treatment facilities. The present study was carried out to establish the applicability of environmental water surveillance (E-wat-Surveillance) of COVID-19 as a potential tool for public health monitoring at the community level. 25.8% and 20% of the urban water samples had detectable SARS-CoV-2 RNA load in AMD and GHY, respectively. N-gene > S-gene > ORF-1ab-gene were readily detected in the urban surface water of AMD, whereas no such observable trend was noticed in the case of GHY. The high concentrations of SARS-CoV-2 genes (e.g., ORF-1ab; 800 copies/L for Sabarmati River, AMD and S-gene; 565 copies/L for Bharalu urban river, GHY) found in urban waters suggest that WWTPs do not always completely remove the virus genetic material and that E-wat-Surveillance of COVID-19 in cities/rural areas with poor sanitation is possible.

Credit author statement

Manish Kumar: Conceptualization, Supervision, Visualization, Project administration, Writing & editing, Data curation and interpretation, and revision. Vaibhav Srivastava: Writing – original draft & editing, sampling and analysis, Data curation and interpretation, illustration plotting and revision Payal Mazumder: Writing original draft & editing, illustration plotting and revision. Jyoti Prakash Deka: Sampling and analysis, illustration plotting and revision. Shilangi Gupta: Analysis & data curation. Ritusmita Goswami: Analysis, Data curation and interpretation Pravin K Mutiyar: Conceptualization and interpretation Shyamnarayan Dave: Project supervision and administration. Chandan Mahanta: Conceptualization, sampling and interpretation. AL. Ramanathan: Conceptualization, and interpretation, Madhvi Joshi:...
Conceptualization & Project administration.

1. Introduction

Viruses are reported to occur in the surface water and believed to impact environmental and human health (Kuroda et al., 2015; Kauppinen et al., 2018; Sekwadi et al., 2018; Kumar et al., 2019). The absence of a sufficient sewage collection and treatment system is likely to exacerbate the situation, especially in developing countries owing to high population density, discharge of (often unregulated) domestic and industrial effluents, and ineffective wastewater treatment (Samaraweera et al., 2019; Guerrero-Latorre et al., 2020; Kozer et al., 2021). It is well recognized that enteric viruses may enter into the aquatic environments through several routes such as water outflows due to heavy rainfall, combined sewer outflows, blockages or sanitation system failures, etc. (Fong et al., 2010; Kumar et al., 2019). The coronavirus that causes severe acute respiratory syndrome (SARS) may infect the gastrointestinal system and be shed in the environment, building the threat of potential faecal-oral transmission (Ding and Liang, 2020; Hindson, 2020). Many studies demonstrated the occurrence of SARS-CoV-2 RNA in wastewater (Medema et al., 2020; Sherchan et al., 2020; Kumar et al., 2020a, 2021a,b,c; Srivastava et al., 2021) and natural water bodies (Fongaro et al., 2021; Kolarević et al., 2021) around the globe. Likewise, Mahbubneet et al. (2021) reported the first study on detecting SARS-CoV-2 RNA in groundwater in Monterrey, Mexico. In another study, de Oliveira et al. (2021) detected SARS-CoV-2 in artificially spiked river water (filtered and unfiltered) at two different temperatures i.e., 4 °C and 24 °C, through plaque assays.

Owing to the enteric involvement, the prevalence of SARS-CoV-2 in the environmental aquatic matrices is likely to increase considerably during the ongoing COVID-19 pandemic situation, posing a severe health risk to humans via faecal-oral transmission or aerosolization of virus-containing water droplets (Yeo et al., 2020; Lodder and de Roda Husman, 2020; Naddeo and Liu, 2020). Mancuso et al. (2021) assessed how SARS-CoV-2 might infiltrate the urban water cycle and subsequently spread from urban to rural water environments, posing a possible risk to agricultural produce and human health. There are several routes for SARS-CoV-2 to enter the ambient environment, including short-circuiting of wastewater discharge into urban waters and inadequate viral elimination during treatment (Kumar et al., 2020b). Many reports suggest that WWTPs can successfully remove SARS-CoV-2 RNA (Randazzo et al., 2020; Sherchan et al., 2020; Arora et al., 2021); however, some studies showed the presence of SARS-CoV-2 genetic material in treated wastewater (Wurtzer et al., 2020; Westhaus et al., 2021; Joshi et al., 2021; Kumar et al., 2021b). The contamination of natural water bodies via the discharge of untreated or partially treated wastewater extends a new dimension to wastewater-based epidemiology (WBE) surveillance by including surface water monitoring. Therefore, it is imperative to include ambient urban water bodies (lakes, rivers, and drains) to improve the efficacy of WBE through environmental water surveillance (E-wat-Surveillance).

The estimates of viral genetic load in aqueous matrices might also be useful for Quantitative Microbiological Risk Assessment (QMRA), as a precautionary approach to optimize public health interventions (Zaneti et al., 2020; Dada and Gyawali, 2021; Kumar et al., 2021a). However, no solid proof of the survival of viable SARS-CoV-2 in ambient waters and its transmissibility from such aqueous matrices has been established till now. Despite this, the World Health Organization (WHO, 2020) has emphasized the need to investigate the presence of novel coronavirus in environmental matrices, including surface water and wastewater (Estimating mortality from COVID-19, 2020).

Overall considering the millions of infections, fatalities, risk of future waves, reports of genetic traces of SARS-CoV-2 in surface water, and potential transmission via contaminated water to animals or humans, we conducted the monitoring of SARS-CoV-2 RNA in different ambient urban water bodies of two Indian cities i.e., Ahmedabad (AMD) (Gujarat) and Guwahati (GHY) (Assam). Cities are selected such that the former (AMD) has nine wastewater treatment plants (WWTPs) of various capacities (35–240 MLD) and a proper drainage system, and the latter (GHY) do not have even a single treatment plant available and no such drainage system to cater its citizens.

The present study aimed at i) understanding the frequency of positive occurrences of SARS-CoV-2 RNA in the urban water bodies of AMD and GHY; as well as ii) comparative assessment of the vulnerability of urban waters (mainly river and lakes) in a city set up amid the silhouette of COVID-19 clinical cases. The present study is critical since numerous developing and underdeveloped countries have poorly maintained sewerage systems, resulting in wastewater leakages and common sewage overflow concerns.

2. Materials and methods

2.1. Study area and sampling location

Ahmedabad (AMD) is the most populous and largest city in Gujarat, comprising a population of 5.5 million as per census 2011. The Sabarmati River runs through the city’s center. The sewerage system of AMD comprises 9 WWTPs, 45 Wastewater Pumping Stations (WWPSs), and a sewerage network of 2500 km. On the other hand, Guwahati (GHY), known as the gateway of north-eastern India, exhibits rapid and unplanned urban growth with around million city residents as per the 2011 census. The Brahmaputra River, an international transboundary, the fifteenth longest and the ninth largest river in discharge, provides one side boundary to the city. While the Bharalu River, a tributary of Brahmaputra River, flows through the dense urban regions of GHY city. Deepor Beel Lake is a natural freshwater lake/wetland system recognized under the Ramsar Convention that provides another side of the city. The Bharal River has now virtually become an urban drain. There is no single WWTP present in GHY city, and wastewater dilution might be the main solution of the wastewater in GHY due to relatively higher annual rainfall (~2054 mm) with 91.9 average rainy days. The Brahmaputra River’s continual flow absolves the municipality of the duty of managing all of its sewage, which is usually discharged directly into the river.

2.2. Sample collection and preparation

In AMD, three lakes i.e., Kankaria, Chandola, and Vastrapur, were sampled weekly from Sep 3, 2020 to Oct 1, 2020, while a river i.e., Sabarmati, was sampled weekly from Sep 3, 2020 to Dec 28, 2020 (Fig. 1a). A total of thirty-one samples were collected and scrutinized for the presence of SARS-CoV-2 RNA in AMD. On the other hand, In GHY, ten samples representing Deepor Beel lake, the Brahmaputra river, the Bharalu river turned drain, the Urban drains (near Covid Centre, Kahanpara and IIT Guwahati) were collected on Oct 27, 2020, followed by analysis (Fig. 1b).

The samples were collected using the grab sampling technique in 500 ml polyethylene sterile bottles (Tarsons, PP Autoclavable, Wide Mouth Bottle, Cat No. 582240, India) and transferred in an ice-box to the laboratory and refrigerated at 4 °C until further process. The sample blanks were prepared and analyzed to control for cross-contamination during transportation. Samples from GHY were transported in a sealed ice-box by air-mail within the same day of sampling, and RNA extraction was performed within 72 h of sampling at Gujarat Biotechnology Research Centre (GBRC), Gandhinagar, Gujarat.

Polyethylene glycol (PEG) based precipitation method was used for concentrating the sample as explained by (Kumar et al., 2020a). Briefly, 30 ml sample was centrifuged (Model: Sorvall ST 40R, Thermo Scientific) at 4000g for 30 min in a 50 ml Falcon tube followed by the filtration of the supernatant with a syringe filter of 0.2μ (Mixed cellulose esters syringe filter, Himedia). The filtrate was then treated with NaCl (17.5 g/L) and PEG 9000 (80 g/L) and incubated at 100 rpm overnight at
To make the pellet, the solution was then subjected to ultra-centrifugation at 14000 g for 90 min (Model: Incu-Shaker™ 10LR, Benchmark). RNase-free water was used to resuspend the pellet containing viral particles, which was then stored in a 1.5 ml Eppendorf tube at a temperature of 4°C until RNA isolation.

The PEG concentration method was standardized considering the influence of various factors, including sample volume, temperature, RCF, and amount of PEG and NaCl, and other working conditions. The detailed workflow concept has been depicted in Fig. 2.

2.3. SARS-CoV-2 viral RNA isolation, RT-qPCR, and gene copy estimation

A commercially ready-for-use kit (NucleoSpin® RNA Virus, Macherey-Nagel GmbH & Co. KG, Germany) was used to isolate SARS-CoV-2 RNA. MS2 phage (10 μL), Proteinase K (20 μL) and RAV1 buffer (600 μL) consisting of carrier RNA were mixed with 300 μL of the concentrated viral particles. MS2 phage serves as the molecular process inhibition control. It was used to monitor the efficacy of RNA extraction and PCR inhibition. It should be remembered that MS2 may spontaneously exist in wastewater, so there is a risk that the retrieved MS2 may consist of both the spiked and the background viral material. As per the
user manual instructions (Macherey-Nagel GmbH & Co. KG), further procedures were carried out. The last elution was done with 30 μL of kit-supplied elution buffer. Using a Qubit 4 Fluorometer (Invitrogen), RNA concentrations were checked.

The nucleic acid was analyzed to identify the S gene, N gene, and ORF1ab of SARS-CoV-2 and the internal control (MS2) with the help of RT-PCR using the TaqPath™ Covid-19 RT-PCR package (Applied Biosystems). Amplification was conducted in a reaction (25 μL) vial containing 7 μL of RNAs derived from each sample, 10.50 μL Nuclease-free Water, 6.25 μL Master Mix, and 1.25 μL COVID-19 RT-PCR Assay Multiplex. 2 μL of the positive control (TaqPath™ COVID-19 Control) and refined 5 μL of negative control were used for the study. Nuclease-free water was applied as a template-free control in this analysis. Additional process steps were executed, as defined in the product guidebook. The RT-qPCR step consisting of 40 cycles, included UNG incubation (25 °C for 2 min), reverse transcription (53 °C for 10 min), and activation (95 °C for 2 min). The reactions were conducted and elucidated as instructed in the handbook of Applied BiosystemsTM 7500 Fast Real-Time PCR. A sample was considered as positive if two out of three genes (N, ORF 1ab, and S) showed amplification in the qRT-PCR assay. Averaging the copies per litre of all three genes in a given sample yielded the effective gene concentration (EGC). The SARS-CoV-2 gene copy was estimated following the approach explained in the author’s previous publications (Kumar et al., 2020a, 2021b, 2021c).

3. Results and discussion

Samples collected from urban surface waters of Ahmedabad (AMD) (Sabarmati River and Kankaria, Chandola, and Vastrapur lakes), Gujarat, India, revealed a considerable variation in SARS-CoV-2 RNA titre. The average Ct value of the internal control i.e., MS2 bacteriophage in urban water samples (n = 31) of AMD was found to be 27.29 ± 0.91. The analogy of qRT-PCR analysis to determine the virus genetic material (N, S, and ORF 1 ab genes) showed 25.8% (8/31) positive samples. The N-gene copies at different sampling points were found to be maximum in Sabarmati River (694 copies/L; Dec 28, 2020), followed by Kankaria (549 copies/L; Sep 10, 2020) and Chandola (137 copies/L; Oct 1, 2020) while, Vastrapur did not show the presence of N-gene (Table 1a). The ORF 1 ab-gene copies were found maximum in samples collected from Sabarmati River (800 copies/L; Oct 29, 2020), followed by Kankaria (87 copies/L; Sep 10, 2020). Chandola and Vastrapur lake samples were negative for the ORF-1ab gene. Similarly, the S-gene copies climbed down from: Sabarmati River (490 copies/L; Dec 14, 2020) > Vastrapur (58 copies/L; Sep 3, 2020) > Chandola (52 copies/L; Sep 3, 2020) > Kankaria (45 copies/L; Sep 24, 2020) (Table 1a). Correspondingly, a higher SARS-CoV-2 effective gene concentration was observed in Sabarmati River (492 copies/L; Oct 29, 2020), followed by Kankaria (318 copies/L; Sep 10, 2020) and Chandola lake sample (75 copies/L; Oct 1, 2020) (Table 1a).

A comparison between the viral genetic load of urban surface water samples with the new confirmed cases on the sampling date didn’t show any conclusive trend. Such results can be attributed to the dilution of virus concentration in surface waters and the low filtration volume of samples (30 mL). These findings are analogous to those of Haramoto et al. (2020), who were unable to detect SARS-CoV-2 RNA in influent wastewater samples due to a greater detection limit and a smaller sample filter volume. It is worth noting that even at a low filtration volume of samples, we found 8/31 positive samples comprising two out of three genes. Similarly, 17/31 samples had at least one SARS-CoV-2 gene in AMD, showing the robustness of the PEG precipitation based viral concentration method. In Dec 2020, there was a substantial increase in viral RNA levels in the Sabarmati River compared to the other months (Table 1a). The N-gene was detected in many samples even though the samples were negative for the ORF-1ab gene and S-gene. These findings can be ascribed to a) sparse concentration of RNA for gene-specific amplification; b) N-gene is more stable in ambient surface
water compared to the ORF-1ab and S-genes; c) sub-genomic N gene messenger RNAs are more abundant than other targets (Ogando et al., 2020).

Similar results were reported by Kumar et al. (2021c), who noticed the resistant nature of N-gene followed by S-and ORF-1ab genes in wastewater samples from AMD, India. Despite the lack of a clear link between the SARS-CoV-2 RNA concentrations in surface water and COVID-19 cases, a weekly examination of wastewater samples from nine different locations in AMD during the same period (Fig. S1) showed a positive association between viral RNA load and COVID-19 cases (Fig. 3). The effective gene concentrations of SARS-CoV-2 were found to be maximum in November 2020, ranging from 3.0 to $25.3 \times 10^3$ copies/L, correlating to a notable increase in new confirmed cases (~245 per day). In contrast, the minimum gene concentrations of $1.06 - 7.42 \times 10^2$ were found in October 2020, equating to ~182 new cases per day (Fig. 3). The descending order of effective gene concentrations in AMD was November > September > December > October (Fig. S2). The authors thoroughly described SARS-CoV-2 RNA loads in wastewater and their relationship to secondary clinical data of AMD city in their previous study (Kumar et al., 2021b).

The average Ct value of MS2 bacteriophage in urban water samples ($n = 10$) of GHY was $27.04 \pm 0.64$. The urban water samples collected from GHY (Deepor Beel lake, Brahmaputra River, and a drain present in an academic institution i.e., IITG) showed negative results for SARS-CoV-2 RNA. While one sample from the drain near a COVID care centre (Kahnapara) and one sample from Bharalu river turned drain (AIDC) tested positive for the presence of the viral genetic material, thus showing 20% (2/10) positive results for the sampled locations. The N-gene, ORF-1ab gene, and S-gene copies were found to be maximum in the COVID care centre i.e., 9169, 4153, and 3580 copies/L, respectively, than that of Bharalu river turned drain (Table 1b). However, in the Bharalu river, the S-gene concentration was found to be the highest ($565$

| Sampling date | Location            | Ct value | Gene copies (copies/L) |
|---------------|---------------------|----------|------------------------|
|               |                     | N-gene   | ORF1ab gene | S-gene | MS2  | N-gene | ORF1ab gene | S-gene | Effective gene concentration |
| 03.09.20      | Kankaria Lake       | 36.01    | ND          | ND     | 27.50 | 90     | 0        | 0     | INC               |
|               | Chandola Lake       | ND       | ND          | 36.94  | 27.88 | 0       | 0        | 52    | INC               |
|               | Vastapur Lake       | ND       | ND          | 36.75  | 28.15 | 0       | 0        | 58    | INC               |
|               | Sabarmati River     | ND       | ND          | 38.34  | 27.61 | 0       | 0        | 24    | INC               |
| 10.09.20      | Kankaria Lake       | 33.10    | 36.08       | ND     | 26.28 | 549     | 87       | 0     | 318               |
|               | Chandola Lake       | ND       | ND          | ND     | 27.65 | 0       | 0        | 0     | 0                 |
|               | Vastapur Lake       | ND       | ND          | ND     | 27.10 | 0       | 0        | 0     | 0                 |
|               | Sabarmati River     | ND       | ND          | ND     | 26.68 | 0       | 0        | 0     | 0                 |
| 17.09.20      | Kankaria Lake       | 37.87    | ND          | ND     | 27.77 | 31       | 0        | 0     | INC               |
|               | Chandola Lake       | ND       | ND          | ND     | 27.16 | 0       | 0        | 0     | 0                 |
|               | Vastapur Lake       | ND       | ND          | ND     | 27.94 | 0       | 0        | 0     | 0                 |
|               | Sabarmati River     | ND       | ND          | ND     | 27.61 | 0       | 0        | 0     | 0                 |
| 24.09.20      | Kankaria Lake       | 37.33    | ND          | ND     | 28.60 | 42       | 0        | 0     | INC               |
|               | Chandola Lake       | ND       | ND          | ND     | 28.18 | 0       | 0        | 0     | 0                 |
|               | Vastapur Lake       | ND       | ND          | ND     | 28.18 | 0       | 0        | 0     | 0                 |
|               | Sabarmati River     | ND       | ND          | ND     | 28.63 | 0       | 0        | 0     | 0                 |
| 01.10.20      | Kankaria Lake       | 35.67    | ND          | ND     | 28.36 | 111      | 0        | 0     | 0                 |
|               | Chandola Lake       | 35.31    | ND          | 39.64  | 28.28 | 137      | 0        | 12    | 75                |
|               | Vastapur Lake       | ND       | ND          | ND     | 27.21 | 0       | 0        | 0     | 0                 |
|               | Sabarmati River     | 35.51    | ND          | ND     | 26.78 | 122      | 0        | 0     | INC               |
| 08.10.20      | Sabarmati River     | 37.70    | 35.78       | 36.86  | 27.79 | 34       | 104       | 55    | 64                |
| 15.10.20      | ND                  | 38.46    | 35.67       | 37.14  | 26.93 | 22       | 110       | 47    | 60                |
| 22.10.20      | ND                  | ND       | ND          | ND     | 25.59 | 0       | 0        | 0     | 0                 |
| 29.10.20      | ND                  | 33.07    | 32.52       | 35.57  | 27.93 | 559      | 800       | 118   | 492               |
| 05.11.20      | ND                  | ND       | ND          | ND     | 27.13 | 0       | 0        | 0     | 0                 |
| 12.11.20      | ND                  | ND       | ND          | ND     | 27.94 | 0       | 0        | 0     | 0                 |
| 19.11.20      | ND                  | ND       | ND          | ND     | 36.96 | 26.94    | 0        | 0     | 52                |
| 26.11.20      | ND                  | ND       | ND          | ND     | 25.30 | 0       | 0        | 0     | 0                 |
| 14.12.20      | ND                  | 34.70    | 35.42       | 33.27  | 25.61 | 199      | 129       | 490   | 273               |
| 21.12.20      | ND                  | ND       | ND          | ND     | 25.80 | 0       | 0        | 0     | 0                 |
| 28.12.20      | ND                  | 32.73    | 33.80       | 39.96  | 26.58 | 694      | 350       | 10    | 351               |

Where; ND= Not detected, and INC= Detected but data inconclusive.

Table 1a
Occurrence of SARS-CoV-2 RNA in the urban surface water samples collected from different locations in Ahmedabad (AMD).
copies/L), followed by N-gene (549 copies/L) and ORF-1ab gene (435 copies/L). Evidently, a larger effective gene concentration was observed in the COVID care facility (5634 copies/L) than the Bharalu river turned drain (516 copies/L) (Table 1b). The negative detection of the SARS-CoV-2 gene in most GHY samples corresponded to the decrease in clinical cases during the sampling time (October 2020). However, samples collected from the drain near the COVID care facility showed positive and highest viral load, presumably due to treatment of diseased cases. A graph illustrating the SARS-CoV-2 effective gene concentration as compared to clinical positive confirmed cases in Ahmedabad (AMD) (Kumar et al., 2021b) and Guwahati (GHY), India. Note: Daily data of new confirmed cases were not available for GHY; Effective gene concentration (EGC) reflects the average value of nine wastewater samples in AMD on each sampling date (See Fig. S1). Likewise, EGC represents the average value of four wastewater samples in GHY. Clinical data of COVID-19 cases obtained from COVID-19 INDIA database (COVID-19 INDIA database, https://www.covid19india.org/).

Table 1b  
SARS-CoV-2 gene concentration in urban water samples collected from Guwahati (GHY) on Oct 27, 2020.

| Source                      | Location         | Ct Value   | Gene copies (copies/L) | Effective gene conc. |
|-----------------------------|------------------|------------|------------------------|----------------------|
| Freshwater lake             | Deepor Beel      | ND         | ND                     | ND                   |
| (L1-L3)                     | (Borgaoan)       | ND         | ND                     | ND                   |
|                            | Deepor Beel-1    | ND         | ND                     | ND                   |
|                            | Deepor Beel-2    | ND         | ND                     | ND                   |
| Bharalu river turned drain | AIDC             | 33.10      | 33.45                  | 33.05                |
| (B1,B2)                     | Bhangagarh       | 33.10      | 33.45                  | 33.05                |
| Brahmaputra river           | Kharguli          | ND         | ND                     | 26.69                |
| (R1-R3)                     | Uzan Bazar       | ND         | ND                     | 27.66                |
|                            | Pandu            | ND         | ND                     | 26.55                |
| Drain (IITG)                | IITG             | ND         | ND                     | 25.91                |
| COVID centre drain          | Khanapara        | 29.01      | 30.11                  | 30.32                |

Where: ND= Not detected, and INC= Detected but data inconclusive.
individuals. Similarly, the Bharalu River, which has now been turned into a drain, showed positive detection in one sample. This is because the Bharalu River flows through the city’s centre and collects resident sewage, including asymptomatic, presymptomatic, and symptomatic individuals, before joining the Brahmaputra River.

Conversely, negative detection of SARS-CoV-2 in surface water samples (i.e., the Brahmaputra River and Deepor Beel lake) can be ascribed to, i) dilution of viral load in natural water bodies; ii) low filtration volume of sample (30 mL) in the concentration method; iii) low rate of clinical cases. The findings imply that the Bharalu river (a river turned to drain) carrying viral genetic material can serve as a core of environmental water surveillance (E-wat-Surveillance) for COVID-19-like pandemics. Because the urban water samples in GHY were only examined on Oct 27, 2020 and clinical data of GHY was not available; therefore, a temporal comparison between SARS-CoV-2 RNA burden and clinical cases was not feasible for GHY. The numbers of new confirmed cases were surprisingly high in Sep 2020 and followed a declining trend over the study period in Assam (Fig. 3). The presence of SARS-CoV-2 RNA signatures in GHY’s urban water bodies was probably owing to the unmediated discharge of sewage water from a population of roughly one million people.

The findings of the present study are comparable to those of Kolarutic et al. (2021), who detected SARS-CoV-2 RNA in the Danube River from Belgrade, Serbia, linked with the discharge of untreated wastewaters. The concentrations of SARS-CoV-2 RNA (N1, N2, and E-genes) varied from $5.97 \times 10^7$ to $1.32 \times 10^4$ copies/L owing to untreated wastewater contamination. Likewise, Fongaro et al. (2021) investigated upstream and downstream river waters in the Minas Gerais State region of southern Brazil (August 2020). The results indicated no evidence of SARS-CoV-2 RNA (N1 and N2 genes) in the upstream samples, but viral RNA averaging $1.1 \times 10^2$ to $1.7 \times 10^3$ genome copies/mL were present in the downstream samples. Mahlknecht et al. (2021) examined the occurrence of SARS-CoV-2 RNA in different freshwater matrices in urban settings, including groundwater, dams, and river waters in Monterrey Metropolitan Area, Mexico. They observed positive detection for SARS-CoV-2 RNA in 44% of the groundwater (2.6 to 38.3 copies/mL), 12% of the dam water (3.3 to 3.8 copies/mL), and 13% of the river water (2.5 to 7.0 copies/mL).

Overall, the present study demonstrated the microbial implications of untreated sewage discharge into natural waterways in both cities (AMD & GHY). WWTPs can remove SARS-CoV-2 RNA, thus, strengthening sanitation and health infrastructure. Also, as per the documented reports, WWTPs do not entirely eliminate SARS-CoV-2 genes and/or gene fragments (Haramoto et al., 2020; Randazzo et al., 2020; Kumar et al., 2021d), thereby adding another dimension to wastewater surveillance and suggest E-wat-Surveillance for COVID-19 management.

At present, the world is on the verge of a third COVID-19 wave, and India faces a slew of natural calamities in 2021, including earthquakes in Assam and cyclones such as Tautkai near the Gujarat coast and Yass in West Bengal and Odisha. In such cases, access to safe water, health, and hygiene during rehabilitation is pivotal. As a result, all potential SARS-CoV-2 RNA exposure paths must be scientifically evaluated, and the point of discharge must be identified and analyzed for microbiological contamination along with basic water quality indices. The findings of our study imply that E-wat-Surveillance may be applied to cities and rural areas as well where sewage is disposed directly into natural waterways. However, it is crucial to highlight that only SARS-CoV-2 genetic material has been traced in urban waters in the current study, and the virus’s survivability in contaminated urban waters is still unknown and debatable. Nonetheless, owing to the prevalence of zoonotic spillover episodes in the Coronavirusidae family, the genetic traces of SARS-CoV-2 and its possible transmission via environmental matrices (soil, air, and water) may have an undisclosed influence on domestic animals, wildlife, and human health (Franklin and Bevis, 2020). The present study supports that estimates of viral gene load in main sewage discharge and urban waters across the city can eventually be used as a surveillance criterion for a prompt warning system, assessing the effectiveness of containment, improving public health interventions, and enhancing decision making in economies with poor health infrastructure and limited clinical sample analysis (Bivins et al., 2020).

4. Conclusion

In the context of intermittent lockdown and progressive rise in COVID-19 cases in India, we attempted to trace the genetic signature of SARS-CoV-2 in ambient urban waters in two metropolitan cities of India viz., Ahmedabad (AMD) (Western zone) and Guwahati (GHY) (North-Eastern zone). The 8/31 and 2/10 urban water samples in AMD and GHY, respectively, showed positive RT-qPCR results for SARS-CoV-2 RNA (N, ORF 1 ab & S-genes). The inability of WWTPs to entirely eliminate viral RNA might be one of the reasons for its detection in AMD, further supporting the surveillance by monitoring ambient waters. In contrast, despite the absence of WWTPs in GHY, surface water samples (lake and river) lacked traces of SARS-CoV-2 RNA, owing to viral load dilution, low filtering volume during the viral concentration method, and fewer COVID-19 cases. The results suggest that surface waters receiving direct sewage or effluent discharge can be targeted for E-wat-Surveillance, providing valuable information on possible COVID-19 transmission, the need for sanitation, potential future risks, and effective management. The knowledge is also helpful to indicate a thorough investigation of the possibility of contagion in places with inadequate sanitation, where people are at risk of being exposed to polluted water or even raw sewage. Nevertheless, future research should focus on a) the modeling approach to assess the transmission risk of SARS-CoV-2 from urban water matrices; b) identifying the chemical and microbiological indicators for tracking SARS-CoV-2 routes, degradation, and inhibitor factors in water/ wastewater (Mahlknecht et al., 2021); c) study on the residence time and related fate of SARS-CoV-2 RNA in urban waters; d) understanding pathogen diversity (viral and bacterial) in water/wastewater in respect to the SARS-CoV-2 RNA load to support and improve WBE prediction; e) whole-genome sequencing of SARS-CoV-2 from urban waters to get information about circulating Variants of Concern (VOCs) and Variants of Interest (VOIs), and track their cryptic transmission.

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.

Acknowledgment

This work is funded by UNICEF, India, and UKIERI. We also acknowledge the help received from Dr. Arbind Patel and Dr. Nirav Raval, IIT Gandhinagar. The authors extend their sincere thanks to GBRC and GPCB staff, who contributed to the sample collection and analyses.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2021.112067.

References

Arora, S., Nag, A., Rajpal, A., Tyagi, V.K., Tiwari, S.B., Sethi, J., et al., 2021. Imprints of lockdown and treatment processes on the wastewater surveillance of SARS-CoV-2: a curious case of fourteen plants in northern India. Water 13 (16), 2265. https://doi.org/10.3390/w13162265.

Bivins, A., North, D., Ahmad, A., Ahmed, W., Alm, E., Beem, F., Bhattacharya, P., Bijlmakers, I., Boehn, A.B., Brown, J., Buttiglieri, G., Calabro, V., Carducci, A.,
