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

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ABSTRACT

COVID-19 pandemic is ongoing for more than a year and has changed priorities and boosted some WBE studies. The aim of this work is to contributed to our knowledge sharing the methodology developed for SARS-CoV-2 detection in wastewater of Madrid region of over six million and a half inhabitants, where the sewer system is a combined system.

At first, a pilot test in a small metropolitan area was carried out in order to define the criteria for the selection of the sampling points to be applied to the entire region. Methodologys for laboratory analysis and statistical analysis and interpretation of data are also presented. This work relies highly on fieldwork, so sewer network safe accessibility is paramount. A total of 289 sampling points were weekly characterised. Each sampling point represents a sewershed, some of them in a cascade distribution. Samples are tested for SARS-CoV-2 concentration (gc/L, genome copies per litre) and physicochemical parameters are also analysed to validate or discard what at first could be an unusual virus presence. Field results are correlated with health indicators such as incidence rates and hospitalisation data.

This information is daily shared with regional health authorities, disaggregated by municipalities, or aggregated for the entire Madrid region. Results have proved to anticipate health indicators. The tool is used as an early warning indicator for COVID-19 pandemic. Further work is planned to apply the current scheme for a permanent epidemiological surveillance system of 87 sampling points to pinpoint infection hotspots and activate the linked sewersheds in the event of an outbreak.

1. 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 were started in multiple countries (Medema et al., 2020; Sherchan et al., 1406; Ahmed et al., 2020a; La Rosa 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 to serve as decision-making tool for the region council.

Preliminary studies reported the detection of SARS-CoV-2 RNA in wastewater in the Netherlands (Medema et al., 2020), USA (Sherchan et al., 1406), Australia (Ahmed et al., 2020a), and Italy (La Rosa et al., 2020). One of the first studies based on surveillance of COVID-19 in wastewater was performed in Australia, and SARS-CoV-2 RNA was detected in two samples within six days of the same WWTP with both qPCR and sequencing (Ahmed et al., 2020a). In the Netherlands, researchers tested sewage of six cities and the airport for SARS-CoV-2 RNA, targeting either the nucleocapsid (N) gene or the envelope (E) (Medema et al., 2020). The results showed that the sewage samples were tested positive for the N gene in March 2020. In Italy, a research group studied twelve influent sewage samples from the WWTPs in Milan and Rome between February and April and reported that 6 out of 12 samples were positive (La Rosa et al., 2020).

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In this study, the goal was to analyse the presence and evolution of SARS-CoV-2 in the sewage sanitation system in Madrid region based on the qPCR method, as a potential tool for early warning detection of SARS-CoV-2 spreading from wastewater-based epidemiology approach. This study has started at the end of March when daily confirmed cases were around 10,000 and 850 deaths in Spain (Worldometer.info, 2020), and the decline of the first wave of the pandemic that affected Spain and in particular Madrid region.

The project started with the definition of each of the basins of Madrid region and made an exhaustive selection of sampling points of the network that would gather relevant data for the investigation, in order to represent the virus load per-capita. To this aim, magnitude of several variables was considered such as distance to the city centre or number of inhabitants. Each sampling point collects wastewater from different sewersheds, this means that six million and a half inhabitants are monitored on a weekly basis. In addition, some of those points are linked to a cascade distribution. At present, this study is carried out across the entire region of Madrid by means of 289 sampling points located throughout the sewer network and wastewater treatment plants.

The above research is based on weekly grab samples from these sampling points, and subsequently on laboratory analysis, data cleaning and validation, statistical analysis, visualization in a platform, and interpretation of results. Results are represented as graphical data (Canal de Isabel II, 2020) by this powerful tool, which acts as an early warning detection system of SARS-CoV-2.

Those results have already been useful to anticipate the second and third waves in Madrid, showing similarities between SARS-CoV-2 concentrations in wastewater ahead of hospitalisations for lockdown (March 15, 2020 to June 21, 2020) and the second (August 27, 2020 to October 22, 2020) and the third (December 07, 2020 to February 16, 2021) waves.

This study, developed by Canal de Isabel II, detects the spread of SASRS-CoV-2 through a wastewater-based epidemiology approach. This data is shared daily with the Health department of Madrid for consultancy and decision-making purposes.

2. Materials and methods

This study has been conducted over the entire urban drainage network of Madrid region, which is mainly combined and provides services to 179 municipalities. The network is made up of 15,000 km of sewerage pipes and 157 wastewater treatment plants, all of which are responsible for cleaning sewage from more than six million and a half inhabitants (Lastra et al., 2019). At present, this study is carried out across the entire Madrid region by means of 289 sampling points located throughout the sewer network and wastewater treatment plants, and they are weekly characterised in terms of SARS-CoV-2 concentration. This is because it has been found that a weekly sampling strategy offers adequate quantification (La Rosa et al., 2020), with fixed sampling hours for every point to reduce the effect of daily variations.

Routes for sample collection are daily planned from upstream to downstream sewershed and to minimise the variability of results during the day, grab samples are taken at a fixed time in every point in order to ensure proper assessment of evolution from week to week (Peccia et al., 2020; The Water Research Founda, 2020). Every single sample must be kept refrigerated and sent to the laboratory as soon as the collection process is over; temperature plays an important role in terms of viral degradation over time (Gundy et al., 2009).

Industrial discharges and rainfall events could potentially affect virus detection, so that additional physicochemical parameters (Ahmed et al., 2020b) are analysed to assess unusual concentrations. In such cases, resampling is required.

2.1. Sewershed definition

Pilot test, based on quantitative PCRs for wastewater in a municipality of 100,000 inhabitants proved that the virus load could be interrelated for each discharging population. A set of tests in 10 different points was launched to assess sampling point location in terms of distance from population centres and hydraulic retention times. This point selection was made so that lower points would include virus loads upstream and to study the aggregation of flow and degradation with time and dilution.

Results allowed researchers to establish sewershed definition criteria to escalate the monitoring system to the whole sanitation network of Madrid. To achieve proper SARS-CoV-2 representativity and where possible, following criteria should be applied in sampling points selection: a maximum of 25,000 equivalent inhabitants, a distance of no more than 3.5 km distance from population centre and no further than 2.5 km distance from the last discharging point (Fig. 1).

Taking into account the particularities of Madrid region (high population density in the centre of the region and many scattered areas with low density around it), and in order to meet the established criteria, a total of 289 sampling points were used to capture data from the entire population (Fig. 2). Of the 289 sampling points, 116 were selected giving priority to Wastewater Treatment Plants when possible, for ease of sampling.

The project significantly relies on the logistical chain for sample collection and transportation, for this reason, priority is given to overground sampling. The functioning is fluent and does not involve workers coming into direct contact with raw sewage. To meet that, 60 % of initial proposed sampling points had to be relocated to guarantee safe access.

2.2. Analysis of results

Samples are not only tested for SARS-CoV-2 presence. Physicochemical parameters such as temperature, COD, chloride levels, and electrical conductivity are also analysed to check the wastewater composition and detect the effect of runoff or industrial discharge.

Canal de Isabel II analyses samples at two laboratories, following the criteria that those samples taken from the same point must be analysed in the same laboratory. Results are available within two days of collection and following this, they are homogenised due to different methodologies are employed to detect SARS-CoV-2 presence in each laboratory. Canal de Isabel II is currently implementing required resources to perform qPCR (Kitajima et al., 2020) in its own wastewater laboratory instead of outsourcing it.

Percent change of SARS-CoV-2 concentration is computed to detect extreme trends, along with physicochemical parameters that are compared with respect to historical data, which must be within the expected limits of urban wastewater. When a new input has an extreme value (Ahmed et al., 2020a; Hart and Halden, 2020), resampling is required at that point, which will be collected in the following 2 to 3 working days (Candel et al., 2021). This counter analysis could either validate or rule out the first sample. During this period, SARS-CoV-2 concentration is provisionally extrapolated (Kumar et al., 2020) 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.

In order to show weekly SARS-CoV-2 presence in wastewater in Madrid region, all sample points are aggregated in one curve, evaluated according to statistical analysis. Results are normalised by 100,000 inhabitants in order to be aligned with the health department and local statistics.

Currently, Canal de Isabel II is also providing data on a daily basis showing SARS-CoV-2 trends, and this is developed thanks to the use of historical counter samples.

3. Results and discussion

Since July 2020, Canal de Isabel II has been testing wastewater to assess SARS-CoV-2 presence on a regular basis. Results show a strong

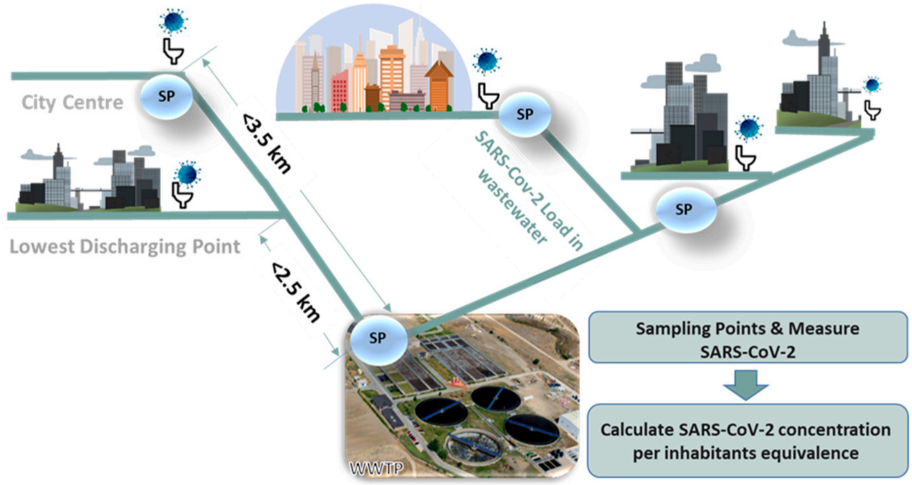

Fig. 1. Criteria for sampling point selection.

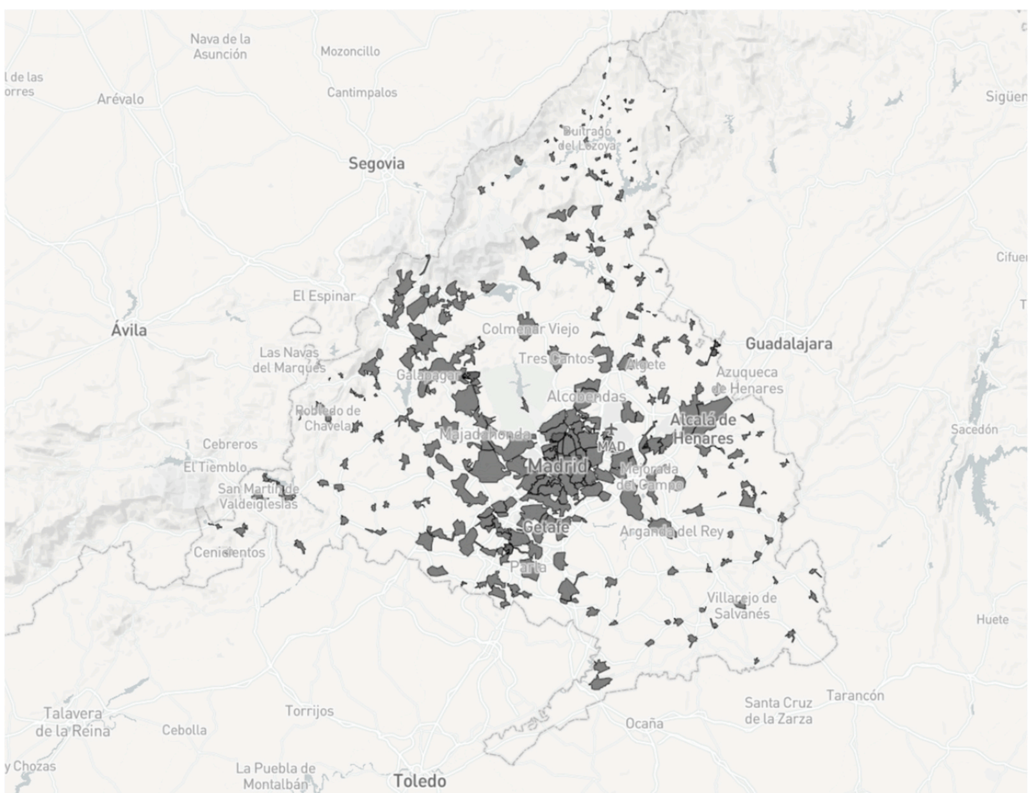

Fig. 2. Overview map of sewersheds.

correlation between SARS-CoV-2 presence in wastewater and different epidemiological indicators. In this section, a comparative analysis with hospitalisation and reported cases is discussed.

3.1. Daily data processing

The Madrid sewerage system is mainly combined, which means that along with domestic waste, sewers collect runoff and industrial waste. It is still unclear how detectable pieces of SARS-CoV-2 virus from infected people interact with above effluents. However, in order to anticipate 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 in industrial effluents). Chloride levels and electrical conductivity are also monitored as additional criteria to detect unusual composition although they are more stable (Fig. 3 and Fig. 4).

Samples with out-of range values are resampled to verify or rule out PCR results within 2–3 days. An example of ruled out samples can be seen in Fig. 5, with both low and high COD values associated with outlier values of gc/L of SARS-CoV-2.

This previous filter for data validation and resampling has not led to a significant increase in fieldwork. Rainfall events have been the main cause of dispersion, as shown in Fig. 6, where monthly discarded samples represented more than 10 % between September and November,

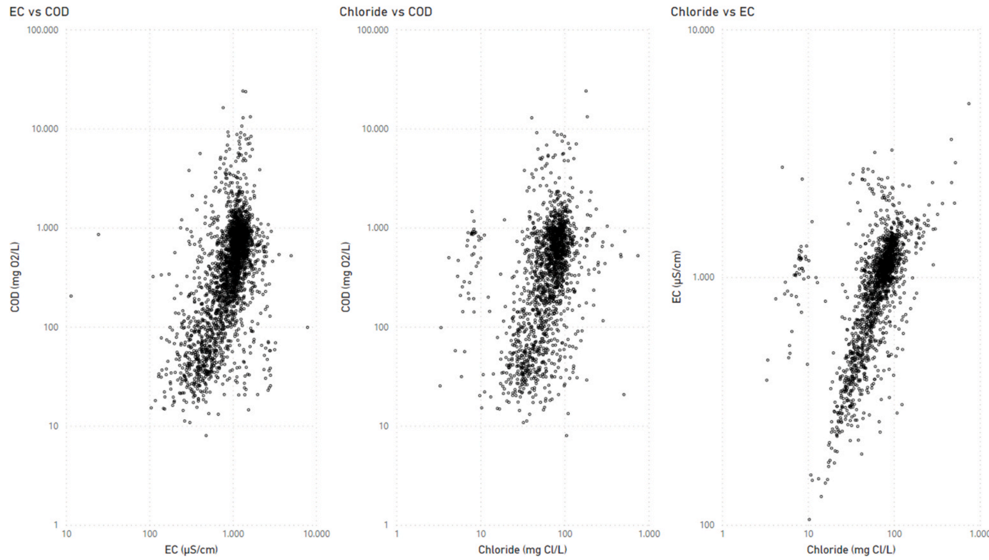

Fig. 3. Dispersion analysis of physicochemical parameters (EC, COD, Chloride).

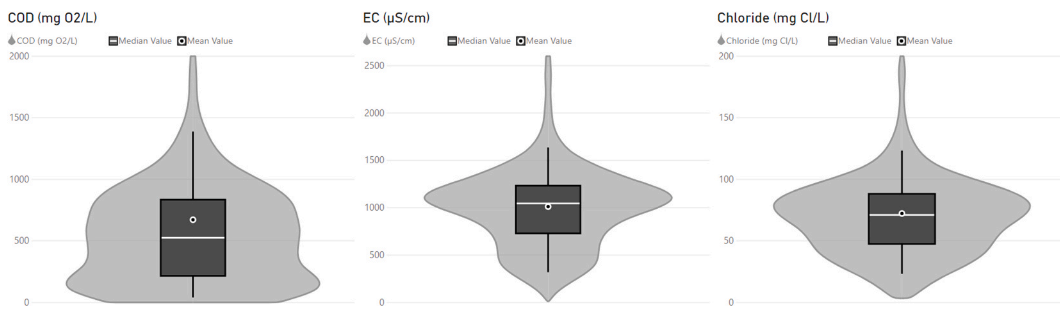

Fig. 4. Observed distribution of physicochemical parameters (EC, COD, Chloride).

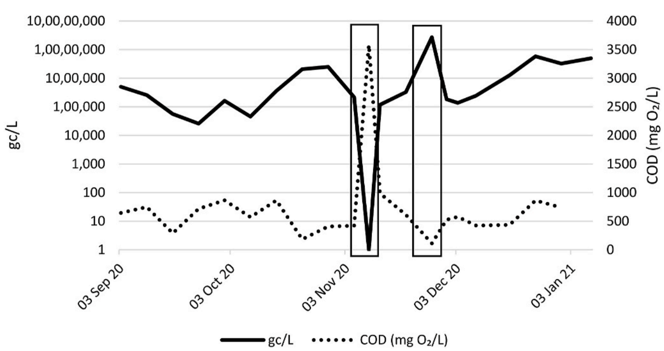

Fig. 5. Outlier detection based on COD.

and less than 5 % in August 2020 and March 2021.

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. Representative COD values are meaningful as long as they are compared to the same sampling point (SP), having each manhole different representativeness.

All historical data are represented in a homogeneous manner, where x-axis (COD concentration) and y-axis (virus concentration) represent normalised values (grey colour). There are some samples that had been ruled out from each available SP (red colour). Data has been rescaled to a standardised variable [Equation 1] to represent each sampled manhole:

As it can be observed on Fig. 7 where there are strong fluctuations along the vertical axis, with shows that raw concentration's comparison between different manholes could not lead to any conclusion.

However, when the percentage change is compared between two consecutive samples, significant distribution of abnormal concentrations are observed as extreme values on the vertical axis for both, high and low values, showing strong point clouds are observed on both extremes of the vertical axis (Fig. 8). However, there is not a clear barrier differentiation between low and high gc/L (genome copies per litre) virus presence in the X-axis. Moreover, it is import at to have in mind that percentage of change in RNA concentration is represented in log of a quotient (Equation 2), being the reason that no presence or concentration 0 are not included in the comparison.

According to historical data, it is observed that at the level of study, there is not temperature variation according to the virus presence or absence. Indeed, Fig. 8 proves that on virus absence samples have lower temperature than when positive cases occur. Furthermore, the peak of negative cases in the histogram curve is retarded in general. This could have been because the towns where there have been more negative samples are in the mountains (small towns), where the temperature is lower, which leads us to assume that there is no obvious effect of temperature on detectability of our results. This situation is also consistent during seasonal analysis (Fig. 9).

All discarding methods mentioned above are supplementary steps to the methodology, the foremost one related to COD representativeness. This means that the decision of ruling out a sample depends also on field observations, being a lot more important than COD values. It had

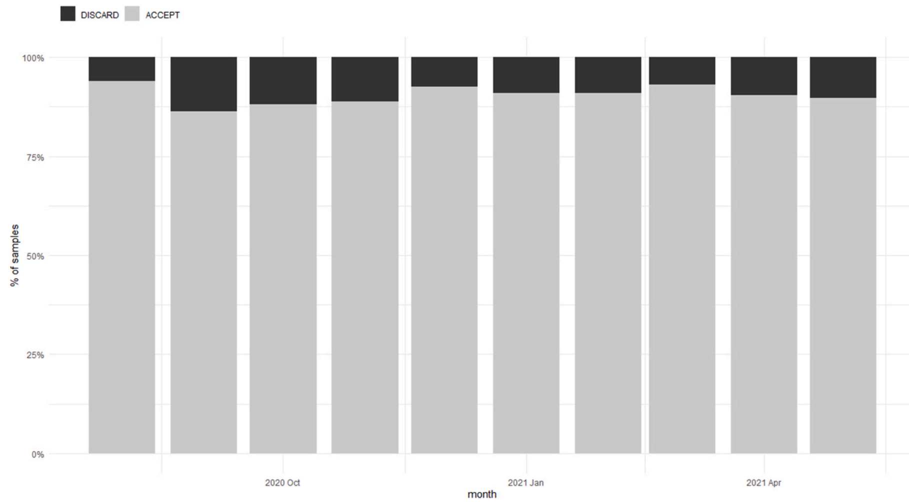

Fig. 6. Monthly discarded samples.

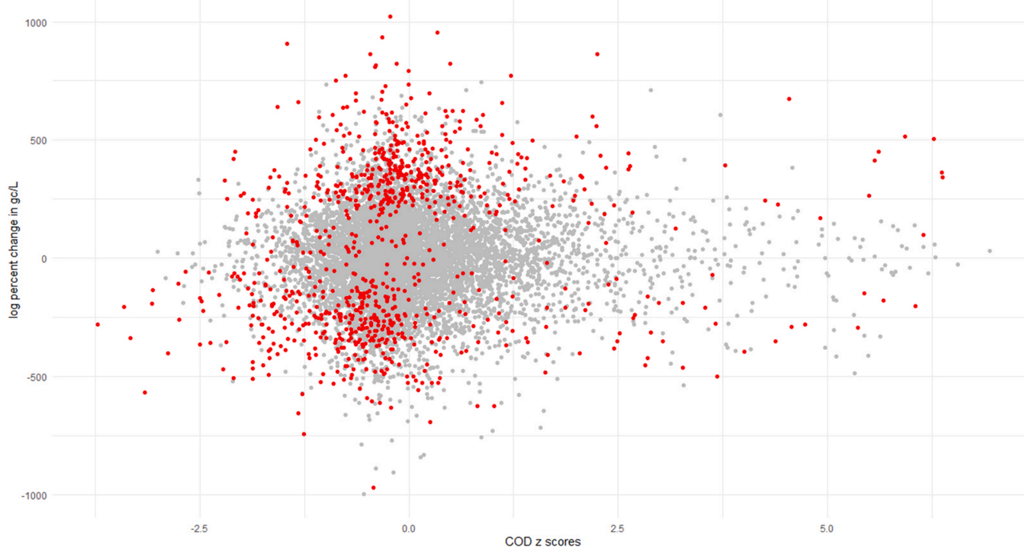

Fig. 7. Unusual discarded samples in terms of percentage of change according to COD values.

already happened those unusual values were related to uncontrolled discharges, unusual sewage colour, or abnormal low water flow related to maintenance operations upstream.

3.2. *Aggregation of timeseries*

Since a subset of points are sampled every weekday, the results are extended to a daily granularity with two considerations:

- Signal is estimated constantly from one sample to the next one.
- When the span between two samples exceeds the 7-day period (due to resampling or any fieldwork difficulties), results are extrapolated to fill the missing information, applying the slope of the moving average series for the two previous results.

Series for different points are then aggregated and normalised based on population data and it is resulting an aggregated global signal is compared to daily hospitalisations and incidence rates.

3.3. *Incidence rates*

An important source of information regarding the evolution of the pandemic are the reported incidence rates. From all available indicators, a significant relation between SARS-CoV-2 concentration in wastewater and 14-day incidence rates with active infection has been found, and reported cases are registered as cases with active infection in either of the following scenarios (Ministerio de SanidadInstituto de Salud Carlos, 2020):

- People with symptoms compatible with COVID-19 and positive Active Infection Diagnostic Test
- Asymptomatic people with positive Active Infection Diagnostic Test and negative IgG or without antibody test.

The information provided by reported active cases offers a better picture in terms of assessing the spread of the infection, although it is more sensitive to testing capacity. A strong correspondence between the 14-day incident rate and SARS-CoV-2 concentration (gc/L per 100.000 inh.) on anticipate infection rates was found.

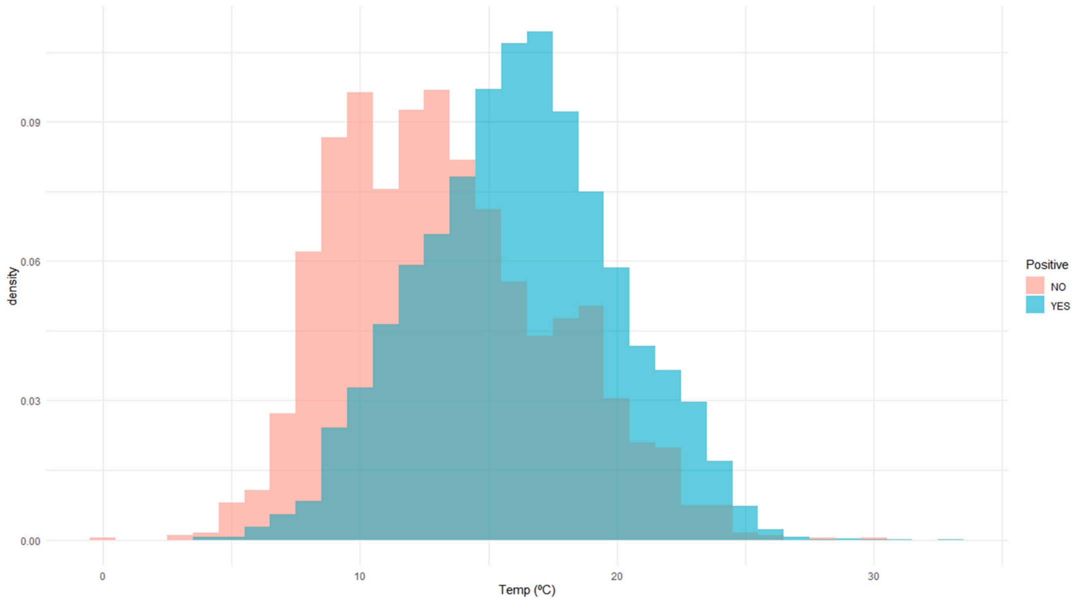

Fig. 8. Histogram of historical temperature data disaggregated according to positive and negative cases.

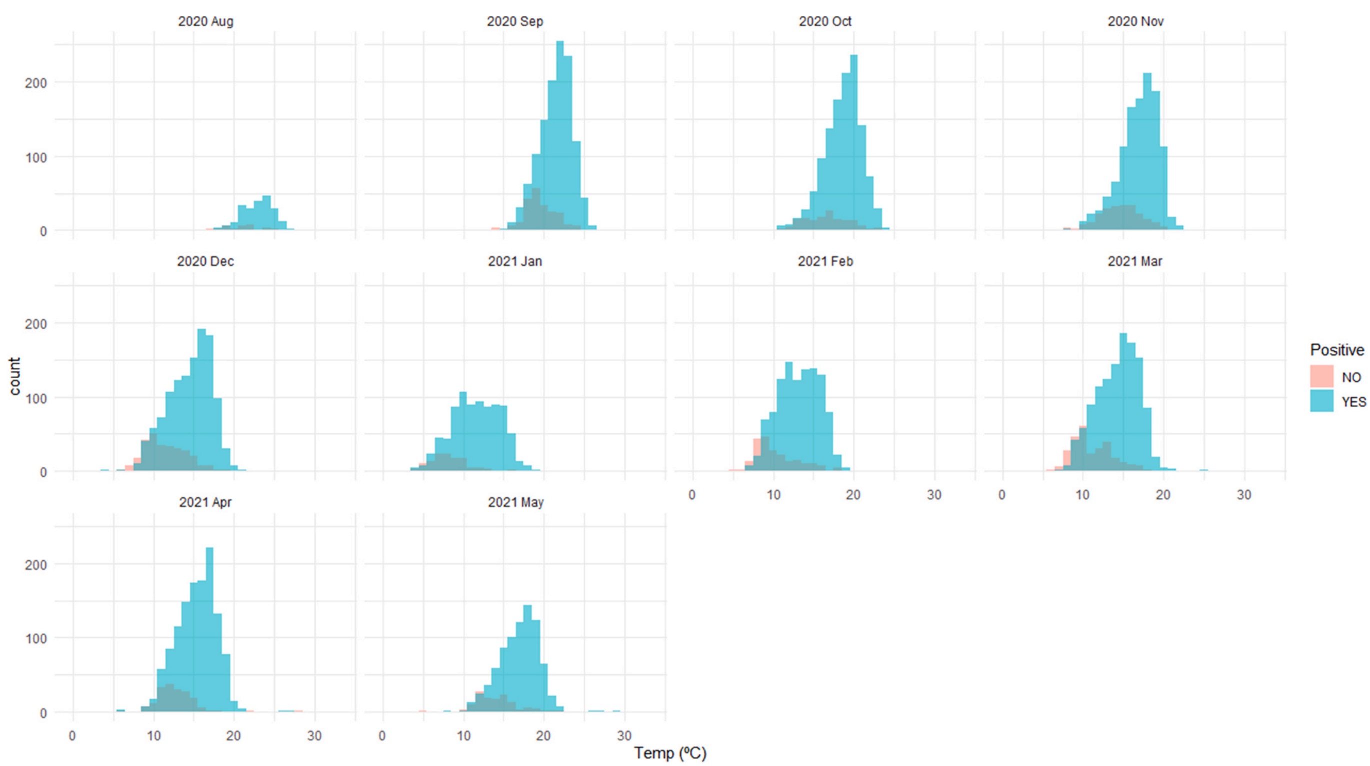

Fig. 9. Histogram of historical temperature data disaggregated according to positive and negative cases and grouped by month (August 2020–May 2021).

3.4. Hospitalisation data – correlation and anticipation

In order to assess wastewater capability as an early warning indicator, aggregation of SARS-CoV-2 concentrations is also compared to reported new COVID-19 hospitalisations. Hospitalisation daily series present strong weekly seasonality, so a 7-day moving average is preferred as a more robust indicator. Following, Fig. 10 shows similarities between both series, with wastewater SARS-CoV-2 concentration ahead of hospitalisation.

The commonly accepted explanation for this lag between wastewater presence and hospitalisations is that SARS-CoV-2 can be found in faeces of patients before symptom onset. Anticipation of wastewater tests may differ depending on location, sewershed size and population, sampling strategies or temperature, among many other factors, as wastewater is in a general a very heterogeneous environment. Results range between 3 and 11 days of anticipation among the studied sewersheds (Larsen and Wigginton, 2020).

4. Conclusions and future work

This study has demonstrated Wastewater-Based Epidemiology capabilities as an early warning tool for the current COVID-19 pandemic in

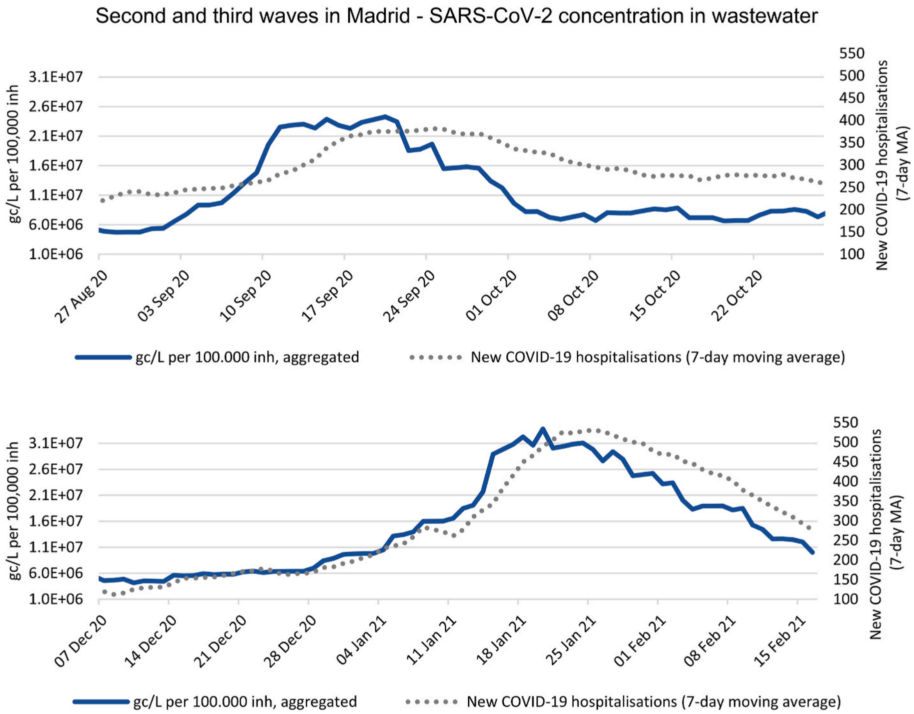

Fig. 10. Second and third waves in Madrid - SARS-CoV-2 concentration in wastewater and reported hospitalisations.

Madrid region. Sampling point selection process was critical to this aim: a total of 289 sample points which represent the same amount of sewersheds are systematically tested for SARS-CoV-2 concentration, following the criteria obtained by a pilot test to define sampling point selection criteria concluding that whenever possible, a maximum of 25,000 equivalent inhabitants, 3.5 km distance to population centres and 2.5 km distance to last discharging point are preferred to obtain optimal results.

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 14-day incidence rates with active infection and reported COVID-19 hospitalisations. Information is daily shared with Health authorities for consultancy and decision-making and results are available as an aggregation for the entire region and for each sewershed.

Future work will include a permanent epidemiological surveillance system where 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. Canal de Isabel II is currently implementing required resources to perform qPCR in its own wastewater laboratory instead of outsourcing it.

A study of 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 the sewer network.

There is also an ongoing pilot test to assess relationships between PCR 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 wastewater treatment plants.

Credit author statement

A.L., J.B. and **A.P**: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Software; Supervision; Validation; Visualization, **J.I.U., J.C.** and **J.F.**: Reviewing of original draft, **J.S.**: Conceptualization; Funding acquisition; Supervision, **P.F.**: Funding acquisition, **M.O.**: Roles/Writing - original draft; Writing - review & editing, **F.J.C.** and **A.Z.**: Resources.

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Policy and ethics

Non-infringement on the ethical principles of the WMA Declaration of Helsinki.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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