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RESEARCH

Urban Wastewater-Based Surveillance of SARS-CoV-2 Virus: A Two-Year Study Conducted in City of Patras, Greece

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Abstract

Wastewater-based epidemiology, during the COVID-19 pandemic years, has been applied as a complementary approach, worldwide, for tracking SARS-CoV-2 virus into the community and used as an early warning of the prevalence of COVID-19 infection. The present study presents the results of the 2-year surveillance project, in the city of Patras, Greece. The purpose of the study was to monitor SARS-CoV-2 and implement WBE as an early warning method of monitoring Public Health impact. The presence of SARS-CoV-2 was determined and quantified in 310 samples using RT-qPCR assays. For the years 2022 and 2023, 93.5% and 78.7% of samples were found positive, respectively. Comparison of detection methods have been conducted to select the method with the highest recovery of the viral load. A seasonal variation of the virus was recorded, showing a recession in summer months confirming the country's epidemiological data as indicated by positive correlation of wastewater viral load with registered cases of COVID-19 infections during these years ($p < 0.05$) and moreover sealed with a significant negative correlation observed with Daily Average ($p < 0.01$) and Daily Maximum Temperature ($p < 0.01$). More research was carried out to elucidate a possible association of physicochemical characteristics of wastewater with viral load showing positive correlation with Chlorides ($p < 0.01$) advocating possible increased use of chlorine-based disinfectants and Electrical Conductivity ($p < 0.01$) indicates that wastewater during periods of increased infections is more heavily loaded with ions from chemical and biological pollutants. No correlation found with rainfall and physicochemical indicators, such as COD, BOD_5, Total Phosphorus, Total Nitrogen, and Total Suspended Solids. According to the findings, WBE represents a useful tool in the management of epidemics based on an environmental approach and it can also shed light on the interacting parameters that capture Public Health since any infections that may lead to epidemics lead to a parallel change in the use of pharmaceuticals, antimicrobials, disinfectants, and microbial load in urban wastewater.

Keywords Urban Wastewater · Wastewater-based epidemiology · COVID-19 · SARS-CoV-2 · Surveillance

Introduction

The outbreak of the Covid-19 pandemic brought to the fore the need for timely detection, prediction, and prevention method for infectious diseases. Wastewater-based epidemiology (WBE) was proposed as a complementary method, an early warning, to track pathogens that are causing infections into the community, in order, the Healthcare systems to take timely measures. The epidemiological surveillance of urban wastewater is now a practice recommended by the European Commission and the World Health Organization (WHO) considered as a complementary method to clinical approach, for COVID-19 surveillance. It can provide important information to Public Health decision-making process helping governments mitigate the health and socioeconomic consequences of outbreaks (Xagoraraki & O'Brien, 2020; World Health Organization, 2022).

COVID-19 first cases were reported in Greece on February 26, 2020. Regarding the multitude of confirmed cases and deaths, during the years 2020–2022 by the infection from SARS-CoV-2, circulation of the virus in the community needed continuous and rapid detection (Kousi et al., 2021).

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Although SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) is a pathogenic virus that infects the respiratory system, it has also been documented to infect the gastrointestinal tract, resulting in excretion through the feces into the sewage system. Many studies have reported gastrointestinal symptoms in patients with COVID-19, such as diarrhea, vomiting, anorexia, and abdominal pain (Guo et al., 2021). Even if there are no gastrointestinal symptoms, the elimination of viral particles is already starting from the beginning of the infection; therefore, they can be detected before the onset of the symptoms of the infection. In addition, asymptomatic carriers shed viral particles, without showing clinical symptoms (Agrawal et al., 2021; Daughton, 2018) and seems to persist longer, about 3 weeks, in the stool than in the respiratory system, as it is recorded in many samples of COVID-19 confirmed cases (Chen et al., 2020; Cho & Ha, 2020; Park et al., 2021; Xu et al., 2020; Zhang et al., 2021). Hence, it is recorded that both asymptomatic and symptomatic individuals, even after recovery from the symptoms, can shed significant copies of SARS-CoV-2 RNA in their feces (Mizumoto et al., 2020; Nishiura et al., 2020; Treibel et al., 2020; Wu et al., 2020). Wastewater samples showed a correlation between SARS-CoV-2 RNA concentrations and the number of COVID-19 clinical cases in many studies (Ahmed et al., 2020; Medema et al., 2020; Peccia et al., 2020). Municipal sewage is sampling material from a wide range of population and mainly healthy population, which would otherwise not be possible to monitor, independent of people's socioeconomic status or ability to have access to healthcare (Sharara et al., 2021). Furthermore, analysis of wastewater samples is a non-invasive method; it does not require informed consent, thus, limiting ethical and bioethical concerns (Anand et al., 2021; Choi et al., 2018). As a result, WBE gives us a complete and reliable picture of the prevalence of SARS-CoV-2 virus, disease spread can be monitored, and the effectiveness of public health interventions can be evaluated.

The number of studies reporting the detection of SARS-CoV-2 RNA in wastewater is ever increasing and nowadays, WBE has been established, across Europe, as an efficient, fast, and reliable source of epidemiological information for national health authorities, local healthcare, national disease control centers, private wastewater operators, and water and environment agencies (Maida et al., 2022; Monteiro et al., 2022). Subsequently, networks have been established for the exchange of information, methods, and results for the purpose of comparability, evaluation, and assessment of methods aiming at more effective and reliable epidemiological surveillance. (https://score-network.eu/) (Publication Map | covid19wbec.org).

Urban wastewater is a complex matrix that includes suspended solids, a variety of organic and inorganic chemicals, pharmaceuticals, metabolites, detergents, small-scale toxic wastes, nutrients, pathogenic microorganisms, and other substances (Balboa et al., 2021). The great diversity of chemical and biological elements contained in wastewater lead to various obstacles in terms of wastewater analysis; for this reason, the physicochemical characteristics of the wastewater must also be taken into account since they can cause inhibition of the viral detection procedure (Bivins et al., 2021). However, inhibition can be limited by the use of good laboratory practices, appropriate selection of the genetic material isolation procedure, and purification of the isolate. Furthermore, questions arise as how much the concentration of the viral RNA can affect the physicochemical characteristics of the wastewater and if certain of them can be additional indicators of the increase of viral load.

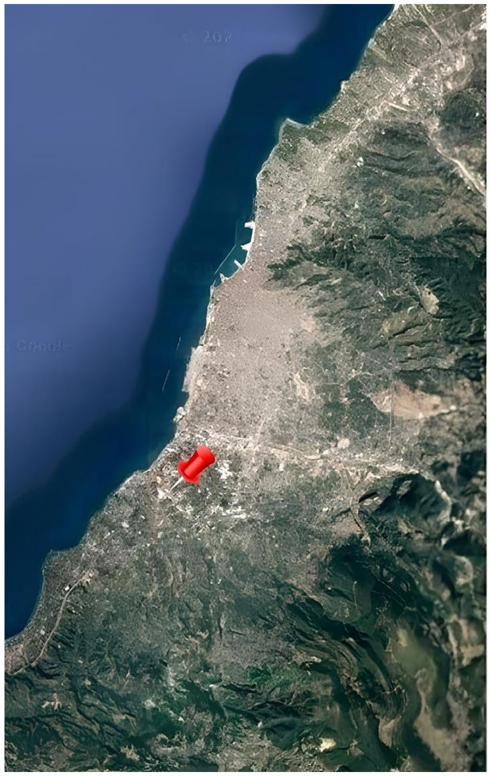
Additionally, the effect of weather conditions, such as rainfall and average and/or maximum day temperature, on the concentration of the virus in wastewater has not yet been thoroughly investigated.

In Greece, at the beginning of 2021, the National Public Health Organization (EODY) created the National Wastewater Epidemiology Network, for the monitoring, recording, and early warning of the spread of the SARS-CoV-2 virus in the community, which is now active on a daily basis in the 5 largest cities of Greece, including Patra. In this study, in the framework of the aforementioned project, the aim was to establish a WBE surveillance system for SARS-CoV-2 in the city of Patras, to assess two RNA recovery methods based on quantitative PCR assays, to monitor the presence and course of the epidemic, and to evaluate the influence of weather conditions on viral load and the correlation with certain physicochemical characteristics with the prospect of extending surveillance knowledge.

Materials and Methods

Collection of Wastewater Samples

This study was carried out in the city of Patras (Greece), accounting 215.922 inhabitants, based on the 2021 population census (Greek Statistical Authority). The samples were collected by the Wastewater Treatment Plant (WTP) located at the southwestern end of the Municipality of Patras with average daily supply 1.500 m^3/h, serving 168.034 inhabitants. Three wastewater samples were collected every week, during 2022 and 2023. 155 samples during 2022 and 155 on 2023 were analyzed, a total of 310 samples. The position of the WTP and the characteristics of sewage are shown in Fig. 1. Five hundred ml of 24 h, composite, sample of raw, untreated wastewater was collected in sterile plastic bottles from the inlet and immediately transferred to the laboratory,



Wastewater Treatment Plant of the Municipality of Patras

Qualitative Characteristics of Wastewater Inlet

Type of Wastewater	**Urban**
Average daily supply	36.000m3/d
BOD5	11700 kg/d
COD	14.400 kg/d
S.S.	14.400 kg/d
Total Nitrogen	2340 kg/d
Phosphorus	468 kg/d

Fig. 1 Satellite map of city of Patras showing the location of the Wastewater Treatment Plant and qualitative characteristics of wastewater inlet, data obtained from Municipal Water Supply and Sewerage Company of Patras

stored at + 5 ℃, and analyzed for the detection of SARS-CoV 2 RNA. Before analysis, samples were pasteurized to inactivate pathogenic microorganisms. Pasteurization was carried out by incubating the sample at 60 ℃ water bath for 30 min and then cooling the sample at 5 ℃ for 30 min. Moreover, data concerning the physicochemical parameters of wastewater samples are obtained from the WTP and meteorological data are obtained from the National Meteorological Agency.

Virus Recovery Methods and RNA Extraction

At first, two concentration and viral RNA recovery methods were compared in 23 positive SARS-CoV-2 wastewater samples. The method of precipitation with polyethylene glycol (PEG)/NaCl, followed by NucliSENS®, easyMAGTM (bioMérieux, Marcy-l'Étoile, France) RNA extraction kit, and Wizard® Enviro Total Nucleic Acid Kit (Promega, Wisconsin, United States). Regarding the first method, 100 mL of each sample was centrifuged for 30 min at 2970 rpm, 4 ℃, followed by sediment disposal. Then, add 3-gr NaCl and 1.75 gr of PEG (Polyethylene Glycol) into the sediment followed by incubation overnight with agitation, centrifuge at 10,000 × g for 30 min at 4◦C using low-binding conical tubes, followed by supernatant disposal, and then resuspend the sediment with 500 μL of PBS. Viral RNA was extracted using the NucliSENS® and easyMAG™ RNA extraction kit (bioMérieux) using Silica magnetic beads as indicated by the manufacturer's instructions, resulting in 50-μL final volume of isolated nucleic acid solution. Regarding Wizard® Enviro Total Nucleic Acid Kit (Promega), 40 mL of each sample were incubated with protease for 30 min and afterward centrifuged for 10 min at 3000xg at room temperature to remove solids, the supernatant was separated in two clean 50-ml conical tubes, and to each tube containing 20 mL of supernatant it was added binding buffers and isopropanol as indicated by the manufacturer. The mixture from each tube was poured into a specific device, which is provided from the company, a Reservoir Extension Funnel and PureYield Binding Columns attached to a Vacuum Manifold port (Vac-Man®), followed by the addition of Column wash buffers and elution, using the Eluator Device® to a total 1 ml of TNA solution. Moreover, extraction procedure is as follows: as indicated by the protocol of the Wizard® Enviro Total Nucleic Acid Kit (Promega) using PureYieldTM minicolumns and centrifugation at 10,000 rpm concluding to 100 μL of total nucleic acid isolate in Nuclease-Free water. As it was indicated by the overall higher recovery rates of the Wizard® Enviro Total Nucleic Acid Kit (Promega) subsequent analyses of the samples continued using the aforementioned method were combined by further treatment with OneStep PCR inhibitor removal kit (Zymo, Irvine, CA, USA) to reduce substances that inhibit the PCR (Dumke et al., 2021). SARS-CoV-2 Recovery Efficiency for Wizard® was calculated based on the genome copies quantified by RT-qPCR, and the equation used is as follows: Recovery Efficiency

(%) = (Virus recovered / Virus seeded) × 100. (Barril et al., 2021), thus, inhibition was evaluated in 12 wastewater samples, one representative sample for each month of the year 2023, in a monthly routine basis. The method used is sterilization at 121℃ of the wastewater sample and spiked it with 3.3×10^6 Genome Copies (GC)/ml of SARS-CoV-2 virus and GC was corrected averagely per month.

Reverse Transcription Real-Time qPCR Assay (RT-qPCR)

To detect and quantify SARS-CoV-2 RNA, RT-qPCR assay was performed using Thermocycler Stratagene Mx30005P (Thermo Scientific, Waltham, MA, USA). The assay was performed according to the recommendations of the manufacturer. Each sample (5 µL) was tested in triplicate. A positive control of Sars-CoV-2 provided by the Greek National Public Health Laboratory, KEDY, and a negative control (grade water) was included, in each run. The reaction mixture consisted of 12.5 µL of reaction mix 2x, 0.4-µL $MgSO_4$, 1 µL of enzyme, all included in Superscript III & Platinum Taq Polymerase One-Step qRT-PCR Kit (Thermo Scientific, Waltham, MA, USA), 1 µL of each primer, 0.8 µL of each probe, and 0.5-µL RNase-free grade water. Sequences of primers and probes of target genes are summarized in Table 1. Primer sets nCoV_IP2 and nCoV_IP4 were multiplexed. Cycling conditions were 15 min at 50 °C for reverse transcription, 2 min at 95 °C for enzyme activation, 50 cycles of 15 s at 95 °C, and 30 s at 58 °C for annealing and extension.

Quantification of SARS-CoV-2 recovery was made using the standard curves for RdRP gene for both extraction methods used and compared. Efficiency was determined based on the equation Efficiency (e%) = 10–1/slope 1 (Mondal et al., 2021). For IP4 primers, set e% = 102.7, Slope = 3.258, y intercept = 38.82 and Rsq = 0.998. For IP2 primers, set e% = 94.6, Slope = 3.458, y intercept = 42.00, and Rsq = 0.994. Standard curve based on control sample of 3.3×10^6 Genome Copies (GC)/ml after testing serial tenfold dilutions. The IP4 Primers and FAM + Probe assay were chosen for quantification of Genome Copies. Limit of Detection (LOD) was determined at 10^2 GC/liter. Mean Ct values was measured in all samples. The virus concentration was expressed as genome copies/liter, using the following equations. According to the instructions of the manufacturer (Promega), if a sample volume of 40 ml is used and total nucleic acid eluted in 40 µl after extraction and clean-up step, the concentration factor is 1000.

(Bustin et al., 2009)

$$\text{Viral Genome (Copies/liter)} = \frac{\text{copies in RT} - \text{qPCR} \times 1{,}000}{\text{volume of nucleic acis extruct used in RT} - \text{qPCR(ml)} \times \text{ concentation factor}}$$

(Mondal et al., 2021)

$$\text{Concentration factor} = \frac{\text{water sample volume used (ml)}}{\text{volume of nucleic acid extracted (ml)}}$$

$$\text{Copies in RT} - \text{qPCR} = \frac{\text{Cq value} - \text{Y intercept}}{\text{slope}}$$

Physicochemical Characteristics of Wastewater Samples

The untreated wastewater samples are characterized by several physicochemical indicators. Physical and chemical analysis of the inlet and outlet of wastewater is essential to monitor the efficient treatment procedure of wastewater by removing contaminants and decreasing pollutants load. The wastewater quality can be measured by analyzing several parameters, such as pH, Total Suspended Solids, Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD_5), Chlorides, Electrical conductivity (EC), Total Phosphorus, Total Nitrogen, copper, iron, nickel, nitrogen, lead, zinc, and more (Roslin & Benit, 2015). The overall

Table 1 Primer and probe sequences of target genes for SARS-CoV-2

Target gene	Primer/Probe name	Sequence (5′–3′)	Reference
RdRP gene[a]	nCoV_IP2-12669Fw	ATGAGCTTAGTCCTGTTG	(Corman et al., 2012)
	nCoV_IP2-12759Rv	CTCCCTTTGTTGTGTTGT	
	nCoV_IP2-12696bProbe(+)	AGATGTCTTGTGCTGCCGGTA [5']Hex [3']BHQ-1	
	nCoV_IP4-14059Fw	GGTAACTGGTATGATTTCG	
	nCoV_IP4-14146Rv	CTGGTCAAGGTTAATATAGG	
	nCoV_IP4-14084Probe(+)	TCATACAAACCACGCCAGG [5']Fam [3']BHQ-1	

[a]National Reference Center for Respiratory Viruses, Institute Pasteur, Paris

organic loading of the samples is described by BOD_5, COD, and Dissolved Organic Carbon (DOC); furthermore, in recent study it was recorded that the ratio of the specific absorption (UV254/DOC) over the Dissolved Oxygen (DO) is the parameter with the highest correlation with SARS-CoV-2 viral copies. (Petala et al., 2021).

For the samples that have been collected for this study, data of the physicochemical characteristics were retrieved in the context of evaluating and correlating them with SARS-CoV-2 viral load. The parameters used to investigate possible correlation of SARS-CoV-2 viral load were chosen to be COD, BOD_5, Chlorides, Electrical conductivity (EC), Total Phosphorus, Total Nitrogen, and Total Suspended Solids. pH was ranging from 6.7 to 7.7. COD ranging from 131 to 530 mg/L, BOD_5 62–414 mg/L, Chlorides 191–988 mg/L, Electrical Conductivity 1127–2029 μScm^{-1}, Total Phosphorus 1.6–4.7 mg/L, Total Nitrogen 23.6–42.5 mg/L, and Total Suspended Solids 60–232 ppm.

Data Analysis

Statistical analysis of data obtained was performed with SPSS Statistics 26 (IBM) and graphs conducted using Excel 2016 (Microsoft). To evaluate the significance of the comparison of the two methods for extracting nucleic acid from wastewater, a paired sample t test were conducted to compare the means of the two methods. Spearman's ρ correlation was conducted to investigate correlation between viral load, physicochemical parameters of wastewater, and meteorological data. Specifically, correlation test conducted for Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD_5), Chlorides, Electrical Conductivity, Total Phosphorus, Total Nitrogen, Total Suspended Solids, Average Daily Temperature, and Maximum Daily Temperature. A Mann–Whitney U test was conducted to investigate the effect of Rainfall on viral load. To evaluate the correlation between the SARS CoV-2 viral load of the wastewater samples and the registered cases of COVID-19, Spearman's ρ correlation was conducted. Spearman's rho was chosen because it is a non-parametric test that is applied either when the variables do not form a normal distribution or if we do not know if they form a normal distribution.

Results

The comparison of concentration and viral RNA recovery methods showed that Wizard® Enviro Total Nucleic Acid Kit (Promega) is more efficient, thus, it was chosen for the wastewater-based epidemiological surveillance of the virus. Using paired samples t test showed a statistically significant difference in means between the two methods for significant level p-value < 0.05. There is a 5:7 ratio between the results compared in 23 samples that interpreted in more satisfactory results for the Wizard® method. Analytical comparison of Genome Copies per liter in 23 samples obtained, with both methods examined, is shown in Fig. 2 and box plot in Fig. 3. SARS-CoV-2 overall Recovery Efficiency for Wizard® method, for RdRP gene, through 12 spiked wastewater samples, was determined at $70.08 \pm 8.5\%$.

The epidemiological surveillance of SARS-CoV-2 virus, during 2022 showed 93.5% ($n = 145/155$) of wastewater samples positive to SARS-CoV-2 and during 2023 showed 78.7% ($n = 122/155$) positive samples with increasing trends in the autumn and winter months and decreasing in the summer months; specifically, we observe an increase during September to January 2022 and February to April 2023, reported in Fig. 4.

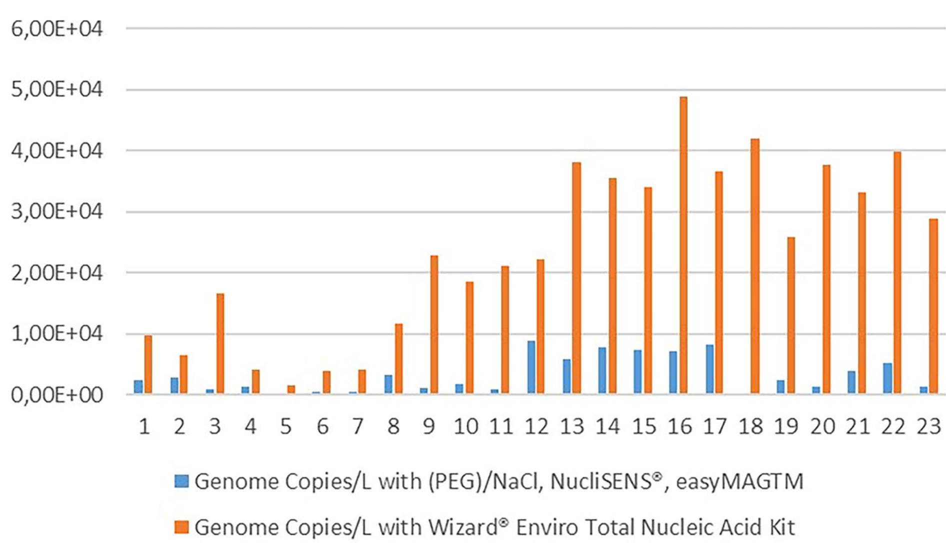


Fig. 2 Graphical comparison of concentration and extraction methods according to 23 samples of untreated wastewater samples

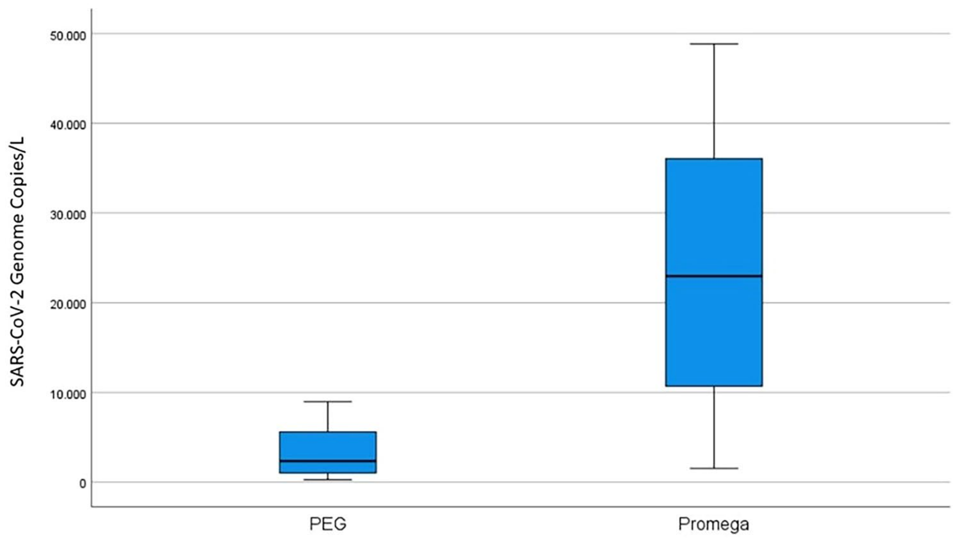


Fig. 3 Box plot for the two viral concentration and extraction methods compared

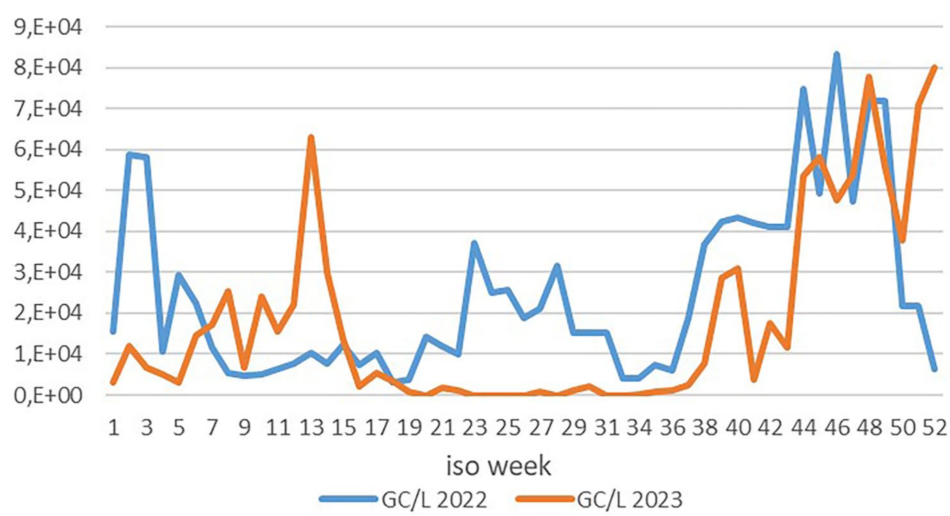


Fig. 4 Epidemiological curve of SARS-CoV-2 during 2022 and 2023 ISO weeks per Genome Copies/Liter based on Wastewater surveillance of city of Patras, Greece

Spearman's ρ correlation test was conducted for Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD_5), Chlorides, Electrical Conductivity (EC), Total Phosphorus, Total Nitrogen, Total Suspended Solids, Average Daily Temperature, Maximum Daily Temperature, correlation found with Chlorides, with significance level $p < 0.01$, negative correlation with Average Daily Temperature, with significance level $p < 0.01$, and negative correlation with Maximum Daily Temperature, with significance level $p < 0.01$. No correlation found on the effect of rainfall.

Furthermore, clinical data of positive registered cases were obtained from the National Public Health Organization (NPHO) of Greece for the years 2022 and 2023. The results are shown in Fig. 5 and using Spearman's ρ correlation test, it was found that there is a positive correlation with statistical significance level $p < 0.05$.

Discussion

There are nowadays many studies that confirm the utility of epidemiological surveillance of municipal wastewater (Monteiro et al., 2022; Gagliano et al., 2023; Kumar et al., 2020; Maida et al., 2023). Wastewater-based surveillance found to be an effective early warning indicator if combined with clinical surveillance. This study confirms the utility of its use which is supported by the positive statistical correlation found ($p < 0.05$). However, despite the positive correlation, the curve of average recorded cases of COVID-19 infection does not fully reflect the viral load peaks found in wastewater samples during the winter months of 2023. This observation may be due to the fact that the illness caused from the prevailing Omicron variant of SARS-CoV-2 virus is more mild than the previous variants prevailed during the 2020–2022 pandemic (Thiruvengadam et al., 2023), despite

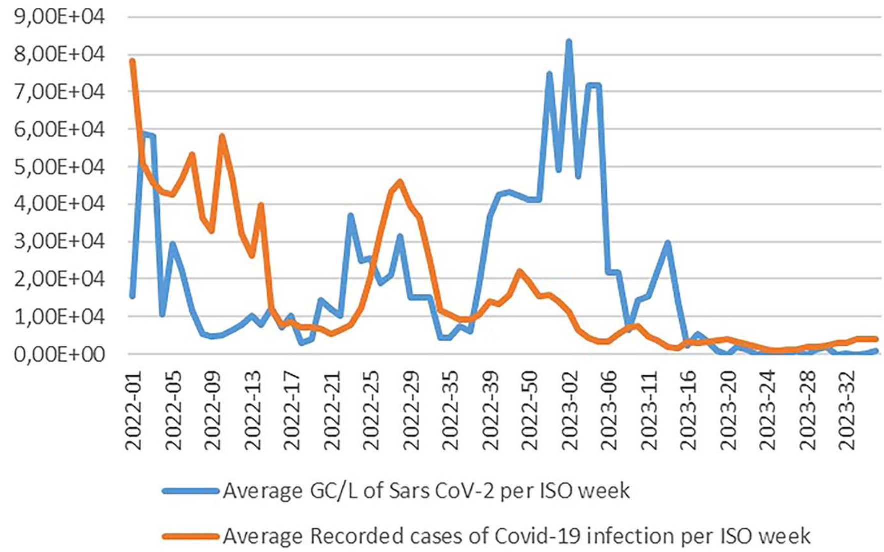


Fig. 5 Average registered cases of COVID-19, in Patras, per ISO week and corresponding viral load trends

its transmissibility. Thus, there is an increased viral load in the community, as human's immunological memory might not work properly against Omicron variant even formerly vaccinated or even if they have been ill before (Dhama et al., 2023), but fewer admissions to hospitals and reduced registrations of the COVID-19 infection.

Wastewater surveillance serves as an early warning system for detecting outbreaks of infectious diseases in communities. If correlations are observed between SARS-CoV-2 concentrations and certain environmental factors, such as temperature and rain precipitation, this information could be used to develop predictive models for identifying periods or regions where viral transmission is likely to increase or decrease. This can facilitate proactive public health responses to prevent or mitigate outbreaks. For that reason, day temperature and rainfall precipitation were investigated.

Seasonality is not recognized in 2022 as there was coexistence and frequent rotation of many variants of SARS-CoV-2; additionally, there was an outbreak in Greece in summer (iso week 23–29) of 2022 as omicron variant, specifically BA.5 subvariant of Omicron, from data published by the World Health Organization in 25th of September 2022; this outbreak is shown in Fig. 4. This variant was more transmissible than other Omicron sub-variants, but not causing more severe forms of COVID-19 disease (WHO Coronavirus Update 80, 2022). Nonetheless, in 2023 there is an obvious seasonality pattern shown in Fig. 4 depicting high GC on colder months and lower in warmer months. However, seasonality was not confirmed by the statistical algorithm SARIMA. On the other hand, there is an important indication of the course of the COVID-19 disease toward seasonality by the statistically significant negative correlation based on Spearman's rho correlation test with the Average Daily Temperature and the Maximum Daily Temperature ($p < 0.01$). As such, we have some important indications to confirm the possible course of the disease toward seasonality. High viral load is observed, specifically from September to January 2022 and February to April 2023. Peaks of virus genome detected obtained on ISO week 2022–45 (November) and 2023–11 (March). The majority of negative or below detection limit samples (25 out of 36 samples) accumulate in the summer months (June, July, August) of 2023, ISO week 23–34. The decrease in summer months reflects the reduced morbidity of the population due to milder weather conditions prevailing in the summer months and additionally, due to the fact that many people leave the cities and head to rural destinations for holidays. Furthermore, a current increase is seen during the fall and December of 2023, which should lead us to a vigilance for a possible future increase in clinical cases. As indicated by the literature, COVID-19 infections follow typical respiratory virus seasonal patterns (Wiemken et al., 2023). Thus, it is confirmed that policy makers should consider SARS-CoV-2 seasonality in managing a possible upcoming outbreak (Nichols et al., 2021; D'Amico et al. 2022).

Rain precipitation and its effect on viral load, based on the literature, are not fully examined. In the literature, we can find few references mentioning that is lowering the level of viral RNA by causing a delusion effect (Saingam et al., 2023). Based on the current research and rainfall data for the year 2022 and 2023, no similar observation was recorded in the city of Patras, as the rainwater is not mostly discharged into the urban wastewater network.

In the use of wastewater as a tool for epidemiological surveillance, there are factors that may affect the validity of the results. The sharp ups and downs of the viral load is due to the fact that wastewater is environmental sample rich in chemical inhibitors and whose chemical composition can vary significantly in each sample causing an uncertainty in the accuracy of the measurement which must be determined. The very different physicochemical composition of wastewater samples affects the isolation of nucleic acids, while the use of ethanol and isopropanol in the isolation process, if not well removed from the isolate, and the possibility of RNA degradation in the wastewater samples has been implied (Kitamura et al., 2021). For this reason, it is recommended to perform further process of the isolate with inhibitor removal (Dumke et al., 2021), implement good laboratory practices, including estimation of the inhibition, as well as to obtain several samples weekly in order to have representative measurements.

In the search for correlations of SARS-CoV-2 viral load with specific physicochemical characteristics of the wastewater, it was found that there is a positive correlation with Chlorides and Electrical Conductivity (EC) of the samples ($p<0.01$). The main source of Chlorides in urban wastewater is salt-based water softeners discharged into the sewer system. In addition, chlorine is one of the most commonly used chemicals for the disinfection of drinking water and water supplies. Another source of Chlorides are disinfectants and antimicrobials used in households. Chlorine solutions, for example, sodium hypochlorite, have been used extensively as disinfectant and antiseptic (Mcdonnell & Russell, 1999). In a recent study, disinfectant such as Diallyldimethylammonium Chlorides (DADMAC) and Benzalkonium Chlorides (BACs) were found in high concentration in wastewater of campus dormitories during Covid-19 pandemic 2021–2022, higher concentrations compared to reported concentrations before the pandemic and showed a relatively strong positive association with the SARS-CoV-2 viral load in wastewater (Mohapatra et al., 2023). Furthermore, during infections the usage of disinfectants in every household is really high (Chen et al., 2021; Gallandat et al., 2021), as a result, high dose of disinfectants is deposited in the sewage system confirming their relative increase during the peaks of SARS-CoV-2 viral load. To confirm this probability, it needs more research to be done on the specific detection of disinfectants in wastewater samples obtained using analytical methods.

Electrical conductivity (EC) is an indicator of the quantity of substances, biological and chemical, that are dissolved in the wastewater. Increasing EC indicates more contaminated and polluted wastewater. Pure water has a low conductivity (5mS/cm or less). EC has been investigated as a parameter used as an alternative normalization method for wastewater-based monitoring of Sars-Cov-2 and is particularly suitable to determine dilution rates relative to dry weather flow concentrations (Langeveld et al., 2023). In our study, positive correlation of SARS-CoV-2 viral load with EC advocates the general aggravated state of the urban wastewater during the period of increased disease in the population from COVID-19 as more ions are dissolved in wastewater from chemical and biological contaminants accompanying the increased morbidity of the population.

WBE findings on negative correlation between SARS-CoV-2 concentrations and average daily temperature and maximum temperature, positive correlation with clinical cases can reveal a pattern that can inform evidence-based policy development and decision-making at the local, national, and international levels, as policymakers can use this information which will lead to knowing what to expect, concerning the course of the disease and to implement timely targeted interventions, allocate resources effectively, and develop strategies for controlling viral spread within communities. Also, understanding the relationship between SARS-CoV-2 concentrations in wastewater and various environmental factors can enhance the utility of WBE as a tool for monitoring and managing infectious diseases, including COVID-19, and then contribute to more effective public health responses.

Despite the upcoming research done in the field of WBE, in recent years, there are still untapped areas and further possibilities on which light can be shed, scoping the better use of epidemiological surveillance of wastewater as a tool for the protection of Public Health and the sustainability of the environment. Beyond the COVID-19 pandemic WBE can also be promising to monitor other infectious agents, antibiotic resistance genes, antibiotic-resistant bacteria, hazardous chemicals, drugs, and other medicinal substances.

Conclusion

Concluding, as WBE continues to expand in the disease monitoring field, any observations and supplementary records need to be taken into account. The most recent findings on wastewater-based epidemiological surveillance indicate a promising community monitoring of many etiological agents, biological and chemical, that can have an impact on Public Health. Data obtained from these practices can complement the clinical evidence and can lead to an integrated management that protects the health of the citizens. A more promising method for SARS-CoV-2 virus extraction is introduced. Moreover, this study comes to confirm the positive correlation of SARS-CoV-2 viral load in wastewater with registered cases of Covid-19 infection during 2022 and 2023. Seasonality observed indicating increase of SARS-CoV-2 infections in autumn, winter, and early spring months and a recession in summer month, confirmed by negative correlation with Daily Maximum and Average Temperature. No effect of rain precipitation observed on viral load. An increase of viral load was observed during December 2023 which needs to be taken into consideration for the upcoming course of time, highlighting the need for vigilance by health management systems. Correlation of viral load with physicochemical parameters of wastewater samples was examined, in search of additional indicators we should take account of. Chlorides and electrical conductivity follow a correlation pattern with viral load on wastewater samples most possibly due to increase of household disinfectants used and more loaded wastewater during peaks of COVID-19 infection.

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Author Contributions Conceptualization: Apostolos Vantarakis; Methodology: Apostolos Vantarakis, Zoi Kotsiri, and Zoi Anastopolou; Formal analysis and investigation: Apostolos Vantarakis, Zoi Anastopoulou, Zoi Kotsiri, Eleftheria Chorti-Tripsa, and Rafail Fokas; Writing—original draft preparation: Zoi Anastopoulou; Review and editing: Apostolos Vantarakis and Zoi Anastopoulou; Funding acquisition: Apostolos Vantarakis; Supervision: Apostolos Vantarakis.

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

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