A novel passive sampling approach for SARS-CoV-2 in wastewater in a Canadian province with low prevalence of COVID-19.

Supplemental Material

RNA Extraction

A volume of 1 mL of sample (wastewater or COSCa eluate) was combined with 6.5 mL of a lysis buffer solution, vortexed at 3000 rpm for 30 sec, and incubated at 30 °C for 10 min. Following incubation, 3.5 mL EtOH was added and thoroughly mixed, then 100 µL of the binding beads mixture was added to the lysed sample, vortexed at 3000 rpm for 30 sec and incubated at 30 °C for 10 min. To precipitate the magnetic beads, a magnet was applied, and the supernatant was discarded. The magnetic beads were washed three times with 1 mL of a wash solution and again three times with 1 mL of another wash solution. Between each wash, the magnetic beads were vortexed for 30 sec, precipitated and the supernatant was discarded. Once washed, the magnetic beads were then left to dry at room temperature for 1 h to evaporate residual EtOH. To elute the RNA from the magnetic beads, 50 µL of preheated (60 °C) elution buffer was added to the magnetic beads, then vortexed at 1500 rpm for 30 sec and incubated at 60 °C for 5 min. Using a magnet, the magnetic beads were separated from the elution, and the eluted RNA was collected and transferred to a separate tube for RT-qPCR analysis.

The LuminUltra RT-qPCR software requires input of the 1 mL RNA extraction sample volume as the RNA concentration calculation is based on the initial amount of sample processed. The concentration is calculated from the standard curve in the software which takes into account the 5-µL volume used in the reaction, 50 µL extracted, and the original 1 mL processed: $y = -3.74x + 40.3$ where y = Ct and x = concentration.

Figure S1. The COVID-19 Sewer Cage (COSCa), a passive sampling device for monitoring SARS-CoV-2 in municipal wastewater.

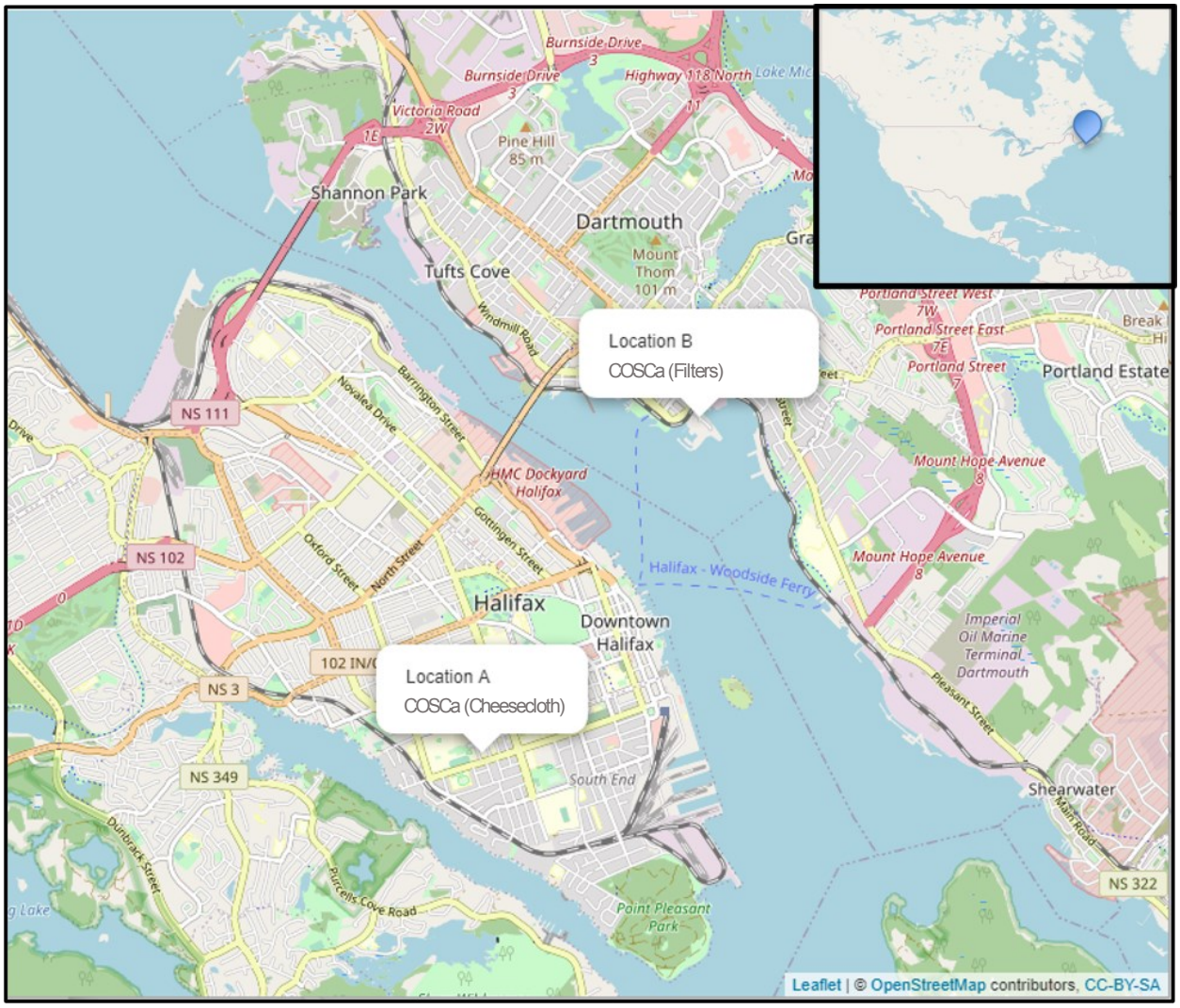

Figure S2. Sewer catchment sampling locations for monitoring SARS-CoV-2 in municipal wastewater using a 3D-printed passive sampling device and two types of adsorbent material. The map in this figure was created using © OpenStreetMap contributors (openstreetmap.org).

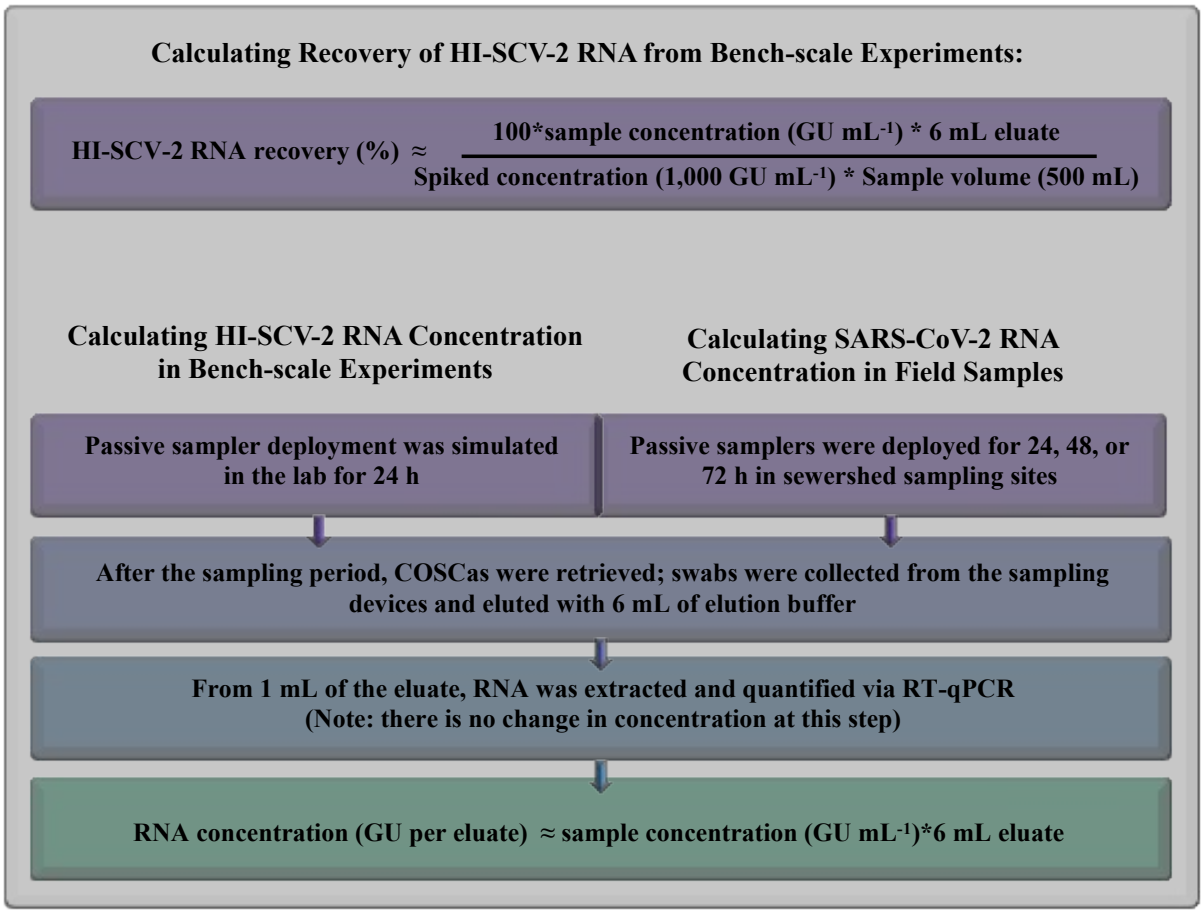

Figure S3. Calculation of RNA recovery (bench-scale experiments) and concentration (bench-scale experiments and field samples) from passive sampling material.

Table S1. Sample eluate and RNA dilutions for each sampling event at Location A.

Sampling Event	SARS-CoV-2 Concentration (GU per eluate)	Days between sampling event 1	Sampling Duration (hrs)	Eluate Dilution	RNA Dilution
1	16380	1	24	None	None
2	0	4	72	None	None
3	0	5	72	None	None
4	0	7	24	None	None
5	1812	8	72	None	None
6	7860	11	72	None	None
7	0	25	48	None	None
8	0	27	168	None	None
9	1932	34	48	1:5	None
10	591	36	24	1:5	None
11	396.6	37	28.5	None	None
12	262.2	38	48	None	None
13	0	40	24	None	None
14	0	41	24	None	None
15	0	42	24	1:1	None

Table S2. Sample eluate and RNA dilutions for each sampling event at Location B.

Sampling Event	SARS-CoV-2 Concentration (GU per eluate)	Days between sampling event 1	Sampling Duration (hrs)	Eluate Dilution	RNA Dilution
1	0	1	48	None	None
2	0	6	144	None	None
3	0	8	48	None	None
4	0	13	144	None	None
5	3276	15	48	None	1:1
6	6120	16	48	None	1:1
7	4512	19	72	None	1:1
8	0	21	48	None	None; 1:1
9	0	23	48	None	None; 1:1
10	0	26	72	None	None; 1:1
11	0	28	48	None	None; 1:1
12	1752	30	48	None	1:1
13	0	33	72	None	None; 1:1
14	3852	35	48	None	1:1
15	0	37	48	None	None; 1:1