Title (and primary focus): Factors driving intra-community variability of wastewater concentrations and export of SARS-CoV-2

Justification

1. The potential (and limitations) of WBE of SARS-CoV-2 (and other viral pathogens) cannot be effectively assessed until we have an improved understanding of factors driving variability in the data collected/measured from WWTFs
2. On-going studies are addressing analytical methodological uncertainties (and those outcomes will be packaged into a separate manuscript) – this paper addresses primarily “field” associated drivers of variability
3. Few studies to date have effectively addressed “field”-based variability issues

Issues/potential factors driving variability that questions and/or hypotheses can be developed around

1. Within sewer factors. Does sewer transit time and or seasonal (temperature?) changes impact virus signal survival
2. Water use contrasts (gallons/day/person) across districts – can we use per capita water usage metrics to normalize concentrations
3. Population demographics/behaviors
4. Environmental factors (refer to separate outline)
5. What drives day-day changes?
6. Fecal marker dynamics – what factors are driving the observed variations in concentrations/export of PMMoV (and others). What are the implications of these finding for use of PMMoV (and others) in “fecal-normalization” of SARS-CoV-2 data?
7. What can weekend versus weekday contrasts in virus levels/export tell us about virus sources and variability?

Other areas of emphasis (that could be a component of the manuscript(s))

1. Which N marker (N1, N2, mean) is most “useful”?
2. How often do we need to sample to characterize/capture trends?
3. Fecal normalization strategies. Are there substantive differences in the efficacy of the various markers? Is the measured noise in the fecal marker data such that normalization is unlikely to reduce SARS-CoV-2 variability?
4. Detailed examination of correlations between SARS-CoV-2 wastewater levels and COVID case prevalence, focusing on lag-time
5. Does the wastewater data allow us to comment of intra-community transmission of COVID-19?
6. Data “spikiness” is likely endemic to this type of data collection. Can be promulgate an “outlier” identification/screening approach for these data streams

Why is Madison a good situation/opportunity to explore these questions?

1. A medium sized city (400K in city/suburbs) serviced by one WWTF and with minimal inflow/infiltration (modern sanitary only sewer lines)
2. Five separate sewershed districts serving the greater Madison area – districts serviced by five main sewer interceptor lines (all of which can be uniquely sampled at the main WWTF)
3. High-resolution (census district level), high quality COVID case data (outline somewhere the granular level of parsed COVID case data), mapped to each separate district
4. Extensive population demographics and geographic GIS-mapped information for each sewershed district (we know a lot about the Madison communities)
5. Relatively small industrial contributions to the wastewater flows
6. High-quality and complete meta-data available from the wastewater utility (MMSD)

Why are the measured data/experimental design especially valuable in addressing these questions?

1. Daily (5-6 days/week) wastewater SARS-CoV-2 data from the full Madison community for a period of 9 months. (relatively long-term data sets)
2. Twice per week Interceptor (district resolved) wastewater SARS-CoV-2 data for a period of 8 months
3. Several virus surges captured within the long-term data sets
4. Similarly robust parallel measurement of the fecal-marker PMMoV (with potential to supplement with additional fecal-marker data (HF-183, crAssphage))
5. Data collected over wide range of seasonal environmental (all four seasons) conditions and seasonal population/community behaviors (e.g. diet)
6. Both weekday and weekend wastewater collections
7. Detailed characterization of the analytical method uncertainties (data that facilitates more robust assessment of community drivers of variability)

Finalize Data Sets

1. Update SARS-CoV-2 and Fecal Marker data where known biases exist
2. Critically examine data sets (“mass-balances” will help here) and identify data points for additional scrutiny. Re-run/re-extract if necessary to resolve.
3. Assemble “final” data. Perform outlier evaluation. Reject outliers.
4. Compile COVID case data, parsed to sewershed district. Consider what level of granularity in COVID demographics to run with.
5. Generate “normalized” data
   1. Wastewater flow
   2. Population
   3. Fecal Marker
6. Develop hypotheses and design/apply appropriate statistical tests

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