Supporting Information

Norovirus Removal and Particle Association in a Waste Stabilization Pond

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Details on Statistical Analysis

All statistical procedures were conducted using the SAS Version 9.0 software package and analyzed using a 95% confidence interval ($p<0.05$). Before detailing the analyses, it is important to describe two preliminary steps.

First, we considered how to treat the concentrations of norovirus genogroup I (GI) and genogroup II (GII) below the detection limit. Due to the fact that nearly half the data was censored (or missing) we could not use the Winsorized mean or trimmed mean methods, and therefore excluded the values below the detection limit from the data set. Exclusion may introduce bias of the mean values; however, our aim is not to examine singular mean values, but differences of means, thereby reducing any bias effect. Many inferential procedures are based on normally distributed data and, therefore, we tested for normality of the data sets of concentrations of GI and GII in four locations of the pond system with the Shapiro-Wilk test. From the p-values of the statistic, it was evident that GI concentrations were normally distributed, while GII concentrations were non-normal. Given this information we performed two statistical procedures. The first was to test the difference in the mean concentrations of GI and GII in the four sampling locations. Analysis of variance (ANOVA) F-Test, relevant for normal data, was utilized to compare the mean concentrations of GI, while the non-normal GII data was analyzed with the non-parametric Kruskal-Wallis H-Test. The H statistic measures the extent to which the GII concentrations differ with respect to their relative ranks.

The second procedure was to conduct multiple comparisons to determine which mean concentration(s) of the four locations is/are significantly different. Again, adhering to the assumptions of normality, the Duncan test, which is a multiple range test for comparing sample means based on a step-wise procedure, was used for the GI data (*1*). The GII data did not have a significant difference of means, and therefore a multiple comparison test was not necessary. The cascade filtration data for both GI and GII did not meet conditions of normality; hence, the Kruskal-Wallis H-test was used to analyze the fractions of GI and GII found in each category. From this test, only GII was found to have significant differences among the filter sizes. Therefore, the Wilcoxon rank-sum test was utilized to compare separate pairs of GII filtration categories and determine which if any of the filters contained mean fractions of viruses that were significantly different.

References

1. Bewick, V.; Cheek, L.; Ball, J. Statistics Review 9: One-way analysis of variance. *Critical Care* **2004**, *8*, 130-136.
2. McClave, J.T.; Dietrich, F; Sincich, T. Statistics. Prentice Hall: Englewood, New Jersey, 1997.
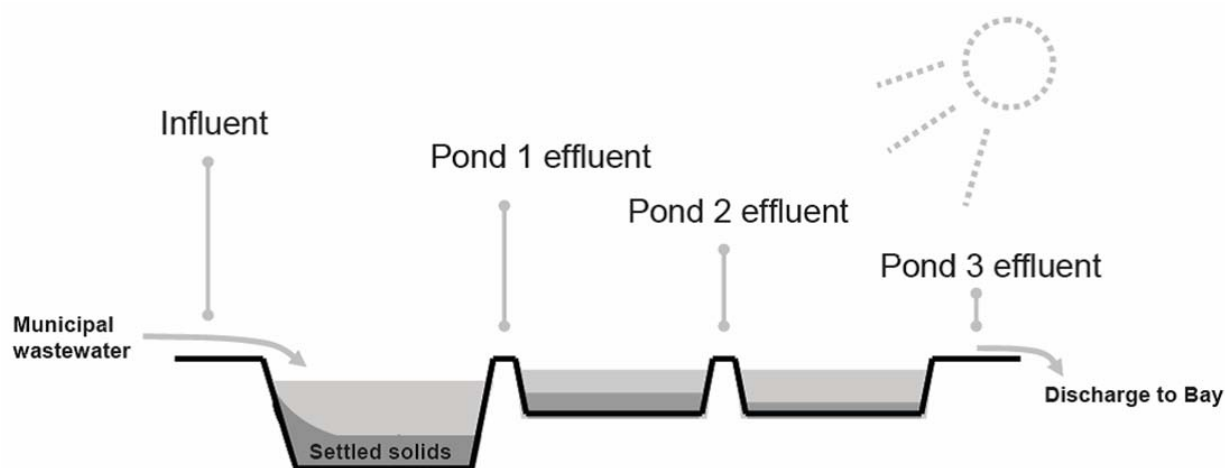
Figure S1. Sampling locations in the waste stabilization pond system. Grab samples (2L) were taken from the following locations: (i) system influent (sampled after the grill which removes large objects), (ii) pond 1 effluent weir, (iii) pond 2 effluent weir, and (iv) pond 3 effluent (final effluent discharged to the estuary).
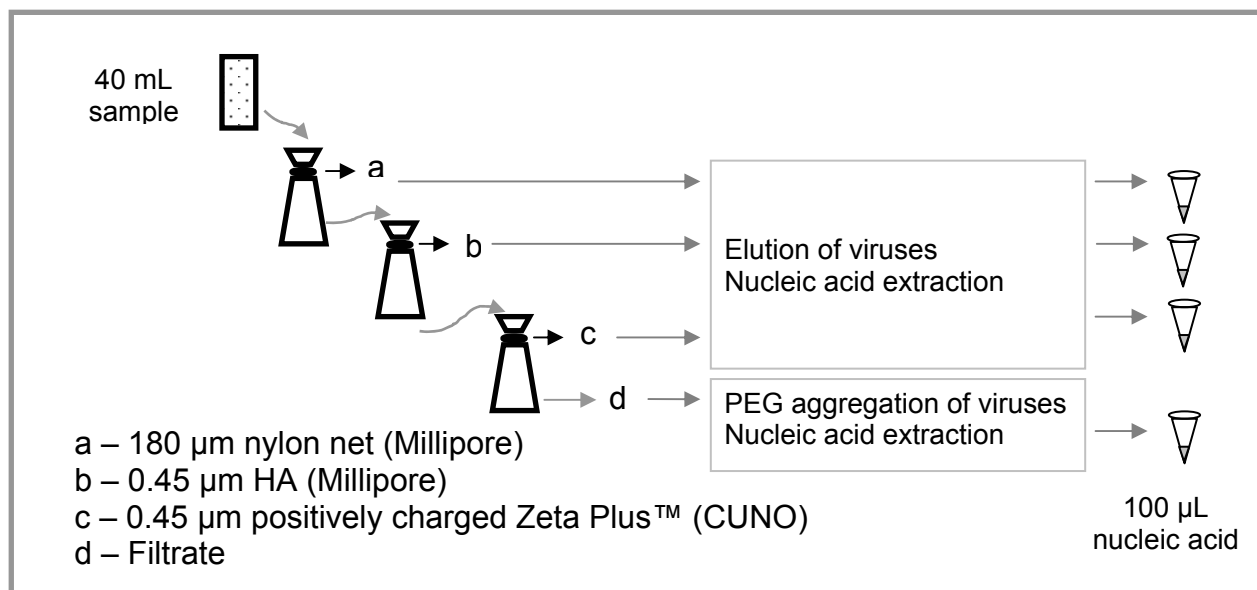

Figure S2. Cascade filtration scheme. Samples were filtered through a series of membrane filters with different pore sizes and surfaces. Subsamples a-d were each spiked with a known quantity of mengovirus to serve as an extraction control.

Table S1. Summary of location and norovirus concentrations in the waste stabilization pond system. A p-value of < 0.05 indicates that one or more of the location have concentrations of detected norovirus GI or GII that are significantly different from the other locations, based on a 95% confidence interval. The p-values were determined using the parametric Duncan test for GI and the non-parametric Kruskal-Wallis H-test for GII.

	Influent	Pond 1	Pond 2	Pond 3	Overall
GI					
Total Samples	16	17	17	13	63
Samples used in Analysis [a]	6	11	9	6	32
Mean concentration ± SD log (genome copies / L)	7.62 ± 1.02	7.01 ± 0.81	6.80 ± 0.94	5.82 ± 0.93	
p-value					0.024 *
GII					
Total Samples	17	16	16	12	61
Samples used in Analysis [a]	16	10	11	2	39
Mean concentration ± SD log (genome copies / L)	6.57 ± 0.81	6.80 ± 0.47	6.15 ± 1.09	5.00 ± 2.12	
p-value					0.183

[a] Only samples with values greater than the detection limit were included in the calculation and comparison of means.

* Means are significantly different.

Table S2. Preliminary filtration analysis of two influent samples**.** Quantities indicate norovirus GI and GII retained on sequential filters.

Date			Log (genome copies per liter)			
	180 µm nylon	100 µm nylon	20 µm nylon	0.45 µm HA	0.45 µm (+)	filtrate
			GI			
1/3/06	--	--	--	5.4	--	NT
1/10/06	--	NT	--	--	--	--
			GII			
1/3/06	3.7	--	3.6	5.0	5.2	NT
1/10/06	3.8	NT	5.0	6.6	4.3	--

NT = not tested.

Table S3. Comparison of fractions of norovirus GI and GII retained on each filter. Table includes p-values calculated using the Kruskal-Wallis test for ranked non-parametric data. A p-value of < 0.05 indicates that one or more of the filter categories have fractions of detected norovirus GI or GII that are significantly different from the other filter categories, based on a 95% confidence interval.

	p-value for Kruskal-Wallis test comparing filter categories	
	Filtered influent samples	Filtered effluent samples
GI	0.3818	0.0568
GII	0.0098 *	0.3020

* Means are significantly different.

Table S4. Matrix of pairwise comparisons of concentrations of norovirus GII in filtered effluent samples. Table includes p-values calculated using the Wilcoxon rank-sum test for non-parametric data. A p-value of < 0.05 indicates that the average fraction of GII found in the two compared filter categories is significantly different, based on a 95% confidence interval.

p values for pairwise Wilcoxon rank-sum tests			
			GII in filtered influent samples
	(b) 0.45 µm	(c) 0.45 µm + charge	(d) filtrate
(a) 180 µm	0.2623	0.0037 *	0.0547
(b) 0.45 µm		0.0278 *	0.0450 *
(c) 0.45 µm + charge			0.2894

* Means are significantly different.