Go With the Flow for Wastewater Data Normalization: Comparing Normalized and Non-Normalized SARS-CoV-2 Viral Load and Physicochemical Parameters in Muskegon and Ottawa Counties

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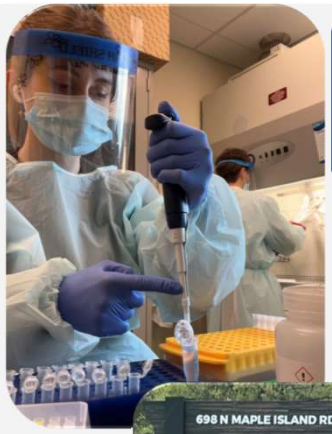
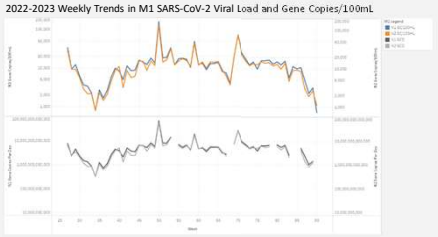


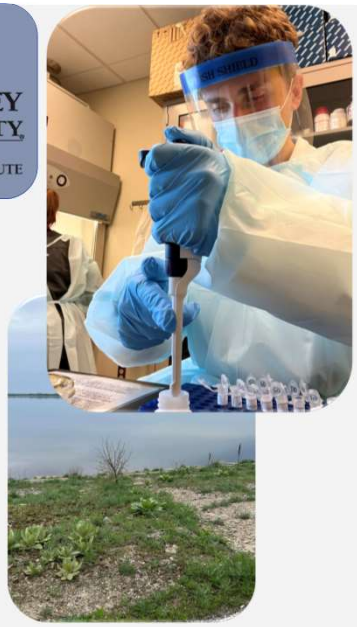
Introduction:

Wastewater is a complex, dynamic matrix that is reflective of the population utilizing the catchment under evaluation. SARS-CoV-2 RNA residing within wastewater has been extracted and analyzed in droplet digital polymerase chain reaction (ddPCR) to estimate viral incidence trends in a population. Flow rate data has been seen by the CDC as an approved variable to normalize ddPCR wastewater surveillance data which may help assess relationships between clinical cases and viral load in SARS-CoV-2 monitoring. Variables such as flow rate can provide accurate quantification and allow for further statistical analyses of wastewater viral load. Wastewater flow rate data is collected routinely by wastewater treatment plants (WWTP) therefore is a readily available data point for viral RNA concentration.

Sampling Methods:

We utilized 2022-2023 historic flow rate data to normalize SARS-CoV-2 RNA concentrations from wastewater in sites exceeding 30,000 individuals serviced and explored how data normalization impacts viral load trends for west Michigan sites by doing a comparative trend analysis of the normalized and non-normalized datasets. We then used additional physiochemical parameters routinely gathered by WWTP's to assess relationships among chemical parameters and our molecular findings.



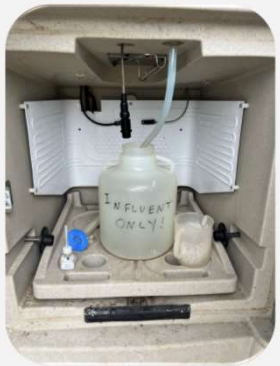


Results:

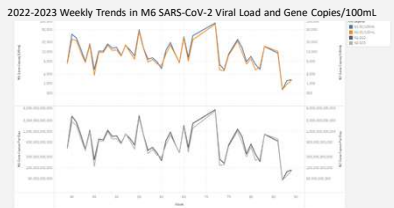
The Wilcoxon Signed Rank Tests indicated that there was a significant difference between flow normalized and non normalized groups ($p < 0.05$). This was additionally true for M8, which utilized a Mann-Whitney U test ($p<0.05$) due to small sample size (n=45).
Spearman's rank correlation compared M1 SARS-CoV-2 Gene Copies/100mL with physicochemical parameters including suspended solids (TSS), conductivity, biochemical oxygen demand (BOD), phosphorus, ammonia, and dissolved metal concentrations. We found a weak negative correlation with 1 parameter, magnesium (n=14).

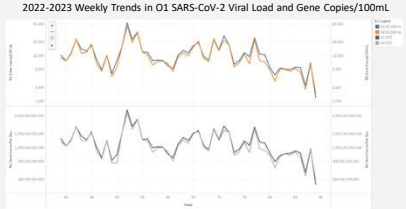
Conclusions:

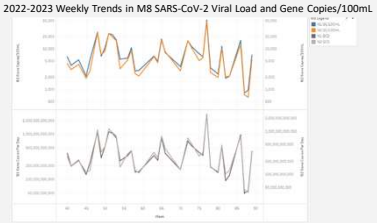
As Wilcoxon Signed Rank Tests showed significant differences between groups, further statistical testing is indicated explore these relationships. Spearman's Rank Correlation indicated no correlations to all but magnesium, which has a weak negative correlation and small sample size. Further collaboration may be informative of this result as metals are surveyed monthly.

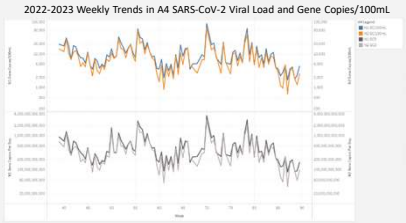


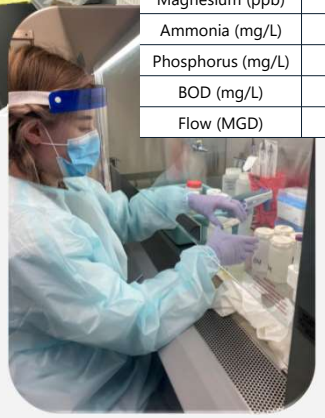
M1 Metinf Compositor at MCCRC





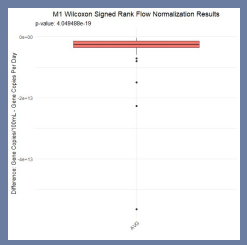


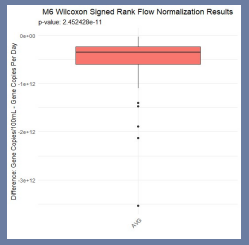


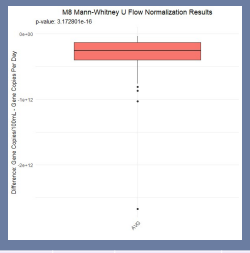


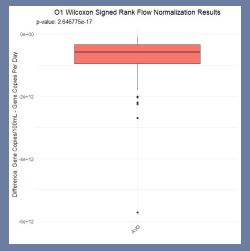
MCRRC Metinf (M1) Physiochemical Parameters to SARS-CoV-2 Gene Copies/100mL Spearman's Rank Correlation Results

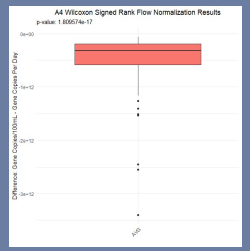
Parameter	Rs Value	Sample Size (n)
Conductivity (uS)	-0.2758996	100
Total Suspended Solids (mg/L)	0.1144841	114
Sodium (ppb)	-0.430303	14
Calcium (ppb)	-0.2363636	14
Magnesium (ppb)	-0.6606061	14
Ammonia (mg/L)	0.2603982	110
Phosphorus (mg/L)	0.1315834	110
BOD (mg/L)	0.2099255	108
Flow (MGD)	-0.2634847	113











Site Name	Group	Sample Size (n)	p Value	Test Type
M1	N1	137	4.05×10^{-19}	Wilcoxon Signed Rank
M1	N2	137	4.05×10^{-19}	Wilcoxon Signed Rank
M1	AVG N1N2	137	4.05×10^{-19}	Wilcoxon Signed Rank
M6	N1	59	2.45×10^{-11}	Wilcoxon Signed Rank
M6	N2	59	2.45×10^{-11}	Wilcoxon Signed Rank
M6	AVG N1N2	59	2.45×10^{-11}	Wilcoxon Signed Rank
O1	N1	95	2.65×10^{-17}	Wilcoxon Signed Rank
O1	N2	95	2.65×10^{-17}	Wilcoxon Signed Rank
O1	AVG N1N2	95	2.65×10^{-17}	Wilcoxon Signed Rank
A4	N1	96	1.81×10^{-17}	Wilcoxon Signed Rank
A4	N2	96	1.81×10^{-17}	Wilcoxon Signed Rank
A4	AVG N1N2	96	1.81×10^{-17}	Wilcoxon Signed Rank
M8	N1	45	3.17×10^{-16}	Mann-Whitney U Test
M8	N2	45	3.17×10^{-16}	Mann-Whitney U Test
M8	AVG N1N2	45	3.17×10^{-16}	Mann-Whitney U Test

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