Supporting information for:

Complementing RNA detection with pharmaceutical monitoring for early-warning of viral outbreaks through wastewater-based epidemiology

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1. Methods

1.1 Pharmaceutical analysis

1.1.1 Chemicals and Reagents

Analytical reference standards of abacavir (ABC), acyclovir (ACV), amantadine (AMT), atazanavir (ATV), chloroquine (CQ), chloroquine-d5 (d5-CQ), darunavir (DRV), dexamethasone (DXM), dexamethasone-d4 (d4-DXM), efavirenz (EFV), emtricitabine (FTC), famciclovir (FCV), ganciclovir (GCV), hydroxychloroquine (HCQ), indinavir sulfate (IDV), lamotrigine (LMT), lamivudine (3TC), lopinavir (LPV), maraviroc (MVC), nelfinavir (NFV), nevirapine (NVP), oseltamivir carboxylate (OC), oseltamivir phosphate (OP), peramivir (PRV), raltegravir (RAL), ribavirin (RBV), remdesivir (RDV), rimantadine (RIM), ritonavir (RTV), saquinavir (SQV), telbivudine (TBV), tenofovir (TDF), zalcitabine (DDC), and zidovudine (AZT) were obtained from Cayman Chemicals (Ann Arbor, MI). Acetaminophen (ACT), acetylsulfamethoxazole (ASMX), acetylsulfamethoxazole-d4 (d4-ASMX), amitriptyline (AMI), anhydro-erythromycin (AERY), azithromycin (AZI), caffeine (CAF), carbamazepine (CBZ), clarithromycin (CLA), dilantin-d10 (d10-DIL), naproxen (NPX), naproxen-d3 (d3-NPX), norfloxacin (NOR), oxolinic acid (OXO), primidone (PMD), sertraline (SERT), sulfamerazine (SMR), sulfameter (SMT), sulfamethazine (SMZ), $^{13}C_6$-sulfamethazine ($^{13}C_6$-SMZ), sulfamethizole (SMI), sulfamethoxazole (SMX), sulfamethoxazole-d4 (d4-SMX), roxithromycin (ROX), trimethoprim (TMP), trimethoprim-d9 (d9-TMP), and were purchased from Sigma Aldrich (Saint Louis, MO). ^{13}C-erythromycin-H_2O (^{13}C-ERY), ciprofloxacin (CIP), desvenlafaxine (DES), diclofenac (DIC), and diclofenac-d4 (d4-DIC) were obtained from Cambridge Isotopes (Tewksbury, MA). Spiramycin (SPI, a mixture of spiramycin II, and III) and sulfathiazole (STZ) were purchased from ICN Biomedicals, Inc (Irvine, CA). Carbamazepine- d10 (d10-CBZ) and ciprofloxacin-d8 (d8-CIP) were purchased from CDN Isotopes (Quebec, Canada). Diphenhydramine-d3 (d3-DPH), bupropion HCl (BUP),

bupropion-d9 HCl (d9-BUP), citalopram HBr (CIT), citalopram-d6 HBr (d6-CIT), norsertraline-$^{13}C_6$ HCl ($^{13}C_6$-NSER) paroxetine maleate (PRX), paroxetine-d6 maleate (d6-PRX), venlafaxine (VEN), venlafaxine-d6 (d6-VEN), desvenlafaxine (DES) and desvenlafaxine-d6 (d6-DES) were obtained from Cerilliant (Sigma-Aldrich, St Louis, MO). Azithromycin-d3 (d3-AZI), ganciclovir-d5 (d5-GCV), lopinavir-d8 (d8-LPV), and oseltamivir phosphate-d3 (d3-OP) were purchased from Toronto Research Chemicals (Toronto, ON, Canada).

The LC-MS grade methanol and acetonitrile were obtained from Omnisolv® through EMD Millipore Corporation (Billerica, MA), formic acid (88%) and ethyl acetate were purchased from Fisher Chemical (Pittsburgh, PA). Ammonium hydroxide and phosphoric acid of ACS grade were obtained through J.T. Baker (Phillipsburg, NJ). Disodium ethylenediamine tetraacetate (Na_2EDTA) was obtained from Fisher Chemical (Pittsburg, PA). 47 mm GF/F glass microfiber filters (pore size of 0.70 µm) and 47 mm nylon filters (pore size of 0.45 µm) were purchased from Sigma Aldrich (Saint Louis, MO) and VWR (Radnor, PA) respectively. AACS-grade ammonium formate (97%) was purchased from Alfa Aesar (Ward Hill, MA). Syringe filters (4 mm diameter, 0.45 µm) were obtained from VWR (Radnor, PA). To obtain 18.2 MΩ water Barnstead Nanopure™ Diamond (Waltham, MA) purification system was used. Oasis hydrophilic-lipophilic balance (HLB),500 mg /6 cc cartridges were supplied by Waters (Milford, MA) for solid-phase extraction (SPE).

1.1.2 Methods of extraction, analysis, and quantification

1.1.2.1 *Pharmaceutical extraction technique*

Upon receiving, the samples of 450 ± 50 mL were acidified to pH 2.5 ± 0.5 with 85% phosphoric acid and filtered through glass microfiber GF/F Whatmann glass fiber filter followed by nylon filter prior to extraction and stored in polypropylene plastic bottles (10% nitric acid pre-rinsed) at −40 °C until further steps of extraction to avoid degradation of analytes. An aliquot of 2 mL of Na_2EDTA (5% w/v in water) was added to each sample followed by 50 µL of 1000 µg/L

isotopically–labeled extraction internal standard mix. Oasis™ HLB cartridges were pre-conditioned with 6 mL of methanol and equilibrated with 6 mL of Nanopure™ water before loading samples. Pretreated samples were then passed at a flow rate of 3-5 mL min^{-1} through SPE cartridges for clean–up and concentration. Once the samples were completely loaded the cartridges were dried under vacuum for at least 1 hour. 8 mL of LC-MS grade acetonitrile was used for elution of the captured analytes. Using a Turbovap®, sample extracts were evaporated for completion under a stream of nitrogen gas. At the end of drying, 50 µL of 1000 µg/L internal standard, d3-DPH was spiked to the evaporated residues of the extracts followed by resuspension of the extracts in 950 µL of starting mobile phase solvent mix. Samples were then filtered using 0.45 µm nylon syringe filters to 2 mL LC-MS amber vials for LC-MS/MS analysis.

1.1.2.2 *Instrumentation and LC-MS/MS conditions*

The mobile phases consisted of (A) an aqueous solution with 10 mM ammonium formate and 0.1% v/v formic acid and (B) 10 mM ammonium formate and 0.1% v/v formic acid in methanol. The optimized chromatographic separation method started with 15% B mobile phase composition for 3 min followed by a linear ramp to 100% B for 22 min. This condition was kept for 3 min, then the mobile phases were switched back to initial conditions within 2 min. The column was re-equilibrated in starting mobile phase ratio before the next sample run making the total run time 46 min. The flow rate used through the entire separation program was 0.2 mL/min and the sample injection volume was set to 10 µL.

87 1.1.3 Quality control

88 Extraction recoveries were determined on matrices of influent wastewater (*n*=*3*) for a
89 volume of 500 mL following the extraction procedure explained above. Pre-spiked and post-
90 spiked samples were prepared separately for the purpose of examining the losses of the analytes
91 encountered during the SPE protocol. Samples labeled as 'pre-spiked' were spiked at a
92 concentration level of 200 µg/L of reference standard mix prior to the SPE process. Side-by-side
93 the extraction recoveries were assessed in matrix-free Nanopure™ water to investigate the matrix
94 effects. Extraction recoveries of the targeted analytes in influent wastewater matrix with
95 comparison of matrix-free Nanopure™ water and the limits of detection are provided in Table S2.

96 1.2 RNA extraction and RT-qPCR analysis protocol

97 The SARS-CoV-2 viral load in the wastewater was quantified using the N1 region of the
98 viral genome according to the manufacturers guide. Twenty-four-hour time proportional 500mL
99 composite samples were preprocessed by addition of 2.6 mg of $MgCl_2$•H2O and adjusting the pH
100 to 3.5 using 2 M HCl. Then, 100mL of each sample was filtered using 0.45µm MCE membrane
101 filter paper. Once filtration was complete, the filter paper was extracted using an RNeasy
102 PowerMicroBiome Kit (Qiagen, Germantown, MD). The samples were then immediately used in
103 RT-qPCR or stored at -80ºC for future use.

104 RT-qPCR was completed with N1 primers for extracted samples using the iTaq Universal
105 Probes One-Step Kit received by Bio-Rad (Hercules, CA) and the 2019-nCoV RUO assays
106 synthesized by Integrated DNA Technologies (IDT) (Kanata, Canada). Reactions consisted of 5
107 µL of the RNA template, 500 nM of forward and reverse primers, and 125 nM of probes for a final
108 reaction volume of 20 µL. Serial dilutions of a 10^5 stock positive standard were used on every 96-
109 well plate to produce a standards curve and quantify the copies of the SARS-CoV-2 gene. RT-
110 qPCR was cycled at 50 ^oC for 10 minutes, 95 ^oC for 1 minute, and then 45 cycles of 95 ^oC for 10

seconds and 60 °C for 30 seconds. The Cq values for each sample were then used according to the manufacturer's instructions to calculate the final unnormalized SARS-CoV-2 gene copy number per 100 mL of wastewater collected.

2. **Tables and Figures**

Table S1. Multiple reaction monitoring (MRM) transitions with corresponding collision energy voltages, fragmentor voltages, and retention times of each targeted analyte.

(HBV= hepatitis B virus, HCV= hepatitis C virus, HCMV= human cytomegalovirus, HIV =human Immunodeficiency virus, HSV= human simplex virus, SARS-CoV-2 = severe Acute Respiratory Syndrome Coronavirus 2, VZV= Varicella-Zoster Virus)

Analyte	**Abb.**	**Therapeutic usage**	**CAS No.**	**m/z Precursor ion**	**m/z product ion (collision energy eV) Quantitative**	**m/z Qualitative product ion (collision energy eV)**	**Fragmentor voltage (V)**	**Retention time (min)**	**log K_{ow} at pH 2.5**
Abacavir	ABC	Antiviral (HIV)	136470-78-5	287.2	191.1 (25)	150.1 (35)	80	14.6	-1.54
Acetaminophen	ACT	Analgesic–antipyretic	103-90-2	152.1	110.1 (14)	65.1 (29)	100	7.4	0.91
Acetylsulfamethoxazole	ASMX	Metabolite	21312-10-7	296.0	198 (10)	134 (10)	100	18.9	0.85
Acyclovir	ACV	Antiviral (HSV, VZV)	59277-89-3	226.1	135.2 (30)	151.9 (5)	100	3.3	0.77
Amantadine	AMT	Antiviral (Influenza)	768-94-5	152.1	135.1 (20)	-	60	15.2	-1.57
Amitriptyline	AMI	Antidepressant	50-48-6	278.0	233 (10)	105 (20)	50	23.6	1.31
Anhydroerythromycin	AERY	Antibiotic	NA	716.5	558.5 (9)	158.1 (20)	164	23.9	-0.90
Atazanavir	ATV	Antiviral (HIV)	198904-31-3	705.4	335.3 (25)	168.1 (50)	60	26.5	3.18
Azithromycin	AZI	Antiviral (SARS-CoV2)	83905-01-5	749.6	591.5 (27)	158.1 (36)	116	24.7	-4.56
Bupropion	BUP	Antidepressant	34911-55-2	240.0	184 (5)	131 (20)	100	19.3	0.03
Caffeine	CAF	Stimulant	58-08-2	195.1	138 (18)	42.2 (33)	126	14.5	-0.55
Carbamazepine	CBZ	Anticonvulsant	298-46-4	237.1	194.1 (18)	179.2 (37)	118	22.4	2.78

Chloroquine	CQ	Antimalarial, Antiviral (SARS-CoV-2)	54-05-7	320.2	247.1 (15)	142.2 (20)	85	12.4	-0.91
Ciprofloxacin	CIP	Antibiotic	85721-33-1	332.1	245.2 (25)	231.1 (41)	136	16.2	-1.69
Citalopram	CIT	Antidepressant	59729-33-8	325.0	262 (15)	109 (25)	150	21.2	0.26
Clarithromycin	CLA	Antibiotic	81103-11-9	748.5	590.5 (14)	158 (25)	196	24.7	-0.26
Darunavir	DRV	Antiviral (HIV)	206361-99-1	548.3	156.1(25)	113.1 (10)	80	23.5	2.57
Desvenlafaxine	DES	Antidepressant	93413-62-8	264.4	159 (20)	133 (25)	122	16.6	-0.91
Dexamethasone	DXM	Antiviral (SARS-CoV-2)	50-02-2	393.2	147 (25)	171.2 (25)	70	24.2	1.68
Diclofenac	DIC	Anti-inflammatory drug	15307-86-5	296.0	250 (10)	214 (34)	100	27.0	4.25
Efavirenz	EFV	Antiviral (HIV)	154598-52-4	316.1	167.9 (25)	244.1 (10)	60	26.6	4.46
Emtricitabine	FTC	Antiviral (HIV)	143491-57-0	248.1	130.1 (5)	-	80	6.5	-0.96
Famciclovir	FCV	Antiviral (HSV, VZV)	104227-87-4	322.2	136.1 (25)	280.2 (20)	70	16.3	-1.50
Ganciclovir	GCV	Antiviral (HCMV)	82410-32-0	256.2	152.1 (15)	134.9 (40)	100	3.0	-2.20
Hydroxychloroquine	HCQ	Antiviral (SARS-CoV-2)	118-42-3	336.2	247.2 (15)	158.2 (20)	100	11.4	-1.96
Indinavir	IDV	Antiviral (HIV)	157810-81-6	614.5	421.1 (35)	465.4 (25)	145	24.0	1.89
Lamivudine	3TC	Antiviral (HBV, HIV)	134678-17-4	230.1	112.1 (15)	-	60	3.3	-2.68
Lamotrigine	LTG	Anticonvulsant	84057-84-1	256.0	211 (20)	109 (60)	140	18.2	-0.84
Lopinavir	LPV	Antiviral (HIV, SARS-CoV-2)	192725-17-0	629.4	183.1 (15)	155.1 (50)	110	27.4	4.69
Maraviroc	MVC	Antiviral (HIV)	376348-65-1	514.3	280.2 (30)	389.2 (15)	75	20.3	-0.36
Naproxen	NPX	Anti-inflammatory drug	22204-53-1	231.1	185.1 (10)	115 (60)	100	25.1	2.98
Nelfinavir	NFV	Antiviral (HIV)	159989-64-7	568.3	330.2 (30)	467.3 (20)	135	25.1	1.46
Nevirapine	NVP	Antiviral (HIV)	129618-40-2	267.3	226.3 (25)	107.3 (25)	80	19.5	1.70
Norfloxacin	NOR	Antibiotic	70458-96-7	319.3	302.1 (20)		135	15.8	-1.70
Oseltamivir carboxylate	OC	Metabolite	187227-45-8	285.2	138.0 (15)	120.1 (30)	80	16.8	-1.87
Oseltamivir phosphate	OP	Antiviral (Influenza)	204255-11-8	313.2	166.1 (15)	120.1 (30)	100	21.4	-1.87
Oxolinic acid	OXO	Antibiotic	14698-29-4	262.2	244.1 (15)	216.1 (30)	60	19.8	1.35
Paroxetine	PRX	Antidepressant	61869-08-7	330.0	192 (20)	123 (25)	100	23.0	-0.09
Peramivir	PRV	Antiviral (Influenza)	330600-85-6	329.2	100.2 (30)	270.2 (15)	80	15.5	-2.84
Primidone	PRM	Anticonvulsant	125-33-7	219.0	162 (10)	119 (10)	90	17.9	1.12
Raltegravir	RAL	Antiviral (HIV)	518048-05-0	445.2	109.2 (35)	361.2 (15)	90	22.7	-0.39
Remdesivir	RDV	Antiviral (SARS-CoV-2)	1809249-37-3	603.3	200.1 (35)	229.1 (15)	80	25.1	2.00
Ribavirin	RBV	Antiviral (HCV)	36791-04-5	245.1	113.1 (5)	-	75	2.5	-2.77
Rimantidine	RIM	Antiviral (Influenza)	13392-28-4	180.2	163.2 (15)	121.2 (25)	100	20.9	-0.82
Ritonavir	RTV	Antiviral (HIV,SARS-CoV-2)	155213-67-5	721.3	268.1 (25)	296.1 (15)	75	26.7	4.83
Roxithromycin	ROX	Antibiotic	80214-83-1	837.6	679.4 (19)	158.1 (34)	215	24.6	-0.71
Saquinavir mesylate	SQV	Antiviral (HIV)	149845-06-7	671.4	570.3 (30)	433 (30)	80	25.2	-0.39
Sertraline	SERT	Antidepressant	79617-96-2	306.0	275 (5)	159 (25)	80	24.8	1.91

Spiramycin II	SPI II	Antibiotic	24916-51-6	443.3	582.3 (9)	174 (18)	125	19.7	-4.06
Spiramycin III	SPI III	Antibiotic	24916-52-7	450.3	596.2 (10)	174 (19)	125	20.7	-3.36
Sulfadiazine	SPD	Antibiotic	68-35-9	271.3	156.1 (9)	92.1 (25)	108	7.7	0.29
Sulfamerazine	SMR	Antibiotic	127-79-7	265.3	108.1 (25)	92.1 (29)	108	12.0	0.40
Sulfameter	SMT	Antibiotic	651-06-9	281.3	108.1 (29)	92.1 (29)	108	14.1	0.12
Sulfamethazine	SMZ	Antibiotic	57-68-1	279.3	124.2 (25)	92.1 (33)	136	14.2	0.53
Sulfamethizole	SMI	Antibiotic	144-82-1	251.3	156.1 (25)	92.1 (13)	108	14.7	0.11
Sulfamethoxazole	SMX	Antibiotic	723-46-6	254.1	156 (10)	108 (22)	120	16.3	0.68
Sulfathiazole	STZ	Antibiotic	72-14-0	256.3	156.1 (9)	92.1 (25)	108	9.7	0.85
Telbivudine	TBV	Antiviral (HBV)	3424-98-4	243.1	127.3 (5)	-	100	5.0	-1.12
Tenofovir Disoproxil Fumarate	TDF	Antiviral (HIV, HBV)	202138-50-9	288.1	176 (25)	135.8 (25)	80	3.3	1.45
Trimethoprim	TMP	Antibiotic	738-70-5	291.2	230.1 (22)	123 (22)	140	13.3	-0.19
Venlafaxine	VEN	Antidepressant	93413-69-5	278.4	215 (10)	121 (30)	114	20.0	-0.76
Zalcitabine	DDC	Antiviral (HIV)	7481-89-2	212.1	112.0 (10)	-	70	2.8	-2.86
Zidovudine	AZT	Antiviral (HIV)	30516-87-1	268.1	127.0 (25)	-	80	15.2	-0.41

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125 **Table S2.** Comparison of extraction recoveries (*n=3)* of the targeted compounds in matrix-free
126 Nanopure™ water and influent wastewater matrix, and their limits of detection (LOD).

127 *[a] Experimental log K_{ow} values obtained from Environmental Protection Agency's (EPA) web-based*
128 *CompTox Chemistry Dashboard*

Analyte	**Abbreviation**	**Recovery (%)**		**Limit of Detection (LOD)**	**log K_{ow} [a]**
		In Nanopure™ water	**In influent wastewater**		
Remdesivir	RDV	60±4	30±2	2.21	-1.85
Emtricitabine	FTC	29±4	28±4	7.39	-1.66
Acyclovir	ACV	4±2	35±20	12.06	-1.56
Chloroquine	CQ	16±5	68±1	0.01	-1.30
Norfloxacin	NOR	80±3	39±8	7.91	-1.03
Lamivudine	3TC	2±2	2±3	0.97	-0.93
Tenofovir Disoproxil Fumarate	TDF	3±0	1±0	12.75	-0.92
Raltegravir	RAL	68±19	41±2	3.66	-0.82
Famciclovir	FCV	54±1	15±3	0.63	-0.43

Peramivir	PRV	34±4	21±1	0.49	-0.17
Ribavirin	RBV	1±0	1±0	1.06	-0.10
Sulfadiazine	SPD	58±0	25±1	2.44	-0.09
Caffeine	CAF	88±7	70±2	15.10	-0.07
Efavirenz	EFV	49±8	43±15	2.57	0.02
Zidovudine	AZT	82±18	67±5	15.33	0.05
Sulfathiazole	STZ	71±7	15±9	0.40	0.05
Sulfamerazine	SMR	39±5	8±1	0.25	0.14
Sulfamethazine	SMZ	91±7	34±7	0.56	0.19
Ciprofloxacin	CIP	78±2	33±5	5.73	0.28
Sulfameter	SMT	58±4	20±2	1.09	0.41
Acetaminophen	ACT	86±6	75±5	3.83	0.46
Sulfamethizole	SMI	93±8	25±11	0.93	0.54
Enrofloxacin	ENRO	74±10	35±5	0.03	0.77
Sulfamethoxazole	SMX	68±1	13±2	27.24	0.89
Primidone	PRM	68±9	96±9	14.59	0.91
Trimethoprim	TMP	87±6	52±7	3.46	0.91
Oxolinic acid	OXO	76±10	28±10	0.26	0.94
Paroxetine	PRX	8±0	42±7	1.30	1.03
Acetyl-Sulfamethoxazole	ASMX	62±1	79±12	8.69	1.09
Abacavir	ABC	55±2	27±2	2.64	1.22
Venlafaxine	VEN	97±8	74±8	6.51	1.42
Oseltamivir phosphate	OP	48±6	11±1	2.51	1.81
Ritonavir	RTV	76±8	16±1	4.92	1.90
Darunavir	DRV	36±10	11±4	56.08	1.92
Amantadine	AMT	38±14	30±3	0.45	2.44
Carbamazepine	CBZ	92±4	69±12	10.76	2.45
Desvenlafaxine	DES	80±4	50±2	10.55	2.49
Lamotrigine	LPV	45±3	31±5	0.49	2.57
Sertraline	SERT	84±7	17±6	1.58	2.60
Zalcitabine	DDC	0±0	1±0	0.63	2.90
Spiramycin II	SPI II	8±0	18±3	0.45	2.94
Citalopram	CIT	29±11	19±1	16.41	3.04
Oseltamivir carboxylate	OC	45±6	38±7	0.29	3.06
Telbivudine	TBV	49±1	28±7	4.20	3.10
Hydroxychloroquine	HCQ	27±5	27±7	0.05	3.12
Clarithromycin	CLA	16±7	39±7	7.97	3.16
Naproxen	NPX	33±4	5±4	36.32	3.18
Roxithromycin	ROX	35±4	17±4	4.52	3.24
Spiramycin III	SPI III	16±0	31±8	0.26	3.28
Anhydroerythromycin	AERY	7±11	51±6	6.29	3.33
Indinavir	IDV	62±10	36±7	0.53	3.49
Ganciclovir	GCV	4±1	1±0	0.64	3.72

Nevirapine	NVP	89±3	36±10	0.38	3.92
Azithromycin	AZI	6±9	40±11	1.89	4.02
Atazanavir	ATV	76±11	44±1	0.08	4.23
Dexamethasone	DXM	59±6	39±10	16.53	4.44
Diclofenac	DIC	6±2	22±4	4.45	4.51
Bupropion	BUP	87±10	56±8	3.06	4.63
Maraviroc	MVC	87±7	78±8	0.45	4.77
Amitriptyline	AMI	50±7	30±7	0.35	4.92
Rimantidine	RIM	56±13	80±8	0.35	5.01
Saquinavir mesylate	SQV	43±3	38±0	4.61	5.03
Lopinavir	LMT	78±3	13±3	0.64	5.42
Nelfinavir	NFV	40±6	33±0	2.22	5.46

Table S3. Spearman correlation coefficients of rolling averages (3-day, 5-day, 7-day, 15-day, and 21-day) of estimated clinical cases and total concentrations of detected COVID-19 approved antiviral drugs in each study location.

	rolling average				
	3-day	**5-day**	**7-day**	**15-day**	**21-day**
Bird Island	0.49**	0.48*	0.58**	0.56**	0.54**
City of Tonawanda	0.29	0.36*	0.37*	0.37*	0.37*
Kenmore-Tonawanda	0.25	0.31	0.27	0.29	0.27
Lackawanna	0.24	0.29	0.36	0.39	0.37

* P <0.05
** P <0.01
*** P <0.001

Table S4. Detected acetaminophen concentrations in Bird Island, City of Tonawanda, and Kenmore-Tonawanda, during sampling campaigns in 2021 (during outbreak) and in 2022 (post-outbreak).

	Acetaminophen Concentration (μg/L)		
Sampling year	**Bird Island (min.-max.)**	**City of Tonawanda (min.-max.)**	**Kenmore-Tonawanda (min.-max.)**
2021	29.35-181.36	15.81- 204.12	22.91- 254.63
2022	14.72- 70.88	7.57- 93.90	6.88- 68.66

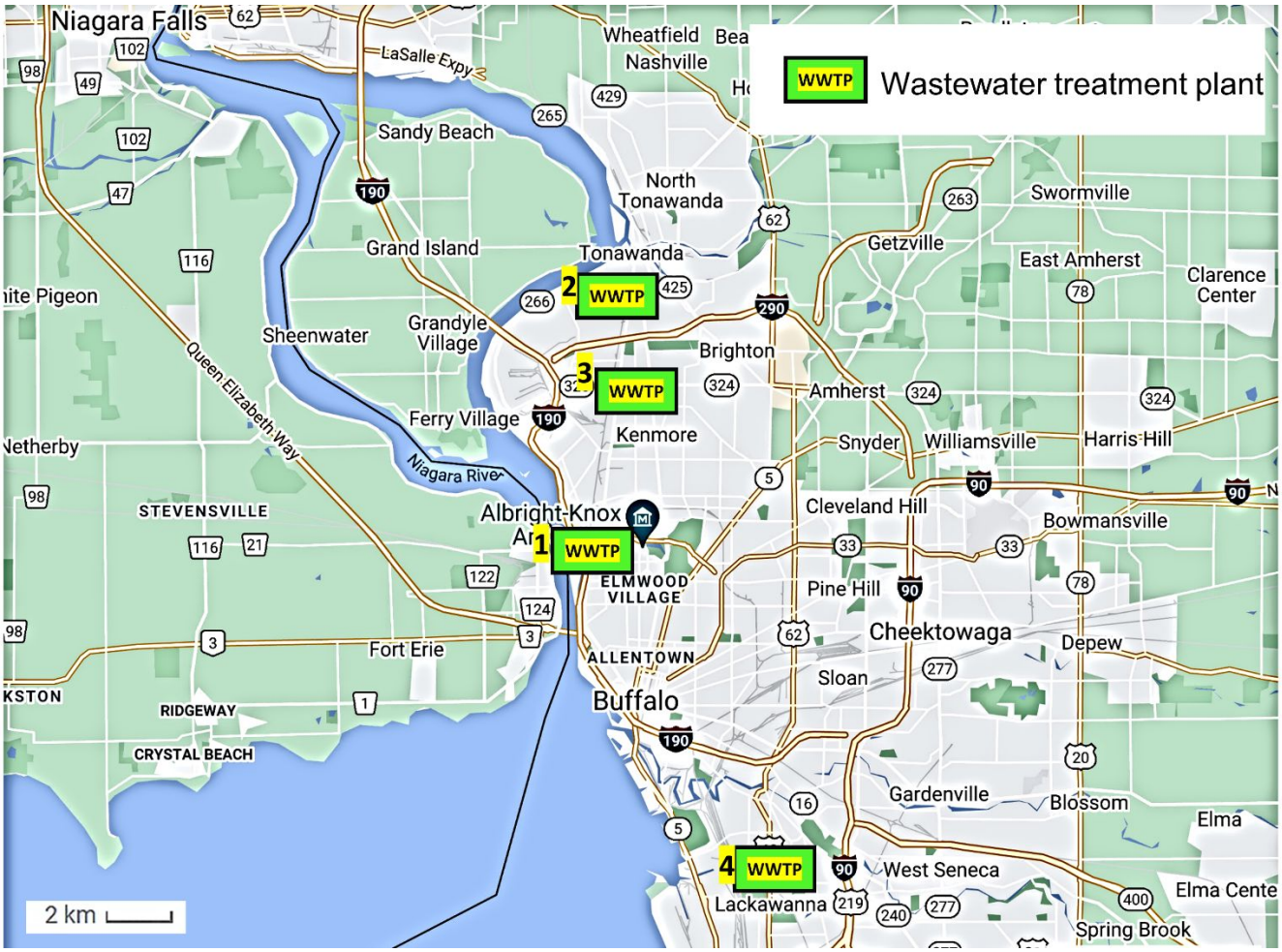

Figure S1. Sampling locations, 1. Bird island (population= 437,357), 2. City of Tonawanda (population=14,875), 3. Kenmore-Tonawanda (population=70,470), and 4. Lackawanna (population=17,859) of the four wastewater treatment plants that participated in the present study. The population estimations are based on 2018 Census data.

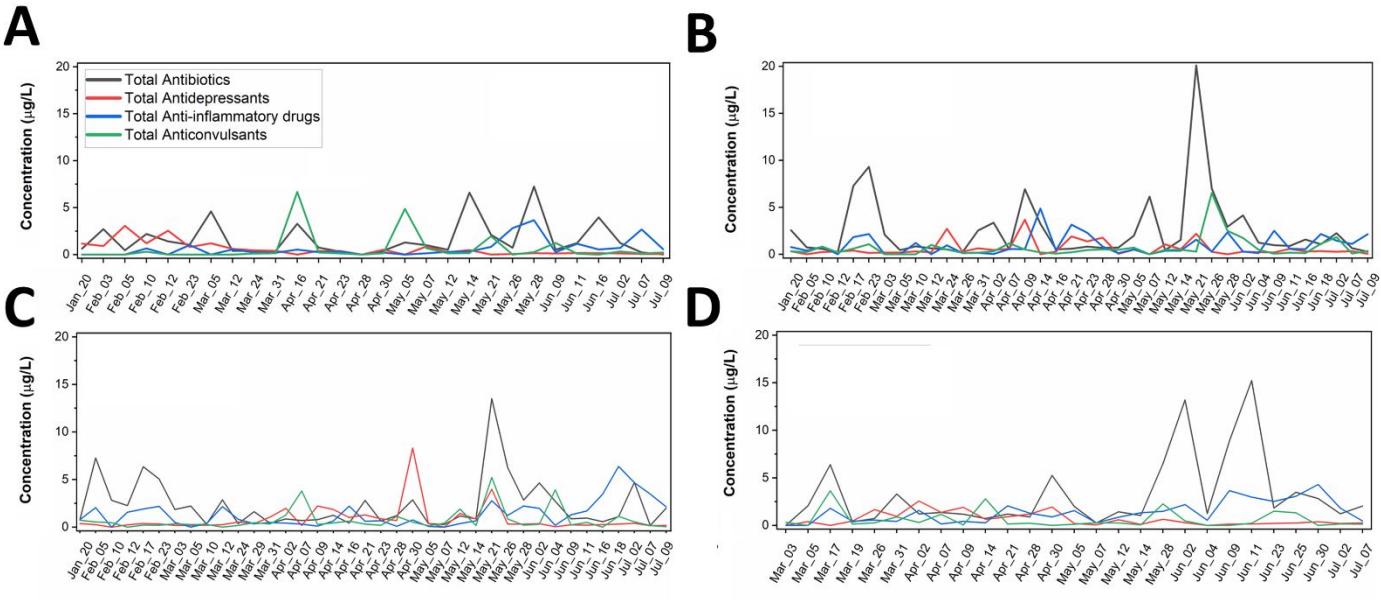

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Figure S2. Detected total concentrations of antibiotics, antidepressants, and anticonvulsants in (A) Bird Island, (B) City of Tonawanda, (C) Kenmore-Tonawanda, and (D) Lackawanna sampling cites of the present study. The sampling campaign was performed in 2021.

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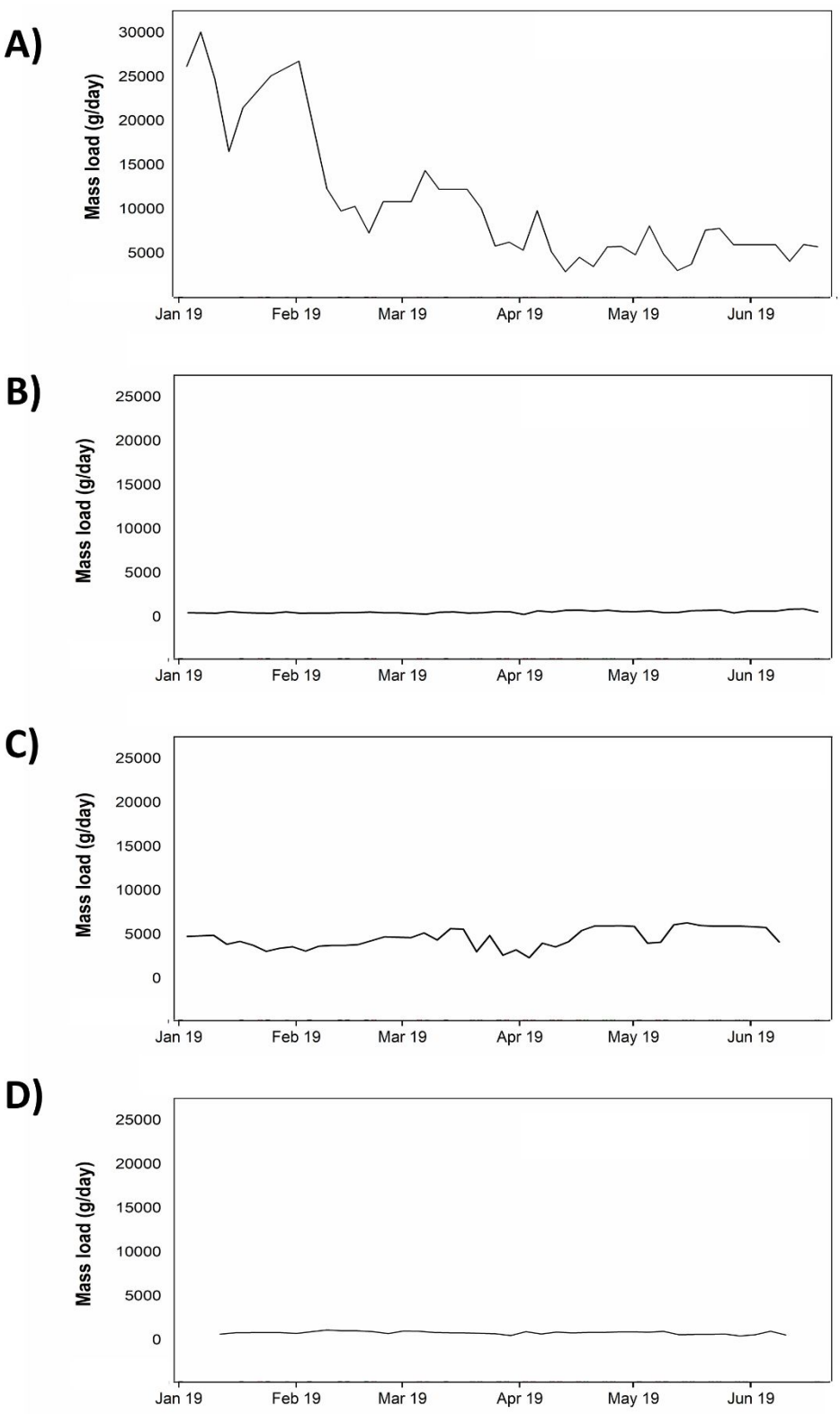

Figure S3. During the outbreak mass loads of caffeine in (A) Bird Island, (B) City of Tonawanda, (C) Kenmore-Tonawanda, and (D) Lackawanna sampling cites. The sampling campaign was performed in 2021.

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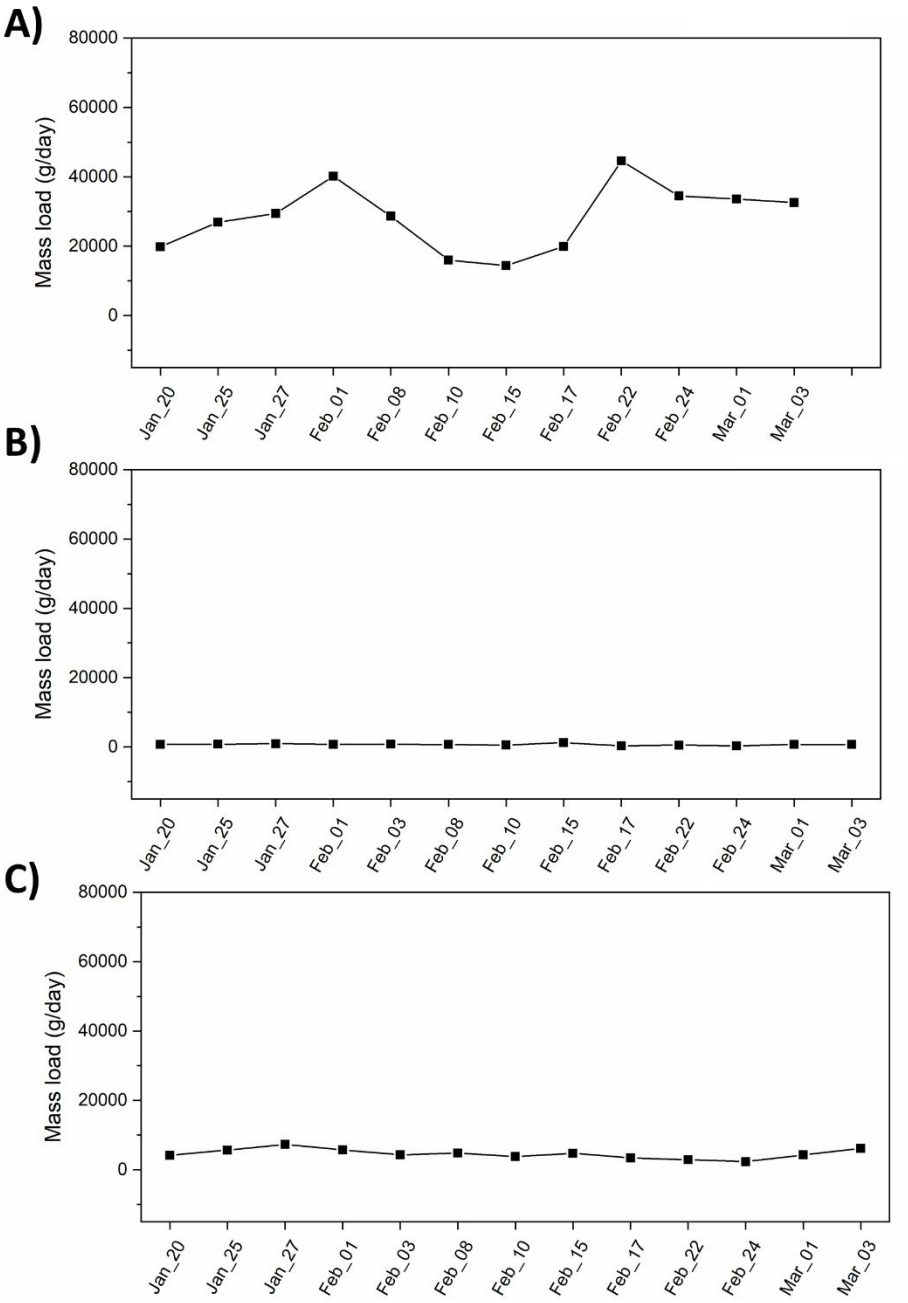

Figure S4. Post-outbreak mass loads of caffeine in (A) Bird Island, (B) City of Tonawanda, and (C) Kenmore-Tonawanda sampling cites. The sampling campaign was performed in 2022.

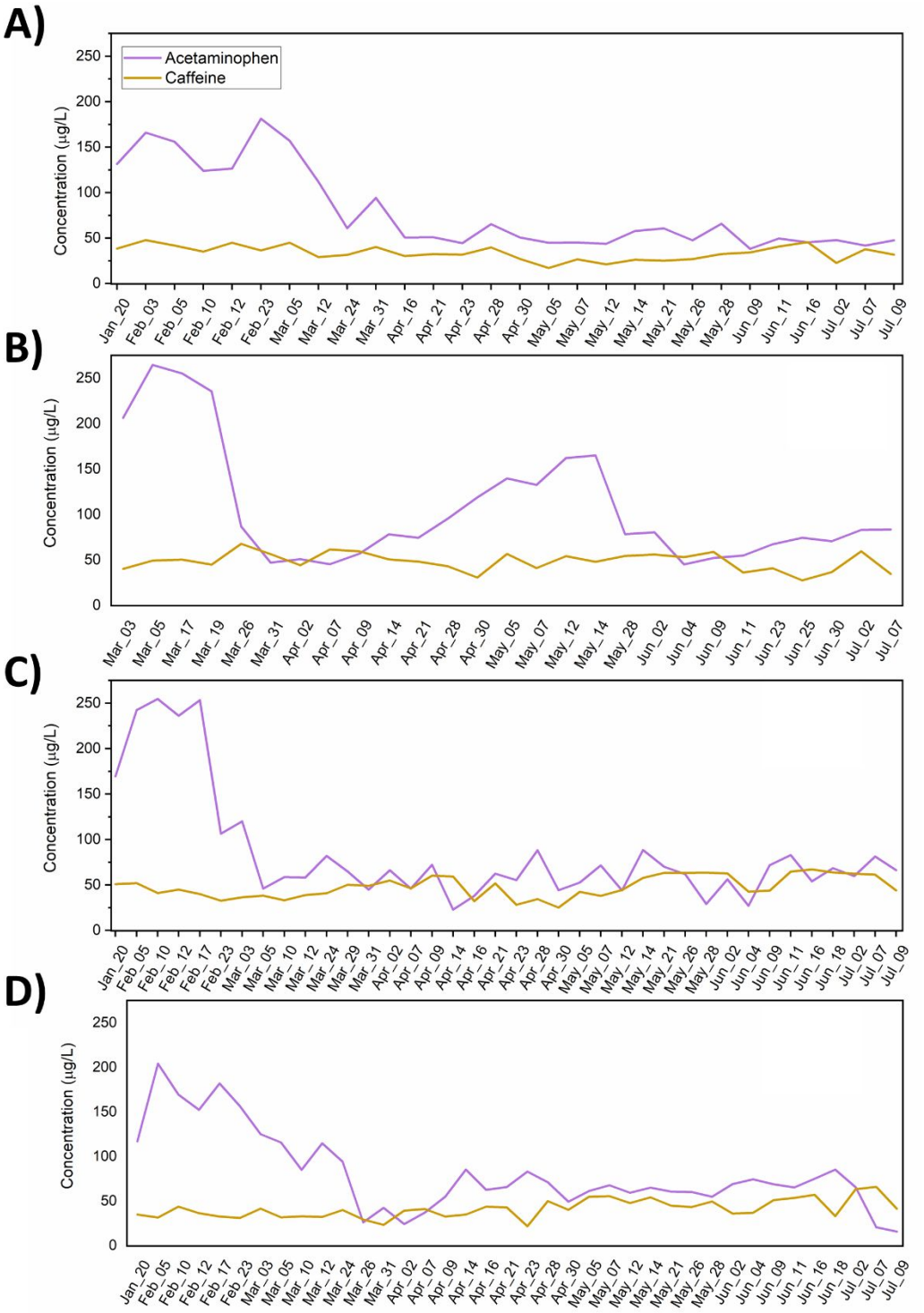

Figure S5. During-outbreak influent wastewater acetaminophen and caffeine concentrations of A) Bird Island, B) City of Tonawanda, C) Kenmore-Tonawanda, and D) Lackawanna sampling locations. Post-outbreak sampling was performed in 2021.

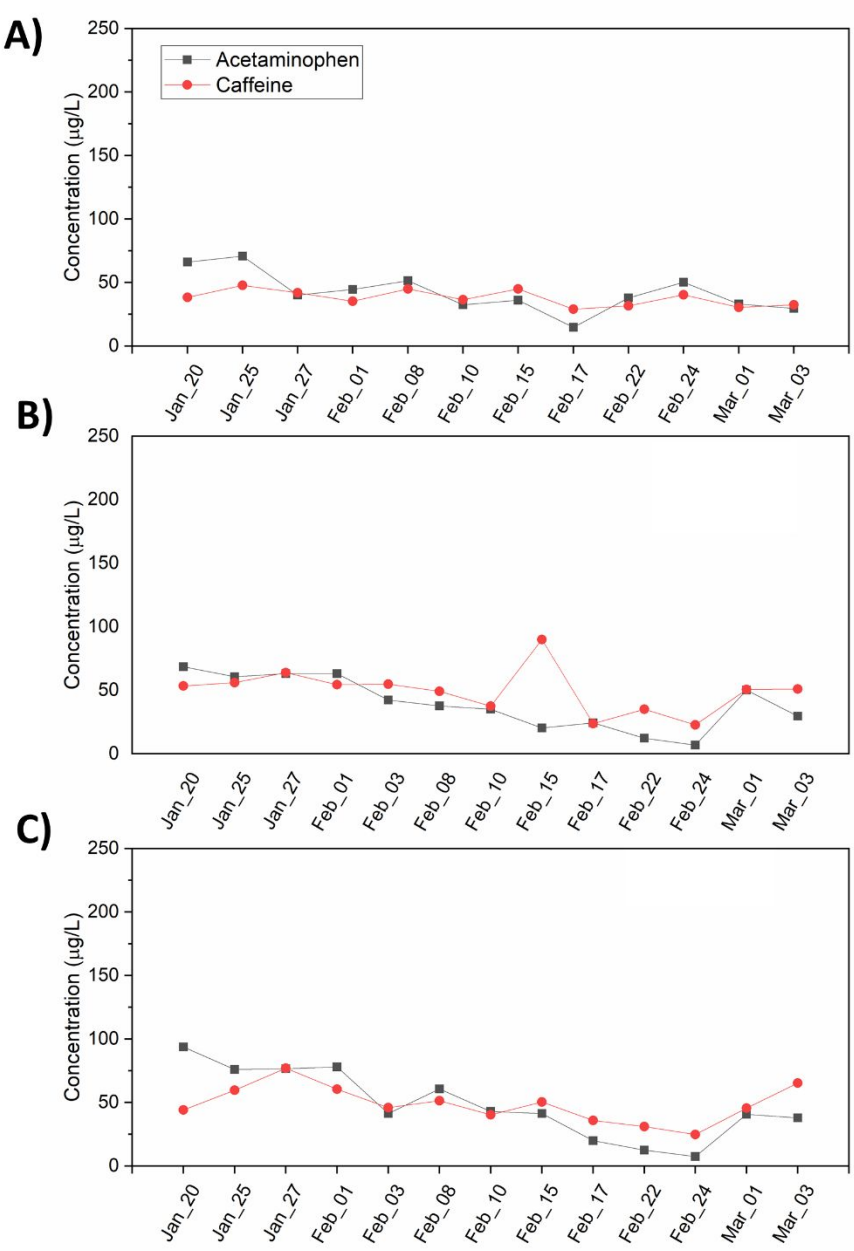

Figure S6. Post-outbreak influent wastewater acetaminophen and caffeine concentrations of i) Bird Island, ii) City of Tonawanda, and iii) Kenmore-Tonawanda sampling locations. Post-outbreak sampling was performed in 2022.

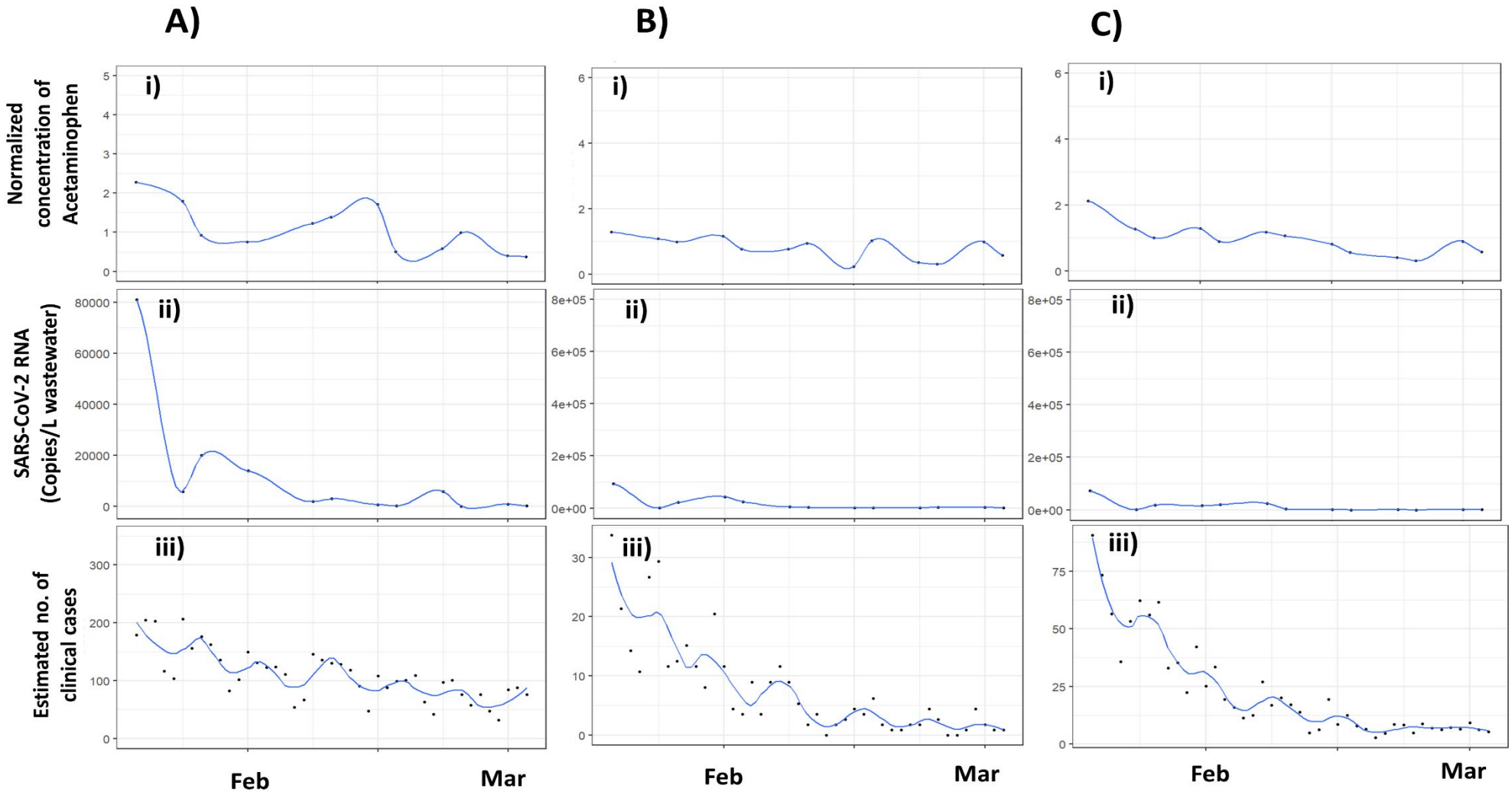

Figure S7. Post-outbreak population-normalized concentrations of detected acetaminophen in influent wastewater, (i), Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) RNA concentrations in influent wastewater, (ii), and estimated clinical cases of coronavirus disease 2019 (COVID-19) (iii) in (A) Bird Island, (B) City of Tonawanda, and (C) Kenmore-Tonawanda sampling sites. Post outbreak samplings were performed in 2022. Concentrations of caffeine in each location were used for the population normalization. Locally weighted scatterplot smoothing (LOWESS) was used to show the trends (blue lines). Estimated numbers of clinical cases and RT-qPCR data were obtained from the wastewater monitoring dashboard of Erie County New York.