Wastewater Surveillance of SARS-CoV-2 at a Canadian University Campus and the Impact of Wastewater Characteristics on Viral RNA Detection

Lena Carolin Bitter, Richard Kibbee, Gabriela Jimenez, Banu Örmeci*

Department of Civil and Environmental Engineering, Carleton University, 1125 Colonel By Dr, Ottawa, ON K1S 5B6, Canada

* Corresponding author: E-mail: banu.ormeci@carleton.ca, Phone: +1 (613) 520-2600, ext. 4144, Fax: +1 (613) 520-3951.

We confirm that all figures and images were created by the authors.

Manuscript List of Figures

Figure 1: Autosampler set-up

Figure 2: Reported university campus cases compared to the reported city cases

Figure 3: Viral copies/mL of the N1 and N2 gene targets compared to reported cases on campus and the city

Figure 4: Wastewater characteristics of Location A: a) Viral copies N1 & N2 (ultrafiltration & centrifugation methods) b) Total and volatile solids c) Turbidity d) Absorbance (190 nm, 254 nm)

Figure 5: Comparison of viral copies to wastewater characteristics at Location A: a) Turbidity and N1&N2 b) Absorbance (190nm) and N1&N2 c) Total Solids and N1&N2 d) Volatile Solids and N1&N2

Figure 6: Wastewater Characteristics of the Campus Residences A, B & C: a) Viral Copies N1 & N2 (Ultrafiltration and Centrifugation Methods), b) Total and Volatile Solids, c) Turbidity, d) Absorbance (190 nm, 254 nm)

Figure 7: Comparison of Viral Copies (identical in all subplots) to Wastewater Characteristics at Residence A: a) Turbidity and N1&N2 b) Absorbance (190nm) and N1&N2 c) Total Solids and N1&N2 d) Volatile Solids and N1&N2

Figure 8: Comparison of Viral Copies to Wastewater Characteristics at Residence B: a) Turbidity and N1&N2 b) Absorbance (190nm) and N1&N2 c) Total Solids and N1&N2 d) Volatile Solids and N1&N2

Manuscript List of Tables

Table 1: Correlations between Wastewater Signals (Centrifugation Method) and Case Counts

Table 2: Correlations between Viral Copies (Raw & PMMoV Normalized) and Wastewater Parameters by Processing Method for Location A

Supporting Information List of Figures

Figure S1: N1 & N2 Viral gene copies/ml at Location A with the Ultrafiltration and Centrifugation Concentration Methods

Figure S2: N1 & N2 Viral gene copies/ml at Location B with the Ultrafiltration and Centrifugation Concentration Methods

Figure S3: N1 & N2 Viral gene copies/ml compared to normalized N1&N2 viral gene copies/ml with PMMoV at Location A with the Ultrafiltration Concentration Methods

Figure S4: N1 & N2 Viral gene copies/ml compared to normalized N1&N2 viral gene copies/ml with PMMoV at Location A with the Centrifugation Concentration Methods

Figure S5: N1 & N2 Viral gene copies/ml compared to normalized N1&N2 viral gene copies/ml with PMMoV at Location B with the Ultrafiltration Concentration Methods

Figure S6: N1 & N2 Viral gene copies/ml compared to normalized N1&N2 viral gene copies/ml with PMMoV at Location B with the Centrifugation Concentration Methods

Figure S7: Comparison of raw viral wastewater signals of the campus and the city

Figure S8: Comparison of PMMoV normalized viral wastewater signals of the campus and the city

Figure S9: Temperature and pH data for Location A

Figure S10: Temperature and pH data for Location B (Residences A, B &C)

Figure S11: Absorbance Scans from 190 nm to 350 nm for Location A

Supporting Information List of Tables

Table S1: Primer and Probe Sequences of N1&N2 Targets and PMMoV

Table S2: Correlations between Case Counts

Supporting Information

Table S1: Primer and Probe Sequences of N1&N2 Targets and PMMoV

Target	Item	Sequence (5' → 3')
N1	Primer-Forward	GAC CCC AAA ATC AGC GAA AT
	Primer-Reverse	TCT GGT TAC TGC CAG TTG AAT CTG
	Probe	FAM-ACC CCG CAT TAC GTT TGG TGG ACC-MGBNFQ
	Linearized Plasmid	TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATG CAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGC CGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTG GCGGGTGTCGGGGCTGGCTTAACTATGCGGCATCAGAGCAG ATTGTACTGAGAGTGCACCAAATGCGGTGTGAAATACCGCA CAGATGCGTAAGGAGAAAATACCGCATCAGGCGCCATTCGC CATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGG GCCTCATCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGC TGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGT CACGACGTTGTAAAACGACGGCCAGTGCAACGCGATGACG ATGGATAGCGATTCATCGATGAGCTGACCCGATCGCCGCCG CCGGAGGGTTGCGTTTGAGACGGGCGACAGATATGTCTGAT AATGGACCCCAAAATCAGCGAAATGCACCCCGCATTACGTT TGGTGGACCCTCAGATTCAACTGGCAGTAACCAGAATGGAG AACGCAGTGGGGCGCGATCAAAACAACGTCGGCCCCAAGG TTTACCCAATAATACTGCGTCTTGGTTCACCGCTCTCACTCA ACATGGCAAGGAAGACCTTAAATTCCCTCGAGGACAAGGCG TTCCAATTAACACCAATAGCAGTCCAGATGACCAAATTGGC TACTACCGAAGAGCTACCAGACGAATTCGTGGTGGTGACGG TAAAATGAAAGATCTCAGTCCAAGATGGTATTTCTACTACC TAGGAACTGGGCCAGAAGCTGGACTTCCCTATGGTGCTAAC AAAGACGGCATCATATGGGTTGCAACTGAGGGAGCCTTGAA TACACCAAAAGATCACATTGGCACCCGCAATCCTGCTAACA ATGCTGCAATCGTGCTACAACTTCCTCAAGGAACAACATTG CCAAAAGGCTTCTACGCAGAAGGGAGCAGAGGCGGCAGTC AAGCCTCTTCTCGTTCCTCATCACGTAGTCGCAACAGTTCAA GAAATTCAACTCCAGGCAGCAGTAGGGGAACTTCTCCTGCT AGAATGGCTGGCAATGGCGGTGATGCTGCTCTTGCTTTGCT GCTGCTTGACAGATTGAACCAGCTTGAGAGCAAAATGTCTG GTAAAGGCCAACAACAACAAGGCCAAACTGTCACTAAGAA ATCTGCTGCTGAGGCTTCTAAGAAGCCTCGGCAAAAACGTA CTGCCACTAAAGCATACAATGTAACACAAGCTTTCGGCAGA CGTGGTCCAGAACAAACCCAAGGAAATTTTGGGGACCAGG AACTAATCAGACAAGGAACTGATTACAAACATTGGCCGCAA ATTGCACAATTTGCCCCCAGCGCTTCAGCGTTCTTCGGAATG TCGCGCATTGGCATGGAAGTCACACCTTCGGGAACGTGGTT GACCTACACAGGTGCCATCAAATTGGATGACAAAGATCCAA ATTTCAAAGATCAAGTCATTTTGCTGAATAAGCATATTGAC GCATACAAAACATTCCCACCAACAGAGCCTAAAAAGGACA AAAAGAAGAAGGCTGATGAAACTCAAGCCTTACCGCAGAG ACAGAAGAAACAGCAAACTGTGACTCTTCTTCCTGCTGCAA TTTGGATGATTTCTCCAAACAATTGCAACAATCCATGAGCA

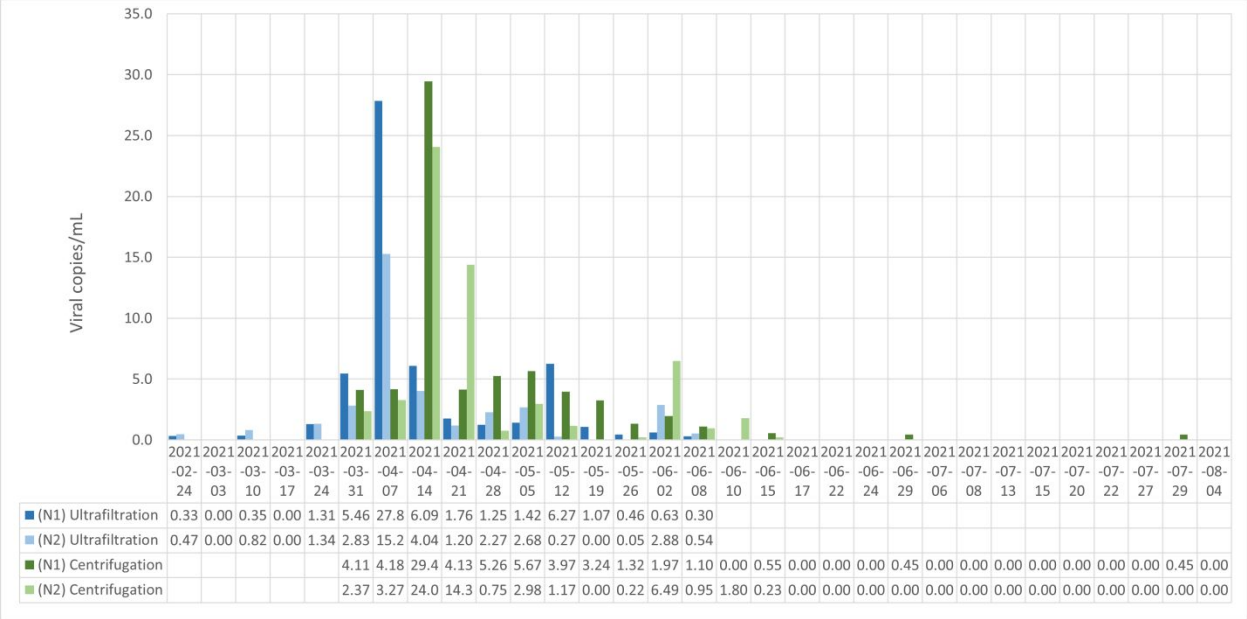
		GTGCTGACTCAACTCAGGCCTAAATCAGTTCTGGACCAGCG AGCTGTGCTGCGACTCGTGGCGTAATCATGGTCATAGCTGTT TCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACAT ACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAAT GAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCC GCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATG AATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGC GCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCG TTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTA ATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGA ACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTA AAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCC CCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGT GGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCC CCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGT CGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGC GTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTC GGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAA CCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTA TCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCAC TGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTA TGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACT ACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTG CTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTG ATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTG TTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCA AGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGT GGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATT ATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAA TGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTG GTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCT CAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCC CCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATC TGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCA CCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAA GGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCC ATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAG TTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTA CAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCA TTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATC CCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTC CGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACT CATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGC CATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACC AAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCT CTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAG CAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGG GGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAG TTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCAT CTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGA AGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGG

		AAATGTTGAATACTCATACTCTACCTTTTTCAATATTATTG AAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATA TTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCG CGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAA CCATTATTATCATGACATTAACCTATAAAAATAGGCGTAT CACGAGGCCCTTTCGTC
	Strings DNA Fragments	ATGTACTCATTCGTTTCGGAAGAGACAGGTACGTTAATAGT TAATAGCGTACTTCTTTTTCTTGCTTTCGTGGTATTCTTGCTA GTTACACTAGCCATCCTTACTGCGCTTCGATTGTGTGCGTA CTGCTGCAATATTGTTAACGTGAGTCTTGTAAAACCTTCTTT TTACGTTTACTCTCGTGTTAAAAATCTGAATTCCACCCATTC AGTACATCGATATCGGTAATTATACAGTTTCCTGTTTACCTT TTACAATTAATTGCCAGGAACCTAAATTGGGTAGTCTTGTA GTGCGTTGTTCGTTCTATGAAGACTTTTTAGAGTATCATGAC GTTCGTGTTGTTTTAGATTTCATCTAAACGAACAAACTAAA ATGTCTGATAATGGACCCCAAAATCAGCGAAATGCACCCCG CATTACGTTTGGTGGACCCTCAGATTCAACTGGCAGTAACC AGAATGGAGAACGCAGTGGGGCGCGATCAAAACAACGTCG GCCCCGTGGTCCAGAACAAACCCAAGGAAATTTTGGGGACC AGGAACTAATCAGACAAGGAACTGATTACAAACATTGGCC GCAAATTGCACAATTTGCCCCCAGCGCTTCAGCGTTCTTCG GAATGTCGCGCATTGGCATGGAAGTCACACCTTCGGGAACG TGGTTGACCTACACAGGTGCCATCAAATTGGATGACAAAG
N2	Primer-Forward	TTA CAA ACA TTG GCC GCA AA
	Primer-Reverse	GCG CGA CAT TCC GAA GAA
	Probe	FAM-ACA ATT TGC CCC CAG CGC TTC AG-MGBNFQ
	Linearized Plasmid	Same as N1
	Strings DNA Fragments	Same as N1
PMMoV	Primer-Forward	GAG TGG TTT GAC CTT AAC GTT TGA
	Primer-Reverse	TTG TCG GTT GCA ATG CAA GT
	Probe	FAM-CCT ACC GAA GCA AAT G-MGBNFQ
	gBlock	5’-TGGCAGCAAAGGTAATGGTAGCTGTGGTTTCAAATGAG AGTGGTTTGACCTTAACGTTTGAGAGGCCTACCGAAGCAAA TGTCGCACTTGCATTGCAACCGACAATTACATCAAAGGAGG AAGGTTCGTTGAAGATTGTG-3’
	Strings DNA Fragments	5’-TGGCAGCAAAGGTAATGGTAGCTGTGGTTTCAAATGAG AGTGGTTTGACCTTAACGTTTGAGAGGCCTACCGAAGCAAA TGTCGCACTTGCATTGCAACCGACAATTACATCAAAGGAGG AAGGTTCGTTGAAGATTGTG-3’

Table S2: Correlations between Case Counts

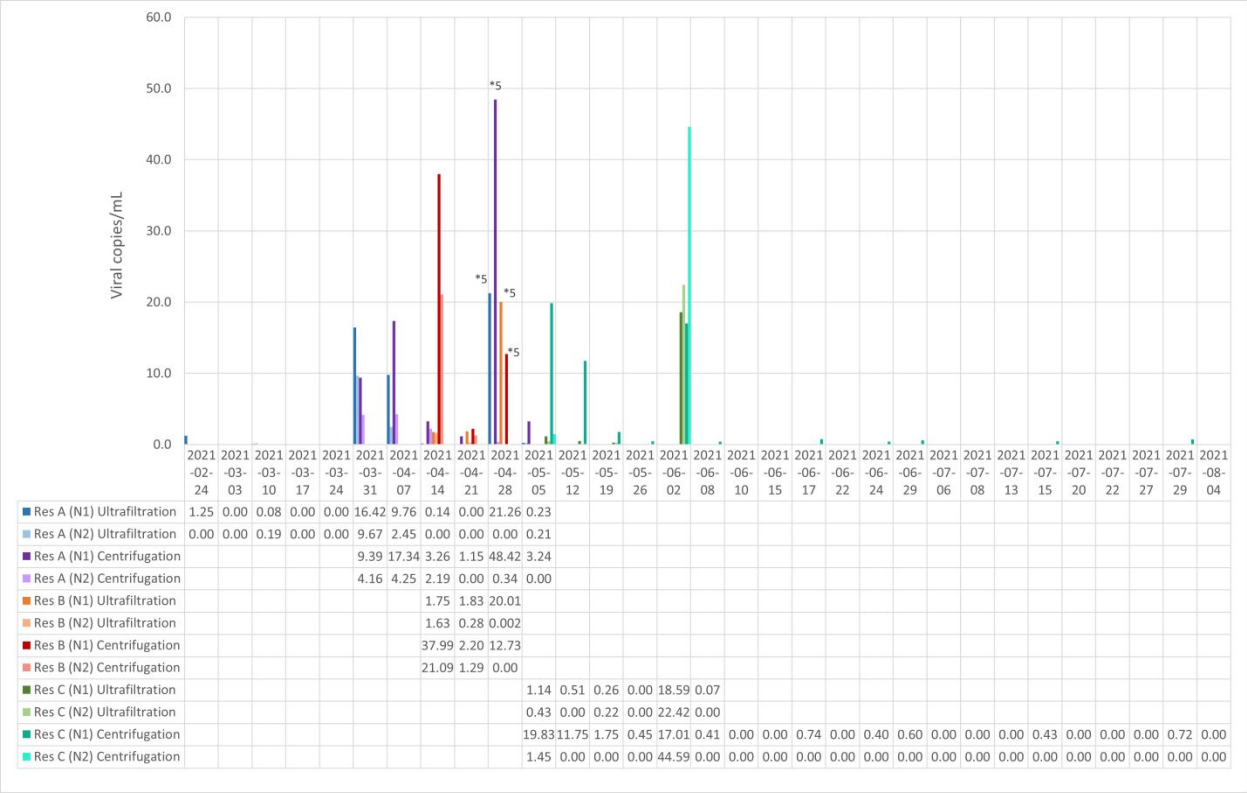
		City-wide Case Count			
	n	Week prior	3 days prior	Week of	Week after
Campus-wide Case Count					
Week prior	26	0.52**			
3 days prior	26		0.51**		
Week of	26			0.61**	
Week after	26				0.69**
Residence A Case Count					
Week prior	6	–			
3 days prior	6		–		
Week of	6			–	
Week after	6				–

Note. Significant correlations reflected as * $p < 0.05$, ** $p < 0.01$, and – reflects nonsignificant correlation



	2021-02-24	2021-03-03	2021-03-10	2021-03-17	2021-03-24	2021-03-31	2021-04-07	2021-04-14	2021-04-21	2021-04-28	2021-05-05	2021-05-12	2021-05-19	2021-05-26	2021-06-02	2021-06-08	2021-06-10	2021-06-15	2021-06-17	2021-06-22	2021-06-24	2021-06-29	2021-07-06	2021-07-08	2021-07-13	2021-07-15	2021-07-20	2021-07-22	2021-07-27	2021-07-29	2021-08-04
(N1) Ultrafiltration	0.33	0.00	0.35	0.00	1.31	5.46	27.8	6.09	1.76	1.25	1.42	6.27	1.07	0.46	0.63	0.30															
(N2) Ultrafiltration	0.47	0.00	0.82	0.00	1.34	2.83	15.2	4.04	1.20	2.27	2.68	0.27	0.00	0.05	2.88	0.54															
(N1) Centrifugation						4.11	4.18	29.4	4.13	5.26	5.67	3.97	3.24	1.32	1.97	1.10	0.00	0.55	0.00	0.00	0.00	0.45	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.45	0.00
(N2) Centrifugation						2.37	3.27	24.0	14.3	0.75	2.98	1.17	0.00	0.22	6.49	0.95	1.80	0.23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Figure S1: N1 & N2 Viral gene copies/ml at Location A with the Ultrafiltration and Centrifugation Concentration Methods



	2021-02-24	2021-03-03	2021-03-10	2021-03-17	2021-03-24	2021-03-31	2021-04-07	2021-04-14	2021-04-21	2021-04-28	2021-05-05	2021-05-12	2021-05-19	2021-05-26	2021-06-02	2021-06-08	2021-06-10	2021-06-15	2021-06-17	2021-06-22	2021-06-24	2021-06-29	2021-07-06	2021-07-08	2021-07-13	2021-07-15	2021-07-20	2021-07-22	2021-07-27	2021-07-29	2021-08-04
Res A (N1) Ultrafiltration	1.25	0.00	0.08	0.00	0.00	16.42	9.76	0.14	0.00	21.26	0.23																				
Res A (N2) Ultrafiltration	0.00	0.00	0.19	0.00	0.00	9.67	2.45	0.00	0.00	0.00	0.21																				
Res A (N1) Centrifugation						9.39	17.34	3.26	1.15	48.42	3.24																				
Res A (N2) Centrifugation						4.16	4.25	2.19	0.00	0.34	0.00																				
Res B (N1) Ultrafiltration								1.75	1.83	20.01																					
Res B (N2) Ultrafiltration								1.63	0.28	0.002																					
Res B (N1) Centrifugation								37.99	2.20	12.73																					
Res B (N2) Centrifugation								21.09	1.29	0.00																					
Res C (N1) Ultrafiltration											1.14	0.51	0.26	0.00	18.59	0.07															
Res C (N2) Ultrafiltration											0.43	0.00	0.22	0.00	22.42	0.00															
Res C (N1) Centrifugation											19.83	11.75	1.75	0.45	17.01	0.41	0.00	0.00	0.74	0.00	0.40	0.60	0.00	0.00	0.00	0.43	0.00	0.00	0.00	0.72	0.00
Res C (N2) Centrifugation											1.45	0.00	0.00	0.00	44.59	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Figure S2: N1 & N2 Viral gene copies/ml at Location B with the Ultrafiltration and Centrifugation Concentration Methods

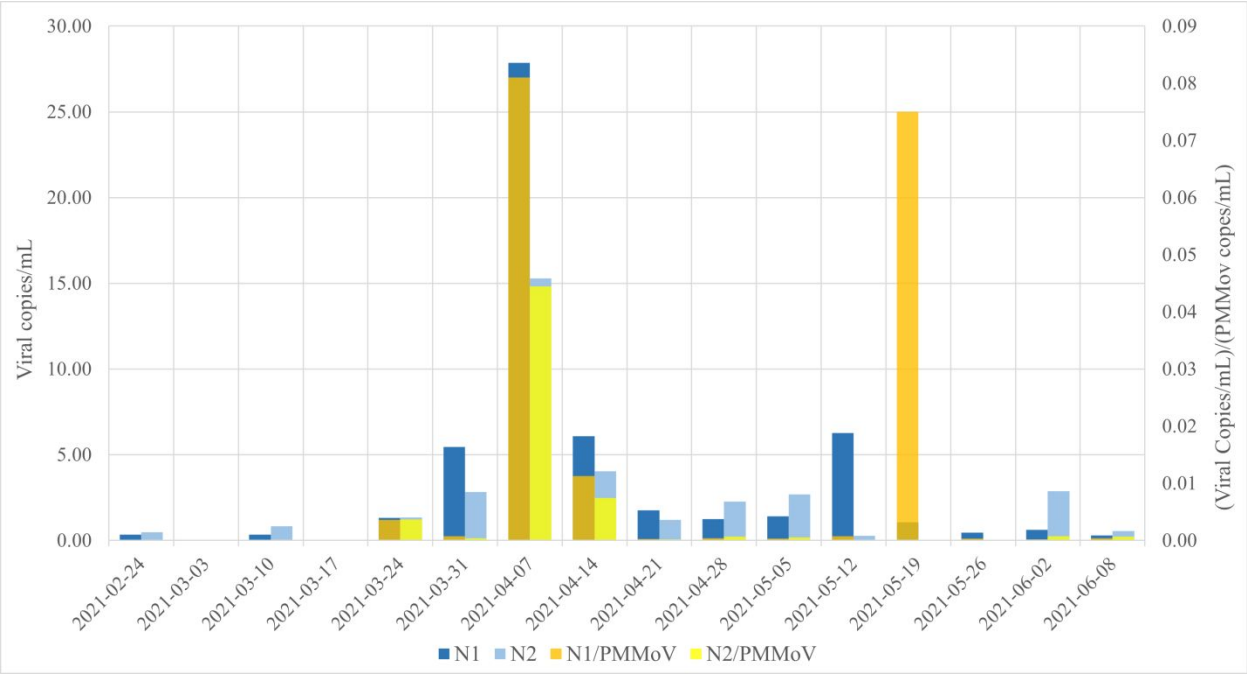


Figure S3: N1 & N2 Viral gene copies/ml compared to normalized N1&N2 viral gene copies/ml with PMMoV at Location A with the Ultrafiltration Concentration Methods

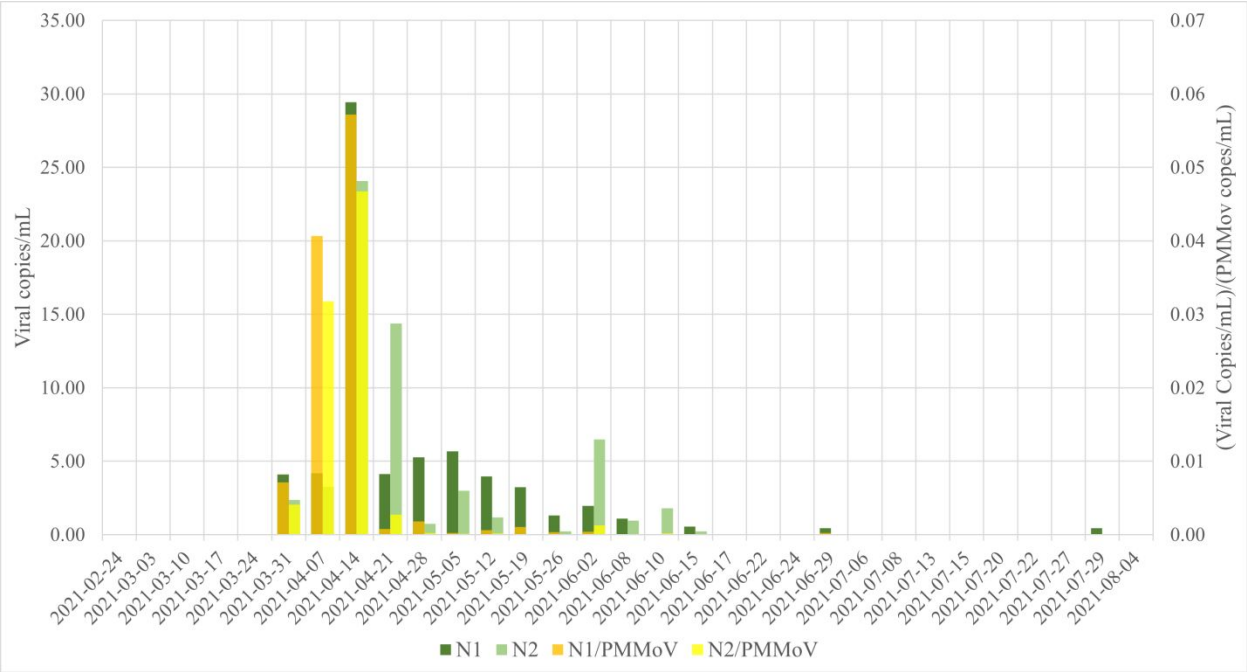


Figure S4: N1 & N2 Viral gene copies/ml compared to normalized N1&N2 viral gene copies/ml with PMMoV at Location A with the Centrifugation Concentration Methods

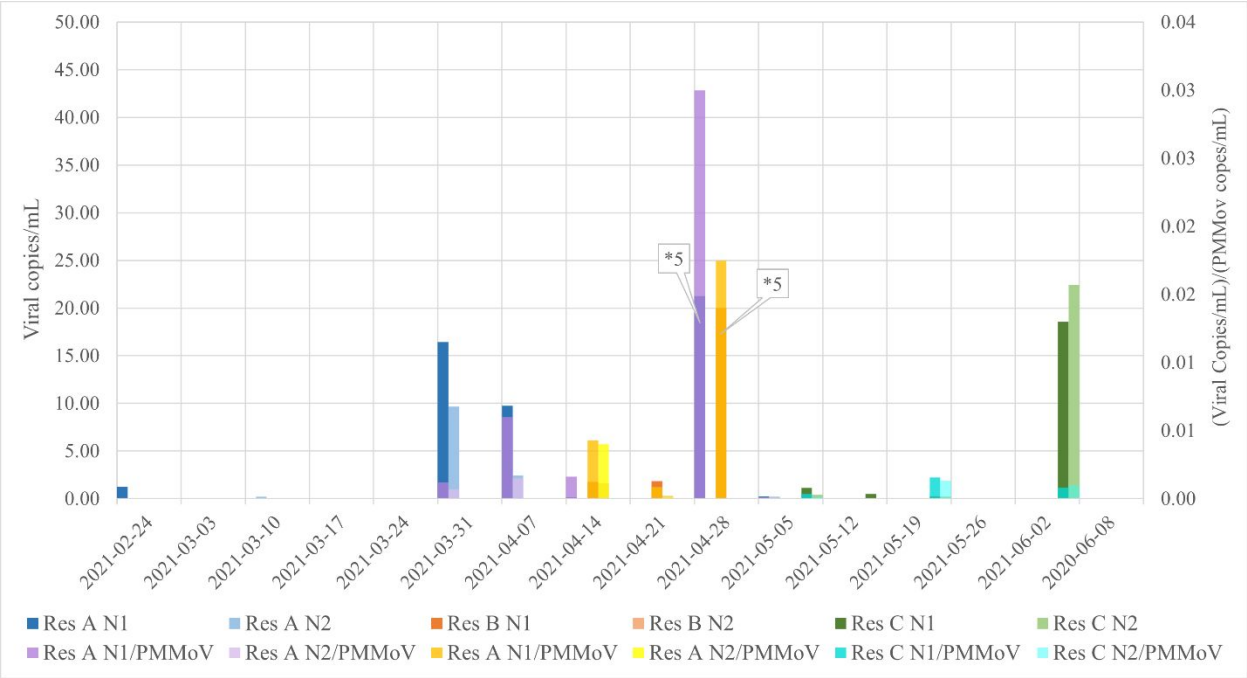


Figure S5: N1 & N2 Viral gene copies/ml compared to normalized N1&N2 viral gene copies/ml with PMMoV at Location B with the Ultrafiltration Concentration Methods

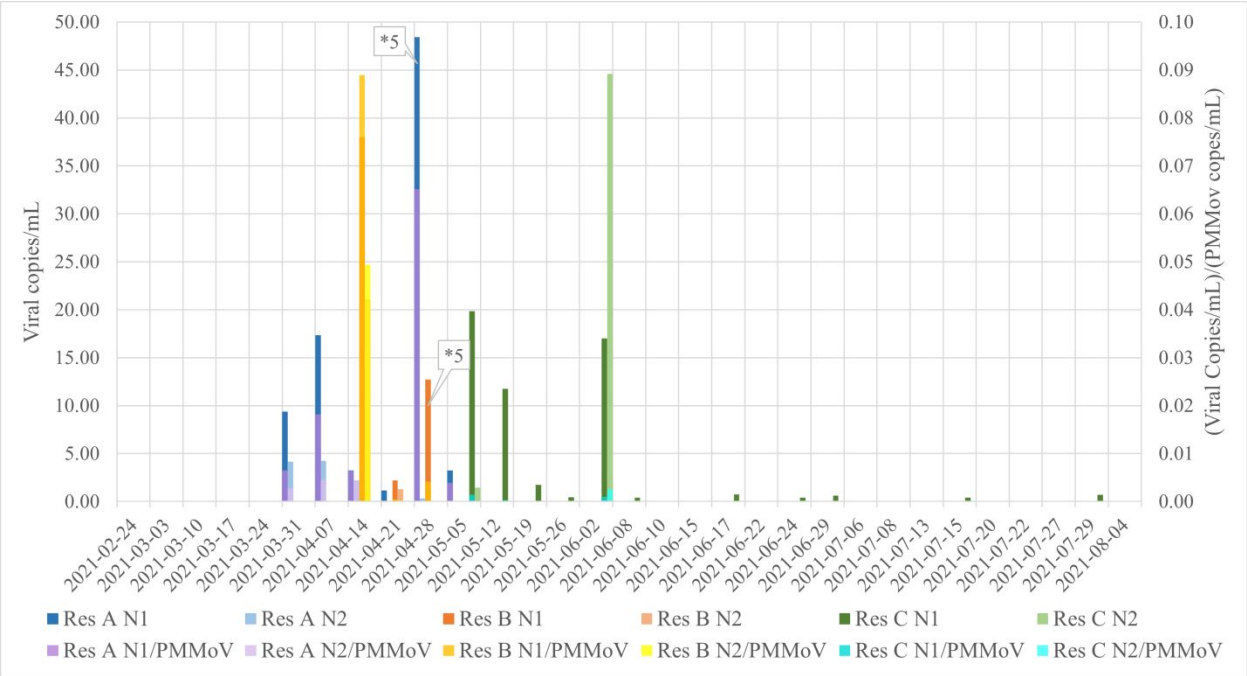


Figure S6: N1 & N2 Viral gene copies/ml compared to normalized N1&N2 viral gene copies/ml with PMMoV at Location B with the Centrifugation Concentration Methods

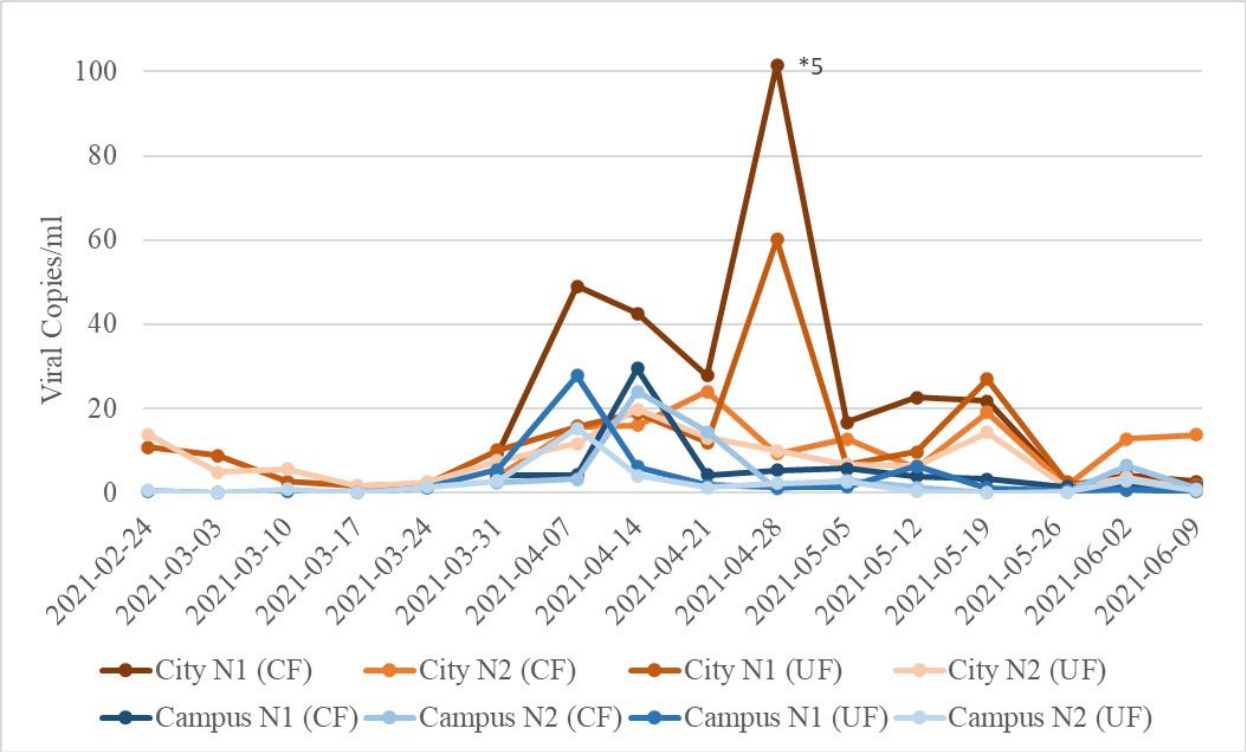


Figure S7: Comparison of raw viral wastewater signals of the campus and the city

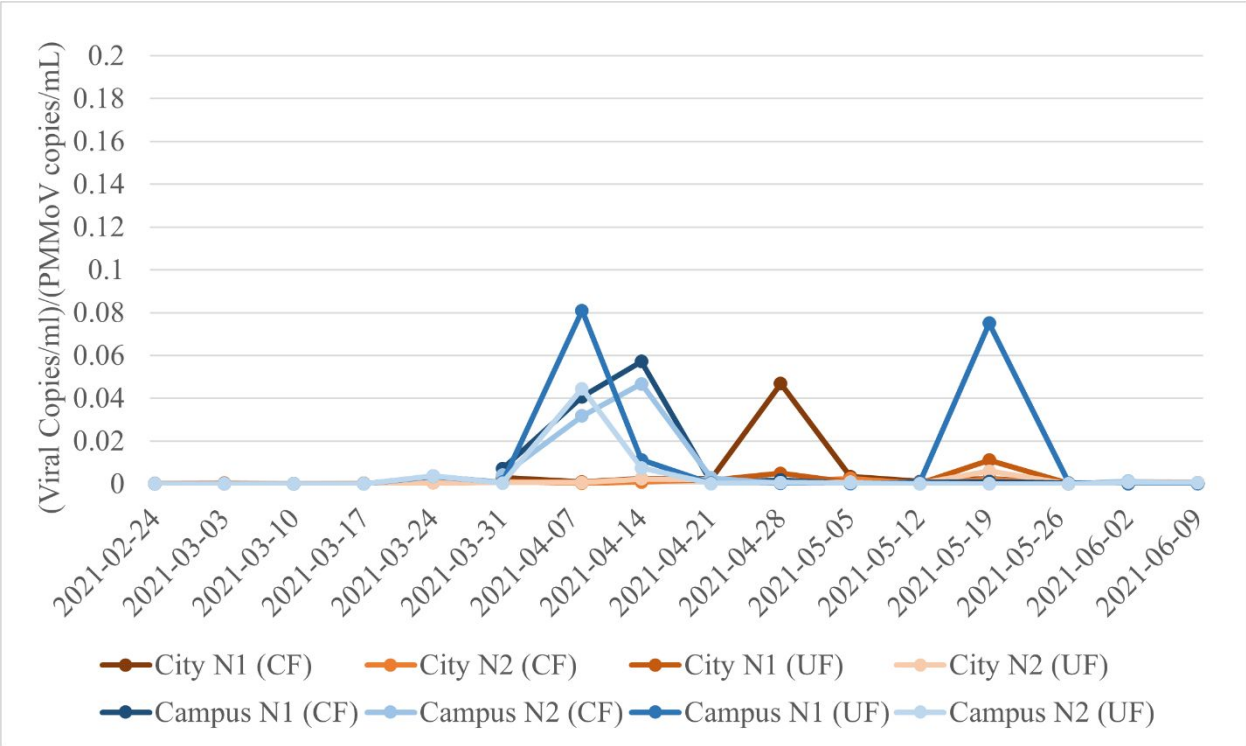


Figure S8: Comparison of PMMoV normalized viral wastewater signals of the campus and the city

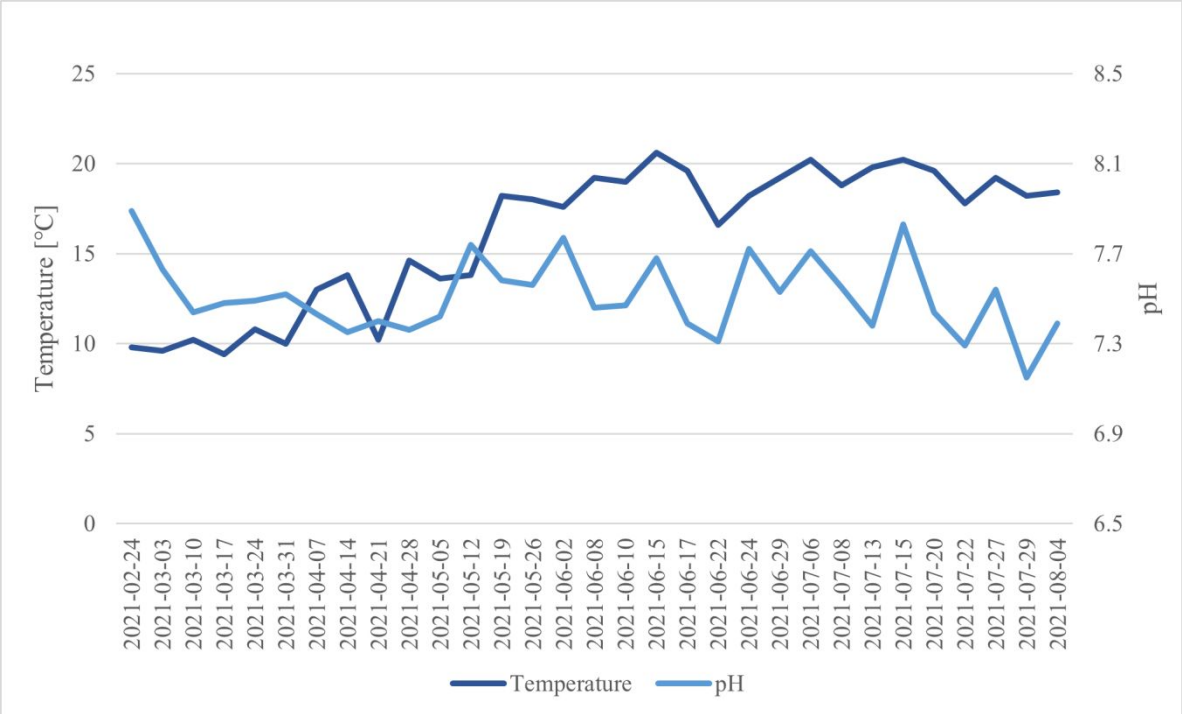


Figure S9: Temperature and pH data for Location A

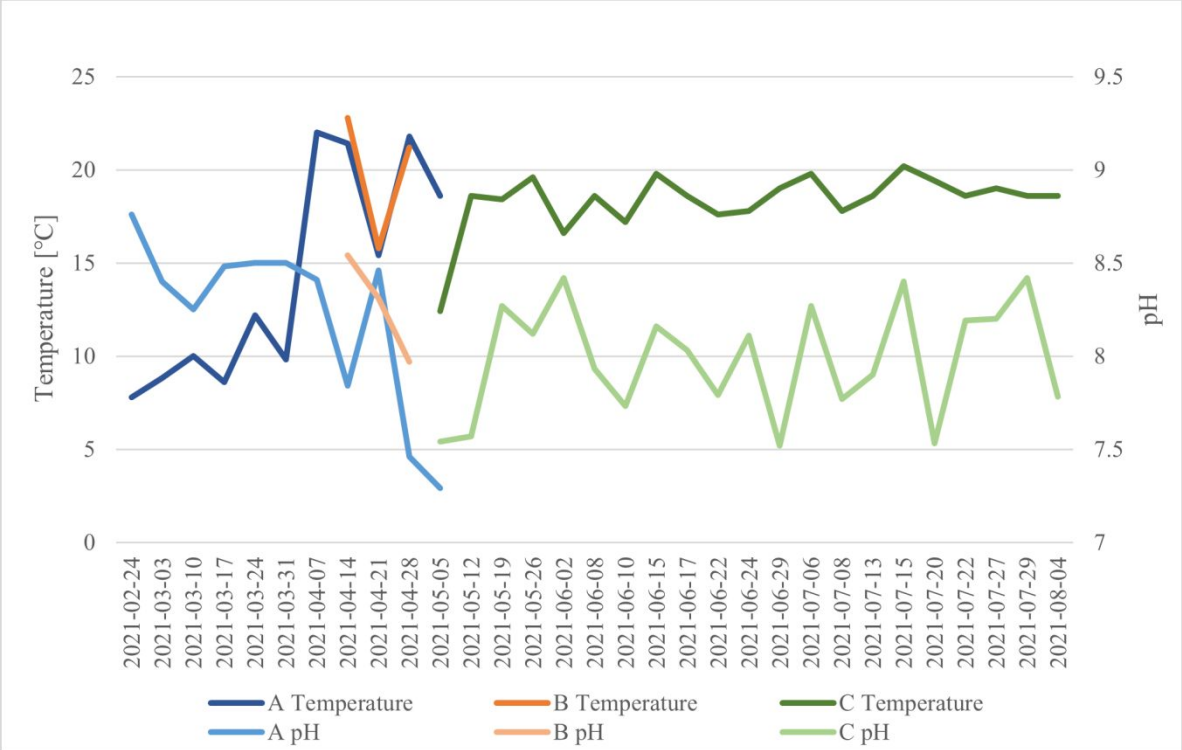


Figure S10: Temperature and pH data for Location B (Residences A, B &C)

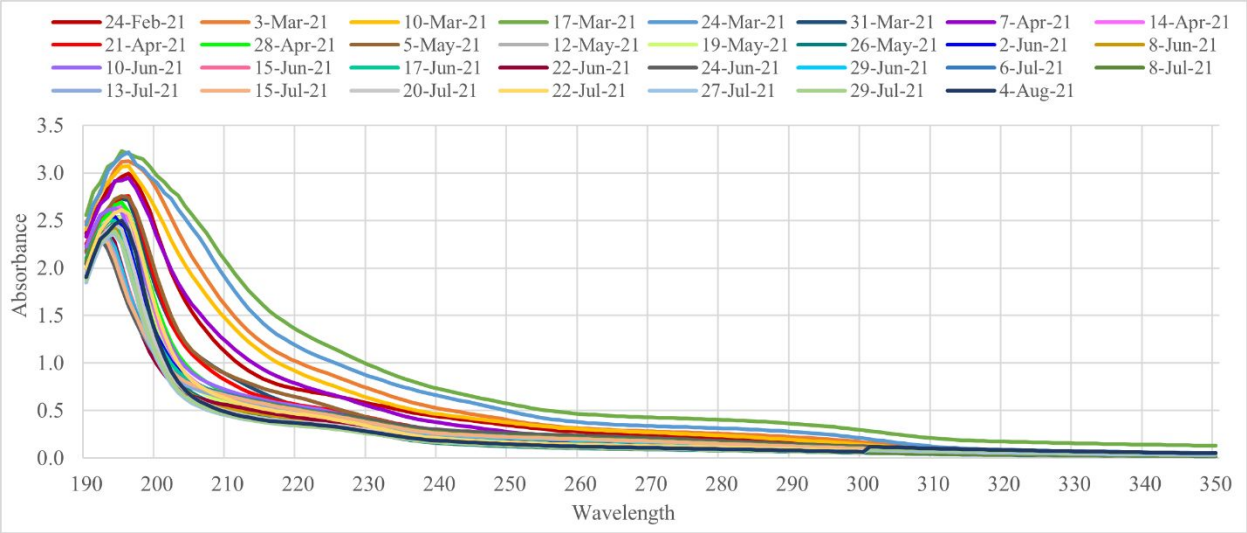


Figure S11: Absorbance Scans from 190 nm to 350 nm for Location A