First Data-Set on SARS-CoV-2 Detection for Istanbul Wastewaters in Turkey

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) started in Wuhan, China, in December 2019 and became a global pandemic [1]. By 26 April 2020, more than 2.9 million people were infected by SARS-CoV-2 and over 203 thousand people lost their life globally. By 26 April 2020, 107773 confirmed cases were reported in Turkey with 2706 deaths. Majority of the cases in Turkey has been observed in Istanbul. In the world, the duration of availability of SARS-CoV-2 was found to be significantly longer in stool samples than in respiratory and serum samples [2]. SARS-CoV-2 was detected in wastewaters in Australia [3], Netherlands [4], USA [5], France [6], Spain [7] and USA [8] by using different virus concentration techniques. In this work, Istanbul metropole with 65 % of Covid-19 cases was chosen as the pilot city. On the 21st of April 2020, 24-hr composite samples were collected from the Ambarli, Pasakoy and Kadikoy wastewater treatment plants (WWTP). On the 25th of April 2020, more wastewater samples were taken from Terkos, Buyukcekmece, Baltalimani and Tuzla WWTPs. These wastewater treatment plants were selected among 81 plants in Istanbul in order to take representative samples from 4 different districts of Istanbul according to the severity of Covid-19 cases, like very serious, serious, moderate and mild. Grab samples were also collected from Bagcilar and Kartal manholes located nearby the pandemic

hospitals on April 21st, 2020. Polyethylene glycol 8000 (PEG 8000) adsorption [5] SARS-Cov-2 concentration method was used for SARS-CoV-2 concentration after optimization. Real time RT-PCR diagnostic panel validated by US was used to quantify SARS-CoV-2 RNA in raw sewage taken from the inlets of treatment plants and manholes. Five samples out of seven from wastewater and all samples from manholes were tested positive. SARS-CoV-2 in raw sewage from Ambarli, Pasakoy, Kadikoy, Terkos, Buyukcekmece, Baltalimani and Tuzla WWTPs were found as $8.26x10^3$, $1.80x10^4$, ND, ND, $3.73x10^3$, $4.95x10^3$, $2.89x10^3$, respectively. The Bagcilar and Kartal manholes nearby pandemic hospitals exhibited $4.49x10^4$ and $9.33x10^4$, respectively. SARS-CoV-2 virus titers of manhole were higher than those of inlet of WWTPs. The observed copy numbers were presented against the number of Covid-19 cases coming to the WWTP per treatment plant capacity. Quantitative measurements of SARS-CoV-2 in wastewater can be used as a tool in wastewater-based epidemiology (WBE) and it can provide information about SARS-CoV-2 distribution in wastewater of various districts of Istanbul which exhibit different scores of Covid-19 cases. The distribution of epidemy was followed not only with blood test but with wastewater monitoring. This may allow us to identify the districts not exhibiting many Covid-19 cases, but under high risk. Continuous monitoring of wastewater for SARS-Cov-2 may provide an early warning signs before an epidemy starts in case of infection resurge.

Keywords

SARS-CoV-2, Covid-19, sewage, wastewater, RT-qPCR, virus concentration, PEG

Value of the Data

- The dataset reports the SARS-CoV-2 detection results in wastewater of Istanbul with 15.5 million inhabitants and a very high population density (2987 persons/km2). Hence, the dataset provides information about SARS-CoV-2 distribution in wastewater at various districts having serious, moderate and mild cases and presents Covid-19 case numbers against wastewater SARS-CoV-2 titers.

- As being the seventh systematic study in the world, the dataset presented is quite beneficial for the governors trying to fight with Covid-19 around the world. They will have chance to adapt the methodology to their own countries and monitor the epidemic not only with the blood test but also wastewater monitoring. They may have chance to catch the districts not exhibiting too many cases but under risk.

- This surveillance will indicate a correlation the Covid-19 cases with SARS-CoV-2 RT-qPCR results in wastewater. It has quite potential for verifying the reported number of Covid-19 cases with the real situation. Additionally, continuous monitoring of wastewater for SARS-CoV-2 may provide an early warning sign before an epidemy starts in case of infection resurge.

Data Description

RT-qPCR raw data and standard curve graphs produced by Biorad CFX96 thermal cycle (Biorad Laborateries, Montreal, Quebec), copy number quantification summary, CQ (CT) results and standard curve results exported from Biorad CFX Manager Version 3.1, CQ were submitted with the Supplementary Data.

SARS-CoV-2 copy numbers per liter and calculated indexes in wastewater samples were summarized in Table 1 and shown in Figure 1 together with case number observed at the sampling district against influent flowrate of WWTPs. The slope of the standards SARS-CoV-2 spike protein gene assay was -3.251 and -with an amplification efficiency of 103%. SARS-CoV-2 virus genome was detected in wastewater samples. Five samples out of seven from wastewater and all samples from manholes were tested positive. SARS-CoV-2 titers were ranged 10^2 to 10^4 in WWTPs influent samples. SARS-CoV-2 in raw sewage from Ambarli, Pasakoy, Kadikoy, Terkos, Buyukcekmece, Baltalimani and Tuzla WWTPs were found as $8.26x10^3$, $1.80x10^4$, ND, ND, $3.73x10^3$, $4.95x10^3$, $2.89x10^3$, respectively. The Bagcilar and Kartal manholes nearby pandemic hospitals exhibited $4.49x10^4$ and $9.33x10^4$, respectively. SARS-CoV-2 virus titers of manhole were higher than inlet of WWTPs. Terkos wastewater sample has the highest Case number (person)/WWTP flow (m3/d), but SARS-CoV-2 virus was not detected.

Table 1. SARS-CoV-2 RT-qPCR results for Istanbul wastewaters

Sampling Point	Cq value	Cq SD	RT-qPCR results	RT-qPCR SD	Virus titer per liter	Virus titer per liter SD	slope	Reaction efficiency %	R^2
Terkos WWTP	ND	ND	ND	ND	ND	ND	-3.25	103	0.99
Ambarli WWTP	38.37	1.38	6.89E+02	5.83E+02	8.26E+03	7.00E+03	-3.25	103	0.99
Pasakoy WWTP	37.23	1.11	1.50E+03	1.03E+03	1.80E+04	1.24E+04	-3.25	103	0.99
Kadikoy WWTP	ND	ND	ND	ND	ND	ND	-3.25	103	0.99
Baltalimani WWTP	38.818	0.44	4.12E+02	1.28E+02	4.95E+03	1.53E+03	-3.25	103	0.99
Buyukcekmece WWTP	39.183	0.1	3.11E+02	2.23E+01	3.73E+03	2.67E+02	-3.25	103	0.99
Tuzla WWTP	39.54	NA	2.41E+02	NA	2.89E+03	NA	-3.25	103	0.99
Bagcilar Manhole	35.91	0.98	3.75E+03	2.83E+03	4.49E+04	3.40E+04	-3.25	103	0.99
Kartal Manhole	34.667	0.36	7.78E+03	1.95E+03	9.33E+04	2.34E+04	-3.25	103	0.99

ND: not detected, NA: not applicable

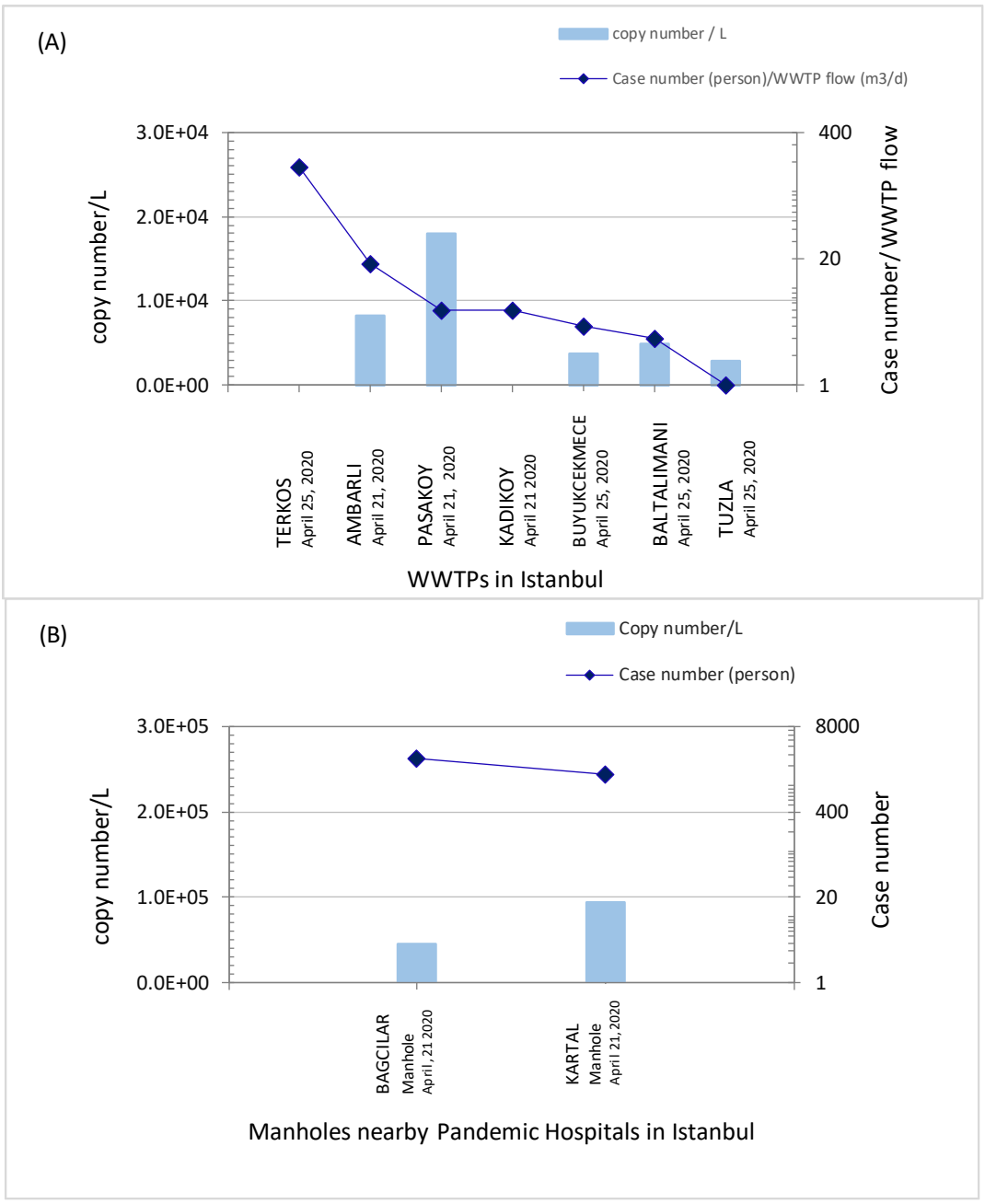

Figure 1. SAR-Cov-2 Levels in Istanbul wastewaters (a) at the inlet of WWTPs, (b) at the manholes nearby pandemic hospitals

Experimental Design, Materials and Methods

2.1 Sampling

The wastewater samples were collected from inlets of seven WWTPs in Istanbul, Turkey and 2 manholes nearby Istanbul epidemic hospitals in 250 ml sterilized bottles. Figure 2 presents a map indicating the first confirmed SARS-CoV-2 detections in the world together with the detection points in Istanbul WWTPs and manholes in Turkey. The map demonstrates sampling points together with the reported Covid-19 cases in the districts of the WWTPs and manholes. The description of sampling points is summarized in Table 2.

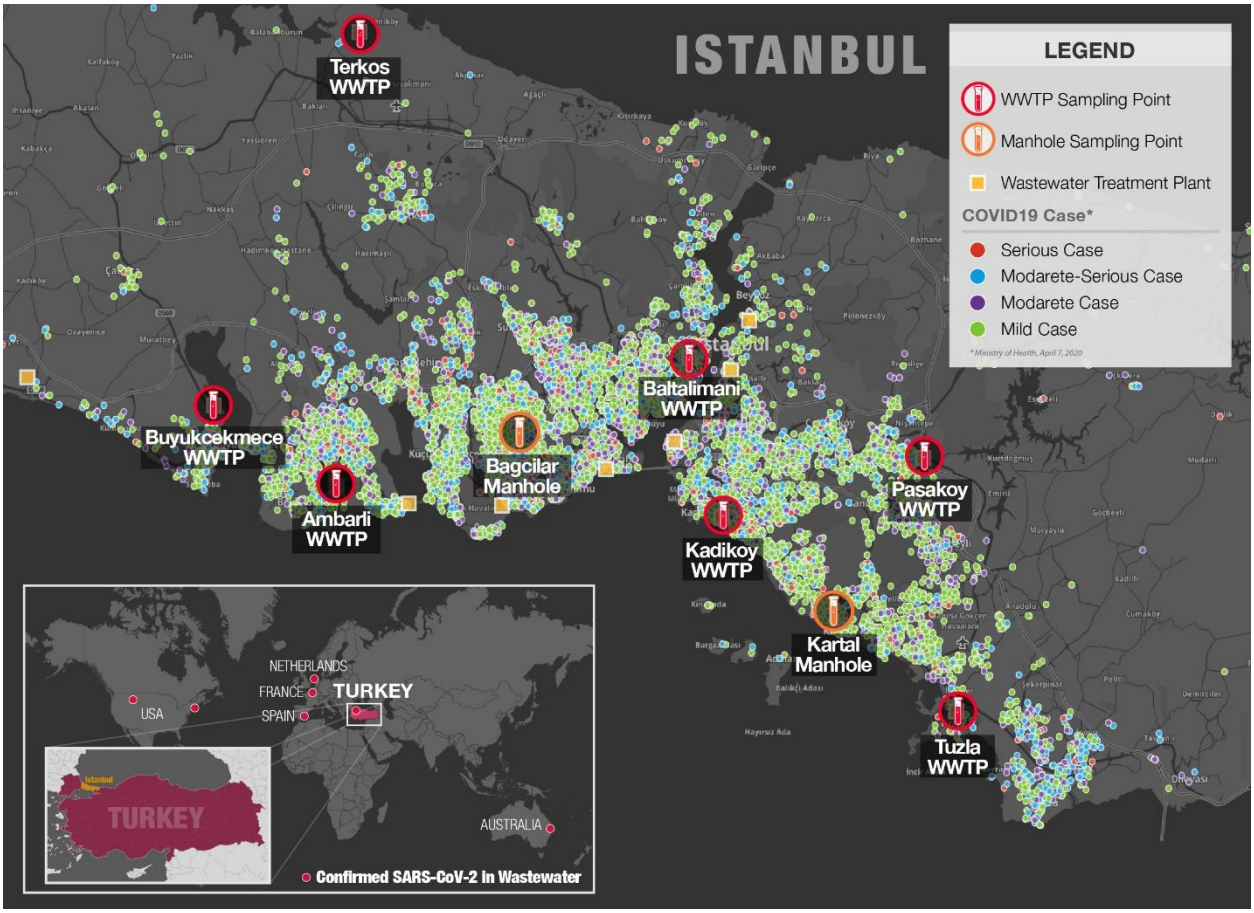

Figure 2. SARS-CoV-2 detection studies in wastewater around the world and in Turkey (Cases from https://geomatic.org/koronovirus on 21st April, 2020.)

Table 2. SARS-CoV-2 wastewater detection points in Istanbul WWTPs

Sampling Point	**Coordinate**	**Type of WWTP**	**Current Flowrate m3/d**	**Covid-19 Cases around the district** (on 8th Ap, 2020)	**Index Covid-19 Case / Flowrate**
Terkos WWTP	41.303137, 28.674010 (41°18'11.3"N, 28°40'26.4"E)	Activated Sludge	1792	320	179
Ambarli WWTP	40.988571, 28.683843 (40°59'39.0"N, 28°40'58.1"E)	Advanced Nutrient removal	342715	6045	18
Pasakoy WWTP	41.015882, 29.273205 (41°00'53.7"N, 29°16'21.8"E)	Advanced Nutrient removal	167690	943	6
Kadikoy WWTP	40.988305, 29.019468 (40°59'18.0"N, 29°01'12.5"E)	Preliminary Treatment	397408	2354	6
Baltalimani WWTP	41.096601, 29.050261 (41°05'47.8"N, 29°03'00.9"E)	Preliminary Treatment	183522	927	5
Buyukcekmece WWTP	41.046486, 28.553018 (41°02'47.4"N, 28°33'10.9"E)	Advanced Nutrient removal	66277	284	4
Tuzla WWTP	40.825605, 29.295600 (40°49'32.2"N, 29°17'44.2"E)	Advanced Nutrient removal	357841	1042	3
Bagcilar Manhole	41.029145, 28.870616 (41°01'47.9"N, 28°52'14.8"E)	N/A	N/A	2658	N/A
Kartal Manhole	40.917165, 29.175142 (40°54'58.3"N, 29°10'31.4"E)	N/A	N/A	1481	N/A

2.2 SARS-CoV-2 concentration

Efficient and reliable virus concentration and recovery from wastewater are the most important step in SARS-CoV-2 detection. So far, ultracentrifugation [6], Polyethylene glycol 8000 (PEG 8000) adsorption [5], electronegative membrane [3] and ultrafiltration [3, 4, 8] methods were used for SARS-CoV-2 concentration from wastewater samples. The work was first started with the use of Amicon® Ultra-15 with

10KDa cutoff ultrafiltration units (Millipore Corporation, Billerica, MA, USA) to concentrate virus [4]. 250 ml wastewater samples were collected from the inlet of Ankara Tatlar WWTP (39.903239, 32.459330, 39°54'11.7"N, 32°27'33.6"E) which collects wastewater from central Ankara. Large particles such as Bacteria and debris were removed by centrifugation at 3200G for 45 mins (Eppendorf 5810R plus swing-out rotor# A4-62; Eppendorf AG, Hamburg, Germany) without brake. Then, a volume of 250 ml supernatant was filtered using ultrafiltration units by centrifugation at 3200G for 25-40 minutes. (Eppendorf 5810R plus swing-out rotor# A4-62; Eppendorf AG, Hamburg, Germany). The concentrate (200 – 600 µl) was collected from the filter unit. Total RNA was extracted with The QIAamp cador Pathogen Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocols. The QuantiNova Pathogen +IC Kit (Qiagen, Hilden, Germany) were added to samples to evaluate PCR inhibition following the manufacturer's protocols. However, a high degree of inhibition was observed in the samples. Expected Ct value of internal control was shifted from 30 to 35. As an alternative to UF method, the polyethylene glycol (PEG) adsorption method [10] was tried. Briefly, 250 ml wastewater samples were collected from the inlet of Ambarli WWTP in Istanbul. Large particles such as Bacteria and debris were removed by centrifugation at 3000G for 45 mins (Eppendorf 5810R plus swing-out rotor# A4-62; Eppendorf AG, Hamburg, Germany) without brake. The supernatant was sequentially filtered with 0.45 µm and 0.2 µm filters to remove remaining particles. 5X PEG Stock Solution were prepared by dissolving 100 g of PEG 8000 (Nordic Chemical Solutions, Norway) and 17.5 g of NaCl in 200 ml distilled water. The pH was adjusted to pH 7.0-7.2. Final stock solution had 50 % (w/v) PEG 8000 and 1.5 M NaCl. The solution was sterilized with 0.2 µm filter. Filtrate was mixed thoroughly with PEG 8000 (10% w/v) by shaking for 1 minute. The mixture was incubated at 4°C at 60 rpm for overnight. Following to incubation, virus was precipitated with Centrifuge (Eppendorf 5810R plus swing-out rotor#F34-6-38; Eppendorf AG, Hamburg, Germany) at 4°C at 5700 G for 120 minutes. Supernatant was removed carefully without disturbing the pellets. Pellets was re-suspended with 600 µl RNA free water and stored at -80°C for a long time. A volume of 200 µl virus concentrate was used for total RNA extraction as described above. Due to high community risk of using SARS-CoV-2 virus, 300 µl of 10^5 copy/ml surrogate avian coronavirus of Infectious Bronchitis Virus were added our samples in order to evaluate the virus recovery efficiency of PEG 8000 adsorption method. Based on RT-QPCR results, 1-1.5 log virus titer loss were observed after PEG 8000 adsorption and RNA isolation.

2.3. Quantitative reverse transcription PCR (RT-qPCR)

Primers taqman probe sets targeting SARS-CoV-2 RdRp gene were used in this study [9]. Serial dilution of SARS-CoV-2 RdRp gene were used as a standard to quantify results. RT-qPCR analysis was performed in 20 µL QuantiNova Pathogen +IC Kit (Qiagen, Hilden, Germany) contained 0.8 nM of forward primer and reverse primer, 0.25 nM probe and 5 µL of template RNA. The RT-qPCR assays were performed using a Bio-Rad CFX96 thermal cycler (Bio-Rad Laboratories, Montreal, Quebec).

Ethics Statement

The work did not involve any human subject and animal experiments.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have, or could be perceived to have, influenced the work reported in this article.

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