Supporting Information

Tracking SARS-CoV-2 RNA through the wastewater treatment process

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A. Chemicals and materials

All chemicals and reagents, unless specified otherwise, were of analytical grade. Milli-Q grade deionized water (18 MΩ-cm) was used wherever needed. FX100 dialysers (Fresenius Medical Care, Bud Hamburg, Germany) with an effective surface area of 2.2 m^2, ultrafiltration coefficient of 73 mL·h^{-1}·mmHg^{-1} and pore size of 3.3 nm [1,2] were used for sample concentration in this study. Phosphate Buffered Saline (PBS) was prepared using phosphate buffered saline tablets purchased from Sigma-Aldrich-Merck, Israel, Commercial 10% w/w sodium hypochlorite (NaOCl) solution, used for sterilization, and ethanol (99.99%) were purchased from Romical chemicals and laboratory equipment.

B. Target wastewater treatment plants (WWTP)

The COVID-19 active cases in the target WWTPs (Figure S1) (Shoket WWTP in Hura and Sorek WWTP in Jerusalem) and the basic physical properties of the collected WW (Table S1) are given below.
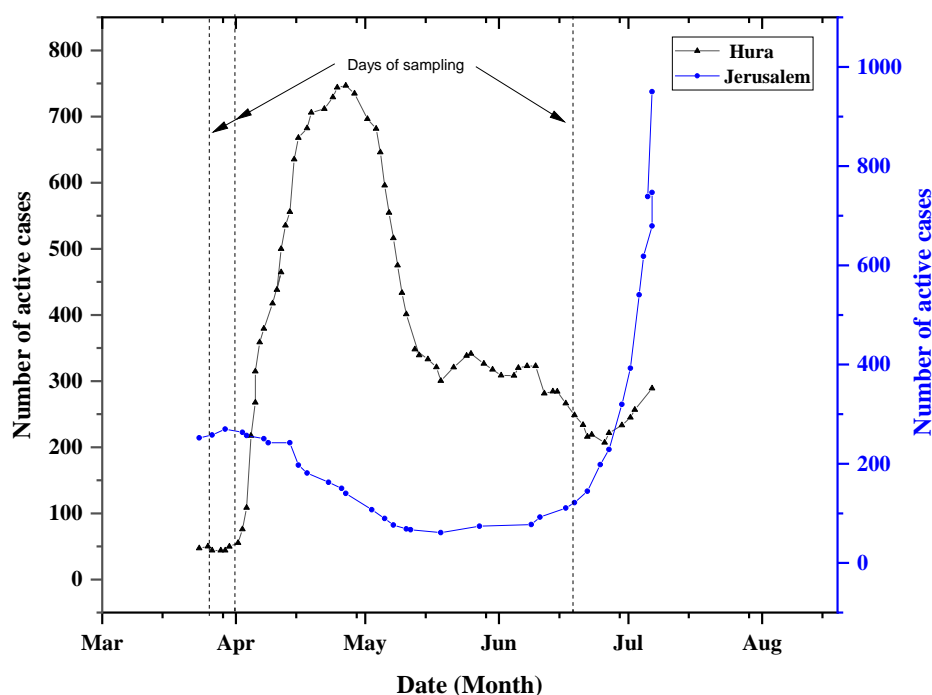

Figure S1. Number of COVID-19 active cases in the areas served by the targeted WWTPs.[3] Numbers of cases are per 100,000 residents. Number of active cases is calculated by removing deaths and recoveries from total cases**.**

Table S1. Characteristics of the Targeted wastewater treatment plants (WWTPs) on the days of sampling.

WWTP	sampling date	average temperature (°C)	wastewater collection point	BOD (mg·L⁻¹)	COD (mg·L⁻¹)	TSS (mg·L⁻¹)	residual chlorine (mg·L⁻¹)	HRT (h)*
Shoket WWTP	21st April, 2020	22°C	influent	-	-	434	-	
			primary sedimentation	260	1194	290	-	3.5
			secondary sedimentation	5	62	6	-	8.5
			sand filtration	5	61	3	-	3.5
			chlorine disinfection	-	-	-	2.3	0.5
	27th April, 2020	22°C	influent	-	-	422	-	
			primary sedimentation	360	613	300	-	3.5
			secondary sedimentation	4	46	2	-	8.5
			sand filtration	-	-	-	-	3.5
			chlorine disinfection	-	-	-	1.7	0.5
	14th July, 2020	29°C	influent	260	357	266	-	
			primary sedimentation	240	294	160	-	3.5
			secondary sedimentation	1	43	8	-	8.5
			sand filtration	5	44	3	-	3.5
			chlorine disinfection	-	-	-	2.2	0.5
Sorek WWTP	21st April, 2020	22°C	influent	373	812	570	-	
			primary sedimentation	232	359	117	-	1.5
			secondary sedimentation	10	76	11	-	7.9
			sand filtration	--	--	--	--	
			chlorine disinfection	-	-	-	-	
	27th April, 2020	22°C	influent	485	885	562	-	
			primary sedimentation	329	519	221	-	1.5
			secondary sedimentation	17	61	18	-	7.9
			sand filtration	--	--	--	--	
			chlorine disinfection	-	-	-	-	
	14th July, 2020	29°C	influent	447	963	788	-	
			primary sedimentation	135	421	155	-	1.5
			secondary sedimentation	18	113	122	-	7.9
			sand filtration	--	--	--	--	
			chlorine disinfection	-	-	-	-	

WWTP characteristics were retrieved from the plants' operators. '-'→ data not available; '--'→ treatment process does not exist in the plant; COD: Chemical Oxygen Demand; BOD: Biological Oxygen Demand; TSS: Total Suspended Solids; HRT: Hydraulic Retention Time (*approximate average timing).

C. Sample processing

C1. Sewage sampling and concentration

The volumes of collected WW samples along with their corresponding concentration factors (wherever applicable) are given in Table S2. The complete method of WW sample collection to RT-PCR detection of SARS-CoV, as followed in the present work, is pictorially presented in a stepwise manner in Figure S2.
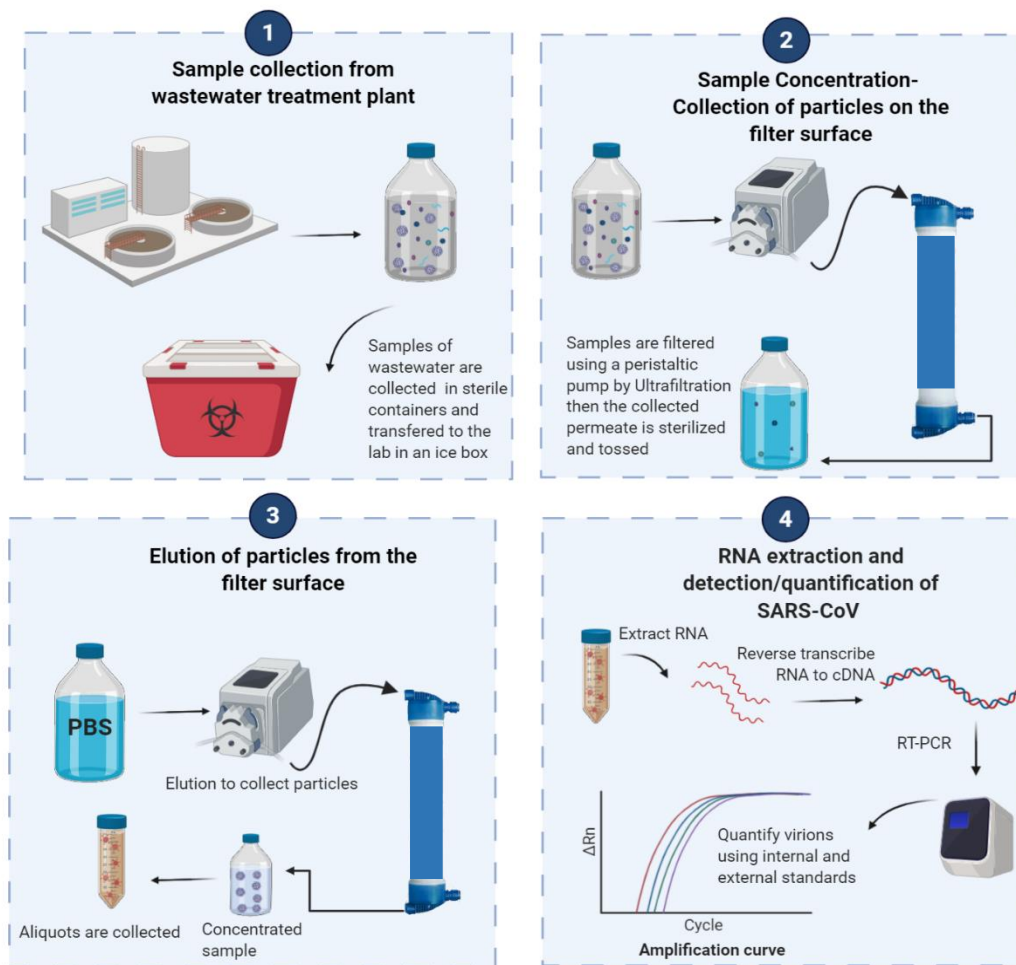

Figure S2. Method used in quantifying SARS-CoV-2 in WW. All components of the figure have been made using BioRender[4]. *Step 1* – WW collections in sterilized plastic containers, S*tep 2* –concentration/volume reduction by ultrafiltration, *Step 3-* elution by PBS (phosphate buffer solution) to collect the accumulated particles on the membrane surface, *Step 4* – RNA extraction and detection of SARS-CoV by RT-PCR.

PBS was chosen based on a previous study in which viral particles were concentrated from large volumes[5]. Other studies are using Tween, glycine, Sodium polyphosphate (NaPP) or beef extract in the elution solution [6] which can be tested in future experiments.

Table S2. Concentrations of WW samples before and after concentrating by ultrafiltration method.

WWTP	sampling date	wastewater collection point	initial volume (L)	final volume (L)	concentration factor
Sorek WWTP	21st April, 2020	influent	0.2	0.2	1
		primary sedimentation	3.8	0.22	17.3
		secondary sedimentation	8.5	0.22	38.6
		sand filtration	--	-	--
		chlorine disinfection	8.5	0.22	38.6
	27th April, 2020	influent	0.2	0.2	1
		primary sedimentation	3.5	0.22	15.9
		secondary sedimentation	8.5	0.22	38.6
		sand filtration	--	--	--
		chlorine disinfection	8.5	0.22	38.6
	14th July, 2020	influent	0.2	0.2	1
		primary sedimentation	9.5	0.27	35.2
		secondary sedimentation	9.5	0.25	38
		sand filtration	--	--	--
		chlorine disinfection	9.5	0.25	38
Shoket WWTP	21st April, 2020	influent	0.2	0.2	1
		primary sedimentation	3.5	0.22	15.9
		secondary sedimentation	8.5	0.22	38.6
		sand filtration	8.5	0.22	38.6
		chlorine disinfection	8.5	0.22	38.6
	27th April, 2020	influent	0.2	0.2	1
		primary sedimentation	3.5	0.22	15.9
		secondary sedimentation	8.5	0.22	38.6
		sand filtration	8.5	0.22	38.6
		chlorine disinfection	8.5	0.22	38.6
	14th July, 2020	influent	0.2	0.2	1
		primary sedimentation	9.5	0.22	43.2
		secondary sedimentation	9.5	0.28	33.9
		sand filtration	9.5	0.22	43.2
		chlorine disinfection	9.5	0.26	36.5

'-'→ data not available; '--'→ treatment process does not exist in the plant.

C2. Quantification of SARS-CoV to copy number per L

For the calibration curve, we used a plasmid that contains the full SARS-CoV-2 N gene sequence as it isolated from Wuhan-Hu-1, complete genome (GenBank: NC_045512.2). We prepared serial dilutions for the plasmid and calculations for the copy number. RT-PCR amplification were performed according to user manual recommendation using CDC's N1 primers and probe set. In parallel to N1 test, each RNA sample was spiked with a N gene in known concentration to rule out any inhibitors affect to the enzymatic reaction. Second quality control was done with adding MS2 phage to the lysis buffer step for RNA extraction indication (See Table s3 for RT-PCR results). To create standard curve, we performed linear regression between the log copy number and the Ct values from the RT-PCR (figure S3). Copy number presented in the calibration graph were divided by two in order to parallel it to RNA quantification. Using the linear equation, we calculated copy number of N1 gene in sewage samples presented in this study.

Table S3: Primers and probes used for RT-qPCR.

primer/ probe[7,8]	sequence (5'→3')	database accession number	position	concentration (nM)
MS2_Forward	TGCTCGCGGATACCCG		3169-3184	300
MS2_Reverse	AACTTGCGTTCTCGAGCGAT	V00642	3229-3210	300
MS2_Probe	[VIC]-ACCTCGGGTTTCCGTCTTGCTCGT-[BHQ1]		3186-3209	200
N1_Forward	GACCCCAAAATCAGCGAAAT		28287 - 28306	500
N1_Reverse	TCTGG TACTGCCAGTTGAATCTG		28335 - 28358	500
N1_Probe	[FAM]-ACCCCGCATTACGTTTGGTGGACC-[BHQ1]	NC_045512.2	28309 - 28332	125

*TaqMan® probes are labelled at the 5′-end with the reporter molecule 6-carboxyfluorescein (FAM) or 2′-chloro-7′phenyl-1,4-dichloro-6-carboxy-fluorescein (VIC) and with the quencher, Black Hole Quencher 1 (BHQ-1).
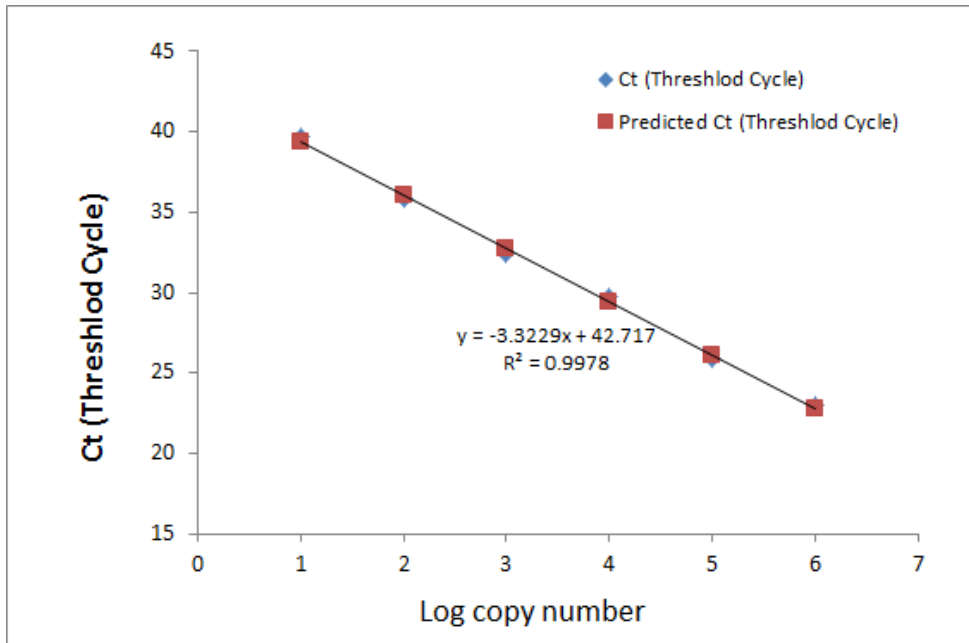
Figure S3: calibration curve for CDC's N1 primers and probe set. Linear regression between the log copy number of SARA-CoV-2 N gene plasmid and the threshold cycle **(**Ct) values from the RT-PCR amplification.

Table S3: Ct values for detecting SARS-CoV-2 in WW samples.

WWTP	date	wastewater collection point	concentration factor	N1	MS2	Spike N gene
Shoket	21st April, 2020	influent	1.0	37.34	29.47	28.86
		primary sedimentation	15.9	37.35	29.95	27.98
		secondary sedimentation	38.6	35.58	28.82	26.54
		sand filtration	38.6	-	29.37	26.35
		chlorine disinfection	38.6	-	29.47	27.04
	27th April, 2020	influent	1.0	34.41	30.24	26.31
		primary sedimentation	15.9	33.58	30.61	26.91
		secondary sedimentation	38.6	34.51	29.53	25.87
		sand filtration	38.6	35.97	29.61	26.41
		chlorine disinfection	38.6	-	29.31	26.21
	14th July, 2020	influent	1.0	35.42	28.66	30.1
		primary sedimentation	43.2	31.47	30.36	26.15
		secondary sedimentation	33.9	-	30.07	26.11
		sand filtration	43.2	-	29.89	26.08
		chlorine disinfection	36.5	-	29.68	26.33
Sorek	21st April, 2020	influent	1.0	-	29.54	25.94
		primary sedimentation	17.3	34.97	30.33	26.31
		secondary sedimentation	38.6	-	29.98	26.24
		sand filtration	--	--	--	--
		chlorine disinfection	38.6	-	29.33	26.65
	27th April, 2020	influent	1.0	-	29.96	26.17
		primary sedimentation	15.9	34.16	29.74	26.71
		secondary sedimentation	38.6	36.59	30.11	25.87
		sand filtration	--	--	--	--
		chlorine disinfection	38.6	-	29.9	26.84
	14th July, 2020	influent	1.0	34.7	28.87	29.89
		primary sedimentation	35.2	32.83	29.45	25.43
		secondary sedimentation	38.0	35.77	29.81	26.31
		sand filtration	--	--	--	--
		chlorine disinfection	38.0	37.14	29.03	26.09

'-'→ data not available; '--'→ treatment process does not exist in the plant.

D. References

(1) Fresenius Medical Care. FX high-flux and FX low-flux dialyzers https://www.freseniusmedicalcare.asia/en/healthcare-professionals/hemodialysis/dialyzers/fx-high-and-low-flux-dialyzers/.

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