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Monitoring of Community Wastewater for Early Signaling the Spread of COVID-19 in Chennai City

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Background

Wastewater has emerged as a good indicator for the presence of **SARS-CoV-2** as confirmed by several research studies across the globe through the detection of SARSCoV-2 Ribo Nucleic Acid (RNA) in faeces of symptomatic and asymptomatic patients. Sewers or sewage treatment plants (STPs) also provide near-real-time outbreak data with the continuous collection of fluids shed by infected population. **Wastewater-based epidemiology (WBE)** thus emerges as a promising approach to comprehend the prevalence of viruses in a given wastewater treatment plant (WWTP) catchment population.

Several research investigations detected novel coronavirus SARS-CoV-2 in wastewater. In Switzerland, École Polytechnique Fédérale de Lausanne (EPFL) researchers, in association with the Swiss Federal Institute of Aquatic Science and Technology (EAWAG) developed a study in March 2020, as an early warning signal for spread of SARS-CoV-2 in communities. In April 2020, sewage sampling across greater Paris (France) for more than one month, detected a rise and fall in novel coronavirus concentrations that correspond to the shape of the SARS-CoV-2 outbreak. Detection of SARS CoV-2 in wastewater has been so far been reported from developed countries such as Australia (Ahmed et al, 2020), Cyprus (Michael-Kordatou et al, 2020), France (Wurtzer et al, 2020), Ireland (Cahill et al, 2020), Italy (La Rosa et al, 2020), Japan (Haramoto et al, 2020), Netherlands (Medema et al, 2020), Spain (Chavarria Miró

et al, 2020), Switzerland (Stringhini et al, 2020), and the USA (Peccia et al, 2020).

In India, the presence of three SARS-CoV-2 genes was detected in wastewater sampled from old Pirana WWTP at Ahmedabad, Gujarat in July 2020 (Kumar et al, 2020). Another study was conducted with wastewater samples from hospitals and WWTPs of Jaipur, Rajasthan, a pandemic hotspot (red zone) since April 2020. COVID-19 WBE Collaborative, an international project by 51 universities and research institutes including IIT Gandhinagar is a Consortium on a global scale for wastewater-based epidemiology of SARS-CoV-2. Further, Biobot Analytics collects and shares valuable information on the presence of SARS-CoV-2 in wastewater through a digital platform to spread awareness about the health of communities.

To analyse the presence of SARS-CoV-2 in the community wastewater of different catchments in Chennai city in India, **SRM Institute of Science and Technology, Mu Gamma Consultants Pvt. Ltd (MGC) and The Energy and Resources Institute (TERI)** jointly implemented an intensive wastewater surveillance study, the first-of-its-kind in the region. The study entails pre-monsoon and post monsoonal survey along the wastewater discharge points in Adayar and Cooum Riverine belt flowing through the densely populated region of **Chennai** to develop early signalling spread of COVID-19 in communities during **partial** and **post-lock down** periods in 2020.

Hypothesis

In the absence of effective measures to combat the spread of SARS-CoV-2, a densely populated country like India requires constant vigilance to contain COVID-19 infection hot spots. Real-time community sewage detection can aid restricting local population movements, thus contributing to minimise the pathogen spread. This could help the timely determination of whether there are COVID-19 carriers (symptomatic and asymptomatic) in an area to enable preventive measures such as rapid screening and quarantine. The possibility of evidence of the virus in city's wastewater even before patients show any symptoms, or prior to clinical diagnosis and even testing supports the hypothesis of this study. A hypothetical concept and motivation of this project is presented in **Figure 1**.
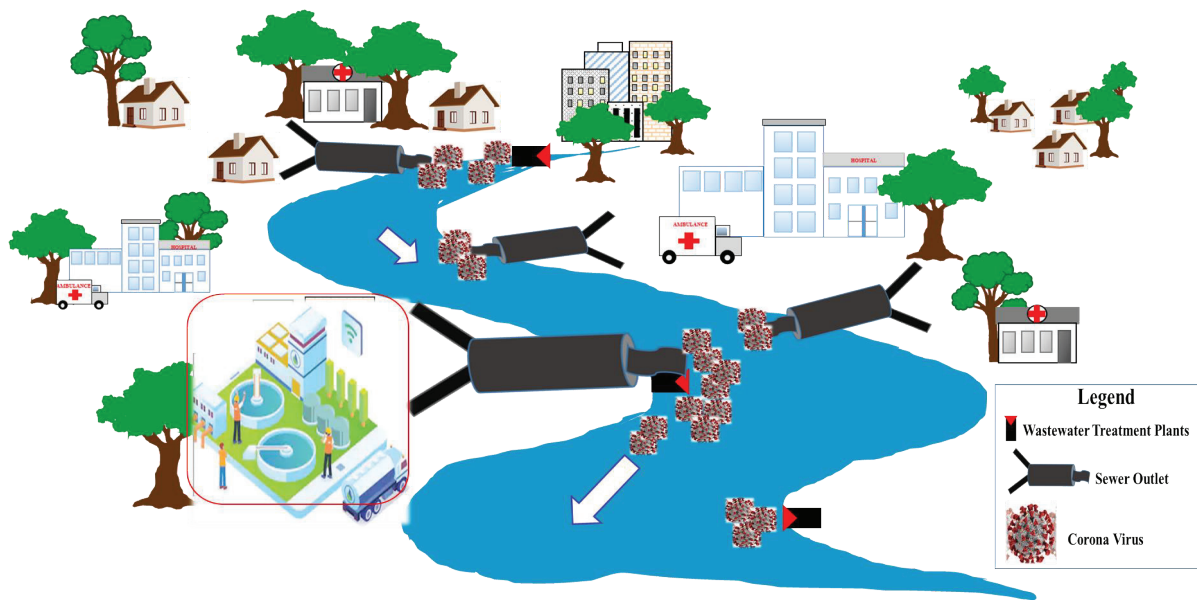

Figure 1: Hypothetical model of the study

Current status of COVID-19 pandemic

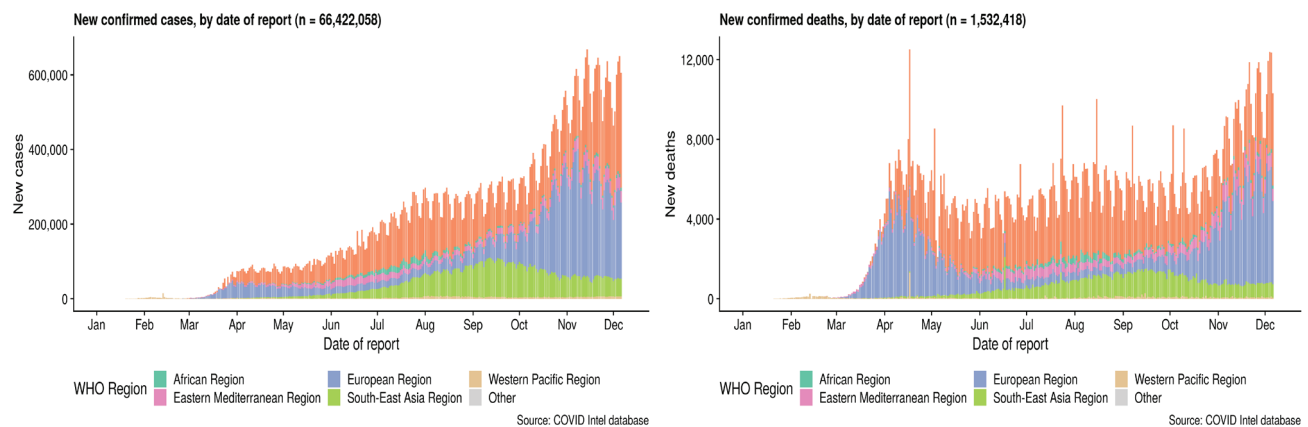### Global and Indian situation

Figure 2: Global situation of COVID-19 confirmed cases and deaths (Source: WHO, 2020)

Since February 2020, the confirmed COVID-19 cases have been on the rise globally as depicted in **Figure 2**. The cumulative cases as on December 8, 2020 are 66,422,058 with large recoveries. However, there has been 1,532,418 deaths.
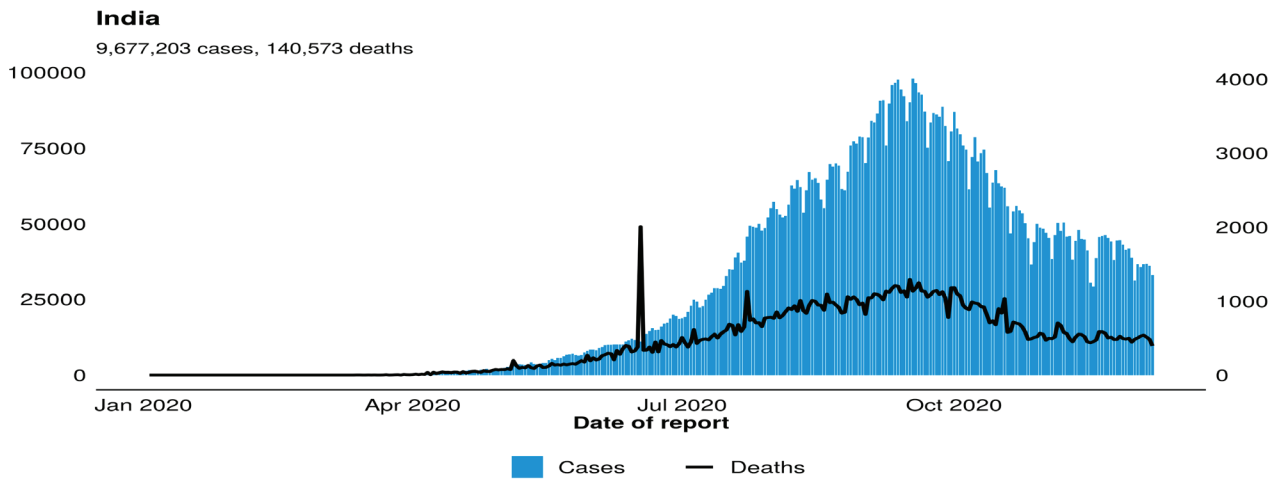

Figure 3: COVID-19 situation in India (Source: WHO, 2020)

In India the pandemic steadily rose from April 2020 with peaking in mid-August 2020 as presented in **Figure 3**. The cumulative confirmed cases as on November 1, 2020 are 9,677,203 with large recoveries. However, the death toll so far has been 140,573. The State-wise distribution of confirmed cases and deaths is presented in **Figures 4** A and B, which shows a comparison in number of cases in June and November 2020 which shows the status of total cumulative cases and deaths in States and UTs of India.
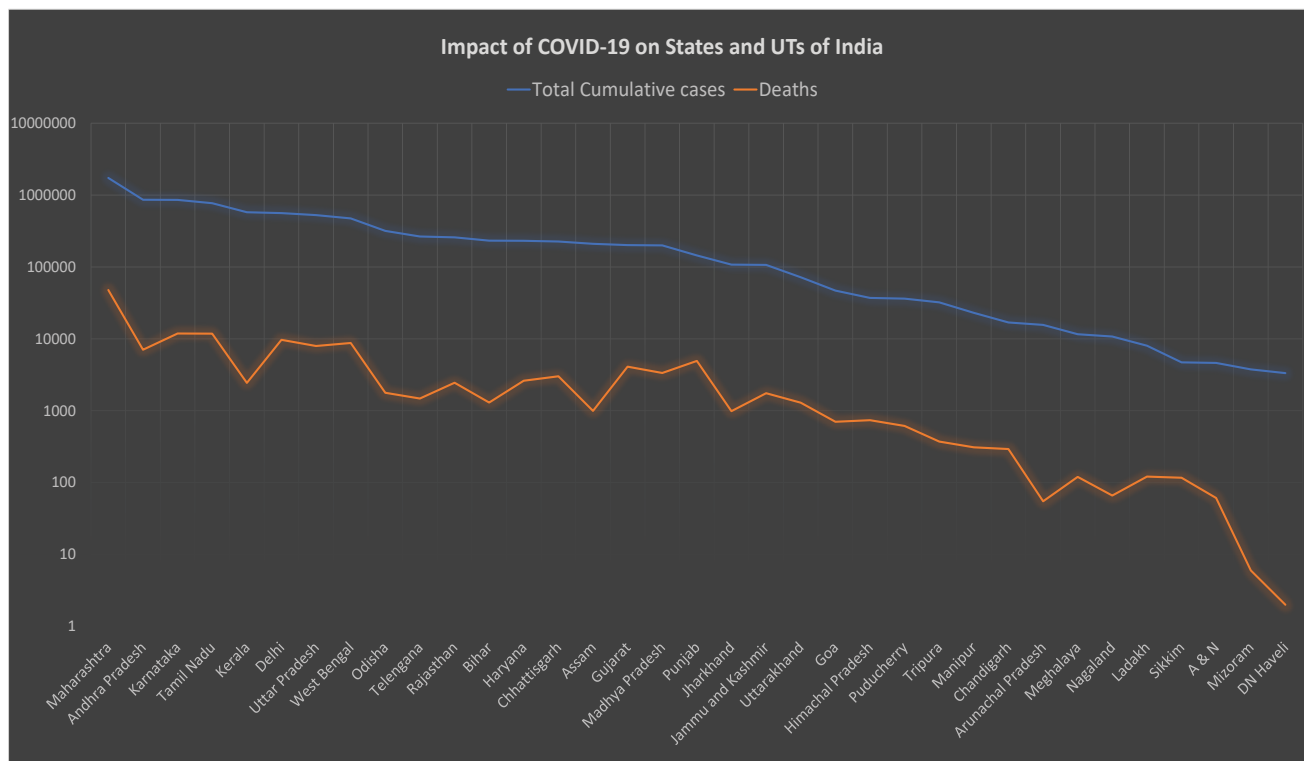

Figure 4 : Distribution of COVID-19 Cases in different States and UTs of India (Source: MoHFW, Government of India, 2020)

Pandemic scenario in Tamil Nadu and Chennai

The total positive cumulative cases in Tamil Nadu, as on December 8, 2020 is 781915 and 11712 deaths. In Chennai, the total cumulative cases are 215360 and recoveries 207761 till 30 November, 2020 as depicted in Figures 5 A & B.

Safety measures, compliances and sampling protocols of SARS-CoV-2 monitoring research

Preparatory steps were taken for SARS-CoV-2 monitoring research with all safety protocols, permissions from Chennai Metropolitan Water Supply and Sewerage Board (CMWSSB) authorities
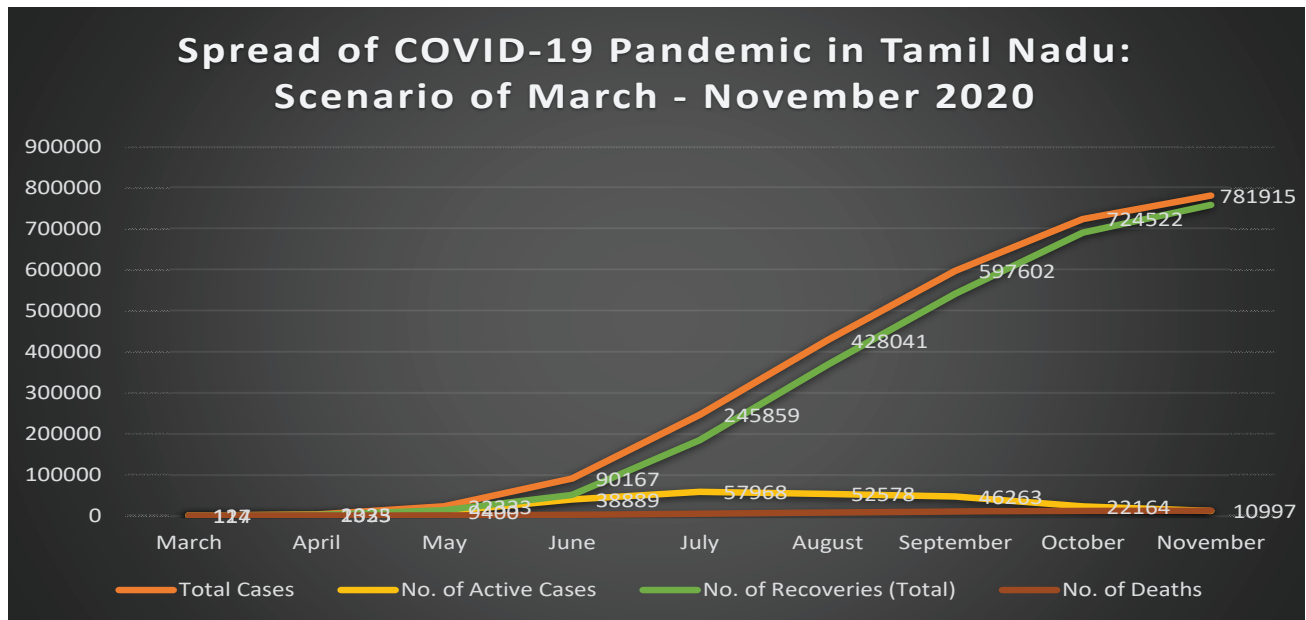

Figure 5A : COVID-19 scenario in Tamil Nadu (Source: Government of Tamil Nadu, 2020 https://tn.data.gov.in/catalogsv2)
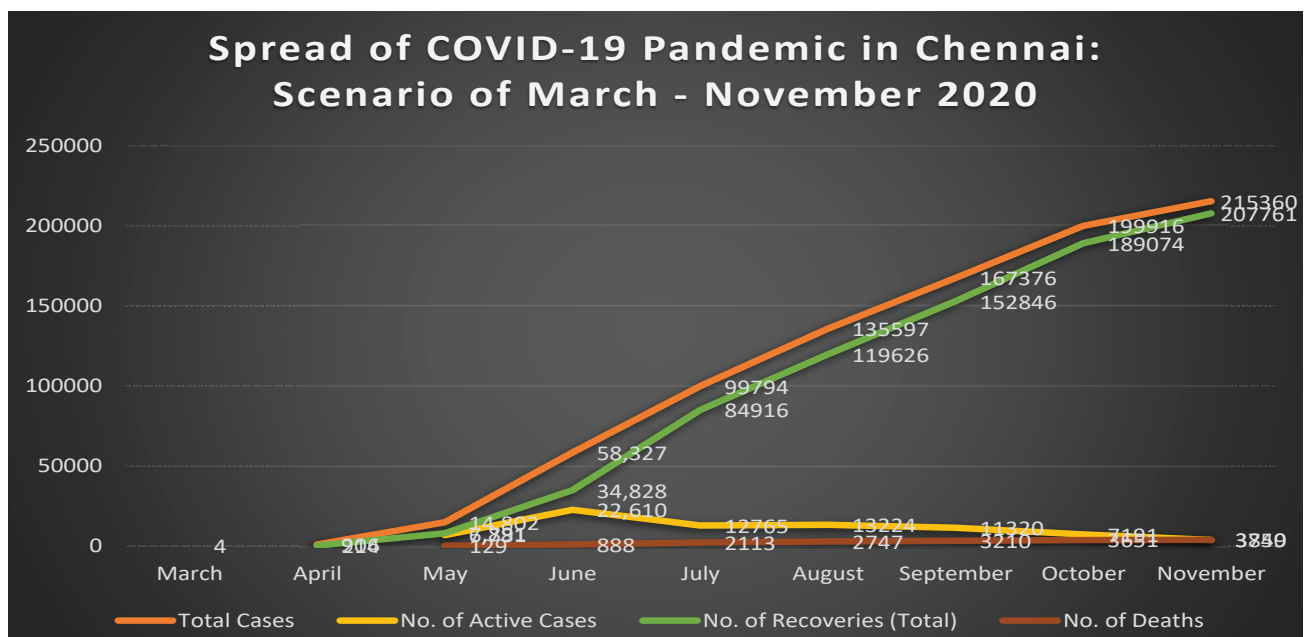

Figure 5B : COVID-19 scenario in Chennai City (Source: Government of Tamil Nadu, 2020)

and compliances to World Health Organisation (WHO) and Indian Council of Medical Research (ICMR) Standards. A Biosafety Committee was set up to regularly monitor the safety standards, compliances and protocols as per WHO standards. Personal protection equipment (PPEs) used during field sampling and laboratory analysis were disposed as per the institutional norms. A Biosafety level 2 cabinet was also procured for this study. The results were cross checked with an NABL accredited laboratory and found to be in agreement.

Sampling design of SARS-CoV-2 monitoring

A total of 156 samples (using four extraction methods) were analysed in the STPs of Chennai City (in February), SRM campus (during partial and post lockdown period) and Chennai Metro Area (during post lockdown period), out of which 48% (75 nos.) was found positive. The sampling sites included 5 sewage pumping stations (SPSs), 4 STPs (inlet, outlet, and primary sludge) of Chennai city and SRM Hospital wastewater.

The sampling design adopted for this study is presented in Table-1 and sampling locations in Figure 6. This gives details of various STPs, the total number of samples, positive and negative samples in this study.

Research on SARS-CoV-2 monitoring in Chennai City and suburban areas
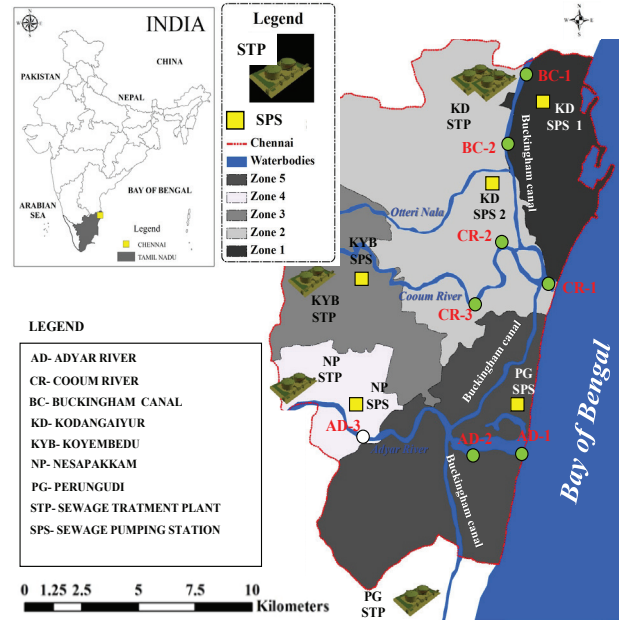
In India, more than 70% of the wastewater

Figure 6: Study site in Chennai City

Table 1. Details of 156 wastewater samples tested in Chennai				
Sample Details			RT-PCR Analysis Details	
Sample Place	Sample type	Total Sample	Positive	Negative
Chennai city STPs (n=1)	During February	24	0	24
SRMSTP	Partial Lockdown	44	12	32
	Post Lockdown	20	20	0
Chennai Metro STP (During Post Lockdown)	Inlet	16	15	1
	Sludge	16	9	7
	Outlet	16	0	16
	Sewage Pumping Station	20	19	1
Total No of positives				75
Total No of negatives				81
Total No of sample				156

generated is directly discharged to waterbodies. Even though the virulence of SARS-CoV-2 in wastewater is not evident, the outflow of sludge and water effluents from WWTPs is a matter of public health concern. An intensive wastewater surveillance during partial and post lockdown phases were conducted to detect the presence of SARS-CoV-2 in the four major STPs and SPS of Chennai city with support from CMWSSB during June to October 2020 (see **Figure 6 and 7**).

Wastewater surveillance was conducted to map the spread of SARS-CoV-2 from the STPs and SPS of Chennai city during the first two weeks of September 2020. Simultaneously, wastewater of SRM University Hospital, were monitored on a weekly basis. The hospital wastewater monitoring was carried out at different time intervals, (7am, 10am, 1pm, 4pm and 7pm during the day). Human wastewater markers such caffeine, carbamazepine and other organic tracers were monitored to track the load of these contaminants along with SARS-CoV-2, which also mirrored the functional efficacy of STPs. This was done by real time polymerase chain reaction (RT-PCR) technique.


Figure 8: Biosafety Level 2 Cabinet

RT-PCR, was used to detect the presence of SARS-CoV-2 in samples by using nucleocapsid (N1 and N2) specific primers and probes to monitor the genetic fragments of the virus present in the wastewater samples. Altogether 17 grab samples were collected (**Figures 6 & 7**) from WWTPs and sewage pumping stations of Chennai city and 43 samples collected from SRM Hospital STP were analysed. The experiment was conducted in a Bio-safety level 2 Cabinet at SRMIST laboratory (**Figure 8**). Wastewater was extracted for SARS-CoV-2 detection from the samples using **composite, supernatant, sediment** and **syringe filtration methods**. The extracted RNA (Figure 9) from each method was concentrated and subjected to RT-PCR analysis.

Figure 7: Wastewater sample being collected from STP in Chennai



Figure 9: RNA Extraction
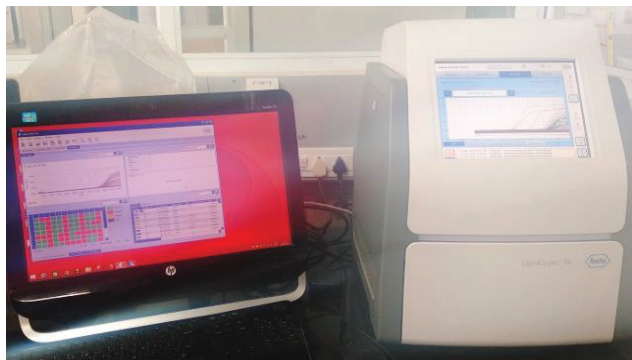
Figure 10: RT-PCR Analysis

Figure 11: RT-PCR Signal

A number of positive and negative samples were detected (**Figures 11**). Out of the 17 STP samples collected and analysed using all the four methods, viz., composite, supernatant, sediment and syringe filtration methods, all the inlets were found to be positive for both N1 and N2 (*Chakraborty et. al., 2020). The semi-quantitative estimation of RNA was done by counting the number of RNA copies in the positive samples using NANODROP.

Chemical markers, such as caffeine and carbamazepine have been preferred as potential markers for untreated wastewater or non-functional STPs (Chen et al., 2014; O'Brien et al., 2017., Chakraborty et al., 2019 and 2020). Human wastewater markers such caffeine, carbamazepine and other organic tracers were monitored to track the load of these contaminants along with SARS-CoV-2. Caffeine is found to be an effective chemical marker and its high level was observed in Koyambedu (population of 14 Lakhs, 90 Million Litres per day (MLD) wastewater), as compared to Kodangaiyur (population 30 Lakhs, 202 MLD wastewater) and Perungudi (population 30 Lakhs, 95 MLD wastewater) reflecting the direct release of higher volume of wastewater directly to the river in Koyambedu. Unlike Koyambedu, Perungudi catchment houses a population of 30 lakhs, yet generates almost similar amount of wastewater as Koyambedu (95 MLD) and the estimated positive cases of SARS-CoV-2 was one-fourth of both Koyambedu and Kodangaiyur.

Limitations and challenges of the study

- Importing the biosafety cabinet during the pandemic amidst several restrictions.
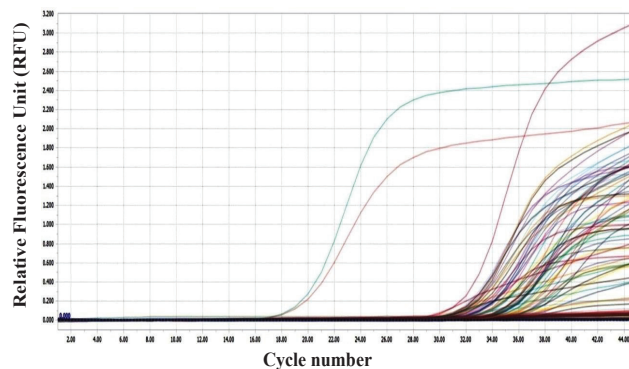
- Being the first-of-its-kind study at SRMIST, development of the Standard Operating Procedures (SOPs), and methods required several rounds of iterations & calibrations.
- Special permission to collect samples during the lockdown under severe restrictions of vehicle movement.
- Procuring permission from the governing bodies while the campus activities were restricted.

Inferences

The wastewater surveillance revealed some very useful insights regarding highly infected communities with high population density within Chennai city, as below:

- Out of four methods of wastewater extraction, composite (COM), supernatant (SUP), sediment (SED) and syringe filtration (SYR), SED and SUP were in line with the results from a NABL accredited laboratory. These findings would be useful to scientific communities for directing efforts in the sample collection.
- Number of estimated infected persons by both SUP and SED methods were comparable with the available data of active cases in the given time frame (**Figure 12**).
- In Hospital Wastewater (HWW), maximum positive response (85%) was seen in samples during partial lockdown phase. This can be due to a greater number of infected patients admitted in SRM hospital during the pandemic.
- Collection of composite grab samples by dedicated skilled personnel, at different time intervals is important to track the status of viral load in a given catchment.

- The study has highlighted that the removal efficiency of caffeine varied between 84%-99% in the STPs, supporting the fact that caffeine can be used as a potential marker for wastewater even under high dilution conditions.

- Unlike other catchments, higher estimated infected persons with lowest population can be related to high population density in the Koyambedu catchment.

- Monitoring of wastewater from treatment plants, sewers and freshwater bodies of Chennai as part of the present study will **go a long way in estimating the number of infected individuals** in the area, and hence provide robust scientific evidence for informed decision making at the policy level.

- This is an early, cost-effective, unbiased community-level indicator of the presence of COVID-19 and also of 'hotspots' within a community.

- It supports **risk mitigation decisions** for the communities.

- The study provides information for effective **sensitization of staff** in the sanitation sector for adopting standard procedures and precautionary measures.

- It **guides decisions** about where and when to impose or relax more targeted restrictions on movement and activity.

- Alerts **asymptomatic infections** in the community through real-time community sewage detection.

- Very useful for **positioning resources and**

triggering public actions for improved health outcomes.

- It can potentially alert a **second** and **subsequent wave of the pandemic.**

- Provides information for preparedness towards outbreak of other **pandemics in the future.**

Way forward

1. Historically, WBE has played an important role in the eradication of polio in 2011. India continues wastewater surveillance at 52 WWTPs and unregulated catchment areas for detection of poliovirus. This surveillance network can be a viable resource for the detection of SARS-CoV-2 in wastewater.

2. Development of protocols for analysing, interpreting, and ways of responding to positive wastewater samples was needed in the early stages of the study. The **Standard Operating Procedures** (SOPs) developed under this study has established and developed methods and protocols for SARS-CoV-2 testing which can be **replicated** in other Districts of Tamil Nadu as well as in **other States within India** for increasing the coverage of wastewater monitoring and surveillance towards COVID-19.

3. The method, volume and frequency of sampling, population demographics of the study area, and local epidemiological factors were key considerations during planning the surveillance. Such aspects must be considered during replication of the study in other areas.
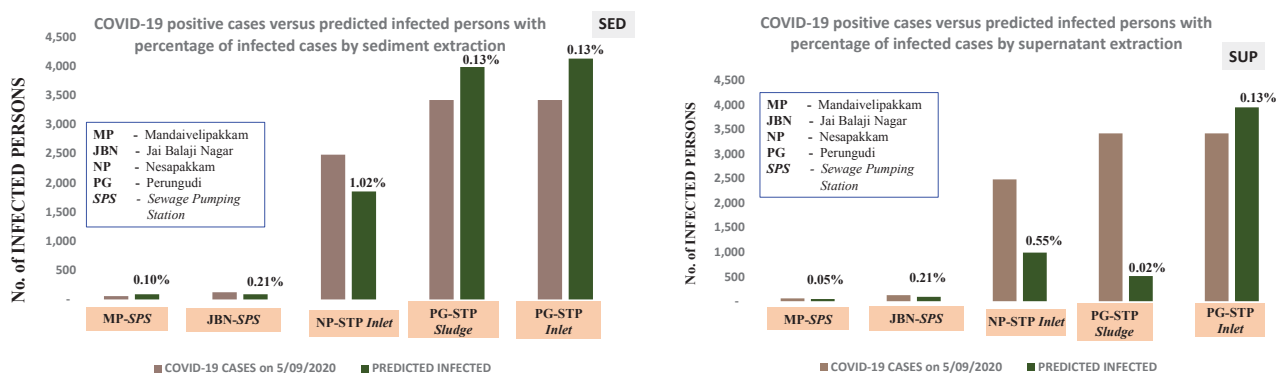
4. In laboratory conditions, suitable process

Figure 12: *Analysis of infected cases using SED and SUP methods*

controls need to be well-defined to determine false negatives (if any), validate results, and reduce cross-contamination, as applicable.

5. With a possibility of some mobile population in the study area, positive test of wastewater samples of a given area may not necessarily indicate the presence of SARS-CoV-2 infection of the local population. Such aspects must be evaluated during (result-driven) decision making.

6. The Biosafety cabinet level-2 and lab set-up developed for COVID-19 monitoring can be utilized for **further surveillance of wastewater for the current pandemic**.

7. Development of a **Guidance document** on the SOPs and methods for capacity building of other laboratories can support and enhance the testing facilities within India.

8. **Need for regular monitoring with intense quantitative analysis** of SARS-CoV-2 in community wastewater and modelled predictions to **assist the water boards** is required.

9. Enhancing the monitoring and surveillance by increasing testing facilities provides opportunity for **skill development and job creation for educated unemployed youth** as well as **entrepreneurial opportunity**.

10. This study could potentially be one of the bases for **scientifically informed decisions to implement public health intervention strategies** consistent with legal and ethical considerations in India.

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