SARS-CoV-2 an extensive monitoring of an Italian full-scale wastewater treatment plant

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ABSTRACT

Wastewater-based epidemiology was adopted to monitor SARS-CoV-2 RNA in Caltanissetta (Sicily, Italy) full-scale wastewater treatment plant (WWTP). The sampling campaign lasted 288 days (from October 11, 2021 to July 26, 2022). Influent wastewater, effluent from the secondary clarifier, and disinfected effluent samples were monitored for SARS-CoV-2 RNA coupled with other conventional pollutants (total suspended solids – TSS, chemical oxygen demand – COD, biochemical oxygen demand – BOD, respectively, *Escherichia coli*). Results showed that the plant performs excellently in removing conventional pollutants (average removal of 94 %, 91 %, and 91 % for TSS, COD, and BOD, respectively). SARS-CoV-2 RNA was detected in all influent wastewater analyzed samples (average 1.1×10^5 copies genomic per litre – GC L^{-1}). Within the biological process, a strong degradation of SARS-CoV-2 RNA was detected.

High correlation between log-transformed SARS-CoV-2 in wastewater and the active cases was obtained (correlation coefficient of 0.85, *p-value* < 0.001when 14 days lag time was considered).

1. Introduction

The COVID-19 pandemic caused severe acute respiratory syndrome of coronavirus (caused by SARS-CoV-2), which forced scientists and public health decision-makers to establish/adopt a method able to quantify SARS-CoV-2 and to support the setting up of measures towards virus spreading prevention [1]. Since SARS-CoV-2 RNA is excreted throughout feces, wastewater surveillance has been identified as an excellent supplementary way (concerning the existing public health surveillance systems) to monitor the SARS-CoV-2 spreading [2,3]. With this regard, wastewater-based epidemiology (WBE) has been promoted as an excellent tool, suggesting the adoption of quantitative PCR (RT-qPCR) based methods [2]. Thus, underlying the key importance of wastewater treatment plants (WWTPs) for environmental and public health. Indeed, if no proper and effective treatment occurs inside the WWTP, effluents may cause serious risks for humans and the environment [4].

The adoption of WBE was already mature in 2020 for monitoring

infectious diseases at the population level (among others, [5]) or the adoption of drug of abuse (among others, [6,7]). Several studies from 2020 have been performed applying WBE to detect SARS-CoV-2 RNA in influent and effluent wastewater of WWTPs (among others, [8–10]). Acosta et al. [8] adopted WBE to monitor the spread and occurrence of SARS-CoV-2 RNA from three Canadian WWTPs. Acosta and co-authors revealed SARS-CoV-2 RNA in 98.06 % of influent WWTP samples finding a good correlation with clinically diagnosed data. La Rosa et al. [9] used WBE for the first time to evaluate the spread of the Omicron SARS-CoV-2 variant in Veneto, North Italy. Maida et al. [10] proposed a correlation between SARS-CoV-2 RNA in wastewater and the prevalence of COVID-19 derived from one-year clinical data in Sicily. Maida and co-authors [10] found that a lag time of 7–14 days can be considered for quantifying active cases to be correlated with surveillance data. Most of the studies on SARS-CoV-2 monitoring in WWTPs focused attention on the quantification in the influent and effluent samples without considering the potential SARS-CoV-2 RNA degradation within the plant [11]. Literature revealed that the duration from positive to negative state

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(namely, "duration of positive state") depends on several factors (e.g., sex, temperature) and can be strongly variable. For example, Klein et al. [12] found that male patients have more negative effects and higher mortality than female ones. While Zheng et al. [13] found that the duration from positive to negative state is strongly affected from the male and female metabolic changes.

Moreover, despite the efforts performed in the literature and the huge advantages of using WBE for quantifying SARS-CoV-2 RNA, some gaps still need to be addressed to improve its usefulness [14,15]. Among the most important gaps from an engineer's point of view are: i) the variability of the positive state duration; ii) the calculation of active cases; and iii) the role of biological treatment in the degradation of RNA in wastewater.

With this regard, further studies are required to understand better the advantages of using WBE as a stand-alone surveillance tool to inform Public Health practice [14]. This study aims at providing deep insights from 288 days SARS-CoV-2 monitoring of Caltanissetta (Sicily, Italy) WWTP focusing the attention on how the collected data correlate with that of public surveillance even during the Omicron variant occurrence. Specifically, this article has the novelty of discussing the impact of biological treatment on SARS-CoV-2 RNA and examines its correlation with public surveillance data by using various approaches to quantify the number of active cases. The number of active cases has been calculated according two approaches. Specifically, the approaches proposed by Maida et al. [10] and that of Swift et al. [16] have been adopted.

2. Materials and methods

2.1. Full-scale wastewater treatment plant

The wastewater treatment plant under study is located in the south of Italy (Sicily) (Caltanissetta). The WWTP has a conventional scheme applying the pre-denitrification process in the water line and the anaerobic digestion in the sludge line. In Fig. 1 the schematic layout of the water treatment line is reported.

More precisely, after the pre-treatments the pre-denitrification process is implemented in the water line according to the modified Ludzack–Ettinger system. Two aerobic reactors equipped with horizontal brush aerators for aeration are adopted for the nitrification process. Mixed liquor from the aerobic reactor is partially recirculated to the aerobic reactor (Q_{ML}). The solid/liquid separation occurs through two circular secondary clarifies having horizontal feeding flux. Sewage sludge collected in the bottom of the secondary clarifiers is partially returned into the aerobic reactors (as return activated sludge, Q_{RAS}). Before being discharged into the water body, the treated wastewater is disinfected by using sodium hypochlorite within a plug–flow disinfection tank.

During the monitoring period (from October 11th, 2021 and ending on July 26th, 2022) the influent wastewater flow rate (Q_{IN}) was equal to 14,802 m³h⁻¹ (67,475 equivalent inhabitants calculated by considering a BOD load of 60 g/inhabitant/day) of urban wastewater. Table 1 summarizes the average concentration for the monitored period in the influent and treated wastewater of total chemical oxygen demand (COD), biochemical oxygen demand (BOD), total nitrogen (TN), total phosphorus (TP) and total suspended solid (TSS).

Table 2 summarizes the water line key flow rates, tank volume, and operating conditions. Measured data (flow rates) reported in Table 2 are the average values of the monitoring period (from October 11th, 2021 and ending on July 26th, 2022).

2.2. Experimental campaign

The experimental campaign lasted for 288 days, starting on October 11th, 2021, and ending on July 26th, 2022. During this period, three samples (1 L per sample) were collected one time per week (every Thursday) from three plant sections: influent wastewater (Section 1), secondary clarifier supernatant (Section 2), and treated disinfected wastewater (Section 3). Samples of sections 1 and 3 were collected using an autosampler able to withdraw a flow proportional 24-h composite samples. While the sample of section 2 was a grab sample. The samples were transported at the temperature of 4 °C to the Sanitary and Environmental Engineering Laboratory and to the Reference Laboratory of Western Sicily for the Emergence of COVID-19 of the University Hospital "P. Giaccone" of Palermo within 12 h from the sampling day and then tested for SARS-CoV-2 RNA within 24 h from sampling. All samples (1, 2, and 3) were analyzed for measuring *Escherichia coli* (Colony Forming

Table 1
Average main features of the influent (IN), effluent (OUT) wastewater and removal efficiency of Caltanissetta WWTP.

Symbol	Description	Influent - IN [mg L⁻¹]	Effluent - OUT [mg L⁻¹]	Efficiency [%]
COD_{TOT}	Total Chemical Oxygen Demand	200	18	91
BOD_5	Biochemical Oxygen Demand	90	8	91
TN	Total Nitrogen	37	18	52
TP	Total Phosphorus	10	2	80
TSS	Total Suspended Solids	100	6	94
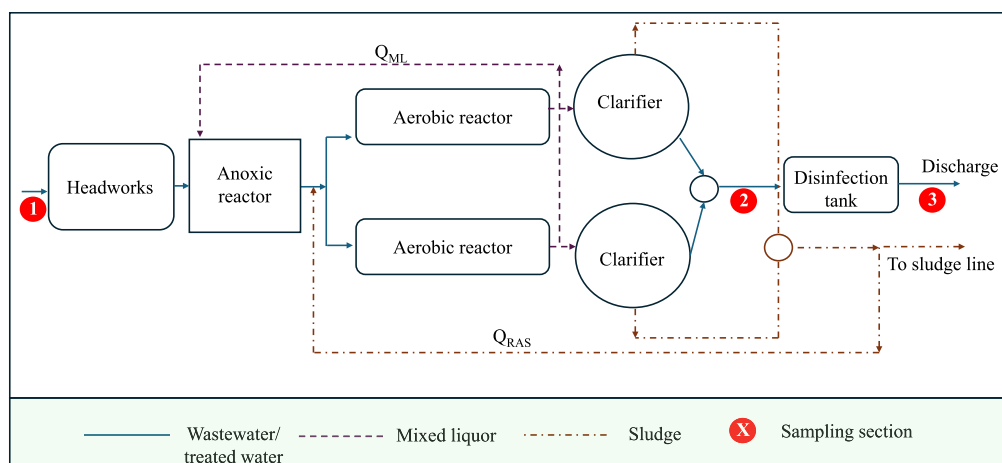
Fig. 1. Schematic layout of the water treatment line of Caltanissetta WWTP.

Table 2

Flow rates, tanks volumes and operating conditions of Caltanissetta WWTP.

Description	Symbol	Value	Unit
Influent flow rate	Q_{IN}	14,802	m^3h^{-1}
Return activated sludge flow rate (from secondary settler to aerobic reactor)	Q_{RAS}	17,500	m^3h^{-1}
Mixed liquor recirculated flow rate (from aerobic to anoxic reactor)	Q_{ML}	29,604	m^3h^{-1}
Anoxic reactor volume	V_{ANOX}	1500	m^3
Aerobic reactor volume	V_{AER}	2×2500	m^3
Secondary clarifier volume	V_{SET}	2×2200	m^3
Disinfection tank volume	V_{DIS}	1200	m^3
Sludge Retention Time	SRT	10	day
Food microorganism ratio	F/M	0.2	kgBOD/ kgTSS/day

Unit - CFU), and SARS-CoV-2 RNA (GC/L). For sample 3, the free chlorine was also analyzed. Moreover, for the entire experimental campaign duration, the water utility society operator (that is the WWTP operator) (Caltaqua Spa) provided the daily data of influent and effluent features in terms of total chemical oxygen demand (COD_{TOT}), 5 days biochemical oxygen demand (BOD_5), and total suspended solids (TSS), total nitrogen (TN) and total phosphorus (TP).

2.3. Analytical methods

2.3.1. SARS-CoV-2 analysis

In view of measuring SARS-CoV-2, the method proposed by Wu et al. [17] was adopted. This method considers three subsequent phases: i) virus concentration; ii) viral RNA extraction; iii) RT-qPCR amplification.

2.3.1.1. Virus concentration. Wastewater samples (45 mL) were centrifuged at 4500 ×g for 30 min; after centrifugation, 40 mL of sample were mixed with polyethylene glycol 8.000, 8 % (wt/vol) and NaCl (0.3 M) (both supplied by Sigma-Aldrich, St. Louis, MO, USA), spiked with a known amount of Murine Norovirus, used as process control. After a centrifugation step at 12,000 x*g* for 2 h, the viral pellet was resuspended in 2 mL of NucliSENS Lysis Buffer reagent (bioMerieux, Marcy-l'Étoile, France) for subsequent RNA extraction.

2.3.1.2. RNA extraction. RNA extraction was done using a semi-automated method with magnetic silica beads (supplied by bio-Merieux, Marcy l'Etoile, France). After an incubation step at room temperature for 20 min, 100 μL of magnetic silica beads were added, and after a further 10 min incubation, an automated procedure was performed by nucleic acid purification system (Auto-Pure96, All Sheng Instruments, Zhejiang, China).The extracted nucleic acids were then purified from potential PCR inhibitors using the OneStep PCR Inhibitor Removal Kit (Zymo Research, CA, USA).

2.3.1.3. RT-qPCR amplification. All RT-qPCR assays for SARS-CoV-2, targeting the ORF1b (nsp14), were conducted on the QuantStudio 6 and 7 Flex Real-Time PCR System (ThermoFisher Scientific) according to [18] with some modifications for quantification that was performed using 10-fold dilutions, ranging from 1.0 to 1.0×10^5 Genomic Copies (GC) per reaction, of a dsDNA SARS-CoV-2 provided by the National Institute of Health (NIH). RT-qPCR standard curves were generated by linear regression of cycle threshold (Ct) values versus log10 standard concentration and used to convert Ct values into ORF1b copies/μL per reaction. SARS-CoV-2 GC/L in wastewater was obtained according to the following formula: C (RNA GC/μL) x 100 (total volume of RNA of the extracted sample) x 25 (ratio factor between analyzed volume and reference volume of 1 L).

2.3.2. Escherichia Coli

To determine the Colony Forming Units (CFU) of *Escherichia coli*, Method F from the manual "Analytical Methods for Waters" [19] is used as the protocol. This method considers the adoption of Tryptone Bile X-Glucuronide Agar – TBX as growing media for *Escherichia coli* bacteria. Samples were first diluted and filtrated through 0.45 μm ester cellulose filters. After filtration, samples were incubated at 44 ± 1 °C for 24 h inside a Petri plate in contact with the growing media. After the incubation, the *Escherichia coli* bacteria are counted and quantified as CFU/100 mL.

2.3.3. Free chlorine

Free chlorine has been measured according to the method proposed by CNR-IRSA [19].

2.3.4. Online measured data

Data provided by the WWTP operator were acquired by using online probes (S::CAN, Vienna, Austria). Further, the hourly influent, effluent, and returned sludge flow rates were acquired by means of flow meters (MJK Automation, Denmark).

2.3.5. SARS-CoV-2 data

Sicilian COVID-19 cases, and their relevant clinical data, were recorded in the web-based integrated national surveillance platform established by the National Institutes of Health -NIH [20].

The aforementioned platform (https://www.epicentro.iss.it/en/coronavirus/sars-cov-2-dashboard) is public only for the aggregated date at national and regional level. The platform summarizes the number of confirmed SARS-CoV-2 infection reported in Italy in the last 30 days, the percentage of SARS-CoV-2 cases notified in Italy in the last 30 days grouped by age and the total number of cases per region and province of residence. SARS-CoV-2 positive patients were considered eligible if they met the following inclusion criteria: resident in Caltanissetta (or temporarily domiciled in Caltanissetta) and having a laboratory-confirmed SARS-CoV-2 positive result from nasal, pharyngeal, or naso-pharyngeal swabs, between 11 October 2021 and 26 July 2022.

Furthermore, in view of establishing the variants present during the monitoring period, data acquired by the NIH during the "flash survey" have been used here. Specifically, the NIH since October 2021 performed national "flash surveys" during which monthly sampling campaigns were done simultaneously in the WWTP under national study (including Caltanissetta) to assess the SARS-CoV-2 in wastewater.

2.3.6. Data analysis

Data from the web-based integrated national surveillance platform regarding SARS-CoV-2 positive patients have been elaborated in view of correlating measured SARS-CoV-2 RNA data with the total number of active infections and the daily new cases. The total number of active infections has been calculated as total positive swabs within 15 days of wastewater sampling data minus that declared negative, according to Maida et al. [10]. The daily number of new cases has been quantified as the sum of positive cases lagged two days after the measured SARS-CoV-2 RNA concentration, according to Swift et al. [16]. Data have been correlated by linear regression between log-transformed measured SARS-CoV-2 RNA concentrations, the total number of active infections and the daily new cases.

3. Results

3.1. WWTP performance – conventional pollutants

Fig. 2 shows the trend of influent (Section 1), effluent (Section 3), and removal efficiencies for COD_{TOT}, BOD_5, and TSS. Data from Fig. 2 shows that the plant is always able to achieve the standard limits imposed by the Italian Regulation [21]. This is also debited to the low influent load, especially for the organic compounds, as corroborated by
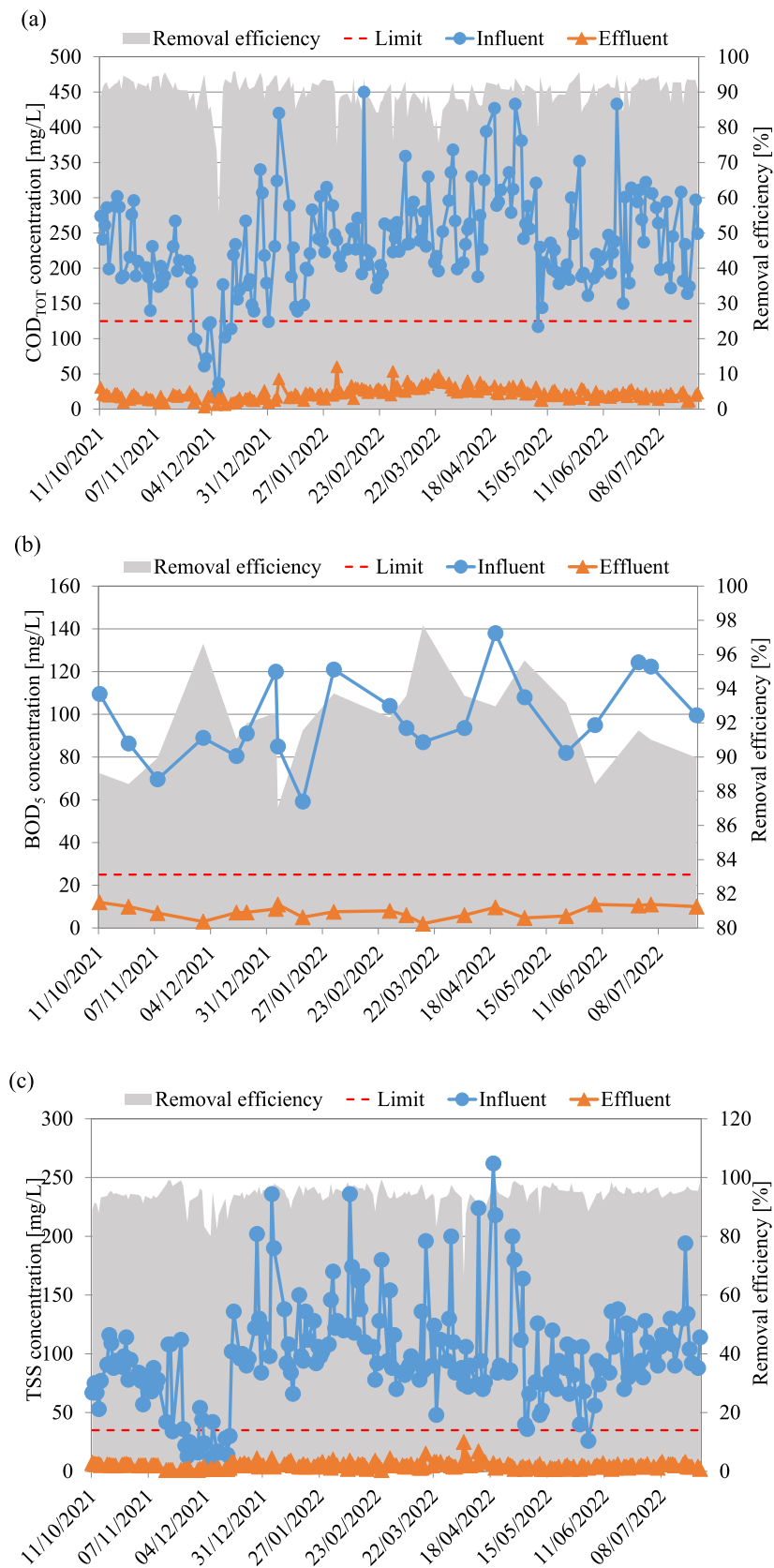
(a)

(b)

(c)

Fig. 2. Trend of influent, effluent and removal efficiency of COD_{TOT} (a), BOD_5 (b), and TSS (c).

the low influent F/M ratio. Fig. 2a shows that the influent COD_{TOT} ranges between 24 and 450 mg L^{-1} with an average value of 234 mg L^{-1}. As occurred for all influent pollutants, the minimum concentrations of conventional pollutants were achieved during rainy periods. The average removal efficiency obtained for COD_{TOT} was equal to 90 %. On average, the influent BOD$_5$ is almost 42 % of the influent COD_{TOT}, which is in good agreement with the typical civil wastewater features. On average, 92 % of the influent BOD$_5$ was removed inside the WWTP, with an average effluent concentration of 8 mg L^{-1} (Fig. 2b). TSS removal performance was also achieved, with an average removal efficiency of 94 % (Fig. 2c).

Fig. 3 shows the trend of influent (Section 1), effluent (Section 3), and removal efficiencies for TP and TN. Data from Fig. 3 shows that the average influent TP and TN are 5 mg L^{-1} and 37.4 mg L^{-1}, respectively. Although the plant was not designed for phosphorus removal, a relatively high removal efficiency occurred (on average 66 %) (Fig. 3a).

This result is likely debited to the growth of phosphors accumulating organisms (PAOs) under anoxic conditions favored by the relatively high amount of influent organic biodegradable compounds and the long

hydraulic retention time within the secondary settler (around 3 h). For TN, 55 % removal efficiency was achieved in the plant (Fig. 3a).

3.2. WWTP performance – SARS-CoV-2 and Escherichia coli

In Fig. 4, data of SARS-CoV-2 RNA load and *E. coli* concentrations are shown for influent wastewater (Section 1), secondary clarifier supernatant (Section 2), and treated disinfected wastewater (Section 3) samples. For Section 1, 100 % of samples resulted positive for SARS-CoV-2 RNA with a concentration ranging between 3.6×10^3 GC/L to 7.5×10^5 GC/L (average value 1.1×10^5 GC/L). The maximum SARS-CoV-2 RNA concentration was obtained during the wave of infection in mid-January (Fig. 4a). By analyzing the data of Fig. 4a, it is apparent that a significant reduction of SARS-CoV-2 RNA concentration occurred in samples of Section 2. Indeed, in Section 2, 24 % of samples resulted positive for SARS-CoV-2 with viral load ranging between 0.0 and 1.1×10^4 GC/L (average value 6.0×10^2 GC/L and average reduction compared to Section 1 of 99.45 %). This outcome indicates that the virus RNA could be damaged as a result of both biological and physical
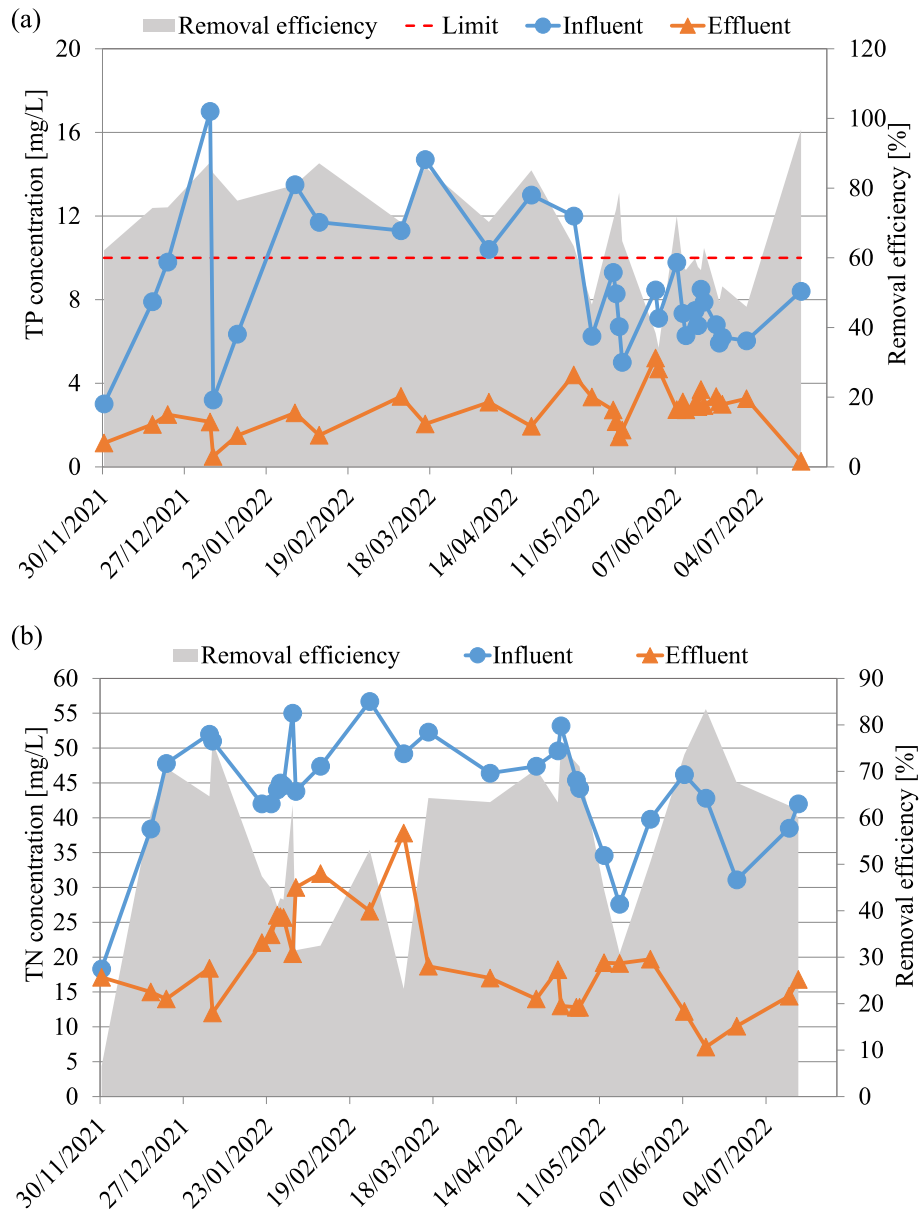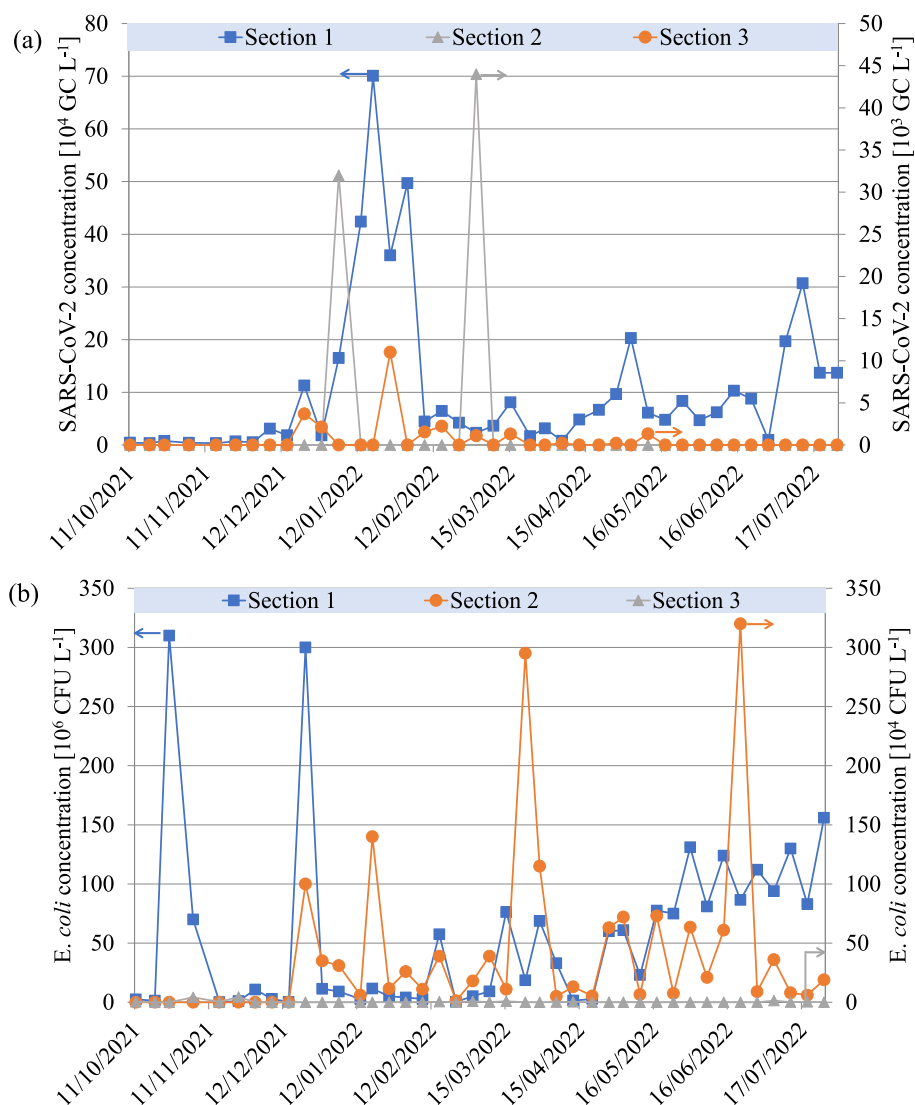

Fig. 3. Trend of influent, effluent and removal efficiency of TP (a) and TN (b).

Fig. 4. Trend of SARS-CoV-2 concentration (GC/L) of influent (Section 1), effluent of clarifier (Section 2), and disinfected effluent (Section 3) samples (a); trend of *E. coli* concentration (CFU/L) of influent (Section 1), effluent of clarifier (Section 2), and disinfected effluent (Section 3) samples (b). Read data of Section 1 on the left y-axes; read data of Section 2 and Section3 on the right y-axes.

processes taking place within the WWTP. Indeed, the activated sludge process represents a barrier for the genetic material of SAR-CoV-2. As suggested in the literature, the hydrophobic nature of SAR-CoV-2 favors its adsorption into large solids [22,23]. Consequently, SARS-CoV-2 virus is eliminated through the gravitational processes inside the settling tanks [24]. For Section 3, 4.5 % of samples were positive for SAR-CoV-2 RNA with concentration values ranging between 0.0 and 4.4×10^1 GC/L (average value 1.9 GC/L) (Fig. 4a). The substantial reduction of SAR-CoV-2 RNA concentration in section 3 is mainly due to its inactivation caused by the disinfection performed by using Sodium hypochlorite (NaOCl) [11]. Mousazadeh et al. [11] summarize several literature experiences demonstrating the inactivation effect of chemical agents such as sodium hypochlorite towards SARS-CoV-2 (among others, [25,26]).

Regarding *E. coli*, a high removal efficiency occurred inside the WWPT. On average 99 % and 100 % of removal efficiency were obtained between Section 1 and Section 2, and Section 1 and Section 3, respectively. *E. coli* concentration in Section 1 ranged between 2.0×10^5 CFU/ L to 3.1×10^8 CFU/L. The effluent concentration (section 3) of *E. coli* ranged between 0.0 and 4.5×10^4 CFU/L.

3.3. Comparison of measured SARS-CoV-2 with surveillance data

Fig. 5 shows the linear regression between log-transformed SARS-CoV-2 RNA concentration, daily new cases (calculated according to [16]) (Fig. 5 a-b), and the total number of active infections (calculated according to [10]) (Fig. 5 c-d). Specifically, according to Maida et al. [10] the total number of active infections represents the total positive swabs within 15 days minus that declared negative. While, according to Swift et al. [16] the number of active infections is the sum of positive cases lagged two days after the measured SARS-CoV-2 RNA concentration.

Important to precise is that the linear regressions of Fig. 5a and Fig. 5c were performed, including all data from 11st October 2021 to 26th July 2022. While the linear regressions of Fig. 5b and Fig. 5d were performed by dividing data into two groups one from 11st October 2021 to 21st December 2021 and the other one from 28th December 2021 to 26th July 2022 (Fig. 5 b and Fig. 5d). This is because national "flash surveys" data revealed the predominance of the Omicron variant from the end of December 2021 [9]. Specifically, national "flash surveys" data show the presence of the Omicron variant in December 2021, BA.1 Omicron sub-variant in January and February 2022, BA.2 Omicron sub-
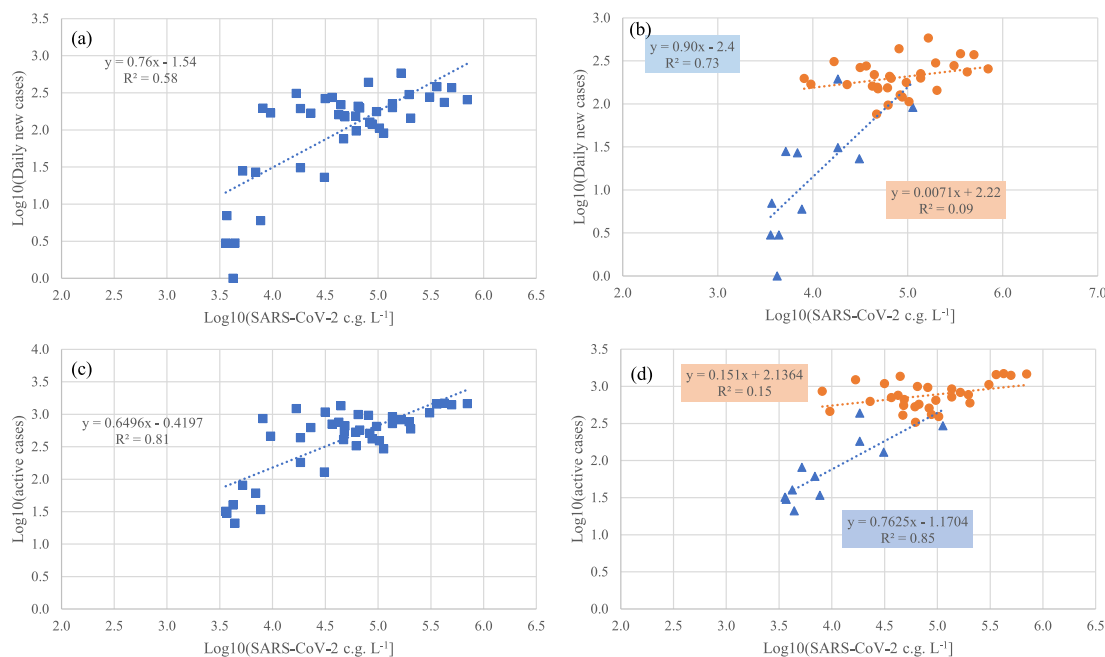
Fig. 5. Linear regression between log-transformed SARS-CoV-2 RNA concentration, daily new cases (calculated according to [16]) considering all data from 11st October 2021 to 26th July 2022 (a) and considering data divided into groups from 11st October 2021 to 21st December 2021 (blue triangle) and from 28th December 2021 to 26th July 2022 (orange circle) (b), and the total number of active infections (calculated according to [10]) considering all data from 11st October 2021 to 26th July 2022 (c) and considering data divided into groups from 11st October 2021 to 21st December 2021 (blue triangle) and from 28th December 2021 to 26th July 2022 (orange circle) (d). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

variant from March to June 2022, and sub-variant BA.4/5 in July 2022; thus, revealing a rapid spread of the Omicron variant and sub-variants in the population. Data from Fig. 5a show a good relationship between daily new cases and SARS-CoV-2 with a linear regression coefficient of log-transformed data equal to 0.58 (*p-value* < 0.001). However, as reported in Fig. 5b, the correlation between daily new cases and SARS-CoV-2 could depend on the spread of the Omicron variant and sub-variants. Indeed, the correlation performed by considering only the data acquired from 11st October 2021 to 21st December 2021, shows a correlation coefficient equal to 0.73 (*p-value* < 0.012) (with a correlation coefficient very low and equal to 0.09 for the period from 28th December 2021 to 26th July 2022) (Fig. 5b).

Data from Fig. 5c-d show a significant improvement in the results when the new cases are replaced with the total active cases calculated according to Maida et al. [10]. Indeed, the correlation between active cases and SARS-CoV-2 shows, in this latter case, a correlation coefficient of 0.81 (*p-value* < 0.001), considering all acquired data (Fig. 5c). When the total active cases are taken into account, the correlation coefficient increases compared to that presented in Fig. 5d. In particular, the correlation coefficient increased to 0.15 (*p-value* < 0.03) from 28th December 2021 to 26th July 2022, and to 0.85 (*p-value* < 0.003) from 11st October 2021 to 26th July 2022 (Fig. 5d). These results suggest that it is better to correlate surveillance data with the total number of active cases, especially when variants can rapidly spread even without symptoms (e.g., Omicron). Important to precise is that the different correlation of the log-transformed data during the period with the Omicron variant could also due to the different capability of SARS-CoV-2 to be transferred in the wastewater depending on the sub-variants and to the different temperatures. Indeed, during the Omicron period (from December 28th 2021, to 26th July 2022) the months with warm temperatures occurred (e.g., May, June and July with average temperature of 22 °C, 26 °C and 27 °C respectively). Literature suggests that with the increase of temperature from 4 °C to 10 °C the decay rate of SARS-CoV-2 RNA doubles [27].

For sake of completeness, Table 3 summarizes the results of the statistical analysis (namely, R^2, *p*-value, standard error and T – test two

Table 3
Results of the statistical analysis.

	Period		
	11st Oct. 2021- 26th Jul. 2022	11st Oct. 2021- 21st Dec. 2021 (blue triangle)	28th Dec.2021 - 26th Jul. 2022 (orange circle)
	According to Swift et al. [16]		
R^2	0.58	0.73	0.09
p-value	6.51E-08	0.01226	0.12920
Standard error	0.43	0.34	0.46
T - test	2.33E-31	1.32E-09	3.30E-27
	According to Maida et al. [10]		
R^2	0.81	0.85	0.15
p-value	2.34E-08	0.00280214	0.031622317
Standard error	0.4	0.29	0.45
T - test	1.54E-26	7.98E-10	4.98E-23

tailed) in view of providing information on the statistical significance of correlations reported in Fig. 5.

4. Conclusions

The study summarizes 288 days of monitoring SARS-CoV-2, *E. coli,* and conventional pollutants removal of Caltanissetta (Italy) WWTP.

The key results revealed that:

✓ SARS-CoV-2 RNA was found in 100 % of analyzed influent wastewater samples;
✓ SARS-CoV-2 inactivation occurs inside the activated sludge process sections of the WWTP, likely due to the capability of the virus to be adsorbed into large solids;
✓ SARS-CoV-2 log-transformed concentrations strongly correlate with active cases calculated with 15 lag days; the correlation coefficients strongly reduce if the new cases are considered;

✓ The correlation between SARS-CoV-2 log-transformed concentrations could depend on the presence of the Omicron variants, revealing the importance of detailed analytical information;

✓ WBE is revealed as a powerful complementary tool for classical clinical surveillance.

CRediT authorship contribution statement

Alida Cosenza: Conceptualization, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Carmelo Massimo Maida:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing – review & editing. **Marta Vullo:** Formal analysis, Visualization. **Giovanni Casamassima:** Data curation, Validation. **Gaspare Viviani:** Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing.

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.

Data availability

Data will be made available on request.

Acknowledgements

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