ACS ES&T Water

pubs.acs.org/estwater

Article

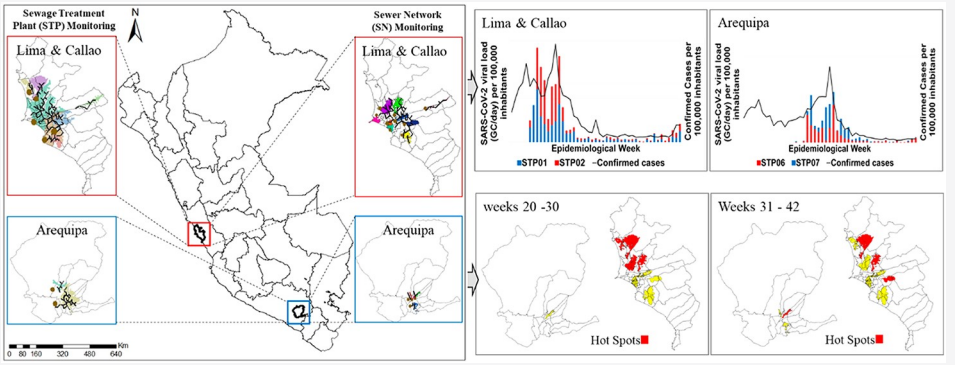
Spatiotemporal Surveillance of SARS-CoV-2 in the Sewage of Three Major Urban Areas in Peru: Generating Valuable Data Where Clinical Testing Is Extremely Limited

Braulio Pardo-Figueroa, Elias Mindreau-Ganoza, Alonso Reyes-Calderon, Sonia P. Yufra, Isabel M. Solorzano-Ortiz, Alberto J. Donayre-Torres, Claudia Antonini, Jose Miguel Renom, Antonio Marty Quispe, Cesar R. Mota, Carlos A. L. Chernicharo, Max A. Carbajal, and Mónica C. Santa-María*

Cite This: *ACS EST Water* 2022, 2, 2144–2157

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information



ABSTRACT: Peru has been severely affected by the COVID-19 pandemic. By January 2022, Peru had surpassed 200 000 COVID-19 deaths, constituting the highest death rate per capita worldwide. Peru has had several limitations during the pandemic: insufficient testing access, limited contact tracing, a strained medical infrastructure, and many economic hurdles. These limitations hindered the gathering of accurate information about infected individuals with spatial resolution in real time, a critical aspect of effectively controlling the pandemic. Wastewater monitoring for SARS-CoV-2 RNA offered a promising alternative for providing needed population-wide information to complement health care indicators. In this study, we demonstrate the feasibility and value of implementing a decentralized SARS-CoV-2 RNA wastewater monitoring system to assess the spatiotemporal distribution of COVID-19 in three major cities in Peru: Lima, Callao, and Arequipa. Our data on viral loads showed the same trends as health indicators such as incidence and mortality. Furthermore, we were able to identify hot spots of contagion within the surveyed urban areas to guide the efforts of health authorities. Viral decay in the sewage network of the cities studied was found to be negligible (<2%). Overall, our results support wastewater monitoring for SARS-CoV-2 as a valuable and cost-effective tool for monitoring the COVID-19 pandemic in the Peruvian context.

KEYWORDS: *wastewater-based epidemiology, SARS-CoV-2, COVID-19, environmental surveillance, epidemiological monitoring, hot spots*

1. INTRODUCTION

COVID-19, a disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first reported in Wuhan, China, in December 2019.[1] Since then, it has caused worldwide public, economic, social, health, and educational crises,[2] accounting for more than 360 million confirmed cases and more than 5.5 million deceased.[3,4] In Peru, the first confirmed COVID-19 case was reported in March 2020.[5] Since then, Peru has been one of the countries most affected by COVID-19 globally, with more than 2.8 million confirmed cases and 204 000 deceased by January 23, 2022.[6] Furthermore, by June 2021, Peru was reported to have had more than 560 deceased per 100 000 inhabitants, the highest excess mortality in the world,[7] which continued until early 2022.[3] At the same time,

Special Issue: Wastewater Surveillance and Community Pathogen Detection

Received: February 1, 2022
Revised: May 6, 2022
Accepted: May 9, 2022
Published: May 20, 2022

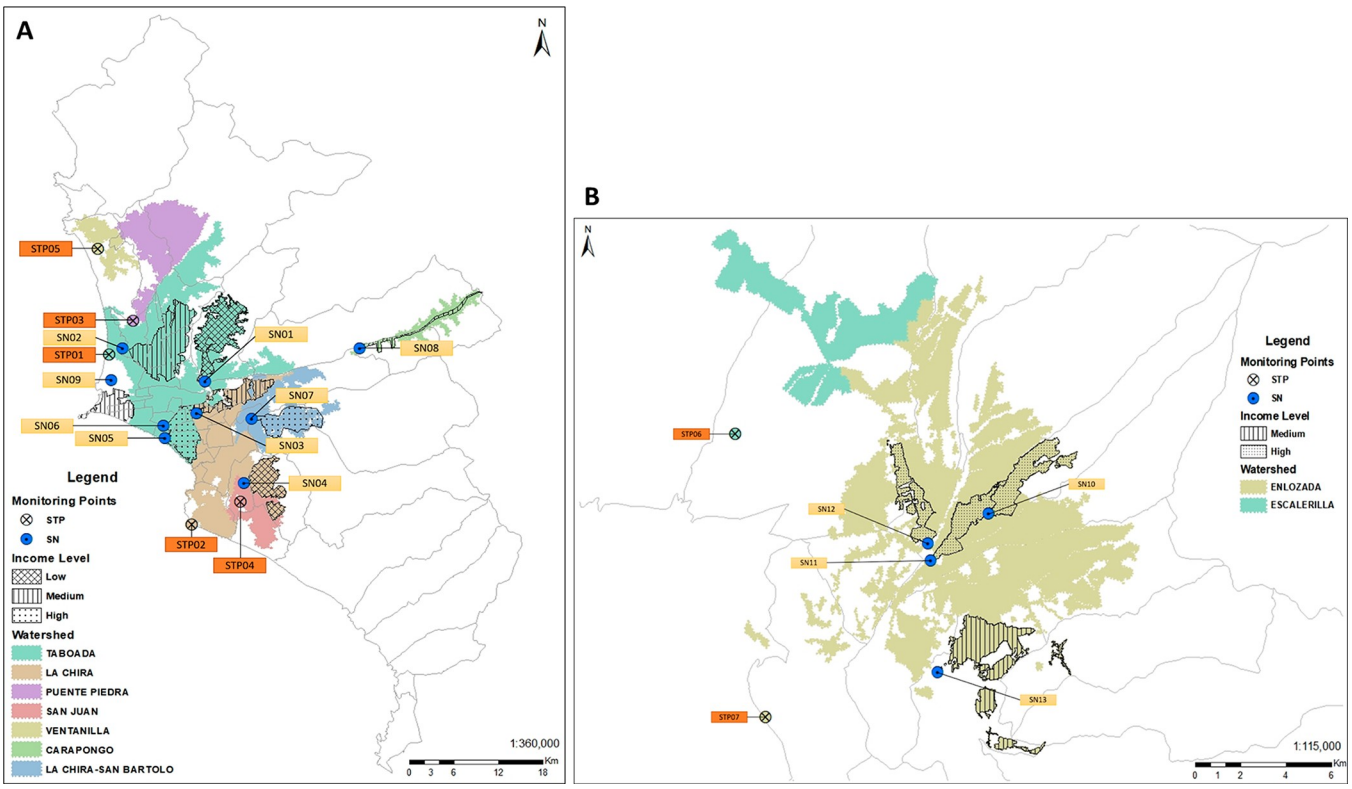


Figure 1. Map of the monitored regions and sampling points in (A) Lima and Callao and (B) Arequipa showing the income level of the contributing populations.

in Peru, there is strong evidence that suggests that the number of confirmed cases may not reflect the real number of infected individuals due to the very limited number of clinical tests performed, the high costs of diagnostic tests (prohibitive for many), and medical care that does not reach all of the population, with deficient infrastructure in most cities and many economic hurdles.[8,9] Therefore, alternative approaches that allow for reliable, unbiased, population-wide, and cost-effective monitoring of the COVID-19 pandemic are needed for the Peruvian context.

SARS-CoV-2 is shed in human feces of infected individuals, including symptomatic, asymptomatic, and presymptomatic cases.[10−14] It enters the sewage network, where it can be stable for 2−4 days at 24 °C,[15] although COVID-19 infections through sewage have not yet been confirmed.[16−18] Alternatively, SARS-CoV-2 RNA can persist in the wastewater system for over 12 days at 25 °C,[19] where it does not replicate, making it a useful bioindicator for estimating the prevalence of the COVID-19 pandemic in a given population.[18,20,21]

Consequently, many groups worldwide rapidly implemented wastewater surveillance for SARS-CoV-2 RNA in the early stages of the pandemic in sewage treatment plants, environmental water bodies, or sewage networks with remarkable success.[22−30] SARS-CoV-2 RNA wastewater surveillance has been used to give an early warning of the pandemic and to identify surges and hot spots. As an early warning system, the presence of the virus in wastewater was reported to precede confirmed cases by up to 63 days,[31] possibly due to SARS-CoV-2 RNA detection in wastewater coming from asymptomatic or misdiagnosed symptomatic cases;[32] overall, 13 studies reported positive samples before the first cases were detected in the community.[30,33−36] Furthermore, Mota et al.[22] were able to identify hot spots within heterogeneous urban areas on the basis of SARS-CoV-2 RNA sewage data, whereas Haak et al.[36] evaluated COVID-19 spatial patterns through spatial monitoring in wastewater basins.

In this study, we monitored SARS-CoV-2 RNA in the sewage of three major cities in Peru: Lima, the capital; Callao, located close to Lima; and Arequipa, the second-largest city in Peru. The estimated total population monitored in this study was 7 million in Lima and Callao (which represents 65% of the population residing in these localities) and 0.8 million in Arequipa (55% of the population residing in the Arequipa metropolitan area).[37] These three cities have georeferenced and consolidated sanitation systems operated by two service providers (SEDAPAL for Lima and Callao and SEDAPAR for Arequipa). We initiated wastewater surveillance in Lima and Callao on February 08, 2021, when Peru was going through the second wave of the contagion, and in Arequipa on April 13, 2021, right before the onset of the second wave in that city. Our objective was to provide health authorities with relevant data, complementary to clinical testing data, to help them take action during the pandemic. Wastewater monitoring in these locations continued beyond the scope of this study.

2. EXPERIMENTAL METHODS

2a. Monitoring Plan. Our monitoring plan included sampling points in sewage treatment plants (STPs) and sewer network (SN) manholes throughout the cities of Lima and Callao (Figure 1A) and Arequipa (Figure 1B). To select sampling points, we considered (1) the population covered, (2) the average income of the contributing population [provided by the Peruvian Institute on Statistics and Informatics (INEI)], (3) high-traffic areas (districts with high levels of tourism or having major markets), and (4) COVID-19 incidence, registered as the

https://doi.org/10.1021/acsestwater.2c00065
ACS EST Water 2022, 2, 2144−2157

total number of deceased (the most reliable indicator of the pandemic in Peru). We selected five STPs and nine SNs for metropolitan Lima and Callao (Figure 1A and Table 1) and two STPs and two SNs for the Arequipa metropolitan area (Figure 1B and Table 1). Later in the study, two additional SN monitoring points were added for Arequipa due to a pronounced increase in COVID-19 cases reported for that city.

2b. Sampling Procedure. In most STPs, composite samples were collected for 24 h periods using automated samplers (Sigma SD900 Portable Sampler and Sigma AWRS Sampler model 3542SDRH, HACH, Loveland, CO). Otherwise, composite samples were collected manually for 4 h periods, between 8 a.m. and 12 p.m. (see Figure 2A for Lima and Callao and Figure 2B for Arequipa). First, 250 mL and 1 L plastic bottles [high-density polyethylene (HDPE)], cylindrical, wide mouth, with a lid and a counter lid, were used for single and composite wastewater samples, respectively. All sampling bottles were sanitized as follows. Bottles were submerged in a 1% (v/v) commercial bleach solution for at least 1 h to overnight; they were then thoroughly rinsed with distilled water (at least three times) and allowed to dry placed upside down inside a clean cabinet. The process for collecting wastewater samples in the field was as follows.

With the help of service provider companies (SPCs) (SEDAPAL for Lima and Callao and SEDAPAR for Arequipa), a perimeter was fenced near the monitoring manhole with cones and rods. Manholes were opened, and the sampling team waited a few minutes for the toxic gases to dissipate. Using a bucket and a rope, two grab samples of 250 mL were taken each hour for a total of 4 h (total composite sample volume of 2 L). Samples were kept in a cooler with ice packs (temperature kept below 8 °C at all times). From this 2 L composite sample, a 500 mL aliquot was used to prerinse the plastic bottles three times. A 1 L sample was used for SARS-CoV-2 RNA quantification at the laboratories of the Universidad de Ingenieria y Tecnologia (UTEC). A 250 mL sample was used to determine the chemical oxygen demand (COD), measured by an accredited commercial analytical laboratory. A third 250 mL aliquot was used to measure temperature, conductivity, and pH in situ using a pH/EC EZDO-7200 multiparameter instrument (GOnDO Electronic Co., Taipei, Taiwan). Samples from STP01, STP02, STP04, STP06, and STP07 were collected with an autosampler with a 24 h sampling time. Ice packs and data loggers were used to monitor temperature. One liter of the composite sample was used for SARS-CoV-2 RNA quantification. The remaining volume was used to measure the parameters of interest for each STP (i.e., COD, BOD, ammoniacal nitrogen, suspended solids, thermotolerant coliforms, etc.). For STP03 and STP05, sampling was done manually, similar to SN points, with 4 h sampling times.

The same sampling day and time were maintained for each sampling point throughout the study. Samples were assigned unique codes, and, overall, methods for ensuring traceability were put in place. The temperature was kept below 8 °C at all times. In all cases, wastewater samples were processed within 48 h of being collected. Samples from Arequipa were filtered at the San Agustin National University (UNSA); filtration membranes with samples were placed in RST1 and Nucleozol buffers (see below) and shipped to UTEC laboratories in Lima for further processing.

2c. CoV-2 RNA Quantification. SARS-CoV-2 viral particles in wastewater were concentrated using previously reported methods.[38] Briefly, 100 mL aliquots were taken from each 1 L composite wastewater sample. One milliliter of 2.5 M $MgCl_2$ (Himedia, Mumbai, India) was added (to afford a final concentration of 25 mM), as well as 100 μL of Bovilis Vista Once SQ (MSD Animal Health), a vaccine containing the bovine respiratory syncytial virus (BRSV), used to evaluate viral recovery as previously described.[39] Viral recovery estimations in this study were used to verify that we were obtaining results comparable to those previously reported[40] and to optimize filtration and transport procedures for the Arequipa samples in our laboratories in Lima. Samples were stirred for 1 min and allowed to sit for 30 min at 4 °C.[41] Then, 50 mL aliquots were filtered through a 0.45 μm pore size, 47 mm diameter mixed cellulose ester (MCE) HAWP04700 electronegative membrane (Merck Millipore, Burlington, MA) and immediately stored in 2 mL ceramic bead tubes containing 770 μL of RST1 and 230 μL of Nucleozol buffers from the NucleoSpin RNA Stool isolation kit (Macherey-Nagel, Düren, Germany). Tubes containing filtered membranes in Nucleozol buffers were stored at −20 °C until RNA extraction was performed.

To obtain total RNA, membranes containing filtered wastewater were cut using sterile scissors (baked for 2 h at 250 °C) inside each bead tube before homogenization. Total RNA was extracted using the NucleoSpin RNA stool isolation kit (Macherey-Nagel) following the manufacturer's instructions, with minor modifications. First, 770 μL of RST1 buffer (instead of 660 μL) and 230 μL of Nucleozol buffer (instead of 200 μL) were added to filtered samples (step 1); then, 165 μL of RST2 buffer (instead of 140 μL) was added to precipitate contaminants (step 3), and 210 μL of RST2 (instead of 180 μL) was added to adjust the binding conditions (step 5). Total RNA was eluted in 100 μL of RNase-free water and stored at −80 °C.

SARS-CoV-2 RNA was quantified by RT-qPCR targeting the nucleocapsid protein (N) gene region N1 and the *Homo sapiens* pibonuclease P protein subunit p30 (Hs_RPP30) gene (to serve as an internal control), using primer and probe combinations included in the 2019-NCOV RUO KIT (IDT, Newark, NJ), following recommendations of the U.S. Centers for Disease Control and Prevention.[42] Amplification reactions were carried out in a 7500 Fast Real-Time PCR System (Applied Biosystems, Waltham, MA). Each 20 μL RT-qPCR mixture was composed of 10 μL of GoTaq Probe 1-Step qPCR Master Mix (Promega, Madison, WI), 3.1 μL of nuclease-free water, 1.5 μL of N1 or Hs_RPP30 primer/probe mix, 0.4 μL of Go Script RT Mix (Promega), and 5 μL of either (1) a commercial plasmid containing the SARS-CoV-2 N gene (2019-nCoV_N_Positive Control, IDT), (2) a commercial plasmid containing the Hs_RPP30 gene (Hs_RPP30_Positive Control, IDT), (3) nuclease-free water (nontemplate control), or (4) 1:5-diluted RNA samples. All samples, positive controls, and nontemplate controls were assayed in triplicate. Standard curves were made by serial 10-fold dilutions of the 2019-nCoV_N_Positive Control (IDT), ranging from 10 to 10^4 copies per reaction, and were included in each RT-qPCR run to avoid interplate variation. Similarly, the Hs_RPP30 target was analyzed in parallel reactions within the same RT-qPCR run, using 10^3 copies for the positive control reactions. Thermal conditions were 45 °C for 15 min, 95 °C for 2 min, and 45 cycles of 95 °C for 3 s and 55 °C for 30 s. In all RT-qPCR experiments, we verified that the amplification efficiency was in the range of 90−110%, the slope was between −3.1 and −3.6, and the R^2 was >0.98. The threshold was set within the exponential amplification phase and adjusted if needed (i.e., to meet

https://doi.org/10.1021/acsestwater.2c00065
ACS EST Water 2022, 2, 2144−2157

Table 1. General Description of the Watersheds Selected for the Cities of Lima, Callao, and Arequipa

watershed code	UTM coordinates[a]	manhole code[b]	city districts covered (% of total district population)	contributing population	sampling period (h)
STP01	267931 (*X*); 8673107 (*Y*)	NA	Bellavista (28%), Breña (100%), Callao (54%), Carabayllo (69%), Carmen de la Legua Reynoso (100%), Comas (99%), El Agustino (69%), Independencia (100%), Jesus María (100%), La Victoria (5%), Lima (96%), Lince (100%), Los Olivos (100%), Lurigancho (11%), Magdalena del Mar (100%), Miraflores (69%), Pueblo Libre (100%), Rimac (99%), San Isidro (84%), San juan de Lurigancho (92%), San Martín de Porres (95%), San Miguel (92%), Surquillo (14%), Ventanilla (2%)	4 288 450	24
STP02	279167 (*X*); 8649657 (*Y*)	NA	Ate (23%), Barranco (100%), Chorrillos (88%), El Agustino (38%), La molina (0.38%), La Victoria (96%), Lima (5%), Miraflores (34%), San Borja (100%), San Isidro (16%), San Juan de Miraflores (44%), San Luis (100%), Santa Anita (87%), Santiago de Surco (97%), Surquillo (93%), Villa Maria del Triunfo (66%)	1 852 682	24
STP03	271206 (*X*); 8677741 (*Y*)	NA	Carabayllo (26%), Los Olivos (6%), Mi Perú (8%), San Martín de Porres (3%), Puente Piedra (88%), Ventanilla (2%)	401 943	4
STP04	285707 (*X*); 8652744 (*Y*)	NA	Villa el Salvador (68%), Villa María del Triunfo (19%)	337 368	24
STP05	266421 (*X*); 8687679 (*Y*)	NA	Ventanilla (75%), Mi Perú (92%), Santa Rosa (4%)	275 592	4
SN01	280938 (*X*); 8669332 (*Y*)	BZ-264976	San Juan de Lurigancho (69%), Rimac (0.06%)	695 164	4
SN02	269810 (*X*); 8673885 (*Y*)	BZ-346543	San Martín de Porres (40%), Independencia (100%), Comas (41%), Los Olivos (52%), Rimac (0.29%), Callao (0.33%)	836 660	4
SN03	279798 (*X*); 8664950 (*Y*)	BZ-108938	Ate (10%), El Agustino (33%), La Victoria (29%), Lima (5%), San Luis (10%), Santa Anita (87%)	357 534	4
SN04	286179 (*X*); 8655354 (*Y*)	BZ-87962	Villa María del Triunfo (65%), San Juan de Miraflores (1%)	259 589	4
SN05	275529 (*X*); 8661486 (*Y*)	BZ-118311	Miraflores (70%), San isidro (76%), Lince (9%), Magdalena del Mar (5%), Surquillo (8%)	125 379	4
SN06	275270 (*X*); 8663220 (*Y*)	BZ-118746	Lince (93%), Jesus Maria (100%), La Victoria (12%), Lima (8%), San Isidro (15%), Breña (0.41%)	169 469	4
SN07	287232 (*X*); 8664167 (*Y*)	BZ-166706	La Molina (25%), Cieneguilla (19%), Pachacamac (6%)	46 174	4
SN08	301720 (*X*); 8673879 (*Y*)	BZ-187126	Chaclacayo (63%), Lurigancho (9%)	47 360	4
SN09	268283 (*X*); 8669550 (*Y*)	BZ-233185	Callao (31%), La Punta (100%), San Miguel (5%), La Perla (99%), Bellavista (74%)	260 932	4
STP06	219187 (*X*); 8188684 (*Y*)	NA	Cerro Colorado (32%), Yura (62%)	81 689	24
STP07	220491 (*X*); 8176654 (*Y*)	NA	Alto Selva Alegre (93%), Arequipa (100%), Cayma (92%), Cerro Colorado (46%), Chiguata (86%), Jacobo Hunter (98%), Jose Luis Bustamante y Rivero (100%), Mariano Melgar (82%), Miraflores (97%), Paucarpata (98%), Sabandía (22%), Sachaca (65%), Socabaya (84%), Tiabaya (71%), Yanahuara (99%)	766 043	24
SN10	230373 (*X*); 8185305 (*Y*)	BZ-48148	Arequipa (0.5%), Miraflores (74.3%)	43 702	4

https://doi.org/10.1021/acsestwater.2c00065
ACS EST Water 2022, 2, 2144−2157

Table 1. continued

watershed code	UTM coordinates[a]	manhole code[b]	city districts covered (% of total district population)	contributing population	sampling period (h)
SN11	227806 (X); 8183314 (Y)	BZ-49336	Arequipa (24.9%), Miraflores (14.8%), Alto Selva Alegre (0.5%)	21 361	4
SN12	227687 (X); 8184041 (Y)	BZ-47207	Arequipa (5.3%), Cayma (0.4%), Cerro Colorado (8.4%), Sachaca (0.3%), Yanahuara (47.9%)	31 118	4
SN13	228125 (X); 8178539 (Y)	BZ-48635	Jose Luis Bustamante y Rivero (17.1%), Jacobo Hunter (0.1%), Sabandía (21.7%), Socabaya (70.6%)	63 927	4

[a]UTM coordinates correspond to the monitoring points. [b]NA, not applicable.

amplification efficiency parameters). Samples with a quantification cycle (Cq) of >40 were considered negative.

The limit of detection (LOD) was estimated by an exponential model[43] using a 95% probability of amplification, similar to that previously described.[22] RT-qPCR mixtures containing 10^4, 10^3, 10^2, 50, 30, 10, 5, and 3 copies of the 2019-nCoV_N_Positive Control were tested in at least eight runs using the N1 RT-qPCR assay described above. The "observed amplification frequency" was estimated as the number of positive reactions over the total reactions assayed for each target concentration. The "predicted amplification frequency" was estimated using the exponential model described by Mota el al.[22] Observed and predicted amplification frequencies were compared using the χ^2 goodness of fit test, run in Microsoft Excel. We obtained a p value of 0.94, indicating that our observed amplification frequency distribution agrees with the exponential model tested. Then, the LOD was estimated as the lowest copy number predicted by the model that meets a 95% probability of amplification, which, for our study, was 22 copies per reaction (8.8 copies/mL of sewage).

To verify the absence of PCR inhibitors in the RNA samples, we performed the Sketa22 spike assay as previously reported.[44,45] Briefly, 10^4 copies of a 77 bp dsDNA sequence from the *Onchorrhynchus keta* ITS2 region (O.keta-ITS2 gBlock; IDT) were spiked into PCR tubes containing (1) 5 μL of nuclease-free water or (2) 5 μL of 1:5 diluted RNA sample (as used in the RT-qPCR experiments) and amplified by real-time PCR using Sketa22 primers and the probe (see ref 44). The Cq values of both reactions were compared. Cq differences over two cycles were indicative of PCR inhibitors in the RNA samples. In this case, RT-qPCRs for SARS-CoV-2 RNA were repeated with a more diluted sample and/or a new RNA extraction was performed. The inhibitor test was performed for every sample in the study.

2d. Estimation of SARS-CoV-2 RNA Decay in the Sewage Network. Viral RNA is labile and may decay as it travels through sewerage networks. Because sewerage networks were sampled in different locations, including upstream (near the contributing population) and downstream (in sewage treatment plants), it is important to estimate SARS-CoV-2 RNA decay throughout the different sampled points. This allows us to distinguish when comparing concentrations in upstream and downstream locations whether there are actual differences in measured SARS-CoV-2 RNA concentrations (due to higher viral loads from the contributing population) or whether such differences are due to RNA decay.

To estimate SARS-CoV-2 RNA decay, the necessary hydraulic variables of the sewerage system (average diameter, length, average slope, and average flow) were provided by the SPC of each city, Lima and Callao (Figure 2A) and Arequipa (Figure 2B). On the basis of these variables, the travel time was estimated (Table 2; detailed calculations are presented as Supporting Information 1). Then, the degradation rate was estimated according to Mota et al.[22] and Bertsch et al.[19] for all monitoring points using eq 1:

$$N_{(0)} = \frac{N_{(t)}}{e^{(-kt)}} \quad (1)$$

where $N_{(0)}$ is the viral load excreted at the beginning of the drainage area, $N_{(t)}$ is the estimated viral load at the monitoring point, t is the travel time of the virus from when it was excreted until it reaches the monitoring point, and k is the decay constant,

https://doi.org/10.1021/acsestwater.2c00065
ACS EST Water 2022, 2, 2144−2157

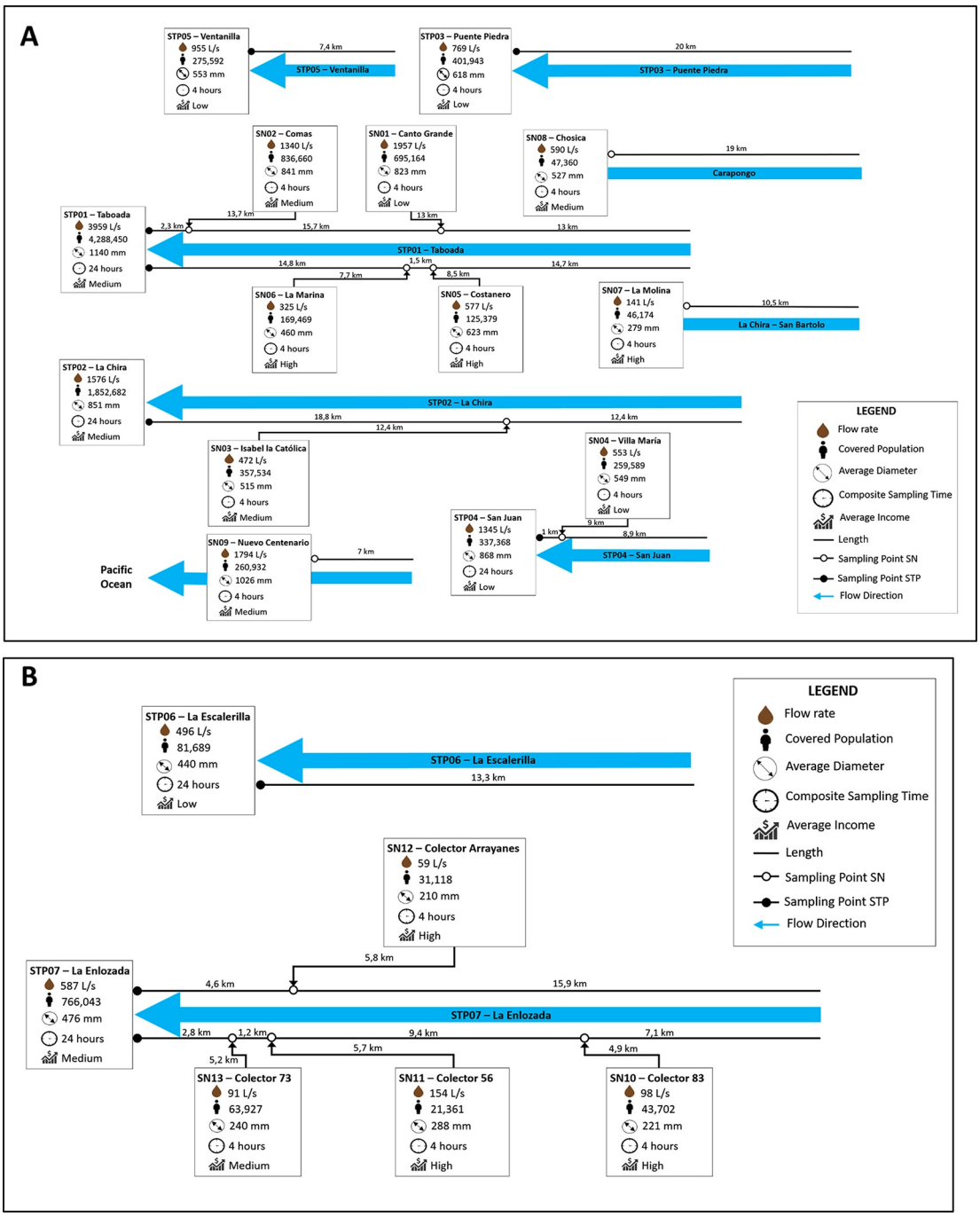


Figure 2. (A) Sewage network diagram used to determine distances among STPs and monitoring sites for viral decay estimations in the cities of Lima and Callao. SN07 and SN08 drain to STPs not included in this study. SN09 drains to the Pacific Ocean without treatment. (B) Sewage network diagram used to determine distances among STPs and monitoring sites for viral decay estimations in the city of Arequipa.

which is the speed at which SARS-CoV-2 RNA decomposes at different temperatures (for 25 °C, its value is 0.183 ± 0.008 day^{-1}).[19]

2e. Estimation of the SARS-CoV-2 Relative Prevalence Index. Mota et al.[22] introduced the relative prevalence index (RPI) to identify COVID-19 hot spots in cities on the basis of sewage data. It is a method that allows the comparison of the concentration of SARS-CoV-2 RNA (gene copies per liter) of an STP with an SN belonging to its area of influence (eq 2; detailed calculations are presented as Supporting Information 2).

$$\mathrm{RPI} = \frac{C_{SN}}{C_{STP}} \tag{2}$$

If the RPI value obtained for a given monitoring point at a given time was >1, then the corresponding sewershed was considered a hot spot of SARS-CoV-2 prevalence.[22] A Johnson *t* test[46] (run in RStudio version 4.1.2.) was used to test the null hypothesis of having an RPI of >1, with 95% confidence. This test is based on population means; it requires that the sample size be ≥10 and assumes that the statistical distribution is unknown (therefore not necessarily symmetric). We had one observation per monitoring point per week; therefore, we

https://doi.org/10.1021/acsestwater.2c00065
ACS EST Water 2022, 2, 2144−2157

Table 2. Design Characteristics of Sampled Trunk Sewers and Interceptors and Results of In-Sewer Travel Time Calculations

sewershed	average diameter (mm)	hydraulic radius (m)	length (m)	average slope (%)	average flow (L s^{-1})	average velocity (m s^{-1})	travel time (h)
STP01	1140	0.338	31075	1.39	3959	5.2	1.7
STP02	851	0.252	35156	1.04	1576	3.7	2.6
STP03	618	0.183	20037	1.37	769	3.4	1.6
STP04	868	0.257	9963	0.68	1345	3.0	0.9
STP05	553	0.164	7478	3.81	955	5.3	0.4
STP06	440	0.130	13273	3.50	496	4.4	0.8
STP07	476	0.141	20530	3.21	587	4.4	1.3
SN01	823	0.244	13012	1.92	1957	4.9	0.7
SN02	841	0.249	13677	0.80	1340	3.2	1.2
SN03	515	0.153	12396	1.36	472	3.0	1.1
SN04	549	0.163	8978	1.33	553	3.1	0.8
SN05	623	0.185	8530	0.74	577	2.5	0.9
SN06	460	0.136	7741	1.18	325	2.6	0.8

grouped our data into 11- and 12-week periods (weeks 20−30 and weeks 31−42, respectively). It would have been ideal to have as many observations as possible in the shortest time period to allow for a weekly determination of hot spots with statistical robustness, but this was limited by budget and logistic constraints. Alternatively, hot spots could be determined on a weekly basis, with one observation per week and statistical robustness using a moving average approximation, as described in ref 47; however, this was beyond the scope of this study. In addition, we did not have enough data to assume symmetry or asymmetry in the RPI distribution, thus meeting the second Johnson *t* test requirement.

The RPI normalizes the uncertainty of the estimated viral load introduced by different indicators such as population density, different water use patterns, rainwater intrusion, etc.[22] The chemical oxygen demand (COD), measured at all monitoring points through all epidemiological weeks (presented as Supporting Information 5), was used to normalize the RPI values. When the median COD of the SN was significantly different from the STP to which it contributes sewage, a correction factor was used (COD of the SN divided by COD of the STP). Wilcoxon's paired statistical test (run in RStudio version 4.1.2.) was used for significance analysis of COD values with 95% confidence. This test does not assume that observations are independent or normally distributed. COD observations meet these conditions because (1) the sample size is small (thus, we need a nonparametric test), (2) the COD of the SN drainage areas contributes to the COD of the STP drainage area, and (3) the samples were taken in the same monitoring week (thus, we cannot assume independence).[48] The RPI for STP03 and STP05, which are smaller watersheds, was calculated by comparing them to STP01 due to geographic proximity and demographic similarities (see Figure 1A). Similarly, the RPI for SN07 and STP04 was estimated by comparing them to the STP02 watershed due to geographic proximity and similar demographics.

2f. Collection of COVID-19 Indicators from the Health Care System. COVID-19 data from the health sector were obtained from the Peruvian national open data platform (available at https://www.datosabiertos.gob.pe/). The following indicators were used: (1) mortality per 100 000 inhabitants, extracted from the "Sistema Nacional de Defunciones" (SINADEF), (2) incidence, as confirmed positive cases per 100 000 inhabitants, (3) occupancy of intensive care unit (ICU) beds per 100 000 inhabitants, (4) occupancy of hospital beds in the COVID-19 area per 100 000 inhabitants, and (5) vaccination status (first and second doses). The data were grouped by date and then summed by week to match SARS-CoV-2 RNA data in wastewater (which was obtained on a weekly basis). The information was processed in RStudio version 4.1.2.[49]

3. RESULTS AND DISCUSSION

3a. SARS-CoV-2 RNA Viral Decay in the Sewage Network. Viral loads estimated not accounting for viral decay $[N_{(t)}]$ for each watershed throughout epidemiological weeks 6−42 are presented as Supporting Information 3. Then, viral loads estimated accounting for viral decay $[N_{(0)}]$ for the same period are presented as Supporting Information 4. Using $N_{(t)}$ and $N_{(0)}$, we estimated the percent RNA degradation for each watershed (Table 3). The maximum degradation measured was 2% for STP02, a watershed in which the virus has the longest travel path. The values obtained in this study are on the same order of magnitude as those reported in the city of Belo Horizonte in Brazil.[22] To see how $N_{(t)}$ and $N_{(0)}$ are related throughout all monitoring weeks evaluated, a graphical comparison for STP02 is presented (Figure 3). Overall, our results indicate that the viral

Table 3. Estimated SARS-CoV-2 RNA Decay in the Sewer Network for All Monitoring Points in This Study

sewershed	estimated SARS-CoV-2 RNA decay in the sewer network (%)
STP01	1.3
STP02	2.0
STP03	1.2
STP04	0.7
STP05	0.3
STP06	0.6
STP07	1.0
SN01	0.6
SN02	0.9
SN03	0.9
SN04	0.6
SN05	0.7
SN06	0.6
SN07	0.7
SN08	1.1
SN09	0.5
SN10	0.3
SN11	0.4
SN12	0.5
SN13	0.4

https://doi.org/10.1021/acsestwater.2c00065
ACS EST Water 2022, 2, 2144−2157

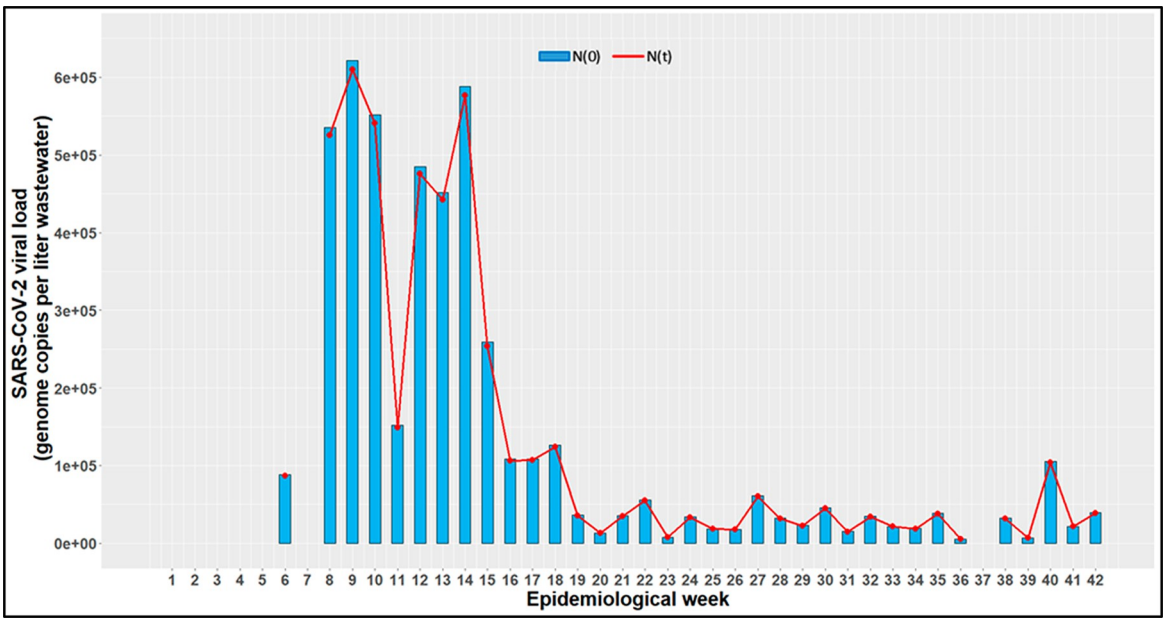


Figure 3. SARS-CoV-2 viral loads measured at STP02, corrected $[N_{(0)}]$ and not corrected $[N_{(t)}]$ for viral decay. STP02 is the monitoring point with the largest trajectory and the highest viral decay estimated in the study.

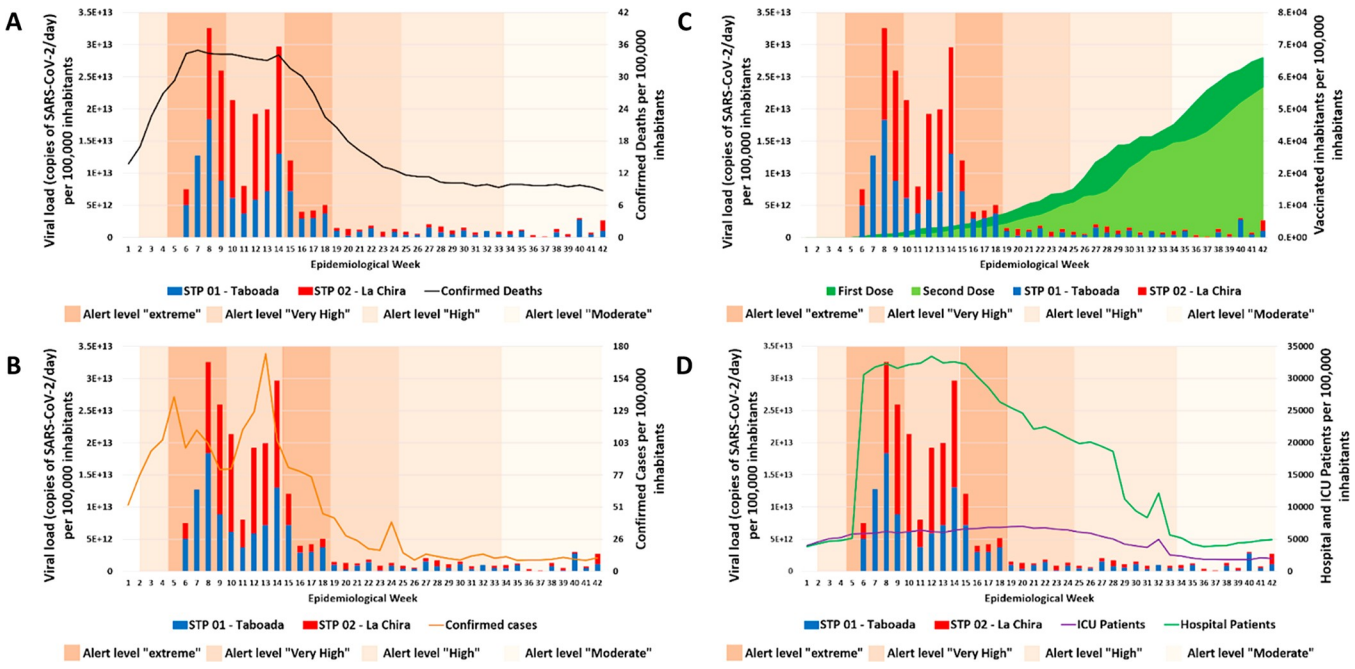


Figure 4. Comparison between epidemiological data and SARS-CoV-2 viral loads in wastewater for metropolitan Lima and Callao. SARS-CoV-2 viral load peaks in this figure correspond to the second wave of infections registered for Lima and Callao.[51] (A) SARS-CoV-2 viral load vs confirmed number of deceased. (B) SARS-CoV-2 viral load vs confirmed COVID-19 cases. (C) SARS-CoV-2 viral load vs scope of vaccination for COVID-19. (D) SARS-CoV-2 viral load vs COVID-19 hospitalizations. Alert level "Extreme", curfew from Monday to Saturday from 9:00 p.m. to 4:00 a.m. and Sunday all day. Alert level "Very high", curfew from Monday to Sunday from 10:00 p.m. to 4:00 a.m. Alert level "High", curfew from Monday to Sunday from 11:00 p.m. to 4:00 a.m. Alert level "Moderate", curfew from Monday to Sunday from 2:00 a.m. to 4:00 a.m.

decay of SARS-CoV-2 RNA in the sewage networks surveyed was negligible. Therefore, $N_{(t)}$, not $N_{(0)}$, was used for further analysis.

3b. In-Sewage SARS-CoV-2 RNA Estimates for Monitoring the COVID-19 Pandemic. Peru faced the COVID-19 pandemic by declaring a countrywide state of health emergency on March 15, 2020, soon after the first COVID-19 case was reported. Earlier than most Latin American countries, Peru implemented a countrywide lockdown, curfew, and several other mobility restrictions to minimize the impact of COVID-19. Nevertheless, Peru counted more than 80 000 deaths during its first wave and more than 110 000 during its second wave despite its efforts.

At the end of 2020, before the peak of Peru's second COVID-19 wave, the Sanitation Division from the Ministry of Housing, Construction, and Sanitation took the initiative to implement a wastewater monitoring system for SARS-CoV-2. Following the success of neighboring countries and others around the world,

https://doi.org/10.1021/acsestwater.2c00065
ACS EST Water 2022, 2, 2144−2157

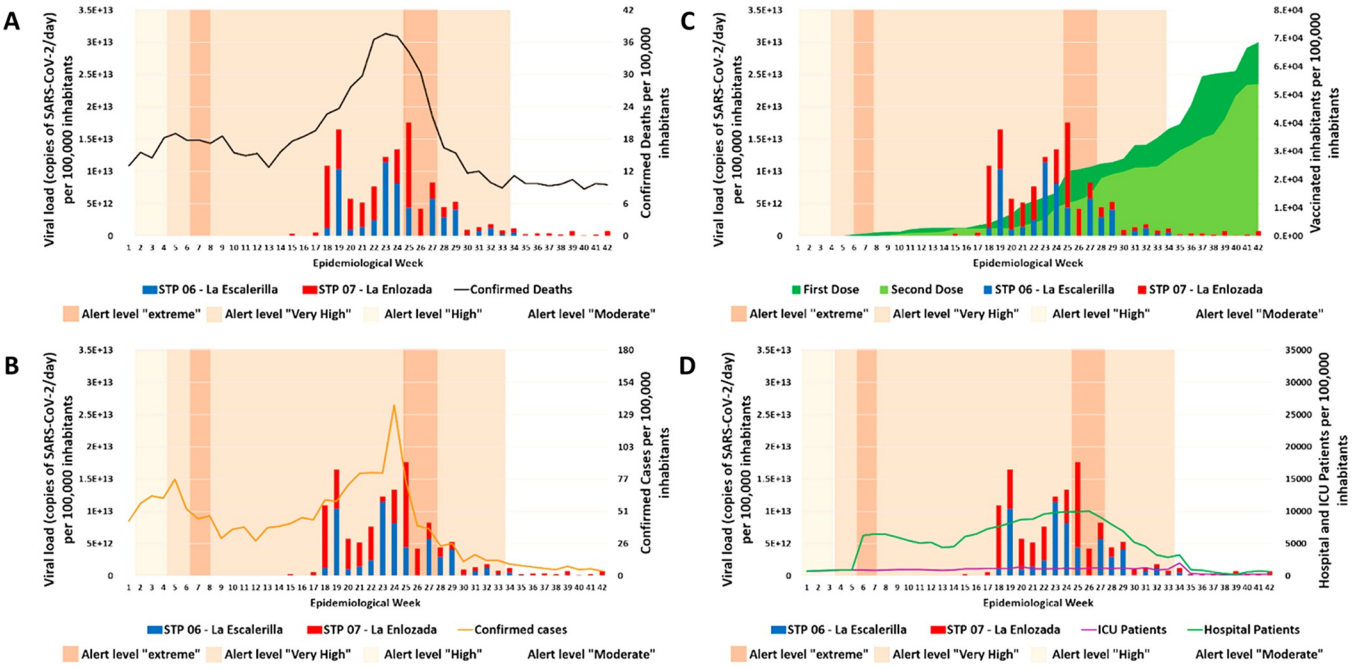


Figure 5. Comparison between epidemiological data and SARS-CoV-2 viral loads in wastewater for metropolitan Arequipa. SARS-CoV-2 viral load peaks in this figure correspond to the second wave of infections registered for Arequipa.[51] (A) SARS-CoV-2 viral load vs confirmed number of deceased. (B) SARS-CoV-2 viral load vs confirmed COVID-19 cases. (C) SARS-CoV-2 viral load vs scope of vaccination for COVID-19. (D) SARS-CoV-2 viral load vs COVID-19 hospitalizations. Alert level "Extreme", curfew from Monday to Saturday from 9:00 p.m. to 4:00 a.m. and Sunday all day. Alert level "Very high", curfew from Monday to Sunday from 10:00 p.m. to 4:00 a.m. Alert level "High", curfew from Monday to Sunday from 11:00 p.m. to 4:00 a.m. Alert level "Moderate", curfew from Monday to Sunday from 2:00 a.m. to 4:00 a.m.

the plan included sites at three of the largest cities in the country: Lima, Callao, and Arequipa. In this plan, we had sampling points in STPs, prioritizing those that collected wastewater from the largest populations, and SNs, selecting sampling points with high spatial resolution. A primary focus was to include wastewater contributions from low-income settings that are often neglected by the health care surveillance systems.

The populations of Lima, Callao, and Arequipa present stark differences in socioeconomic levels based on average household incomes. This goes along with limited health care coverage and testing access among low-income people. Thus, monitoring SARS-CoV-2 through the aforementioned COVID-19 indicators is not enough to have a broad view of the evolution of the virus in the population. Local researchers highlighted the need to increase the level of contact tracing that focuses on symptomatic and asymptomatic cases in Peru.[50] With the aim of implementing creative and effective alternatives to gather more COVID-19 prevalence data, SARS-CoV-2 RNA monitoring in wastewater was implemented. Our results allowed us to generate information complementary to the indicators previously used to assess the COVID-19 situation in the monitored regions.[6]

This study successfully presents the first Peruvian SARS-CoV-2 RNA wastewater monitoring report. This report corresponds to the period between the second wave peak and the third wave's beginning, between the 2021 epidemiological weeks 6 (February 8−14) and 42 (October 18−24). During this period, we contrasted the main COVID-19 epidemiological indicators, including COVID-19 incidence and mortality, with the estimated SARS-CoV-2 RNA viral load in wastewater per 100 000 inhabitants as recommended[31] for metropolitan Lima and Callao (Figure 4) and metropolitan Arequipa (Figure 5). Herein, we observed that the estimated concentration of SARS-CoV-2 RNA in the wastewater followed the same trend as the COVID-19 epidemiological indicators.

In Lima and Callao, the beginning of the second wave of infections and deaths from COVID-19 occurred before the study. Therefore, we were unable to statistically determine how SARS-CoV-2 RNA wastewater data provided an early warning. However, the peak in SARS-CoV-2 RNA in the sewage can be observed between week 7 (February 15−21) and week 14 (April 5−11) of 2021, which showed trends similar to those of mortality and the number of confirmed cases (Figure 4A,B). For Arequipa, it was possible to see the second wave of infections and deaths from COVID-19 between week 18 (May 3−9) and week 29 (July 19−25) (Figure 5A,B). Here, too, SARS-CoV-2 RNA measured in the sewage showed trends similar to those of mortality and confirmed COVID-19 cases. Similar to the case for Lima and Callao, our wastewater monitoring started after the onset of the second wave of the contagion for Arequipa; thus, we were unable to statistically determine how wastewater data provided an early warning. It is important that authorities maintain wastewater monitoring, at least at sentinel points (i.e., STPs, ground transportation hubs, airports, etc.), regardless of the fluctuation in epidemiological data, to better use this approach as an early warning tool.

As one can see in the graphs presented, the SARS-CoV-2 RNA wastewater data corresponded better with the mortality data (confirmed deaths per 100 000 inhabitants), followed by the number of confirmed cases, and to a lesser degree with hospitalizations and ICU occupancies (Figures 4 and 5). The trends observed may support the thesis that wastewater surveillance certainly can produce reliable information, which could help overcome the limitations of the health care system of Peru (i.e., insufficient testing and limited access to health infrastructure). Also, the data indicate that the increased

https://doi.org/10.1021/acsestwater.2c00065
ACS EST Water 2022, 2, 2144−2157

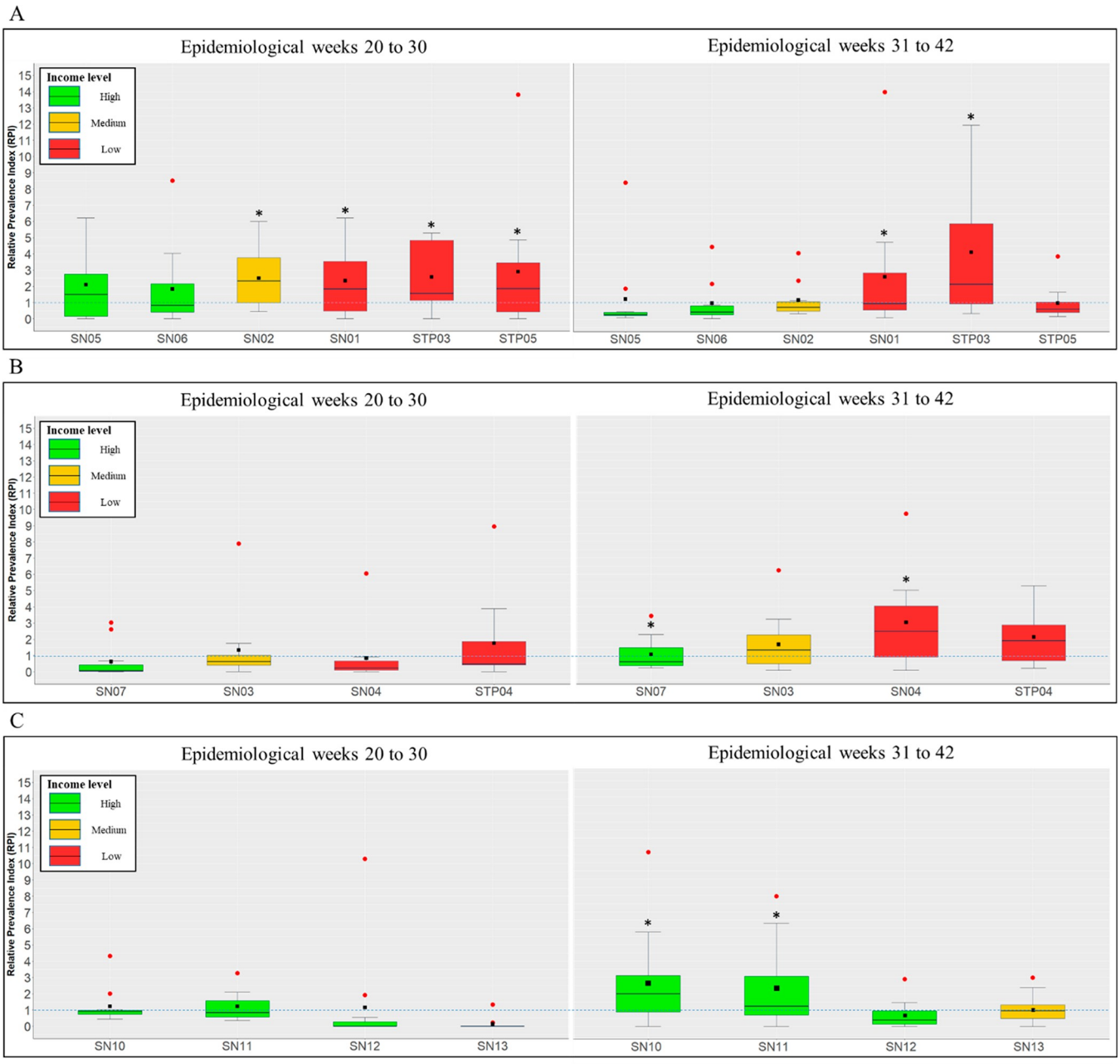


Figure 6. Identification of hot spots through the relative prevalence index (RPI) during epidemiological weeks 20−30 and 31−42. (A) STP01 sewershed (also known as Taboada, located in Callao). (B) STP02 sewershed (also known as La Chira, located in Lima). (C) STP07 sewershed (also known as la Enlozada, located in Arequipa). The average household income of the contributing population is presented. Means (■), medians (−), outliers (red circles), and hot spots (*) (RPI values of >1 with 95% confidence) are indicated.

vaccination coverage among the population impacted the epidemiological indicators and the viral load in wastewater (Figures 4C and 5C). These findings should be verified in further studies.

Molecular biology laboratories equipped for SARS-CoV-2 RNA estimation by RT-qPCR are not readily available throughout Peru. Therefore, this study also tested how basic laboratory facilities could be implemented in some cities to do sampling and initial processing and then ship samples to a centralized facility for further analysis. This was assayed for the city of Arequipa, where minimal infrastructure was put in place to facilitate field work and perform sample filtration. Using data on viral recovery of the BRSV, we were able to optimize sample processing and transport from Arequipa to the Lima facility. We were able to determine that viral loads corresponding to weeks 15−17 were suboptimal (see Figure 5), which was overcome by week 18 (using the methods presented in this study), when BRSV recovery values comparable to those of the samples from Lima and Callao were obtained (data not shown).

3c. Estimating the Relative Prevalence Index (RPI) to Identify Hot Spots. While encouraging results were obtained, estimating the number of cases of COVID-19 with only wastewater data is not yet possible, with acceptable levels of uncertainty.[22] In the current context of the pandemic, we must find catchment areas that we know are more infected than others, i.e., to implement epidemiological fences. Spatial resolution will also allow us to compare populations within urban areas to find trends and varied needs in the cities studied,

https://doi.org/10.1021/acsestwater.2c00065
ACS EST Water 2022, 2, 2144−2157

considering Peru's stark socioeconomic heterogeneity in urban populations.

The RPI analysis was performed to address these limitations and identify hot spots on the basis of sewage data in the drainage network of STP01 (Taboada) and STP02 (La Chira), corresponding to metropolitan Lima and Callao (panels A and B of Figure 6, respectively), and the drainage network of STP07 (La Enlozada), corresponding to metropolitan Arequipa (Figure 6C). The RPI data were estimated for two periods; the first period covers week 20 (May 17−23) to week 30 (July 26 to August 1), and the second period covers week 31 (from August 2−8) to week 42 (October 18−24) of 2021. This was set to allow for sufficient statistical robustness for the COD and RPI analysis and to contain the second wave of the contagion in Arequipa. To facilitate the visual interpretation of the data, the RPI for each catchment area was color-coded according to the average household income of the contributing population.

For basin STP01 [which collects sewage from Lima and Callao (see Figure 1A)], we observed that most catchment areas selected (SN01, SN02, STP03, and STP05) were hot spots during epidemiological weeks 20−30 with p values of 0.04, 0.0112, 0.0176, and 0.0264, respectively (Figure 6A). However, for weeks 31−42, when COVID-19 prevalence was at its lowest (see Figure 4), hot spots were identified for catchment areas with the lowest household income [SN01 and STP03 with p values of 0.043 and 0.0215, respectively (Figure 6A)]. Similar observations were reported in previous studies.[22,52] Interestingly, for the STP02 basin [which collects from metropolitan Lima only (see Figure 1A)], all SN catchment areas selected were not identified as hot spots of SARS-CoV-2 RNA prevalence during epidemiological weeks 20−30. During this period, COVID-19 indicators in the population were relatively low (see Figure 4A,B). Then, similar to the STP01 basin, SN catchment areas became hot spots during weeks 31−42 [SN04 and SN07 with p values of 0.00156 and 0.0449, respectively (Figure 6B)]. At this time, we were approaching a third wave of contagion, which took place in Lima and Callao by epidemiological week 47.[53]

In Arequipa, only SN catchment areas contributing to the STP07 basin were surveyed (see Figure 1B). During epidemiological weeks 20−30, none of the SN catchment areas were identified as hot spots. This period corresponds to the second wave of COVID-19 infections in that city. It is possible that during this period, COVID-19 prevalence was averaged in the population, given that the number of confirmed cases was very high and that metropolitan Arequipa is much smaller than Lima and Callao. Another possibility is that the areas of highest SARS-CoV-2 incidence were not included in our monitoring plan. This is why, toward weeks 29 and 30, monitoring points SN12 and SN13 were added, respectively, to increase the spatial resolution of our wastewater survey. However, due to the few observations obtained, the RPI analysis for SN12 and SN13 during epidemiological weeks 20−30 was not representative.

During epidemiological weeks 31−42, SN11, which collects wastewater from Arequipa's tourist area, and SN10, whose catchment area is contiguous to SN11, sharing areas of high-income households, but also collects sewage from population settled in the upper areas (surrounding mountains) with lower average incomes, were identified as hot spots (p values of 0.0012 and 0.038 for SN10 and SN11, respectively). This agrees with a previous observation indicating a higher incidence of SARS-CoV-2 in high-traffic (i.e., touristic areas) and low-income areas.[22]

Several difficulties were faced in this study. Implementing wastewater monitoring requires an advanced geographic information system (GIS) that allows for a geospatial view of the sewage system. However, this type of information was not always available. It is known that advanced GIS in developing countries is limited, and inadequate sewage infrastructure can make this type of study even more challenging.[31] Another critical aspect of our study was that not all of the population in the cities surveyed contributed to the sewage network, especially those living in poorer parts of the city. This made it more challenging to translate sewage data to health care estimates. Alternative approaches should be considered to collect information from off-the-grid populations.[54] Other limitations encountered included the relatively small sample volumes that could be collected from the sewage, the lack of automatic samplers to collect 24 h composite samples at all locations, and the smaller volume from each composite sample that could be filtered. Collecting and filtering larger volumes will provide greater representability; however, they will bring new operational challenges both in the field and in the laboratory. In any case, our SARS-CoV-2 RNA data proved to be reliable and valuable, showing trends similar to those of the health care indicators on the COVID-19 pandemic at all sampled locations.

For the RPI estimation, the main limitation was the use of COD data to normalize for the contributing population. While this parameter proved to be robust and was able to discern between low- and high-income households (see Supporting Information 5), we could not discard industrial contributions to the sewage. Different methods of normalizing for contributing populations are being developed and tested, which could be used in future WBE applications.[55]

Finally, detecting SARS-CoV-2 RNA during the lower points of the pandemic was challenging using the methods available. Several studies have shown that the lack of detection of SARS-CoV-2 RNA in a sewer probably suggests a low prevalence of COVID-19 cases.[56] Alternative viral particle concentration methods, improved RNA extraction protocols, and/or alternative amplification techniques (i.e., digital PCR[57]) should be explored to overcome this limitation.

4. CONCLUSIONS

This study demonstrates the usefulness of wastewater-based epidemiology analysis in controlling the COVID-19 pandemic, especially in the context of extremely scarce clinical data. The information provided by the analysis of SARS-CoV-2 RNA in wastewater has been shown to be reliable. (1) It follows the same trends as the health sector indicators. (2) It offers a more economical, sensitive, and far-reaching alternative to anticipate the appearance of new waves of the contagion. (3) It makes it possible to identify community contagion hot spots within large and heterogeneous metropolitan areas. (4) It can be used as a general approach to understanding the spread of the pandemic in a city. (5) It can ensure public health resilience beyond this pandemic.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestwater.2c00065.

Calculations used to determine the travel time for SARS-CoV-2 particles in the sewer system (Supporting Information 1), calculations used to determine the RPI

https://doi.org/10.1021/acsestwater.2c00065
ACS EST Water 2022, 2, 2144−2157

in the sewer system (Supporting Information 2), SARS-CoV-2 RNA concentrations in genome copies per liter of wastewater determined for each sewershed, not corrected for viral decay [$N_{(t)}$] (Supporting Information 3) and corrected for viral decay [$N_{(0)}$] (Supporting Information 4), and COD values presented as notched box plots corresponding to epidemiological weeks 20−30 and 31−42 (PDF)

AUTHOR INFORMATION

Corresponding Author

Mónica C. Santa-María − *Universidad de Ingenieria y Tecnologia (UTEC), Centro de Investigación y Tecnología del Agua (CITA), Lima 15063, Peru; Universidad de Ingenieria y Tecnologia (UTEC), Departamento de Ingeniería Ambiental, Lima 15063, Peru;* orcid.org/0000-0003-4789-7444; Phone: +51 956175269; Email: msantamaria@utec.edu.pe

Authors

Braulio Pardo-Figueroa − *Universidad de Ingenieria y Tecnologia (UTEC), Centro de Investigación y Tecnología del Agua (CITA), Lima 15063, Peru;* orcid.org/0000-0001-7555-7610

Elias Mindreau-Ganoza − *Universidad Nacional Mayor de San Marcos, Facultad de Ciencias Biológicas, Lima 15081, Peru*

Alonso Reyes-Calderon − *Universidad de Ingenieria y Tecnologia (UTEC), Centro de Investigación y Tecnología del Agua (CITA), Lima 15063, Peru*

Sonia P. Yufra − *Universidad Nacional de San Agustin de Arequipa, Departamento de Ingeniería Metalúrgica e Ingeniería Ambiental, Arequipa 04001, Peru*

Isabel M. Solorzano-Ortiz − *Universidad de Ingenieria y Tecnologia (UTEC), Departamento de Ingeniería Ambiental, Lima 15063, Peru*

Alberto J. Donayre-Torres − *Universidad de Ingenieria y Tecnologia (UTEC), Departamento de Bioingeniería, Lima 15063, Peru*

Claudia Antonini − *Universidad de Ingenieria y Tecnologia (UTEC), Departamento de Ingeniería Industrial, Lima 15063, Peru*

Jose Miguel Renom − *Universidad de Ingenieria y Tecnologia (UTEC), Departamento de Ciencias, Lima 15063, Peru*

Antonio Marty Quispe − *Universidad de Ingenieria y Tecnologia (UTEC), Departamento de Bioingeniería, Lima 15063, Peru; Universidad Continental, Escuela de Posgrado, Huancayo 12001, Peru;* orcid.org/0000-0003-2100-7423

Cesar R. Mota − *Universidade Federal de Minas Gerais, Departamento de Engenharia Sanitária e Ambiental, Escola de Engenharia, 31270-901 Belo Horizonte, Brazil*

Carlos A. L. Chernicharo − *Universidade Federal de Minas Gerais, Departamento de Engenharia Sanitária e Ambiental, Escola de Engenharia, 31270-901 Belo Horizonte, Brazil;* orcid.org/0000-0001-7226-8266

Max A. Carbajal − *Ministerio de Vivienda Construcción y Saneamiento, Dirección de Saneamiento, Lima 15073, Peru*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsestwater.2c00065

Author Contributions

B.P.-F. executed and supervised sample collection in Lima and Arequipa, significantly contributed to the design and execution of the monitoring plan, analyzed and summarized project data into reports and publications, and prepared the manuscript. E.M.-G. executed sample collection in Lima, executed laboratory analysis, and contributed to manuscript preparation. A.R.-C. designed, supervised, and executed laboratory analysis and contributed to manuscript preparation. S.P.Y. supervised and executed sample collection in Arequipa, was in charge of sample filtration and shipping to our laboratory in Lima, and contributed to the design and execution of the monitoring plan for Arequipa. I.M.S.-O. supervised sample collection in Lima and Arequipa, was in charge of all coordination with the sewage network operators, contributed to the design of the monitoring plan, and contributed to manuscript preparation. A.J.D.-T. supervised and executed laboratory work and contributed to reports and publications. C.A. designed and executed statistical analysis, contributed to manuscript preparation, and participated in group discussions. J.M.R. designed and executed statistical analysis, contributed to manuscript preparation, and participated in group discussions. A.M.Q. contributed to the design of the monitoring plan and interpretation of the data from an epidemiological perspective and contributed to manuscript preparation. C.R.M. contributed to the design of the monitoring plan and data analysis and substantially contributed to manuscript preparation. C.A.L.C. contributed to the design of the monitoring plan and data analysis and contributed to manuscript preparation. M.A.C. was responsible for the SARS-CoV-2 RNA wastewater monitoring initiative within the Peruvian government, secured the funds needed to execute this study, and facilitated coordination with the sewage network operators. M.C.S.-M. was responsible for the grant application and for the entire execution of the project and prepared the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was funded by the Swiss Cooperation (SECO) through the Interinstitutional Agreement between the Swiss Confederation, represented by the Secretary of State for Economic Affairs (SECO) and the Peruvian Agency for International Cooperation (APCI), the Ministry of Housing, Construction and Sanitation (MVCS), the Technical Agency for the Administration of Sanitation Services (OTASS), and the National Superintendence of Sanitation Services (SUNASS), regarding the "Support Program for the Strengthening of the Water and Sanitation Sector (W&S) in Peru"; executed by the Technical Assistance Fund (SECOSAN), with Contract 12-2020-SECOSAN. The author acknowledges the outstanding assistance and constant support provided by the Water and Sanitation Service Provider Companies for Lima and Callao (SEDAPAL) and Arequipa (SEDAPAR).

REFERENCES

(1) Lu, H.; Stratton, C. W.; Tang, Y. W. Outbreak of pneumonia of unknown etiology in Wuhan, China: The mystery and the miracle. *Journal of medical virology* **2020**, *92* (4), 401.

(2) Barron, G. C.; et al. Safeguarding people living in vulnerable conditions in the COVID-19 era through universal health coverage and social protection. *Lancet Public Health* **2021**, DOI: 10.1016/S2468-2667(21)00235-8.

(3) WHO Coronavirus (COVID-19) Dashboard. World Health Organization. https://covid19.who.int/ (accessed January 2022).

(4) Adam, D. The pandemic's true death toll: millions more than official counts. *Nature* **2022**, *601* (7893), 312−315.

(5) Cáceres-Bernaola, U.; Becerra-Núñez, C.; Mendívil-Tuchía de Tai, S.; Ravelo-Hernández, J. *Primer fallecido por COVID-19 en el Perú*, 2nd ed., Vol. *81*; UNMSM, Facultad de Medicina, 2020; pp 201−204.
(6) Sala Situacional COVID-19 Peru. Ministerio de Salud, República de Perú. https://covid19.minsa.gob.pe/sala_situacional.asp (accessed January 2022).
(7) Karlinsky, A.; Kobak, D. Tracking excess mortality across countries during the COVID-19 pandemic with the World Mortality Dataset. *eLife* **2021**, *10*, No. e69336.
(8) Hernández-Vásquez, A.; Barrenechea-Pulache, A.; Azañedo, D. COVID-19 testing in Peru: low access and inequalities. *Journal of Public Health (Oxford, England)* **2021**, *43*, e504−e506.
(9) Moreira-Soto, A.; et al. High SARS-CoV-2 Seroprevalence in Rural Peru, 2021: a Cross-Sectional Population-Based Study. *mSphere* **2021**, *6* (6), No. e00685-21.
(10) Collivignarelli, M. C.; Collivignarelli, C.; Miino, M. C.; Abbà, A.; Pedrazzani, R.; Bertanza, G. SARS-CoV-2 in sewer systems and connected facilities. *Process Saf. Environ. Prot.* **2020**, *143*, 196−203.
(11) Tian, Y.; Rong, L.; Nian, W.; He, Y. Gastrointestinal features in COVID-19 and the possibility of faecal transmission. *Alimentary pharmacology & therapeutics* **2020**, *51* (9), 843−851.
(12) Wu, Y.; et al. Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. *lancet Gastroenterology & hepatology* **2020**, *5* (5), 434−435.
(13) Wang, W.; et al. Detection of SARS-CoV-2 in different types of clinical specimens. *JAMA, J. Am. Med. Assoc.* **2020**, *323* (18), 1843−1844.
(14) Xiao, F.; et al. Infectious SARS-CoV-2 in feces of patient with severe COVID-19. *Emerging infectious diseases* **2020**, *26* (8), 1920.
(15) de Oliveira, L. C.; et al. Viability of SARS-CoV-2 in river water and wastewater at different temperatures and solids content. *Water research* **2021**, *195*, 117002.
(16) Heller, L.; Mota, C. R.; Greco, D. B. COVID-19 faecal-oral transmission: Are we asking the right questions? *Sci. Total Environ.* **2020**, *729*, 138919.
(17) Giacobbo, A.; Rodrigues, M. A. S.; Zoppas Ferreira, J.; Bernardes, A. M.; de Pinho, M. N. A critical review on SARS-CoV-2 infectivity in water and wastewater. What do we know? *Sci. Total Environ.* **2021**, *774*, 145721.
(18) Mohapatra, S.; et al. The novel SARS-CoV-2 pandemic: Possible environmental transmission, detection, persistence and fate during wastewater and water treatment. *Science of The Total Environment* **2021**, *765*, 142746.
(19) Ahmed, W.; et al. Decay of SARS-CoV-2 and surrogate murine hepatitis virus RNA in untreated wastewater to inform application in wastewater-based epidemiology. *Environmental Research* **2020**, *191*, 110092.
(20) Gonzalez, R.; et al. COVID-19 surveillance in Southeastern Virginia using wastewater-based epidemiology. *Water research* **2020**, *186*, 116296.
(21) Sherchan, S. P.; et al. First detection of SARS-CoV-2 RNA in wastewater in North America: a study in Louisiana, USA. *Science of The Total Environment* **2020**, *743*, 140621.
(22) Mota, C. R.; et al. Assessing spatial distribution of COVID-19 prevalence in Brazil using decentralised sewage monitoring. *Water Res.* **2021**, *202*, 117388.
(23) Medema, G.; Heijnen, L.; Elsinga, G.; Italiaander, R.; Brouwer, A. Presence of SARS-Coronavirus-2 RNA in sewage and correlation with reported COVID-19 prevalence in the early stage of the epidemic in the Netherlands. *Environ. Sci. Technol. Lett.* **2020**, *7* (7), 511−516.
(24) Nemudryi, A.; et al. Temporal detection and phylogenetic assessment of SARS-CoV-2 in municipal wastewater. *Cell Reports Medicine* **2020**, *1* (6), 100098.
(25) Peccia, J.; et al. Measurement of SARS-CoV-2 RNA in wastewater tracks community infection dynamics. *Nat. Biotechnol.* **2020**, *38* (10), 1164−1167.
(26) Ahmed, W.; et al. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: A proof of concept for the wastewater surveillance of COVID-19 in the community. *Science of The Total Environment* **2020**, *728*, 138764.
(27) La Rosa, G.; et al. First detection of SARS-CoV-2 in untreated wastewaters in Italy. *Science of The Total Environment* **2020**, *736*, 139652.
(28) Barril, P. A.; et al. Evaluation of viral concentration methods for SARS-CoV-2 recovery from wastewaters. *Science of The Total Environment* **2021**, *756*, 144105.
(29) Hillary, L. S.; et al. Monitoring SARS-CoV-2 in municipal wastewater to evaluate the success of lockdown measures for controlling COVID-19 in the UK. *Water Res.* **2021**, *200*, 117214.
(30) Randazzo, W.; Truchado, P.; Cuevas-Ferrando, E.; Simón, P.; Allende, A.; Sánchez, G. SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area. *Water Res.* **2020**, *181*, 115942.
(31) Shah, S.; Gwee, S. X. W.; Ng, J. Q. X.; Lau, N.; Koh, J.; Pang, J. Wastewater surveillance to infer COVID-19 transmission: A systematic review. *Science of The Total Environment* **2022**, *804*, 150060.
(32) La Rosa, G.; et al. SARS-CoV-2 has been circulating in northern Italy since December 2019: Evidence from environmental monitoring. *Science of the total environment* **2021**, *750*, 141711.
(33) Farkas, K.; Hillary, L. S.; Malham, S. K.; McDonald, J. E.; Jones, D. L. Wastewater and public health: the potential of wastewater surveillance for monitoring COVID-19. *Curr. Opin. Environ. Sci. Health* **2020**, *17*, 14−20.
(34) Mallapaty, S. How sewage could reveal true scale of coronavirus outbreak. *Nature* **2020**, *580* (7802), 176−177.
(35) Colosi, L. M.; et al. Development of wastewater pooled surveillance of SARS-CoV-2 from congregate living settings. *Appl. Environ. Microbiol.* **2021**, *87*, e0043321.
(36) Haak, L.; et al. Spatial and temporal variability and data bias in wastewater surveillance of SARS-CoV-2 in a sewer system. *Science of The Total Environment* **2022**, *805*, 150390.
(37) Gutierrez-Espino, C.; et al. *Peru: Estado de la población en el año del bicentenario, 2021*; Instituto Nacional de Estadística e Informática (INEI): Lima, 2021.
(38) Katayama, H.; Shimasaki, A.; Ohgaki, S. Development of a virus concentration method and its application to detection of enterovirus and Norwalk virus from coastal seawater. *Appl. Environ. Microbiol.* **2002**, *68* (3), 1033−1039.
(39) Boxus, M.; Letellier, C.; Kerkhofs, P. Real Time RT-PCR for the detection and quantitation of bovine respiratory syncytial virus. *Journal of virological methods* **2005**, *125* (2), 125−130.
(40) Ahmed, W.; et al. Comparison of virus concentration methods for the RT-qPCR-based recovery of murine hepatitis virus, a surrogate for SARS-CoV-2 from untreated wastewater. *Science of The Total Environment* **2020**, *739*, 139960.
(41) Kantor, R. S.; Nelson, K. L.; Greenwald, H. D.; Kennedy, L. C. Challenges in measuring the recovery of SARS-CoV-2 from wastewater. *Environ. Sci. Technol.* **2021**, *55* (6), 3514−3519.
(42) 2019-novel coronavirus (2019-nCoV) real-time rRT-PCR panel primers and probes. U.S. Centers for Disease Control and Prevention, 2020; Vol. *24*.
(43) Verbyla, M. E.; et al. Managing microbial risks from indirect wastewater reuse for irrigation in urbanizing watersheds. *Environ. Sci. Technol.* **2016**, *50* (13), 6803−6813.
(44) Haugland, R. A.; Siefring, S. C.; Wymer, L. J.; Brenner, K. P.; Dufour, A. P. Comparison of Enterococcus measurements in freshwater at two recreational beaches by quantitative polymerase chain reaction and membrane filter culture analysis. *Water research* **2005**, *39* (4), 559−568.
(45) Ahmed, W.; et al. Surveillance of SARS-CoV-2 RNA in wastewater: Methods optimization and quality control are crucial for generating reliable public health information. *Current opinion in environmental science & health* **2020**, *17*, 82−93.
(46) Johnson, N. J. Modified t tests and confidence intervals for asymmetrical populations. *Journal of the American Statistical Association* **1978**, *73* (363), 536−544.

https://doi.org/10.1021/acsestwater.2c00065
ACS EST Water 2022, 2, 2144−2157

(47) Lucas, J. M.; Saccucci, M. S. Exponentially weighted moving average control schemes: properties and enhancements. *Technometrics* **1990**, *32* (1), 1−12.
(48) Marusteri, M.; Bacarea, V. Comparing groups for statistical differences: how to choose the right statistical test? *Biochemia medica* **2010**, *20* (1), 15−32.
(49) Allaire, J. RStudio: integrated development environment for R. 2012.
(50) Munayco, C. V.; et al. Early transmission dynamics of COVID-19 in a southern hemisphere setting: Lima-Peru: February 29th−March 30th, 2020. *Infectious Disease Modelling* **2020**, *5*, 338−345.
(51) Vargas-Herrera, N.; Araujo-Castillo, R. V.; Mestanza, O.; Galarza, M.; Rojas-Serrano, N.; Solari-Zerpa, L. SARS-CoV-2 Lambda and Gamma variants competition in Peru, a country with high seroprevalence. *Lancet Regional Health−Americas* **2021**, DOI: 10.1016/j.lana.2021.100112.
(52) Adhikari, S.; Pantaleo, N. P.; Feldman, J. M.; Ogedegbe, O.; Thorpe, L.; Troxel, A. B. Assessment of community-level disparities in coronavirus disease 2019 (COVID-19) infections and deaths in large US metropolitan areas. *JAMA network open* **2020**, *3* (7), No. e2016938.
(53) *MINSA confirma tercera ola ante incremento de casos de contagio por la COVID-19*; Plataforma digital única del estado Peruano, Ministerio de Salud, 2021.
(54) Guerrero-Latorre, L.; Ballesteros, I.; Villacrés-Granda, I.; Granda, M. G.; Freire-Paspuel, B.; Ríos-Touma, B. SARS-CoV-2 in river water: Implications in low sanitation countries. *Science of the Total environment* **2020**, *743*, 140832.
(55) Sims, N.; Kasprzyk-Hordern, B. Future perspectives of wastewater-based epidemiology: monitoring infectious disease spread and resistance to the community level. *Environ. Int.* **2020**, *139*, 105689.
(56) Weidhaas, J.; et al. Correlation of SARS-CoV-2 RNA in wastewater with COVID-19 disease burden in sewersheds. *Science of The Total Environment* **2021**, *775*, 145790.
(57) Suo, T.; et al. ddPCR: a more accurate tool for SARS-CoV-2 detection in low viral load specimens. *Emerging microbes & infections* **2020**, *9* (1), 1259−1268.

https://doi.org/10.1021/acsestwater.2c00065
ACS EST Water 2022, 2, 2144−2157