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A Snapshot of SARS-CoV-2 in Wastewater Treatment Plants in Northwest Arkansas

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Abstract

Because SARS-CoV-2, a coronavirus, is released in the fecal shedding of covid-positive patients, the virus enters the municipal waste stream and flows to wastewater treatment plants (WWTPs). The goal of this research is to quantify the effectiveness of wastewater treatment processes at removing SARS-CoV-2 from wastewater and preventing its release into the environment. Sampling at Paul R. Noland and West Side WWTPs in Fayetteville, Arkansas, took place from August 2020 to May 2021, and samples were tested for the presence of SARS-CoV-2 using N1 and N2 primers via RT-qPCR. Sampling days which returned positive detection of one or both of the target genes in the wastewater influent were assessed for log removal of viruses between the influent and final effluent. Sampling after intermediary treatment steps was completed to assess the efficacy of specific treatment components at removing the virus. While the complete treatment processes at both plants were able to reduce the virus to below the detection limit of this study, primary clarification did not reduce target gene concentrations consistently to below the detection limit. Further research with a surrogate virus may be necessary for finding the maximum capabilities of viral removal for these treatment processes.

Introduction

Wastewater-based epidemiology is a field of study used to detect and understand the presence of chemicals and pathogens of interest in communities serviced by municipal wastewater treatment plants (WWTPs). The process of screening wastewater sewage for a target chemical or pathogen has been applied to regulated drugs as well as waterborne pathogens to quantify the presence of these public health indicators. For pathogens, wastewater epidemiology can also be applied to prevent further transmission of waterborne illnesses into a community (Xagoraraki, 2019). Wastewater epidemiology originated with the Cholera epidemic, when John Snow used Cholera case data to trace the origins of the illness to a single drinking water source (Sedlak, 2015). The same principle that allowed Snow to identify a community's source of illness has been reversed so data collected from wastewater sewage can provide information on community health.

Most pathogens that are known to cause illness via water transmission are required to be removed in wastewater and drinking water treatment processes. Because of the complex treatment requirements of viral illnesses, the EPA has many viral species included on the contaminant candidate list (CCL) (2021). The EPA will continue to review research to validate if removal requirements for viruses should be included in National Pollutant Discharge Elimination System (NPDES) permits.

SARS-CoV-2 has been identified in the fecal shedding of covid-positive patients, even when gastrointestinal symptoms are not present, and when respiratory symptoms have subsided (Zhang, 2020). While coronavirus cases began to spread around the world in 2020, shortcomings in the healthcare system led researchers to explore wastewater-based epidemiology to understand community SARS-CoV-2 presence. Proof of concepts for detection of SARS-CoV-2 genes in wastewater were achieved around the world including Australia, The Netherlands, France, and the United States (Ahmed, 2020; Izquierdo-Lara, 2021; Wurtzer, 2020; Wu, 2020). SARS-CoV-2 gene quantification in

wastewater has been applied by researchers to understand a variety of potential risks or opportunities of the virus.

While coronavirus is not being considered on the most recent EPA CCL, its designation as a global pandemic is cause for concern over potential contamination of water sources. The WWTPs of Fayetteville, AR, release treated effluent into Waters of the State where the water travels throughout the waterbody system of Arkansas. The state of Arkansas has complex and interconnected waterbody systems where surface waters interact with groundwaters through caves, springs, disappearing streams, and sinkholes, and this creates a challenge for tracking the extent a contaminant may travel after discharging to the environment. SARS-CoV-2 is a zoonotic illness which can be transmitted between humans and some animal species, so its presence in the environment has the potential to threaten environmental and human health (Zhang, 2021).

The goal of this research is to quantify the effectiveness of wastewater treatment processes at removing SARS-CoV-2 from wastewater. This was accomplished by sampling the effluents of consecutive wastewater treatment processes at two municipal wastewater treatment plants in Fayetteville, AR. The water samples were then extracted for RNA and measured for the relative abundance using quantitative reverse-transcription polymerase chain reaction (RT-qPCR).

Methods

Sample Collection

To quantify the effectiveness of the wastewater treatment process at removing SARS-CoV-2 from wastewater, samples were taken from WWTPs across Arkansas. For this study, the two WWTPs in Fayetteville, AR, were sampled at five treatment locations. The treatment processes for the two sites vary from each other, so several treatment processes were able to be assessed. At both treatment plants, raw sewage (influent), post-primary clarification, post-secondary clarification, post filtration, and

post disinfection (final effluent*)* were sampled. Samples for raw sewage and final effluent from both WWTPs were collected using 24-hr composite samples while other locations were collected with day-of grab sampling. Noland WWTP uses sand filtration and ozone disinfection following secondary clarification while West Side WWTP uses sand filtration and UV disinfection following secondary clarification. The volume collected for each sample was 250 mL, and the samples were transported on ice to the laboratory. To prevent any potential viral degradation, minimizing time between sampling and performing RNA extraction is ideal, but for temporary storage, the sample bottles were stored at -4 °C.

RNA Extraction

To prepare the samples for RT-qPCR, viral RNA must be removed from the viral envelope and suspended in fluid. To extract RNA in the samples, the QIAmp Viral RNA Mini Kit (Qiagen, Germantown, MD) was used with 140 µL of sample. Procedures followed the standard manufacturer guidelines, and all steps were performed in quick succession. The procedure yields 60 µL of fluid with suspended RNA which was stored at –80 °C.

RT-qPCR

The target gene copy number in each sample was quantified by applying RT-qPCR techniques in a Bio-Rad CFX Connect system. Each sample was prepared for PCR by combining an RNA supermix with a primer/probe mix according to proportions recommended by the supermix protocols. The supermix used was the Reliance One-Step Multiplex Supermix (BioRad, Hercules, CA). The primer/probe mixes included a SARS-CoV-2 N1 gene target mix, a SARS-CoV-2 N2 gene target mix, and a human RP gene target mix from the SARS-CoV-2 Research Use Only qPCR Primer & Probe kit by Integrated DNA Technologies (IDTDNA, Coralville, IA). The human RP gene target mix acted as the positive control for the methods.

For analysis of the samples, each RNA sample was mixed into a 20 µL reaction which contains 5 µL of the sample, 8.5 µL of DNA-free water, 1.5 µL of primer mix, and 5 µL of the Multiplex Supermix. These reactions were arranged in a 96-well PCR plate along with standard dilutions of control plasmids and a negative control. The standard curve included dilutions from 2×10^6 gene copies/mL to 20 gene copies/mL. The negative control contains no sample RNA and instead 5 µL of DNA free water. Each sample and control was duplicated for each gene target mix.

Data Analysis

Assessment of WWTP removal effectiveness was accomplished by reviewing target gene removal between plant influent and effluent as well as reviewing the intermediary treatment step samples. Sampling days which have detection in the influent were used for assessment, while sampling days which have no detection in the influent were not used in calculations. Log removal of the target gene was calculated using detected gene concentration or the lowest limit of detection (LLoD) when RT-qPCR yielded no detection. LLoD was determined by the minimum virus concentration detected by qPCR. Performance of treatment steps was assessed by normalizing the gene copy concentration as a percentage of the influent concentration on a particular sampling day.

Results

At Noland WWTP between the dates of November 20, 2020 and January 4, 2021, 8 sampling days were assessed for virus removal in this study due to having detection of one or both target genes in the plant influent. At West Side WWTP, 11 sampling days from the same window showed the presence of one or both target genes in plant influent. Influent concentrations for Noland and West Side on the selected dates are shown in Figures 1 and 2, respectively. For all sampling days at both plants reviewed in this study, there is no detection of either target gene in the effluent of the plants.

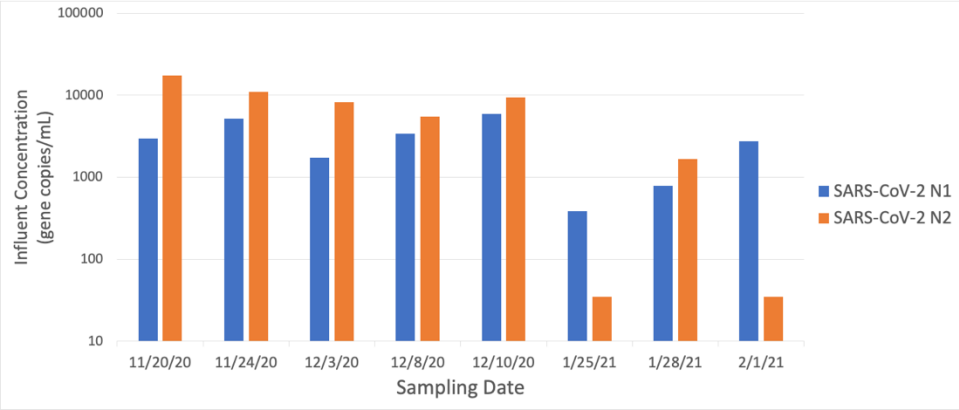

Figure 1. Concentration of target gene in influent at Noland WWTP on sampling date assessed

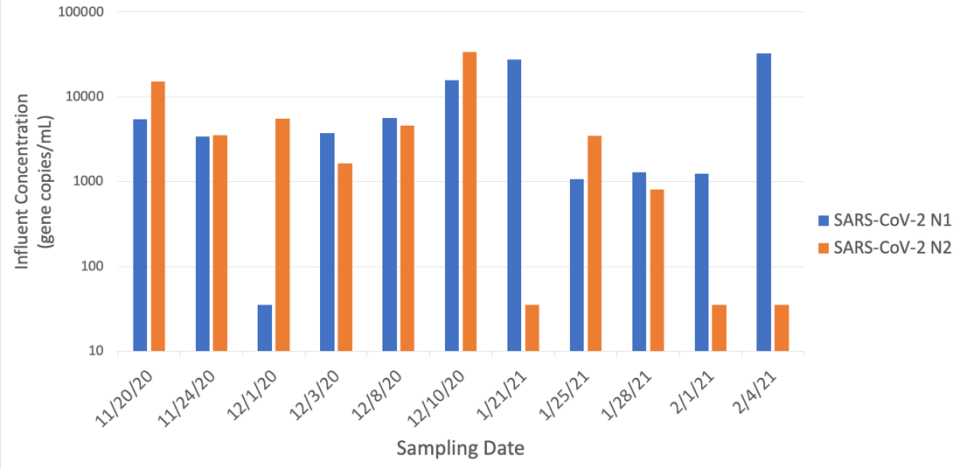

Figure 2. Concentration of target gene in influent at West Side WWTP on sampling date assessed

For removal efficiency calculation, the LLoD of 3.49×10^{1} was used for effluent concentration with no detection. The log removal for Noland WWTP is illustrated in Figure 3, and the log removal for West Side WWTP is illustrated in Figure 4. Days that report as zero log removal of a target gene are the result of no detection for the gene in both the influent and the effluent.

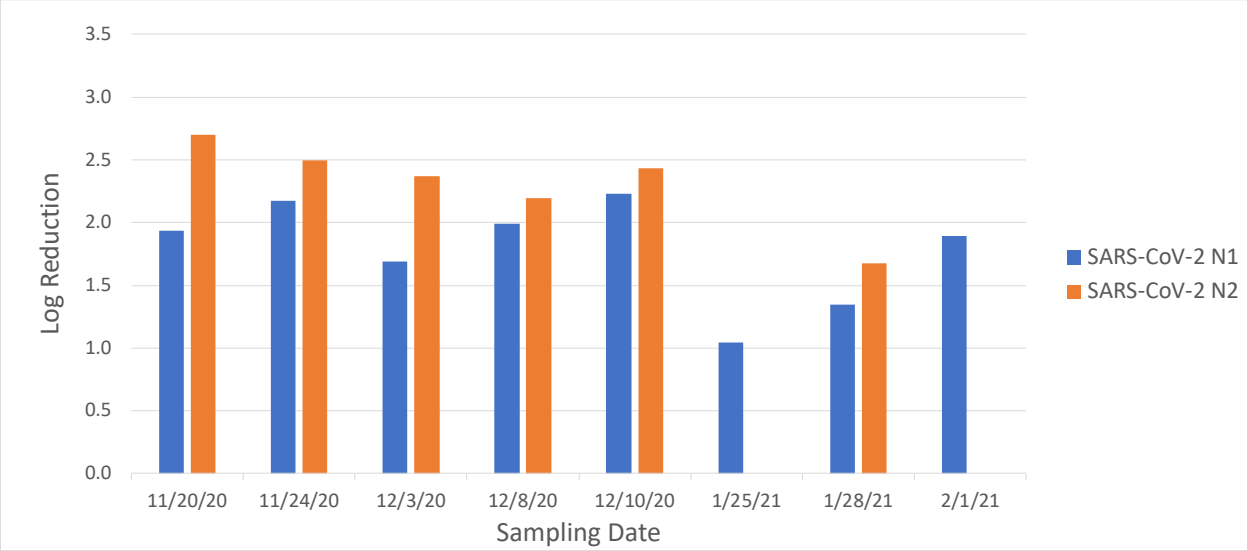

Figure 3. Log reduction between influent and effluent at Noland WWTP

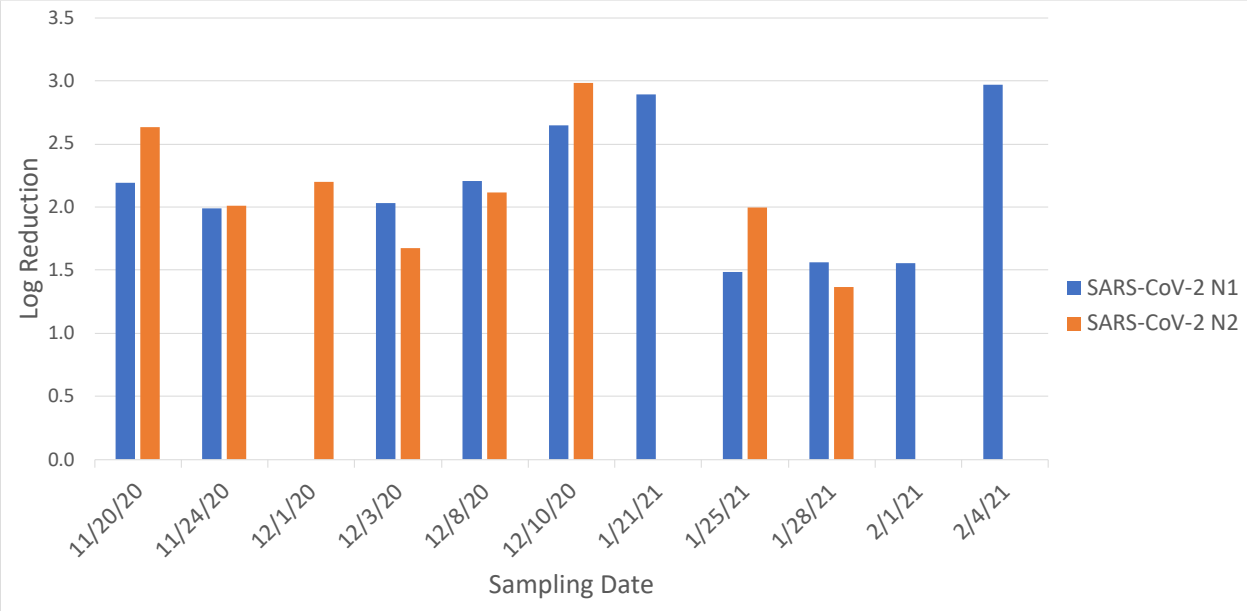

Figure 4. Log reduction between influent and effluent at West Side WWTP

The maximum log removals achieved by Noland WWTP are 2.23 log for N1 and 2.70 log for N2. At West Side, the maximum log removals are 2.97 log for N1 and 2.98 log for N2. The performance of log removals for each day was directly related to the influent concentration as no viruses were detected in effluent.

The results of the RP gene (Figure 5) as positive control were inconsistent. The gene was expected to be found in the influent for all sampling days, but it was not detected in many influent samples. It could be a combination of non-optimized gene target selection and loss of gene target due to sample storage.

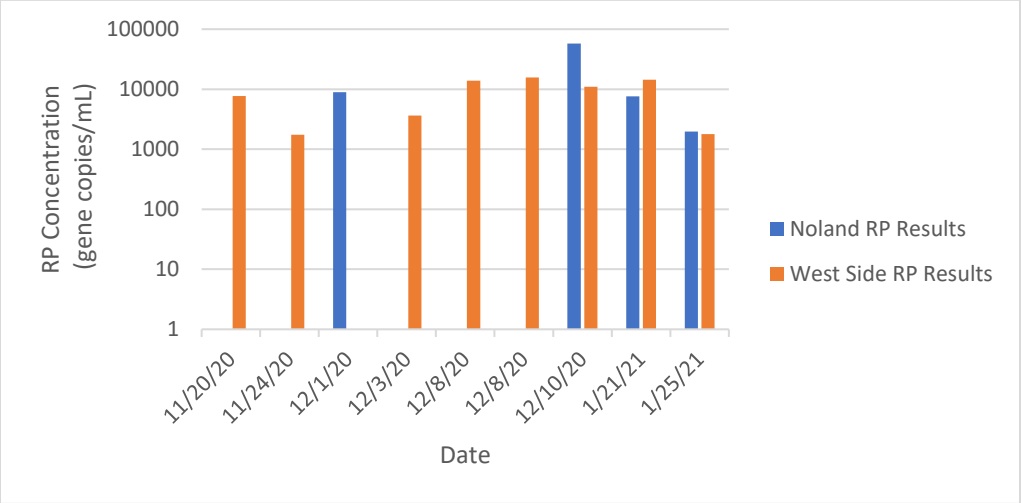

Figure 5. RT-qPCR results for Human RP gene

For the intermediary steps of treatment, one or both target genes were detected in primary clarification effluent in 8 out of 8 sampling days at Noland WWTP. At West Side, 10 out of 10 sampling days had detection of one or both target genes in primary clarification effluent. There was also detection on some sampling days of the N1 gene in the activated sludge effluent. Table 1 summarizes the average percentage of influent concentration detected in the treatment procession at each WWTP.

Table 1. Removal Efficiency of SARS-CoV-2 during wastewater treatment. N/A=Detected without available removal efficiency; N.D.=No detection.

WWTP	**Primary Clarification**	**Activated Sludge**	**Filtration**	**Disinfection**
Noland	188%(N1), 53% (N2)	130% (N1)	N/A	N.D.
West Side	16%(N1), 200% (N2)	60% (N1)	N.D.	N.D.

Discussion

The two WWTPS monitored in this study both reduced SARS-CoV-2 target genes to below the LLoD on all sampling days. This suggests that Noland WWTP and West Side WWTP are both capable of

removing SARS-CoV-2 loads at the necessary capacity for the municipality, but no maximum log removal that can be achieved by each treatment step is identified in this study. The greatest removal capacities measured in this study are 2.7 log removal of N2 gene at Noland WWTP and 3.0 removal of N2 gene at West Side WWTP. Cases of coronavirus in the community fluctuated in waves, leading to inconsistent sampling opportunities for identifying WWTP virus removal capacities. Additionally, treatment of viruses by WWTPs can be affected by temperature, solids ratios, pH, and other environmental factors (Foladori, 2020). For future study, a surrogate virus could be identified which has similar properties as SARS-CoV-2. In a study by Tandukar, et al. (2020), a WWTP in Southern Louisiana treated four human enteric viruses for an average of 2-log removal. The same study also found that the plant virus PMMoV is a good indicator virus for treatment of enteric viruses by WWTPs (Tandukar et al., 2020). The surrogate virus for SARS-CoV-2 could be monitored through the treatment processes to identify if there is a reduction in WWTP removal efficacy at higher viral concentrations.

In this study, the human RP gene is used as a positive control for the methods, but it is found to be inconsistent than expected. The RP gene may not be the best positive control, or continued adjustment of the methods to increase the accuracy of RP gene measurement may be necessary to improve results.

Target genes are detected in the primary clarifier effluent of both plants, sometimes at higher concentrations than that of the influent. Other studies measured the concentration of SARS-CoV-2 in primary clarifier sludge because sludge has been shown to harbor many common viruses (Peccia, 2020). In a study by Westhaus, et al. (2021), the solid phase of the influent was shown to contain a one-log higher virus concentration, and the solids are settled during primary clarification (Westhaus et al., 2021). The virus is removed before release from the WWTP. The removal during the activated sludge treatment could indicate that the processes of biodegradation, photo-degradation, adsorption, and enzymatic degradation are contributing to the removal of SARS-CoV-2 from wastewater.

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