

1 **Supplementary Tables**

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3 **Somatic Coliphages as a Promising Fecal Viral Surrogate for Wastewater**  
4 **Treatment Performance Evaluation in Tropical Coastal Region**

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18 Table S1 - Wastewater Treatment Plant Influent and Effluent Physico-Chemical Parameters by  
 19 Sampling Date  
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Parameter	Unit	9/21/ 23	9/20/ 23	9/19/ 23	7/13/ 23	7/12/ 23	7/11/ 23	5/18/ 23	5/17/ 23	5/16/ 23
BOD Influent	mg/L	98.1	106.1	117.3	116.6	120	82.5	106.3	97.4	*
BOD Final Effluent	mg/L	2.5	2.4	2.3	3.6	3.2	2.9	4.4	3.4	*
TSS Influent	mg/L	83	105.3	160	117	117	105.5	115	120	159.5
TSS Final Effluent	mg/L	2.6	2.2	6.2	3.5	3.6	3	4.3	4.1	4.2
pH Influent	-	7.59	7.5	7.53	7.56	7.6	7.56	7.57	7.58	7.54
Temperature	C	24.4	26.3	25.9	25.7	27.2	27.1	27.5	27.4	27.2
pH Final Effluent	-	6.97	6.87	6.93	6.95	6.93	6.96	7.02	6.92	6.92
Temperature	C	26.5	26.7	26.6	27.4	26.5	27.4	26.9	26.9	26.8
Enterococcus	CFU/100 mL	16.6	29.7	22.5	14.5	9.4	6.8	1	1	1
Ammonia Influent	mg/L as N	17	18	19	16	18	16	16	18	18
Ammonia Final Effluent	mg/L as N	4.7	5.2	4.6	10.9	4.2	2.1	9.9	8.5	8.2
Flow Rate Final Effluent	MGD	3.503	3.41	3.443	3.551	3.532	3.486	3.644	3.739	3.748

\* No measured data for this day

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25 Table S2 - Somatic and F-Specific Coliphage Recovery Efficiencies (%) Across Treatment  
 26 Processes and Dates  
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Date	Wastewater Process	Somatic Recovery (%)	F-Specific Recovery (%)
5/16/23	Primary	21%	4.1%
	Secondary	18%	*
	Final	78%	*
5/17/23	Primary	37%	40.0%
	Secondary	5%	4.2%
	Final	*	NA
5/18/23	Primary	8%	0.2%
	Secondary	55%	1.0%
	Final	7%	*
7/11/23	Primary	51%	*
	Secondary	13%	*
	Final	*	*
7/12/23	Primary	19%	0.12
	Secondary	5%	1.67
7/12/23	Final	106%	0.17
7/13/23	Primary	18%	0.71
	Secondary	7%	0.1
	Final	*	*
9/19/23	Primary	4%	5.63%
	Secondary	58%	0.00%
	Final	0%	0.00%
9/20/23	Primary	43%	0.01%
	Secondary	14%	0.07%
	Final	0%	0.00%
9/21/23	Primary	4%	0.01%
	Secondary	11%	0.37%
	Final	0%	0.69%

\* coliphage concentration below limit of detection.

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30 Table S3 - Total RNA and DNA Concentrations (ng/ $\mu$ L)

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Date	Wastewater Process	RNA (ng/uL)	DNA (ng/uL)
5/16/23	Primary	16.6	5.5
5/16/23	Secondary	5.5	8.5
5/16/23	Final	8.5	28.9
5/17/23	Primary	28.9	7.6
5/17/23	Secondary	7.6	8.0
5/17/23	Final	8.0	22.3
5/18/23	Primary	22.3	7.7
5/18/23	Secondary	7.7	8.4
5/18/23	Final	8.4	0.0
7/11/23	Primary	15.7	3.6
7/11/23	Secondary	3.6	5.4
7/11/23	Final	5.4	8.7
7/12/23	Primary	8.7	2.6
7/12/23	Secondary	2.6	2.6
7/12/23	Final	2.6	22.0
7/13/23	Primary	22.0	2.5
7/13/23	Secondary	2.5	3.1
7/13/23	Final	3.1	0.0
9/19/23	Primary	6.7	6.3
9/19/23	Secondary	6.3	4.9
9/19/23	Final	4.9	7.7
9/20/23	Primary	7.7	6.1
9/20/23	Secondary	6.1	8.5
9/20/23	Final	8.5	44.6
9/21/23	Primary	44.6	3.1
9/21/23	Secondary	3.1	9.1
9/21/23	Final	9.1	0.0

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33 Table S4 - qPCR Assay Oligonucleotides, Thermal Cycling Conditions, and Calibration Metrics for Viral Targets  
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Virus	Oligo Name	Sequence (5' to 3')	Thermal Cycling Condition	Amplification Efficiency	Calibration Equation	R-squared	Limit of Detection (GC/rxn)	Reference
Norovirus GI (Nov GI)	COG1F	CGYTGGATGCGNTTYCATGA	1 cycle of 95°C for 15 mins; 45 cycles of 95°C for 15s, 56°C for 60s	101.2%	$y=-3.2941x+38.746$	0.9975	$10^1$ to $10^6$	(Fout et al., 2016)
	COG1R	CTTAGACGCCATCATCATTYAC						
	RING1(a)-TP	FAM-AGATYGCGATCYCCTGTCCA-BHQ1						
	RING1(b)-TP	FAM-AGATCGCGGTCTCCTGTCCA-BHQ1						
Norovirus GII (Nov GII)	COG2F	CARGARBCNATGTTYAGRTGGATGAG	1 cycle of 95°C for 15 mins; 45 cycles of 95°C for 15s and 56°C for 60s	99.5%	$y=-3.3338x+40.577$	0.9986	$10^1$ to $10^6$	(Fout et al., 2016)
	COG2R	TCGACGCCATCTTCATTCACA						
	RING2-TP	FAM-TGGGAGGGCGATCGCAATCT-BHQ1						
pepper mild mottle virus (PMMoV)	PMMV-FP1-rev	GAGTGGTTTGACCTTAACGTTTGA	1 cycle of 95°C for 15 mins; 45 cycles of 95°C for 5s and 60°C for 60s	98.4%	$y=-3.3614x+46.264$	0.9979	$10^1$ to $10^7$	(Haramoto et al., 2013)
	PMMV-RP1	TTGTTCGGTTGCAATGCAAGT						
	PMMV-Probe1	FAM-CCTACCGAAGCAAATG-BHQ1						
Adenovirus (AdV)	AQ2	GCCCCAGTGGTCTTACATGCACATC	1 cycle of 95°C for 15 mins; 45 cycles of 95°C for 3s and 55°C for 10s and 65°C for 60 s	101.8%	$y=-3.2803x+41.84$	0.9891	$10^1$ to $10^6$	(Heim et al., 2003)
	AQ1	GCCACGGTGGGGTTTCTAAACTT						
	AQP	FAM-TGCACCAGACCCGGGCTCAGGTACTCCGA-BHQ1						
Enterovirus (EV)	EV1F	CCCTGAATGCGGCTAA	1 cycle of 95°C for 15 mins; 45 cycles of 95°C for 15s and 60°C for 60s	97.5%%	$y=-3.5185x+37.211$	0.999	$10^1$ to $10^6$	(Fout et al., 2016)
	EV1R	TGTCACCATAAGCAGCCA						
	EV Probe	FAM-ACGGACACCCAAAGTAGTCGGTTC-BHQ1						
tomato brown rugose fruit virus (ToBRFV)	ToBRFV F	GGTGGTGTGTCAGTGTCTGTTT	1 cycle of 25°C for 10 mins and 95°C for 30s ; 45 cycles of 95°C for 5s and 60°C for 30s	102.5%	$y=-3.225x+41.571$	0.9929	$10^1$ to $10^6$	(Caruso et al., 2022)
	ToBRFV R	GCGTCCTTGGTAGTGATGTT						
	ToBRFV P	FAM-AGAGAATGGAGAGAGCGGACGAGG-ZEN/IBFQ						

36 Table S5 – Primer and Probe Specificity Analysis

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Virus	Primer/Probe	Nucleotide Position	Match Ratio (Match/Sample Size)
Enterovirus	F Primer	1	399/400
		10	277/400
		11	399/400
		13	399/400
		14	399/400
	R Primer	4	354/400
		10	392/400
		11	385/400
		12	392/400
		13	367/400
		14	367/400
		15	140/400
		16	35/400
	Probe	17	146/400
		18	321/400
		3	399/400
		6	399/400
		8	399/400
	Adenovirus	F Primer	19
23			201/400
4			134/200
7			131/200
11			107/200
R Primer		13	107/200
		22	199/200
		3	186/200
		6	94/200
		12	176/200
		15	150/200
Probe		18	174/200
		21	182/200
	6	199/200	
	9	174/200	
	12	184/200	
		15	107/200
		18	119/200
		21	66/200

Virus	Primer/Probe	Nucleotide Position	Match Ratio (Match/Sample Size)
		22	198/200
		27	198/200
PMMoV	F Primer	17	46/47
		19	46/47
		22	46/47
		2	46/47
	R Primer	4	46/47
		5	46/47
		3	46/47
	Probe	6	46/47
ToBRFV	F Primer	2	151/152
	R Primer	9	151/152
	Probe	2	114/152
		20	151/152
Norovirus GI	F Primer	3	78/86
		12	30/86
		13	85/86
		15	81/86
		18	79/86
	R Primer	1	85/86
		20	81/86
		9	60/86
	Probe Ring 1a	15	85/86
		17	84/86
	Probe Ring 1b	5	53/86
		9	60/86
		12	78/86
15		85/86	
17		84/86	
Norovirus GII	F Primer	3	287/400
		6	213/400
		7	33/400
		9	233/400
		14	398/400
		15	376/400
		16	396/400
		18	194/400
	R Primer	2	399/400

Virus	Primer/Probe	Nucleotide Position	Match Ratio (Match/Sample Size)
		12	397/400
		21	393/400
	Probe	9	397/400
		15	399/400

39 Table S6 – Somatic and F-Specific Coliphage Concentrations (PFU/mL)

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WWTP Process Area	Season	Date	Somatic (PFU / 100 mL)	F Specific (PFU / 100mL)	Somatic Log10(PFU / 100 mL)	F Specific Log10(PFU / 100mL)	
Primary	Fall	9/19/2023	395,500	200	5.60	2.30	
		9/20/2023	213,600	24,150	5.33	4.38	
		9/21/2023	473,200	1,900	5.68	3.28	
	Spring	5/16/2023	245,200	1,100	5.39	3.04	
		5/17/2023	612,000	2,000	5.79	3.30	
		5/18/2023	546,800	19,440	5.74	4.29	
	Summer	7/11/2023	503,333	ND	5.70	ND	
		7/12/2023	567,667	8,880	5.75	3.95	
		7/13/2023	683,000	1,300	5.83	3.11	
	Winter	1/31/2023	381,333	1,000	5.58	3.00	
		2/14/2023	231,667	200	5.36	2.30	
		2/15/2023	385,200	10,100	5.59	4.00	
	Average (PFU/100mL)		-	436,542	5,856	5.612	3.080
	Standard Deviation (PFU/100mL)		-	154,431	8,214	0.171	1.190
	Coefficient of Variation (%)		-	35%	140%	3%	39%
Secondary	Fall	9/19/2023	2,111	12.0	3.32	1.08	
		9/20/2023	16,009	7.5	4.20	0.88	
		9/21/2023	14,040	3.0	4.15	0.48	
	Spring	5/16/2023	4,500	ND	3.65	ND	
		5/17/2023	15,265	240.0	4.18	2.38	
		5/18/2023	32,960	50.0	4.52	1.70	
	Summer	7/11/2023	19,685	2.0	4.29	0.30	
		7/12/2023	22,284	1.5	4.35	0.18	
		7/13/2023	11,075	135.0	4.04	2.13	
	Winter	1/31/2023	526	135.0	2.72	2.13	
		2/14/2023	10,680	ND	4.03	ND	
		2/15/2023	3,300	ND	3.52	ND	
	Average (PFU/100mL)		-	12,703	49	3.915	0.937
	Standard Deviation (PFU/100mL)		-	9,502	79	0.516	0.923
	Coefficient of Variation (%)		-	75%	160%	13%	98%
Final	Fall	9/19/2023	101.8	51.0	2.01	1.71	
		9/20/2023	20.5	1.5	1.31	0.18	
		9/21/2023	62.0	2.0	1.79	0.30	
	Spring	5/16/2023	8.0	ND	0.90	ND	
		5/17/2023	10.0	ND	1.00	ND	
		5/18/2023	19.5	ND	1.29	ND	
	Summer	7/11/2023	55.5	ND	1.74	ND	
		7/12/2023	25.5	28.5	1.41	1.45	
		7/13/2023	ND	574.0	ND	2.76	
	Winter	1/31/2023	ND	ND	ND	ND	
		2/14/2023	13.0	ND	1.11	ND	
		2/15/2023	6.0	ND	0.78	ND	
	Average (PFU/100mL)		-	27	55	1.112	0.533
	Standard Deviation (PFU/100mL)		-	31	164	0.637	0.922
	Coefficient of Variation (%)		-	114%	297%	57%	173%
<b>t-test</b>	<b>p-value</b>	<b>Interpretation</b>					
Primary	4.81E-04	Significant					
Secondary	7.35923304549921e-06	Significant					
Final	0.252	Not significant					

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42 Table S7 - Viral Detection Rates and Observed Mutation Frequencies (%)

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Virus	Detection	Mutation Rate
PMMoV	100%	0.28%
ToBRFV	100%	0.42%
Norovirus GI	100%	3.40%
Norovirus GII	100%	4.00%
Adenovirus	41%	5.90%
Enterovirus	30%	6.70%

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45 Table S8 - Coefficients of Variation (%) for Viral Target Quantification  
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Viral Target	Primary Influent Coefficient of Variation	Secondary Effluent Coefficient of Variation	Final Effluent Coefficient of Variation
Norovirus GI	8.0%	7.9%	8.8%
Norovirus GII	6.7%	7.9%	6.4%
Enterovirus	11.2%	DATA BELOW LOQ	DATA BELOW LOQ
ToBRFV	3.2%	3.4%	2.2%
PMMoV	3.1%	3.2%	2.1%
Adenovirus	15.7%	13.7%	DATA BELOW LOQ

\* No data

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49 Table S9 – Normality Test by Shapiro-Wilk of Viral Targets

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**Overall Treatment Process**

Virus	P Value	W	Normality
Norovirus_GI	0.211	0.950	Normal
Norovirus_GII	0.025	0.912	Non-parametric
Enterovirus	0.663	0.814	Non-parametric
ToBRFV	0.050	0.924	Normal
PMMoV	0.168	0.946	Normal
Adenovirus	0.727	0.722	Non-parametric
Somatic	0.006	0.883	Non-parametric
F_specific	0.006	0.884	Non-parametric

**Individual Treatment Processes**

Treatment Process	Virus	P Value	W	Normality
Primary	Adenovirus	0.031	0.816	Non-parametric
Primary	Enterovirus	0.556	0.938	Normal
Primary	Norovirus_GI	0.096	0.860	Normal
Primary	Norovirus_GII	0.872	0.967	Normal
Primary	PMMoV	0.397	0.921	Normal
Primary	ToBRFV	0.395	0.920	Normal
Primary	log10_F_specific	0.046	0.831	Non-parametric
Primary	log10_Somatic	0.111	0.866	Normal
Secondary	Adenovirus	0.00004	0.531	Non-parametric
Secondary	Enterovirus	0.0007	0.675	Non-parametric
Secondary	Norovirus_GI	0.777	0.958	Normal
Secondary	Norovirus_GII	0.811	0.961	Normal
Secondary	PMMoV	0.210	0.892	Normal
Secondary	ToBRFV	0.635	0.945	Normal
Secondary	log10_F_specific	0.355	0.915	Normal
Secondary	log10_Somatic	0.167	0.883	Normal
Final	Adenovirus	NA	NA	Non-parametric
Final	Enterovirus	NA	NA	Non-parametric
Final	Norovirus_GI	0.135	0.874	Normal
Final	Norovirus_GII	0.252	0.900	Normal
Final	PMMoV	0.344	0.914	Normal
Final	ToBRFV	0.819	0.962	Normal
Final	log10_F_specific	0.007	0.757	Non-parametric
Final	log10_Somatic	0.394	0.920	Normal

Table 10 – Wastewater Parameter Spearman Correlations

**Final Effluent Only Correlations**

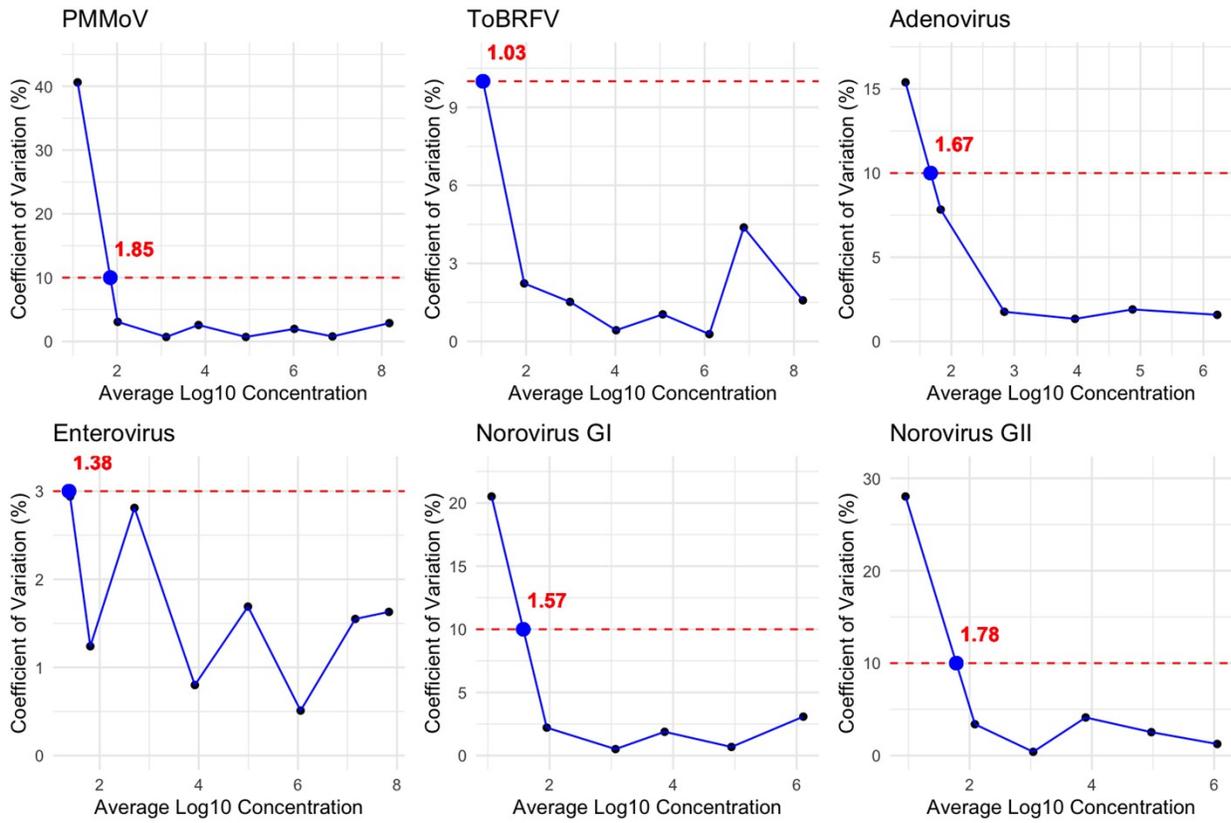
Group	Parameter	n	rho	p value	Significance
F-specific	BOD	7	0.185	0.691	Not Significant
F-specific	TSS	9	-0.191	0.622	Not Significant
F-specific	pH	9	0.391	0.298	Not Significant
F-specific	Temperature	9	0.200	0.606	Not Significant
<b>F-specific</b>	<b>Enterococci</b>	<b>9</b>	<b>-0.726</b>	<b>0.027</b>	<b>Significant</b>
F-specific	Ammonia	8	-0.101	0.811	Not Significant
F-specific	Flow	9	0.453	0.221	Not Significant
<b>Somatic</b>	<b>BOD</b>	<b>7</b>	<b>0.750</b>	<b>0.052</b>	<b>Not Significant</b>
Somatic	TSS	9	0.333	0.381	Not Significant
Somatic	pH	9	0.017	0.965	Not Significant
Somatic	Temperature	9	0.119	0.760	Not Significant
Somatic	Enterococci	9	-0.593	0.092	Not Significant
Somatic	Ammonia	8	0.619	0.102	Not Significant
<b>Somatic</b>	<b>Flow</b>	<b>9</b>	<b>0.767</b>	<b>0.016</b>	<b>Significant</b>

**Removal Correlations**

Group	Parameter	n	rho	p value	Significance
Somatic Log Removal	BOD LRV	7	0.393	0.383	Not Significant
Somatic Log Removal	TSS LRV	9	0.233	0.546	Not Significant
<b>Somatic Log Removal</b>	<b>Ammonia LRV</b>	<b>8</b>	<b>0.714</b>	<b>0.047</b>	<b>Significant</b>
F-specific Log Removal	BOD LRV	7	0.536	0.215	Not Significant
F-specific Log Removal	TSS LRV	9	-0.250	0.516	Not Significant
F-specific Log Removal	Ammonia LRV	8	0.429	0.289	Not Significant

55 Figure 1 – Limit of Quantification

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