

Evaluation of physico-chemical factors affecting Wastewater-Based Epidemiology of SARS-CoV-2 in a southern Philippine sewershed

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Abstract Wastewater-Based Epidemiology as a complementary Public Health Surveillance tool in Davao City proved useful as the basis of further improvements in disease surveillance. Weekly sampling from November to December in 2020 identified 91% sewer water samples with SARS-CoV-2 RNA despite the lack of reported positive clinical cases in areas identified as moderate to high risk for COVID-19 transmission. Detections of SARS-CoV-2 N, RdRP, and E genes concentrated from PEG-NaCl precipitation method and analyzed using RT-PCR were evaluated in conjunction with physico-chemical and fecal parameters, particularly coliform count, flow rate, surface water temperature, salinity, and total dissolved solids. The physico-chemical parameters provide insight on the preservation of organic matter and residence time of the waters that can impact the recovery of SARS-CoV-2. From whole genome sequencing, single nucleotide polymorphisms (SNPs) were detected and spatially clustered in areas with high population density and mobility. The mutations include previously reported and not yet detected to which the SNPs P383L and V21L in wastewater were detected a month before they were reported from clinical surveillance. Refining the sewershed map is highly recommended and is currently surveyed in more detail. For future improved WBE, the sites were narrowed down to fast flowing channels in densely populated areas with more established sewer lines.

Keywords: low-sanitation areas, low-income country wastewater-based surveillance, untreated wastewater, sewershed, COVID-19

1. Introduction

Wastewater-Based Surveillance and Epidemiology are methods successfully used for various diseases, such as

polio in the Philippines in 2019 (Snider et al., 2022), hepatitis A in Detroit, Michigan (McCall et al., 2021). During the COVID-19 pandemic, many countries tested the use of WBE and found advantages in this approach and is highly recommended as a complementary community-based tool to clinical testing. Compared to individual clinical tests which are slower and covers a smaller number of individuals, WBE sample sets cover community population and when combined with geo-referencing, hotspots of infected populace are mapped out including asymptomatic and non-tested symptomatic individuals, which are more common in the second or third surges as symptoms are milder. This pilot study in Davao City, Philippines uses physico-chemical parameters to evaluate other interfering factors on viral RNA and its applications on SARS-CoV-2 WBE (Fig. 1).

2. Methods

Weekly COVID-19 transmission risk categories for 182 barangays (site unit) in Davao City are evaluated from new and active cases calculated against average daily/weekly attack rate. Of the 182 barangays, six were selected for weekly wastewater sampling in waterways chosen with the assistance of barangay officials based on continuous flow of wastewater, accessibility of the area and representations for low, moderate and high/critical risk classification.

Within November 8 to December 12, 2020, weekly 250 mL water samples were collected into sterile high-density polyethylene bottles from the determined sites in 23-C, 76-A Bucana, Leon Garcia Sr., Tomas Monteverde, Mintal (slow to moderate flowing and natural creek), and Matina Crossing (moderate to fast flowing sewer pipe).

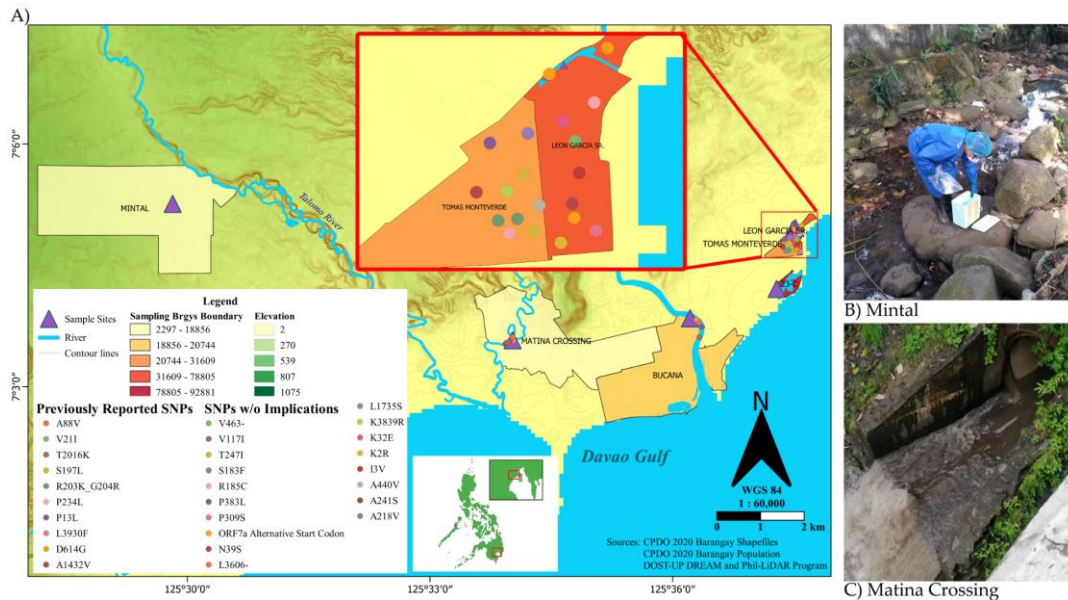


Figure 1. (A) Map of sampling sites with top view photographs of (B) slow flowing creek in Mintal, and (C) fast flowing Matina Crossing water sample collection sites. Single Nucleotide Polymorphisms (SNPs) (inset) locate mutations previously reported elsewhere and unreported SNPs in the GISAID and literatures.

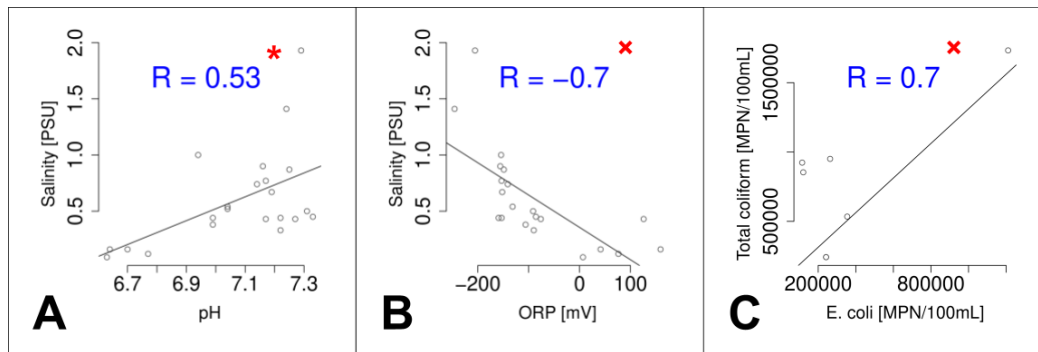


Figure 2. Relationships of salinity with pH (A) and ORP (B) and of total coliform with *E. coli* (C). The red * beside the correlation coefficient denotes statistical significance at least in pairwise comparison whereas a red × denotes significance in multiple comparisons, all to at least 95% confidence.

Detailed RNA extraction and RT-PCR analyses, whole genome sequencing, bioinformatics analyses, SNP mapping and tracking are discussed in Otero et al (2022). Briefly, 40 mL aliquot was sonicated and centrifuged with the supernatant decanted and filtered through 0.22 μm polyethersulfone syringe filter. Pellets were formed using the PEG-NaCl precipitation method.

A YF-S201 G1/2 water flow meter sensor was used to measure discharge rate (Liters per minute) for three sampling days. The fast flowing Matina Crossing attained an average of 23.3 ± 6.5 LPM and Mintal only 2.3 ± 1.3 LPM. Handheld Hannah HI98194 multimeter was used to measure pH, Oxidation-Reduction Potential (ORP), total dissolved solids (TDS), temperature, and salinity.

A 100 mL water sample was collected into a flame-sterilized stainless-steel cup. Aliquots of 0.1 mL and 1 mL were used for total coliform (yellow) and *E. coli* (fluorescent) enumeration using IDEXX™ Colilert™-18 and their reagent mix and Quanti-Tray™ 2000, sealed and incubated dry at 35 ± 0.5 °C.

Canonical correlation analysis was used to simultaneously estimate the relationship between physico-chemical parameters and SARS-CoV-2 indicator genes. The freeware R 4.2.3 (R Core Team, 2023) was used to perform Pearson product-moment correlation, principal component analysis, and to generate their corresponding plots. Statistical significance was considered to be greater than or equal to 95%.

3. Results

Two water samples that coincided with high tide during sample collection had salinities 9.79 PSU from 23-C (13 November) and 3.81 PSU from Tomas Monteverde (27 November). The coastal zone in this area have houses on stilts

very close to the coastline and likely do not have sewer systems that would route the untreated wastewaters to a treatment plant. Hence, coastal waters are expected to have untreated wastewaters, but the complexity of tidal action bringing back the brackish coastal with untreated wastewaters where we still do not completely understand the dynamics of coronavirus viral

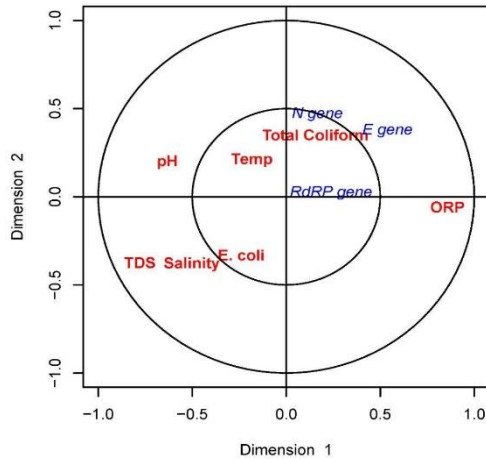


Figure 3. Canonical correlation plots showing the relationships between the physicochemical variables and indicator genes.

RNA preservation compels us to omit from the statistical analyses these two samples. The streamlined roster of samples exhibit significant positive correlations between the SARS-CoV-2 indicator genes, RdRp and N genes with the more broadly defined Sarbecovirus E genes yielding statistically significant (to at least 95% confidence) correlations of $R=0.70$ and 0.55 , respectively (Figure 3). The SARS-CoV-2 indicator genes clustered together in the positive PC1 and negative PC2 quadrant. The direction of SARS-CoV-2 gene variables is towards decreasing viral RNA CT count, thus yielding a slight positive correlation to *Escherichia coli* at least in pairwise comparison (Figure 3). Because *E. coli* is also significantly correlated, although only moderately in this study, with total coliform (Figure 2C) and both are indicators of wastewater contamination, wastewater indicators could potentially be a proxy of substantial SARS-CoV-2 presence. The relationships between the physico-chemical and indicator genes are further explored through Principal Component Analysis (PCA; Figure 4).

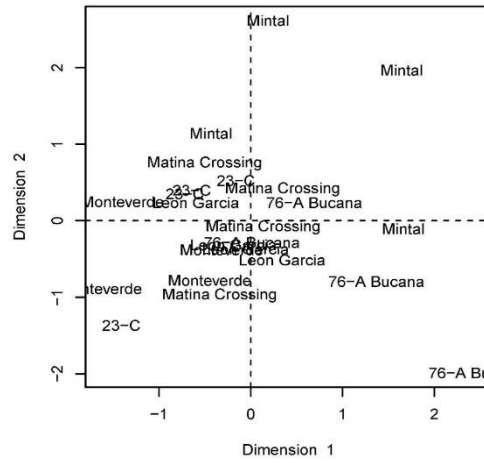
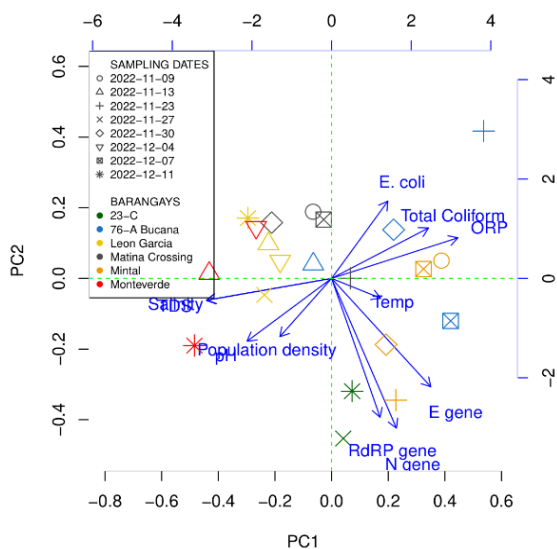


Figure 4. PCA biplot of the sampling sites (observed, points) based on physico-chemical, microbiological, SARS-CoV-2 indicator gene parameters (variables, arrowed).

4. Discussion

Principal component 1 (PC1) axis could be regarded as water freshness (*i.e.*, the inverse of salinity, and is also inversely related to Total Dissolved Solids) because it could explain most of the salinity variance (*i.e.*, the salinity vector is almost parallel to PC1 but in the negative direction). In the same inverse manner, PC2 could be regarded as WBE SARS-CoV-2 detection. Positive sampling site scores on PC2 indicate more SARS-CoV-2 RNA present in the local surface waters. pH and salinity have a similar direction towards the quadrant where PCs 1 and 2 are both negative, which could be expected since freshwater is naturally more acidic than seawater. *E. coli* counts decrease with salinity and TDS. This suggests a major source of these wastewater indicators in the uplands, possibly agricultural with less human settlements but more animal waste discharge. The significant (95% confidence in pairwise comparison) inverse correlations between salinity and ORP and between TDS and ORP (significant in multiple comparisons; Figure 2B) could suggest that even though there could be considerable amounts of organic matter input into these waters, there would still be sufficient O_2 exchange with the atmosphere to maintain the ORP value possibly due to shallow depth of the flowing (non-stagnant) river streams. This is supported by the inverse correlations between salinity and pH (significant at least in pairwise comparison; Figure 2A). On the other hand, the SARS-CoV-2 indicator genes E, N, and RdRp agree with each other and with the environmental context of the sampled sites. All but stations 23-C (27 Nov) and Mintal (23 Nov) report positive clinical SARS-CoV-2 detection. These



two sites also have the highest detected E, N, and RdRp indicator genes, indicating that SARS-CoV-2 is not substantially present in these two localities. These two stations also plot farthest along the vectors of SARS-CoV-2 (inverse) indicator genes in the PCA biplot.

Exposure to high temperature, oxidative and photolytic degradation in addition to microbial degradation of organic matter are important mechanisms that eventually decrease and underestimate gene counts used for WBE or WBS and are not usually factored in. Discharge and residence times of sewer waters are also critical factors to consider in the preservation of organic matter. The fast flowing Matina Crossing retains suitable wastewater material despite exposure to oxidative and microbial degradation. The more stagnant Tomas Monteverde may contain more preserved organic matter, but the residence times are longer and thus the direct relationship to current wastewater is not strong and can present problems in the applications for WBE.

In some other countries, spikes in WBE SARS-CoV-2 viral RNA counts predate spikes in clinical data. More recent studies combined viral RNA counts to pharmaceuticals used for COVID-19 symptoms. Acetaminophen peaked with viral RNA counts before positive clinical COVID-19 cases peaked, exhibiting useful multi-faceted assessments for future studies (Halwatura et al., 2022). Only a small percentage of population are connected to sewers in low-resource areas to wastewater treatment plants, such as in India (Basu et al., 2022) and the Philippines (Otero et al., 2022), hence natural and engineered waterways catch untreated wastewaters and may be used for detection and semi-quantitation of SARS-CoV-2 indicator gene sequences as long as the physico-chemical context is also realized.

4.0 Conclusions and Recommendations

The physico-chemical parameters of waters sampled around Davao City are consistent with their environmental contexts since their correlations are in agreement with what is expected. The use, however, of wastewater physico-chemical signals as proxy for the presence of substantial SARS-CoV-2 in the locality is currently inconclusive given the parameters available, sampling frequency, and number of monitoring sites. A good contextual understanding of SARS-CoV-2 gene abundances and their interaction with the rest of the wastewater and the environment (*e.g.* wastewater age, preservation of viral RNA) is key in using natural and engineered wastewaters for WBE or WBS. Because of the observed decrease in E genes (*i.e.* possibly more presence of SARS-CoV-2) with increasing *E. coli* (statistically significant at least to 95% confidence on pairwise comparison), this study highlights the potential of WBE as a complementary Public Health Surveillance tool. Therefore, it is recommended, on one hand, that more sites should be monitored also at higher frequencies to better elucidate the link between the SARS-CoV-2

detection and wastewater physico-chemical signals. On the other hand, geochemical markers robust against variations in physico-chemical parameters but still directly associated with human waste, such as fecal steroids, can be explored and linked with wastewater SARS-CoV-2 indicators and clinical detections. This pilot study could also be extended into other highly-transmissible and virulent diseases.

Acknowledgements

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