SARS-CoV-2 detection in wastewater as an early warning indicator for COVID-19 pandemic. Madrid region case study

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Highlights

- Sewage surveillance ahead of health indicators between 3 to 11 days.
- 289 sampling points locations are weekly tested at fixed hours.
- SARS-CoV-2 trends and COD monitoring for outlier detection and resampling.

Introduction

During the first quarter of 2020, due to the evolution of the COVID-19 pandemic, several studies on the validation process for monitoring and detecting traces of SARS-CoV-2 in wastewater started in multiple countries (Ahmed et al., 2020; La Rosa et al., 2020; Medema et al., 2020). Canal de Isabel II, as the company responsible for water infrastructure management in Madrid region, started to monitor the spread of SARS-CoV-2 in wastewater in March 2020, when there were around 10,000 daily confirmed cases and a total of 850 deaths had already occurred in Spain. Finally, a graphical data management tool (named VIGÍA project, [Canal de Isabel II,2020]) was developed for early detection of SARS-CoV-2 spread from a sewage-based epidemiological approach. VIGÍA project is based on weekly sampling, data validation, statistical analysis and representation. It covers the entire region of Madrid through 289 sampling points located in the sewerage network and wastewater treatment plants (WWTP) so that almost seven million inhabitants are monitored weekly. The results are shared daily with the Health Department of Madrid for consultancy and decision-making purposes (Candel et al., 2021).

Methodology

This study has been conducted over the entire urban drainage network of Madrid region, which is mainly combined and provide service for 179 municipalities. There are 15.000 km of sewer pipes and 157 WWTP.
To minimize the variability that results show during the day, grab samples are taken at a fixed time for every point to better capture the evolution from week to week. Every single sample must be kept refrigerated and sent to a laboratory as soon as the collection process is over because temperature plays an important role in virus degradation over time (Gundy et al., 2009). Analytical results are received in the next two days at latest. Industrial discharges and rainfall events could potentially affect virus detection, so additional physicochemical parameters are analysed to assess unusual concentrations and resample points when required.

Sewershed definition and selection criteria for sampling locations

A balance must be sought between the number of sampling points, laboratory and fieldwork capacity while ensuring sample representativity. These criteria along with conclusions drawn from a pilot test carried out in May 2020 define that, whenever it is possible, a maximum of 25.000 equivalent inhabitants, a 3,5 km distance to population centres and a 2,5 km distance to the last discharging point are preferred to obtain optimal results. The determination of these points to cover the entire population was fundamental, taking into account that the Community of Madrid has a high population density in the centre (Madrid capital). The total monitoring pointed out a total of 289 sampling points, following all the above criteria, and giving priority to the WWTPs when possible for ease of sampling.

Methodology for analysis of results

Percent change of SARS-CoV-2 concentration is computed to detect extreme trends (Medema et al., 2020), along with physicochemical parameters that are compared to historical data (temperature, COD, chloride levels and electrical conductivity) to check wastewater composition and detect the effect of runoff or industrial discharge. When a new input has an extreme value, resampling is required at that point, which will be collected in the following 2 to 3 working days. This counter analysis could either validate or rule out the first sample. During this period, SARS-CoV-2 concentration is provisionally extrapolated using previous results to give a global overview for the whole region and missing data is filled in by using a moving average value taken over the last two weeks.

Results and discussion

Madrid sewerage system is mainly combined (mixed sewage, stormwater, and industrial effluents). It is still unclear how detectable pieces of SARS-CoV-2 virus from infected people interact with above effluents. However, to detect unusual dilution that could potentially affect virus detectability, physicochemical parameters are also monitored for outlier detection. COD appears to be most relevant in detecting unusual wastewater composition, given that it is more sensitive to both scenarios (high dilutions due to rainfall episodes and higher than usual pollutant presence from industrial effluents). Chloride levels and electrical conductivity are also monitored as additional criteria to detect unusual composition although they are more stable. Samples with out-of-range values are resampled to verify or rule out qPCR results within 2 to 3 days. An example of discarded samples can be seen in Figure 1. Not every extreme trend detected in virus concentration is related to unusual COD results but is a good indicator of wastewater composition.

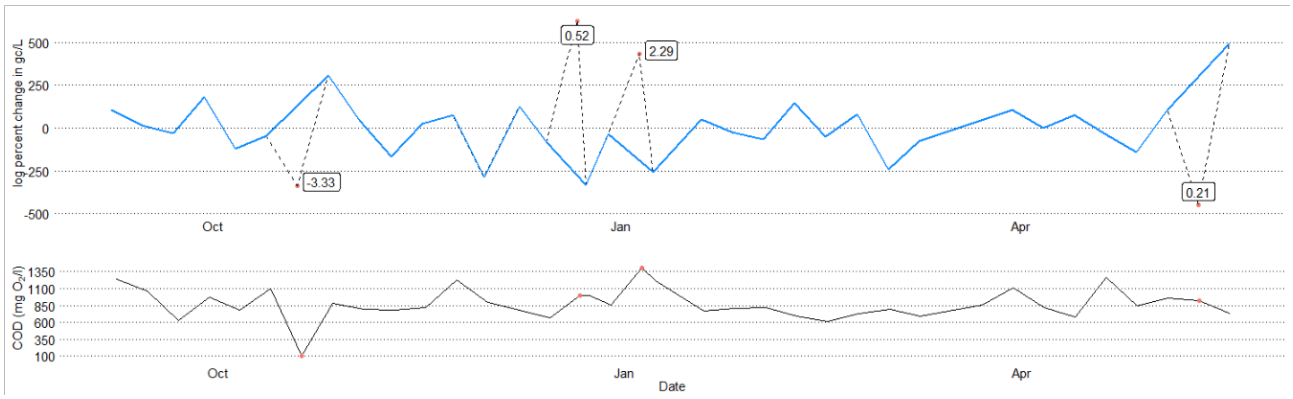

Figure 1. Outlier detection based on COD. Log percent change of SARS-CoV-2 concentration in one sampling point (blue). Labels represent z-scores of COD for resampled and ruled out tests.

Highly diluted samples are often related to inconsistent SARS-CoV-2 concentrations; however, this does not mean concentrations are necessarily low. In fact, some results showed high SARS-CoV-2 concentrations along with low COD in samples taken on rainy days; this could be due to first flush or resuspension phenomena.
Since a subset of points was sampled each day of the week, the results were extended to a daily frequency with two considerations:

(i) The signal was estimated constant from one sample to the next,
(ii) When the interval between two samples exceeds the 7-day period (due to re-sampling or any fieldwork difficulties), the previous results were extrapolated to fill in the missing information by applying the slope of the moving average series of the two previous results.

In order to assess wastewater capabilities as an early warning indicator, aggregation of SARS-CoV-2 concentrations is also compared to new reported cases and new COVID-19 hospitalisations. These daily series present strong weekly seasonality, so a 7-day moving average is preferred as a more robust indicator.

Following Figure 2 shows similarities between series, with wastewater SARS-CoV-2 concentration in wastewater ahead of both series.
The commonly accepted explanation for this lag is that SARS-CoV-2 can be found in faeces of patients before symptom onset. Anticipation of wastewater tests may differ depending on locations, sewershed size and

population, sampling strategies or temperature among many other factors, as wastewater are, in general, a very heterogeneous environment. Results range between 3 to 11 days of anticipation among the studied sewersheds (Larsen & Wigginton, 2020).

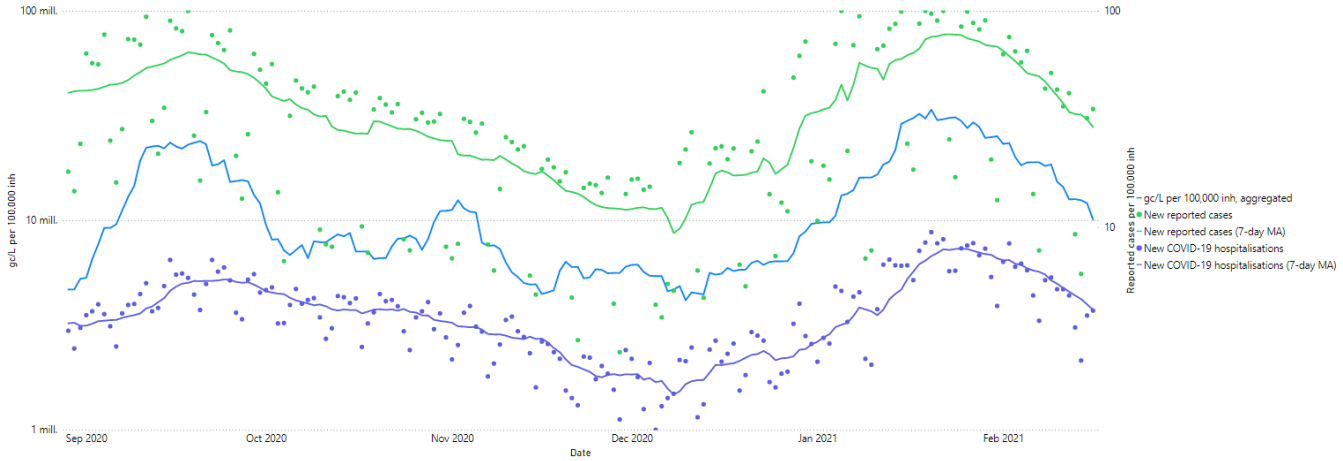

Figure 2. Second and third waves in Madrid - SARS-CoV-2 concentration in wastewater (blue) and public health indicators.

Conclusions and future work

This study has demonstrated Wastewater-Based Epidemiology capabilities as an early warning tool for the current COVID-19 pandemic in Madrid region. Sampling points selection criteria were essential to this aim.

It has been found that a weekly sampling strategy offers adequate quantification with fixed sampling hours for every point to reduce the effect of daily variations. However, laboratory results must be validated with physicochemical parameters to detect unusual compositions. SARS-CoV-2 presence and evolution in wastewater show a strong connection with both reported cases and COVID-19 hospitalisations.

Future work will include a permanent epidemiological surveillance system in which a subset of 87 out of the current 289 sampling points will be monitored and in the event of virus detection, more detailed surveillance points will be activated in that sewershed to pinpoint infection hotspots.

A study SARS-CoV-2 decay in raw wastewater is also under development, based on a 1D sewer network model for water quality, where a set of new theoretical pollutants are declared to model its evolution along with the sewer network.

There is also an ongoing pilot test to assess relationships between qPCR results for grab and composite samples with automatic refrigerated samplers. The goal is to determine if composite samples can offer a more detailed analysis of virus loads where power and security requirements are met for automatic sampler installation, mainly in WWTP.

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