Investigating Environmental Epidemiology Applications and Characterization of its Molecular Markers

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Introduction

Background.

Over the last decades, new approaches in international pathogen monitoring programs have led to recent advances in the detection of pathogen molecular markers in wastewater. This approach, termed wastewater-based epidemiology (WBE), has been recognized over the years for its potential to obtain an overall snapshot of the health status of a community. Since the emergence of the COVID-19 disease, WBE has gained increasing interest in monitoring the spread of SARS-CoV-2 at the community level. WBE is a useful pre-screening tool to target clinical testing needs within a community.

Despite the multitude of studies involving detection and quantification of RNA markers in wastewater and body fluids, very little information is available about the factors that affect the stability of free RNA molecules in aqueous media.

There is a critical need to characterize the effect of the wastewater composition on the RNA degradation kinetics, if an accurate prediction of the pathogen abundance in wastewater is to be achieved.

Relation to Green Chemistry.

WBE and other environmental epidemiology methods have been recognized for its potential to provide insights into community health and environmental exposure, including illicit drugs consumption, pharmaceuticals use/abuse, water pollution, and the occurrence of pathogens and antimicrobial resistance.

These studies investigate the link between human health outcomes and environmental exposures, including chemicals. This data helps identify harmful chemicals and their potential impacts.

Aim 1 – Optimizing a WBE method for SARS-CoV-2 RNA and perform comparison with campus clinical saliva cases

Objectives

- Determine the prevalence of SARS-CoV-2 RNA in untreated wastewater in the Main Campus of George Mason University using reverse transcription quantitative PCR (RT-qPCR)
- Compare the SARS-CoV-2 RNA concentration in wastewater with the number of positive cases among GMU resident students when detected by individual testing

1. Sample collection with autosamplers

- Autosamplers were installed at 12 sites on campus.
- Wastewater was collected every 30 minutes for 24 hours

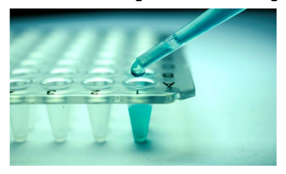
2. RNA concentration with magnetic hydrogel particles

- RNA was concentrated by adding magnetic hydrogel particles (Ceres Nanotrap®) to the heat-inactivated sewage sample
- The concentration process was automated using a Kingfisher Flex robot

3. RNA extraction

- RNA was extracted using a commercial the MagMax® RNA Isolation Kit
- The RNA extraction process was automated using a Kingfisher Flex Robot

4. Reverse-transcription quantitative PCR



- RT-qPCR detected SARS-CoV-2 RNA based on the CDC N1 and N2 assays
- Human fecal maker, fecal marker, pepper mild mottle virus (PMMoV) was used for quality control wastewater detection

5. Matrix recovery controls

- Raw wastewater samples were spiked with a known amount of bacteriophage MS2, which was detected by RT-qPCR to calculate the percent recovery.

Comparison between sewage and individual testing

Weekly RT-qPCR SARS-CoV-2 detection in sewage (RNA copy numbers) and number of GMU positive COVID-19 cases from saliva testing are shown for the period February to December 2022.

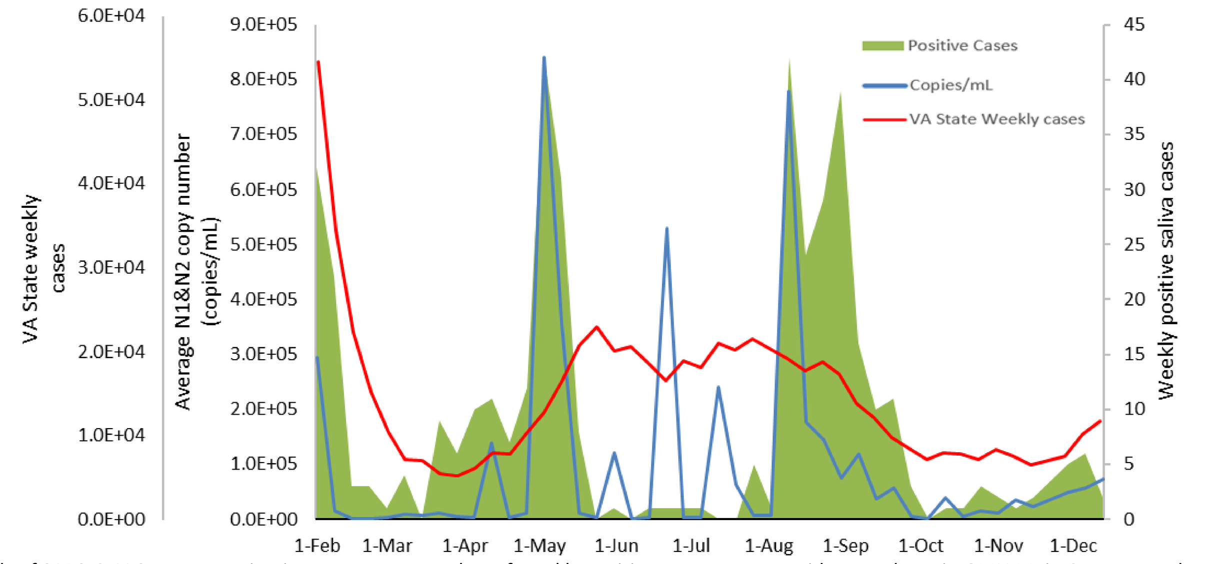


Figure 1. Trends of SARS-CoV-2 concentration in wastewater, number of weekly positive cases among resident students in GMU Main Campus, and weekly news cases in the state of Virginia during the period of study (February to December 2022). The results are based on the analysis of 362 wastewater samples.

Table 2. Correlation matrix between selected wastewater copy numbers mL^{-1} and saliva testing metrics for the period involving all 12 sampling sites (August to December 2022).

		Weekly positives[a]	Weekly positivity rates (%)	Weekly isolation at GC	Fairfax County weekly cases[b]	VA State weekly cases[b]	USA nationwide cases[c]
Average N1/ N2	Spearman's Correlation	0.779**	0.588*	0.321	0.779**	0.792**	0.789**
	Sig. (2-tailed)	0.000	0.013	0.209	0.000	0.000	0.000
Average normalized N1/ N2	Spearman's Correlation	0.681**	0.444	0.293	0.363	0.615**	0.588*
	Sig. (2-tailed)	0.003	0.074	0.254	0.152	0.009	0.013
Weighted average N1/N2	Spearman's Correlation	0.635**	0.404	0.284	0.637**	0.674**	0.625**
	Sig. (2-tailed)	0.006	0.107	0.269	0.006	0.003	0.007

**Correlation significant at the 0.01 level.
*Correlation significant at the 0.05 level.
[a] Number of observations, *n* = 17.
[b] Data obtained from the Virginia Department of Health (USA), COVID-19 Dashboards.
[c] Data obtained from the Centers for Disease Control and Prevention (USA), COVID Data Tracker.

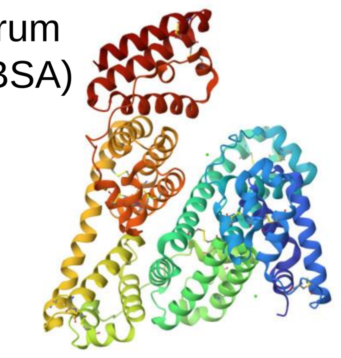
Aim 2 – Characterizing the protective effect of various macromolecules on the degradation of viral RNA for WBE applications

Objectives

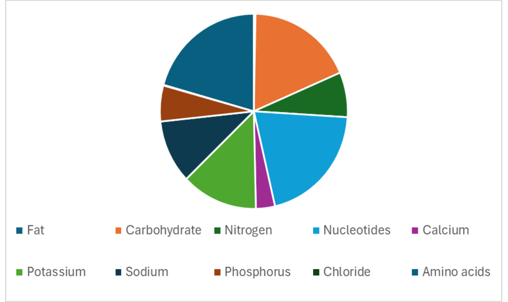
- Characterize and model the effect of various macromolecules on the degradation of viral RNA in wastewater for the purpose of improving the accuracy of wastewater-based epidemiology studies.
- Assess the effect of amplicons of differing length from the same target on the degradation of viral RNA in synthetic wastewater.

1. Macromolecules with potential protective effect on RNA included**: proteins, lipids, carbohydrates, and nucleic acids**.
2. Samples were spiked with known concentration of synthetic viral RNA

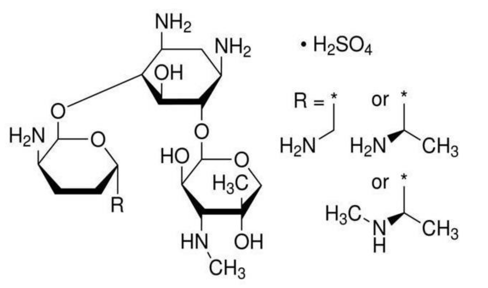
Bovine serum albumin (BSA)



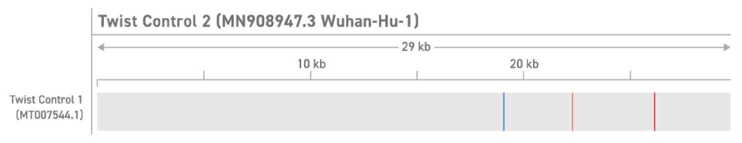
Yeast extract Composition



Soy peptone



*Synthetic SARS-CoV-2 RNA target



- Aliquots of the samples were collected at different timepoints, the remaining RNA was quantified using reverse transcription quantitative PCR (RT-qPCR).
- Aliquots were spiked with the recovery control Human coronavirus 229E.
- The concentration of RNA was expressed in number of copies/μL of sample.
- Relative RNA abundance was calculated by normalizing the CT (cycle threshold) values of the target RNA by the CT of the matrix recovery control to account in differences in RNA extraction efficiencies

Results

- The collected RNA at each timepoint was quantified as the cycle threshold (CT).
- The matrix recovery control normalized the CT as relative amplification.

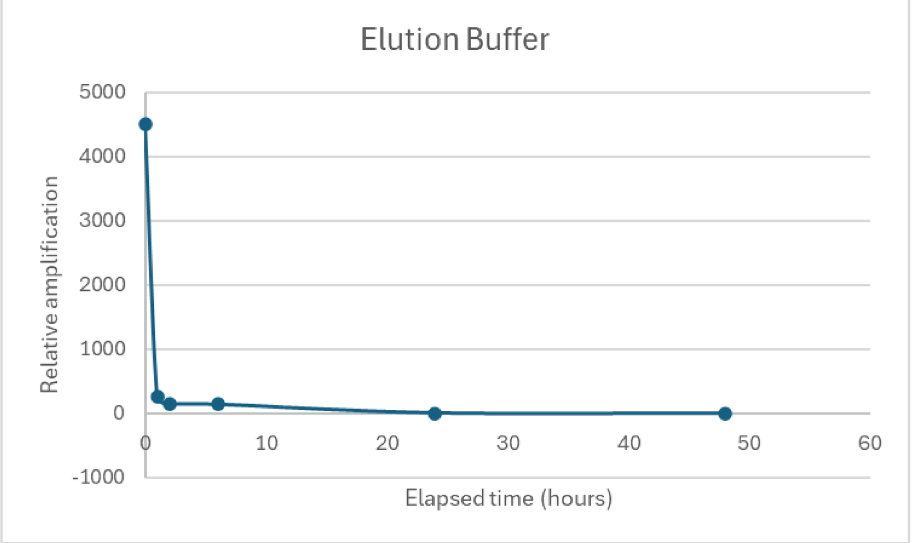


Figure 2: Relative amplification plot of elution buffer sample from quantified synthetic viral RNA

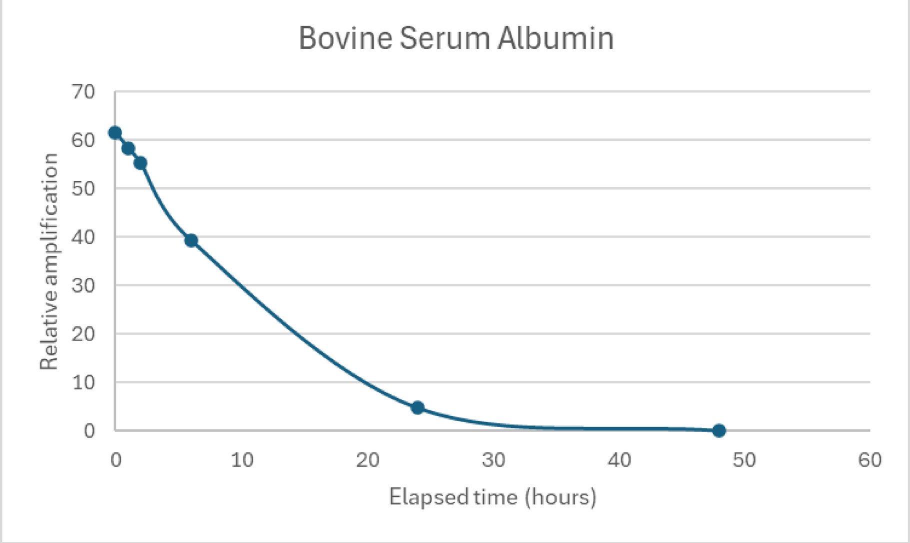


Figure 3: Relative amplification plot of BSA synthetic wastewater sample from quantified synthetic viral RNA

Degradation Kinetics

One phase exponential decay:

$$Y = Plateau + (Y_0 - Plateau) \cdot \exp(-K \cdot X)$$

Description:
X: Time
Y: Starts at Y_0 and decays (with one phase) down to Plateau.
Y_0 and Plateau: Same units as Y
K: Rate constant equal to the reciprocal of the X axis units.

Aim 3 – Mitigating contaminants of emerging concern in stormwater of disadvantaged communities

Objectives

- Determine the overall efficiency of contaminants of emerging concern (CEC) removal in selected stormwater best management practices (BMPs) located in disadvantaged communities in Northern Virginia in different compartments of the BMPs, including bulk water, porewater, sediments, and plants using liquid chromatography with tandem mass spectrometry (LC-MS/MS)
- Engage disadvantaged communities about BMPs in their neighborhood through outreach programs, which will include installation of explanatory signages, educational programs with a local high school, and community meetings.

Three BMPs (stormwater wet ponds) were selected. We choose to focus on wet ponds because they will allow sampling during both dry-weather and storm conditions. In addition, wet ponds are more susceptible to remove stormwater contaminants through microbial activity in water and aquatic plants.

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