Surveillance of SARS-CoV-2 in urban wastewater in Italy
1° Report
(Study period: 01 October 2021 - 31 March 2022)

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Main findings:

- Report on the first six months (01 October 2021 - 31 March 2022) of SARS-CoV-2 surveillance in urban wastewaters in Italy.
- As on 31 March 2022, 19/21 Regions/Autonomous Provinces are actively producing data within the environmental surveillance program and the environmental network includes a total of 168 wastewater treatment plants throughout Italy.
- A total of 3.797 wastewater samples have been analysed so far and 84.6% of them showed the presence of SARS-CoV-2 RNA.
- The national trend of SARS-CoV-2 concentrations in wastewater over the six-month period, represented using Quiver graphs, showed two waves, a major one peaking in January and a minor one peaking in March, mirroring the two Omicron waves recorded to date.
- The data analysis confirms that the environmental surveillance approach can be successfully integrated with the tools used for COVID-19 surveillance, although additional validation steps are needed. Thus, it is not currently used for public health decision-making processes.

Introduction

Faecal shedding of SARS-CoV-2 by infected individuals (symptomatic and asymptomatic) has been established early into COVID-19 pandemic[1]. As a consequence, SARS-CoV-2 can be detected in wastewater, enabling its use for different purposes: (i) as an early warning system capable of predicting COVID-19 outbreaks days before clinical cases; (ii) as a tool capable of establishing trends in current outbreaks; (iii) to estimate the prevalence of infections; and (iv) to study SARS-CoV-2 genetic diversity[2] . Therefore, wastewater surveillance can be a reliable indicator for supporting prevention and management strategies of the pandemic.

On 14th February 2022 WHO published an interim guidance for public health surveillance for COVID-19[3] highlighting the usefulness of environmental surveillance to detect unrecognized transmission and provide an additional source of information to support decision-making about whether to adjust public health and social measures.

Italy was one of the first EU countries searching for SARS-CoV-2 RNA in urban wastewaters. Viral RNA detection was first accomplished in areas of both high (Milan) and low (Rome) epidemic

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[1] Zhang Y, Chen C, Zhu S, Shu C, Wang D, Song J, et al. Isolation of 2019-nCoV from a stool specimen of a laboratory-confirmed case of the coronavirus disease 2019 (COVID-19). China CDC Wkly. 2020;2(8):123–4. https://doi.org/10.46234/ccdcw2020.033.

[2] Bonanno Ferraro G, Veneri C, Mancini P, Iaconelli M, Suffredini E, Bonadonna L, Lucentini L, Bowo-Ngandji A, Kengne-Nde C, Mbaga DS, Mahamat G, Tazokong HR, Ebogo-Belobo JT, Njouom R, Kenmoe S, La Rosa G. A State-of-the-Art Scoping Review on SARS-CoV-2 in Sewage Focusing on the Potential of Wastewater Surveillance for the Monitoring of the COVID-19 Pandemic. Food Environ Virol. 2021 Nov 2:1–40. doi: 10.1007/s12560-021-09498-6. Epub ahead of print. PMID: 34727334; PMCID: PMC8561373.

[3] https://www.who.int/publications/i/item/WHO-2019-nCoV-SurveillanceGuidance-2022.1

circulation between February and May 2020[4]. In July 2020 a pilot study, the "SARI project" (Epidemiological Surveillance for SARS-CoV-2 in urban sewage), coordinated by Istituto Superiore di Sanità (ISS), was launched in Italy[5] and a national network was built on a voluntary basis with the cooperation of Regions, Autonomous Provinces, wastewater service providers, regional environmental protection agencies, local health authorities, zooprophylactic institutes (IZS), universities, and research institutions.
On 17th March 2021, the "EU Commission Recommendation 2021/472 on a common approach to establish a systematic surveillance of SARS-CoV-2 and its variants in wastewaters in the EU", strongly encouraged Member States to put in place, no later than the 1st October 2021, national wastewater surveillance systems aimed at the collection of data on SARS-CoV-2 and its variants[6]. For the implementation of the above EU Recommendation, an Italian governmental funding was granted (Decree Law n. 73 of 25.05.2021, art. 34).
Since October 2021, existing research activities within the SARI project were transformed into a surveillance system, coordinated by ISS.

Aim

The aim of this report is to summarize the activities and the results of the first six months of SARS-CoV-2 environmental surveillance in Italy, covering the period between 01 October 2021 and 31 March 2022.
The present report focuses on the trend analysis of SARS-CoV-2 detection in urban wastewaters over time, as a descriptor of the dynamic of excretion of the virus in human faeces and, therefore, of the epidemiological trends in the population. The second main goal of the environmental surveillance, the study of SARS-CoV-2 variants spread over time, is accomplished through regular monthly national "flash surveys", systematically published on the ISS official website[7] since October 2021.

Methodology

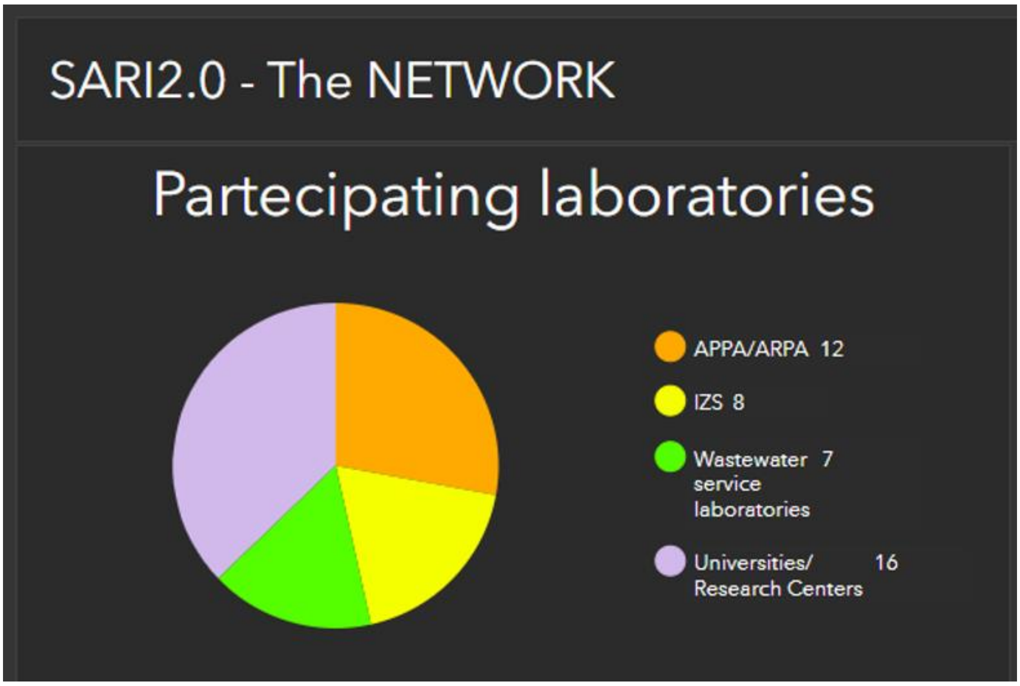
Enrolled Regions/Autonomous provinces

All the 21 Italian Regions/Autonomous Provinces (A.P.) have officially joined the national surveillance system, however, as on the 31st March 2022, only 19 Italian Regions/A.P. are actively producing data within the environmental surveillance program. Concerning the two missing Regions, Calabria and Sardinia, the Region of Calabria is going to start the systematic analytical activities in April 2022, while the Region of Sardinia has not yet provided an operational plan to activate the surveillance.

[4] La Rosa G, Iaconelli M, Mancini P, Bonanno Ferraro G, Veneri C, Bonadonna L, Lucentini L, Suffredini E. First detection of SARS-CoV-2 in untreated wastewaters in Italy. Sci Total Environ. 2020 Sep 20;736:139652. doi: 10.1016/j.scitotenv.2020.139652. Epub 2020 May 23. PMID: 32464333; PMCID: PMC7245320.
[5] ISS, al via la rete 'sentinella' di sorveglianza epidemiologica del coronavirus nelle acque reflue (salute.gov.it)
6 Commission Recommendation (EU) 2021/472 of 17 March 2021 on a common approach to establish a systematic surveillance of SARS-CoV-2 and its variants in wastewaters in the EU. (https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32021H0472&qid=1628798981209)
[7] https://www.iss.it/cov19-acque-reflue

Each Region/A.P. participating to the surveillance has selected and designated the laboratories for analytical activities. The laboratory network includes APPA/ARPA (regional/local environmental protection agencies), IZS, Universities and other research institutions, and wastewater service providers, for a total of 43 Laboratories (Figure 1).

Figure 1. Laboratories participating to the surveillance network



Sampling sites and frequency

In agreement with EU Commission Recommendation 2021/472, the monitoring network includes Wastewater Treatment Plants (WTPs) located in all urban centers with more than 150.000 inhabitants. Based on the data of the Italian National Institute of Statistics (http://dati.istat.it/Index.aspx?QueryId=18460), 25 urban centres with that size are present in Italy, accounting for approximately 11.5 million inhabitants (≈19% of the national population), distributed among 13 of the 21 Italian Regions/A.P.

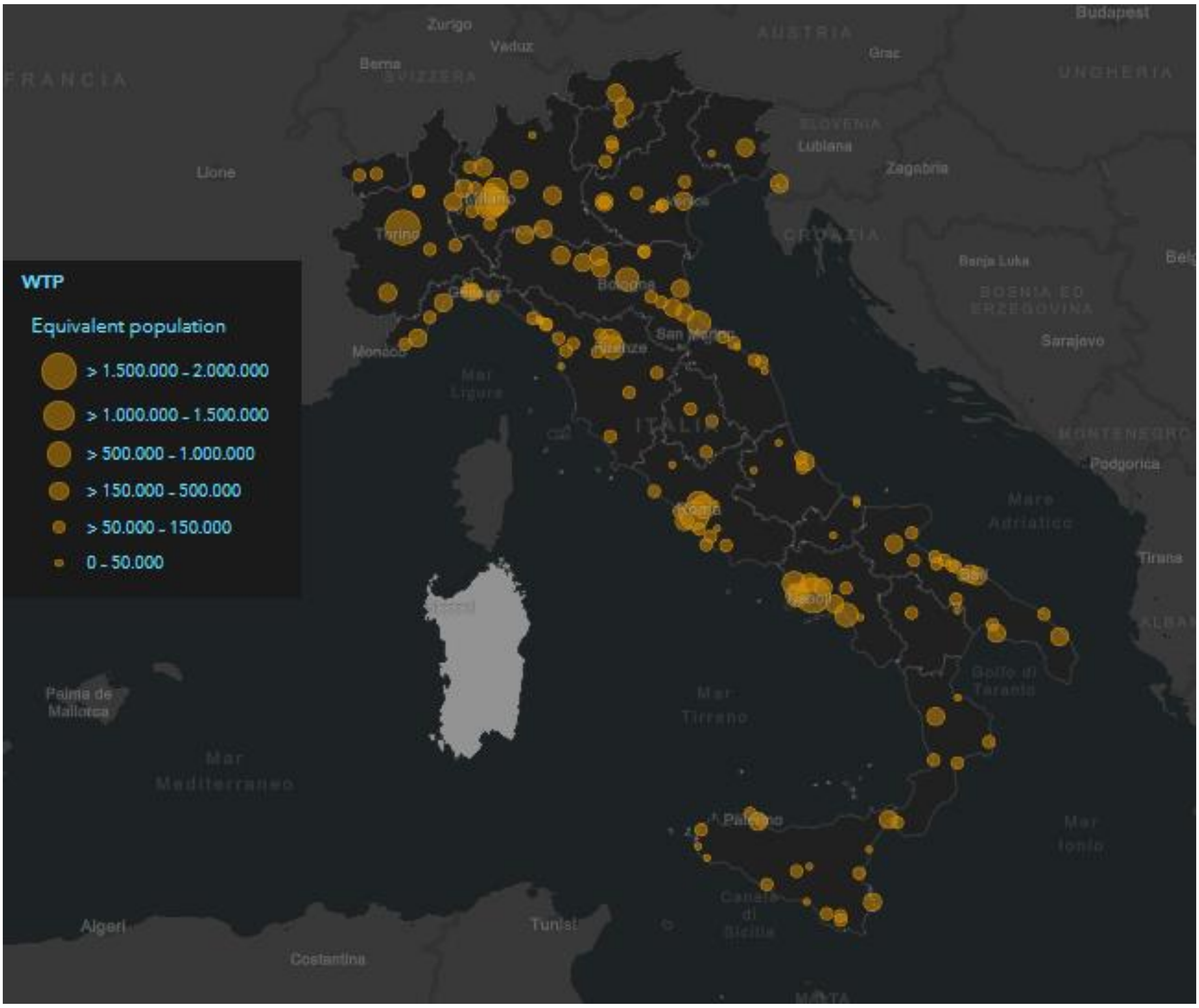
Considering the distribution of the Italian population in medium-size centres, urban centres with a population between 50k and 150k inhabitants were added to the sampling network to improve both the population and territorial coverage and the level of detail compared to the minimum requirements of Rec. 2021/472. According to the data of the Italian National Institute of Statistics, this led to the inclusion of additional 122 urban centres. Moreover, additional sampling points (smaller municipalities with less than 50k inhabitants) were included in the surveillance based on the evaluation of Regions/A.P., due to their touristic relevance.
This resulted in the inclusion of a total of **167 WTPs** within the environmental surveillance network (see Figure 1 and Table 1), serving a total of 31.734.984 population equivalent[8]. The WTPs serving urban centres with more than 150k inhabitants are monitored twice per week as per Rec. 2021/472,

[8] Parameter describing the design treatment capacity of WTPs. It is a measure of total organic biodegradable load in a WTP, including industrial, commercial and domestic organic load, converted to the equivalent number of population (population equivalents)

while WTPs collecting wastewaters from centres with a population between 50k and 150k are monitored once per week.

Since October 2021, both the number of sampling points and of samples tested per week progressively increased, as the Regions/A.P. gradually activated the systematic sampling from the different WTPs over the first six months of surveillance. As on 31 March 2022, the **samples are regularly collected from 154 of the 167 WTPs.** These remaining WTPs points include the ones currently being activated and the small centres sampled only during the touristic season. This sampling scheme results in **approximately 200 samples per week**.

Figure 2. WTPs sampling points: 167 WTPs throughout Italy



The size of each dot is scaled on the population equivalent of the WTP

Table 1. Sampling sites and characteristics of the WTPs studied

Region /A. P.	Metropolitan City	WTP	Population equivalent‡
Abruzzo	Chieti	S. Martino	114.500
	Pescara	Via Raiale	160.000
	Pescara	Montesilvano - Villa Carmine	140.000
	L'Aquila	Pile	48.000
	Teramo	Villa Pavone	41.824
Basilicata	Potenza	Tiera di Vaglio	95.000
	Matera	Pantano	24.000
Calabria	Crotone	Crotone - località Papaniciaro	60.000
	Cosenza	Cosenza - Code di volpe	191.000
	Catanzaro	Catanzaro - Zona industriale	120.000
	Cosenza	Cosenza - Sant'Angelo	45.000
	Reggio di Calabria	Ravagnese - località Aeroporto	120.000
	Catanzaro	Catanzaro Lido - Loc. Verghello	90.000
Campania	Salerno	Salerno	700.000
	Eboli	Eboli	30000
	Salerno	Nocera Sup	299.121
	Avellino	Manocalzati	140.000
	Napoli	Napoli EST	1.750.000
	Napoli	Area Nolana	400.000
	Napoli	Napoli OVEST - ex ingresso Camaldoli	250.000
	Napoli	Napoli OVEST - Ingresso Principale	950.000
	Caserta	Villa Literno	631.714
	Caserta	Area Casertana	370.769
Emilia-Romagna	Reggio Emilia	Mancasale	280.000
	Parma	Parma ovest	168.000
	Bologna	IDAR	800.000
	Modena	Naviglio	500.000
	Ferrara	Ferrara - Linea 1	120.000
	Ferrara	Ferrara - Linea 2	120.000
	Modena	Carpi	200.000
	Piacenza	Borgoforte	163.333
	Forlì-Cesena	Cesena	197.500
	Forlì-Cesena	Forlì	250.000
	Ravenna	Faenza	100.000
	Bologna	Imola	75.000
	Ravenna - Forlì-Cesena	Ravenna	240.000
	Rimini - Forlì-Cesena	S. Giustina	560.000
Friuli-Venezia Giulia	Udine	Udine	200.000
	Pordenone	Cordenons	15.000
	Trieste	Servola	190.000
Lazio	Viterbo	Viterbo - Strada Bagni	30.000
	Roma	Guidonia-Ponte Lucano	50.000
	Roma	Pomezia - Via Cincinnato	60.000

	Roma	Velletri (LA CHIUSA-SORBO)	36.700
	Roma	Anzio - Colle Cocchino	75.000
	Latina	Aprilia (Via del Campo)	66.000
	Latina	Latina Loc Latina Est	90.000
	Roma	Civitavecchia Fiumaretta	86.400
	Roma	Roma Est (linea 1 + 2)	900.000
	Roma	Roma Nord	780.000
	Roma	Roma Sud	1.100.000
	Roma	Ostia	350.000
	Fiumicino	Fregene	76.000
Liguria	Savona	Savona	256.203
	Genova	Pegli	20.507
	Genova	Voltri	40.496
	Genova	Quinto	48.748
	Genova	Rapallo	90.000
	Genova	Sestri P	51.368
	Genova	Sturla	43.573
	Savona	Borghetto Santo Spirito	140.000
	La Spezia	Camisano	40.840
	La Spezia	Silea	17.500
	La Spezia	La Spezia	82.000
	Imperia	Sanremo - località Capo Verde	80.000
	Imperia	Imperia	160.000
	Genova	Darsena	118.276
	Genova	Punta Vagno Genova	75.000
	Genova	Valpolcevera	157.650
Lombardia	Milano - Varese	Lonate Pozzolo	450.000
	Milano - Varese	Canegrate	137.950
	Varese	Varese	74.402
	Milano - Monza e Brianza	Peschiera Borromeo	566.000
	Milano	Bresso	220.000
	Milano	Milano Nosedo	1.250.000
	Milano	Milano San Rocco	1.036.000
	Como	Como	196.000
	Pavia	Pavia	132.912
	Bergamo	Bergamo	220.000
	Como - Lecco - Milano - Monza e della Brianza	Monza	600.000
	Sondrio	Sondrio	49.500
	Pavia	Vigevano	57.925
	Cremona	Citta di Cremona	180.000
	Brescia	Verziano	296.000
Marche	Pesaro-Urbino	Borgheria	116.000
	Pesaro-Urbino	Ponte Metauro	60.000
	Pesaro-Urbino	Ponte Sasso	18.000
	Ancona	Zipa	100.000
	Ancona	Falconara	85.000

	Ancona	Camerano	33.000
Molise	Campobasso	Campobasso - San Pietro	50.000
	Campobasso	Termoli - località Porto	25.000
	Campobasso	Termoli - località Pantano Basso	25.000
Piemonte	Torino	Castiglione Torinese	1.934.099
	Biella	Biella Nord	67.000
	Biella	Biella Sud	53.000
	Novara	Novara	184.000
	Cuneo	Cuneo	185.000
	Asti	Asti	95.000
	Alessandria	Alessandria	110.000
Puglia	Bari	Altamura	95.414
	Brindisi	Brindisi Fiume Grande	93.013
	Lecce	Lecce	195.368
	Taranto	Taranto Bellavista	116.723
	Taranto	Taranto Gennarini	226.667
	Foggia	Cerignola	56.355
	Foggia	Foggia	208.000
	Foggia	Manfredonia	77.000
	Bari	Molfetta	84.803
	Barletta-Andria-Trani	Andria	130.000
	Barletta-Andria-Trani	Barletta	129.356
	Barletta-Andria-Trani	Bisceglie	85.714
	Barletta-Andria-Trani	Trani	83.667
	Bari	Bari Ovest	360.000
	Bari	Bari Est	389.000
	Bari	Bitonto	79.332
Sicilia	Agrigento	Agrigento	55.000
	Enna	Enna	34.000
	Ragusa	Modica	50.400
	Ragusa	Ragusa	98.000
	Ragusa	Vittoria	55.000
	Palermo	Bagheria	75.000
	Caltanissetta	Caltanissetta e San Cataldo	76.700
	Palermo	Acqua dei Corsari	314.973
	Palermo	Fondo Verde	53.886
	Caltanissetta	Gela Macchitella	12.000
	Messina	Mili Marina	227.000
	Trapani	Trapani	118.500
	Trapani	Mazara del Vallo	17.000
	Trapani	Marsala	40.000
	Catania	Pantano d'Arci	68.434
	Catania	Giarre	47.600
	Siracusa	Siracusa	180.000
Toscana	Siena	Ponte a Tressa	99.000
	Grosseto	San Giovanni - Pianetto	100.000
	Prato	Baciacavallo	900.000

	Arezzo	Casolino - San Leo	90.000
	Pistoia	Centrale Pistoia	120000
	Livorno	Rivellino	21.000
	Lucca	Pontetetto	95.000
	Pisa	Pisa Nord - S. Jacopo	52.000
	Firenze	Empoli Pagnana	88.670
	Firenze	San Colombano	600.000
	Massa	Lavello 2	120.000
	Lucca	Viareggio	93.000
	Massa	Lavello 1	87.000
Umbria	Perugia	Perugia - Pian della Genna	90.000
	Perugia	Foligno Casone	90.000
	Terni	Terni	150.000
Valle d'Aosta	Aosta	La Salle	60.000
	Aosta	Brissogne	150.000
Veneto	Padova	Padova Ca' Nordio - centro storico	98.500
	Padova	Padova Ca' Nordio - zip	98.500
	Padova	Padova Guizza	13.000
	Padova	Abano Terme	35.000
	Treviso	Treviso	70.000
	Venezia	Venezia Fusina	400.000
	Vicenza	Vicenza Casale	92.000
	Verona	Verona_collettore 1M	82.000
	Verona	Verona_collettore 3M	102.000
	Verona	Verona_collettore 8M	226.000
A.P. Bolzano	Bolzano	IDA Bolzano	372.410
	Bolzano	IDA Merano	356.520
	Bolzano	IDA Termeno	68.945
A.P. Trento	Trento	Trento nord	120.000
	Trento	Trento sud	100.000
	Trento	Rovereto	95.000

[ǂ] Parameter describing the design treatment capacity of WTPs. It is a measure of total organic biodegradable load in a WTP, including industrial, commercial and domestic organic load, converted to the equivalent number of population (population equivalents)

Analytical Methods

The analytical protocols for determining SARS-CoV-2 concentrations in wastewaters, elaborated by ISS, were shared with the laboratories (https://doi.org/10.5281/zenodo.5758725)[9]. The reference materials for the protocol implementation (standard DNA for quantification, process control for recovery, RNA inhibition control) were also produced by ISS and sent to all the SARI network participants, to ensure comparability of results. Briefly, composite 24 h wastewater samples are

[9] Protocollo della Sorveglianza di SARS-CoV-2 in reflui urbani (SARI) - rev. 3 [Technical protocol (including analytical methods) of the national SARI project (Surveillance of SARS-CoV-2 in wastewaters in Italy)]. Doi: 10.5281/zenodo.5758725

collected at the inlet of WTP and delivered under refrigerated condition to the reference laboratories, where samples are concentrated using polyethylene glycol (PEG) precipitation by centrifugation[10]. After genome extraction, purified RNAs are subjected to a quantitative real time RT-PCR to assess SARS-CoV-2 concentration, expressed in genome copies/L. Analytical calculations are done using a standardized spreadsheet[11]. Analytical data and samples metadata are uploaded to the national database (SARI 2.0, see section below). Quantitative data are normalized for the WTP flow rate at sampling time (to account for fluctuations of the precipitations) and for the population equivalent of the WTP (to account for the different sizes of the contributing population). To ensure data quality, a proficiency test for all laboratories participating to the surveillance network was organized in September 2021, and another control sample (currently under investigation) was distributed in March 2022, to assist laboratories in assessing their performance.

Data collection and visualization: GIS database SARI 2.0

As a part of the SARI project, the National Centre for Innovative Technologies for Public Health in ISS developed a system based on GIS technology for a centralized collection of data provided by regional authorities, creating the dashboards for real-time data visualization. The database (current version, SARI 2.0) includes:

- Facilities/laboratories of the network, according to their role (sample collection/analysis) and the laboratories and Regional/A.P. coordinators (name, contact)
- Data associated with WTPs sampling points (location, catchment area, equivalent inhabitants, sampling frequency, etc.)
- Sample unique ID assignment, including associated sampling point and collection date, analytical data (procedures, laboratory involved, starting and closing dates of analyses, etc.), SARS-CoV-2 detection and quantification (raw amount and normalized loads accounting for WTP flow rate and population equivalent), and other accessory information.

The laboratory coordinators and the regional managers have direct access to the dashboards containing the results obtained at regional level. To ensure data management harmonization, a detailed manual ("User manual for data entry in the SARI.2.0 database") was elaborated and shared with the network to detail sampling, data entry and data management[12].
As a part of the continuous improvement of wastewater surveillance at European level and in agreement with the mandate of Rec. 2021/472, IT requirements for the interoperability of the SARI 2.0 database with the DEEP platform (Digital European Exchange Platform; https://wastewater-observatory.jrc.ec.europa.eu/) under development, have also been tested and verified between February and March 2022.

[10] Wu F, Zhang J, Xiao A, Gu X, Lee WL, Armas F, Kauffman K, Hanage W, Matus M, Ghaeli N, Endo N, Duvallet C, Poyet M, Moniz K, Washburne AD, Erickson TB, Chai PR, Thompson J, Alm EJ. SARS-CoV-2 Titers in Wastewater Are Higher than Expected from Clinically Confirmed Cases. mSystems. 2020 Jul 21;5(4): e00614-20. doi: 10.1128/mSystems.00614-20. PMID: 32694130; PMCID: PMC7566278.
[11] Foglio_di_lavoro_Progetto_SARI_Protocollo_rev3_25.07.2021. Doi: 10.5281/zenodo.5950147
[12] Technical protocol for data entry and data management of the national SARI project (Surveillance of SARS-CoV-2 in wastewaters in Italy). Doi: 10.5281/zenodo.5950261.

Trend Analysis

An approach to study trends has been developed using Quiver graphs (see Figure 5) to highlight the trends of viral concentrations over time and represent the direction (increase / stationary / decrease) and intensity of the changes in concentrations weekly.

Data downloaded from the SARI national database were processed to assess trends of SARS-CoV-2 viral loads in wastewater. In accordance with the European Recommendation 2021/472, for each WTP results were normalized for both the flow rate and the population equivalents (genome copies/day*inhabitant). Then, for each Region/A.P., a weekly average of the daily SARS-CoV-2 genomic copies, weighted for the equivalent inhabitants, was calculated considering all the sampled WTPs of the respective geographic area. The same procedure was performed at country level (i.e. weekly average of the ~150 WTPs sampling points), in order to provide a description of trends at national level. The weekly averages were used to estimate trends through a Smoothing Spline function[13].

Changes over time were assessed through the computation of the weekly variance percentage on the fitted values and were represented by different coloured arrows in the Quiver graphs. The green arrows represent a decrease in the number of viral genomic copies, the orange horizontal arrows represent a stationary phase, and (light to dark) the red arrows represent an increase in the concentration of the genomic copies.

Results

The data on SARS-CoV-2 concentrations in wastewater were produced by the SARI network laboratories (see Acknowledgement section). During the last six months (1 October 2021 - 31 March 2022), **3.864 wastewater samples** have been collected. As on 31st of March 2022, analyses have been completed on 3.797 samples, and **3.211** of them **(84.6%) tested positive** for SARS-CoV-2 RNA. Table 2 summarises the number of samples collected monthly by each Region/A.P. over the investigated period.

[13] D.S.G. Pollock, Chapter 11 - Smoothing with Cubic Splines. Editor(s): D.S.G. Pollock, In: Signal Processing and its Applications, Handbook of Time Series Analysis, Signal Processing, and Dynamics; Academic Press, 1999, Pages 293-322.

Table 2. Samples collected in each Region/A.P. between 1 October 2021 and 31 March 2022

Region/A.P	Oct. 2021	% pos	Nov. 2021	% pos	Dec. 2021	% pos	Jan. 2022	% pos	Feb. 2022	% pos	Mar. 2022	% pos	Total sampled	Total analysed	Total pos	% pos
Abruzzo	20	10%	25	16%	20	0%	20	20%	20	55%	25	40%	**130**	**130**	**31**	**23.8%**
Basilicata	7	14%	8	88%	8	63%	6	100%	8	100%	8	100%	**45**	**45**	**35**	**77.8%**
Campania	48	60%	58	67%	51	82%	60	87%	71	83%	70	99%	**358**	**358**	**290**	**81.0%**
Emilia-Romagna	24	75%	56	68%	68	88%	70	93%	76	91%	81	84%	**375**	**375**	**318**	**84.8%**
Friuli-Venezia Giulia	12	42%	12	83%	12	100%	11	100%	12	100%	12 [b]	100%	**71**	**70**	**61**	**87.1%**
Lazio	36†	89%	35	69%	52	87%	59	98%	52	100%	53 [c]	100%	**287**	**286**	**263**	**92.0%**
Liguria	72	11%	87	45%	75	96%	67	100%	72	100%	55	98%	**428**	**428**	**312**	**72.9%**
Lombardia	38	79%	41	88%	40	98%	43	100%	90	96%	104	96%	**356**	**356**	**334**	**93.8%**
Marche	-	-	19	79%	24	100%	24	96%	24	96%	30	93%	**121**	**121**	**113**	**93.4%**
Molise	-	-	6	17%	9	0%	12	25%	12	67%	15	20%	**54**	**54**	**15**	**27.8%**
Piemonte	12	58%	13	77%	26	100%	35	100%	32	94%	36 [d]	92%	**154**	**142**	**130**	**91.5%**
Puglia	13	69%	13	38%	18	100%	64	100%	73 [a]	100%	94 [e]	100%	**275**	**233**	**221**	**94.8%**
Sicilia	13	54%	40	80%	51	92%	53	100%	84	89%	97 [f]	95%	**338**	**328**	**297**	**90.5%**
Toscana	22	41%	25	52%	23	43%	20	70%	29	62%	27	70%	**146**	**146**	**83**	**56.8%**
Umbria	-	-	-	-	7	100%	16	100%	16	100%	19	100%	**58**	**58**	**58**	**100.0%**
Valle d'Aosta	18	61%	16	75%	18	100%	18	100%	16	94%	17	100%	**103**	**103**	**91**	**88.3%**
Veneto	44	93%	51	98%	48	100%	44	100%	44	100%	56 [g]	100%	**287**	**286**	**282**	**98.6%**
A.P. Bolzano	16	100%	18	100%	16	100%	24	100%	24	100%	24	100%	**122**	**122**	**122**	**100.0%**
A.P. Trento	24	96%	27	100%	27	100%	27	100%	24	100%	27	100%	**156**	**156**	**155**	**99.4%**
Total	**419**	**59.2%**	**550**	**69.1%**	**593**	**87.0%**	**673**	**93.2%**	**779**	**92.3%**	**850**	**92.1%**	**3.864**	**3.797**	**3.211**	**84.6%**

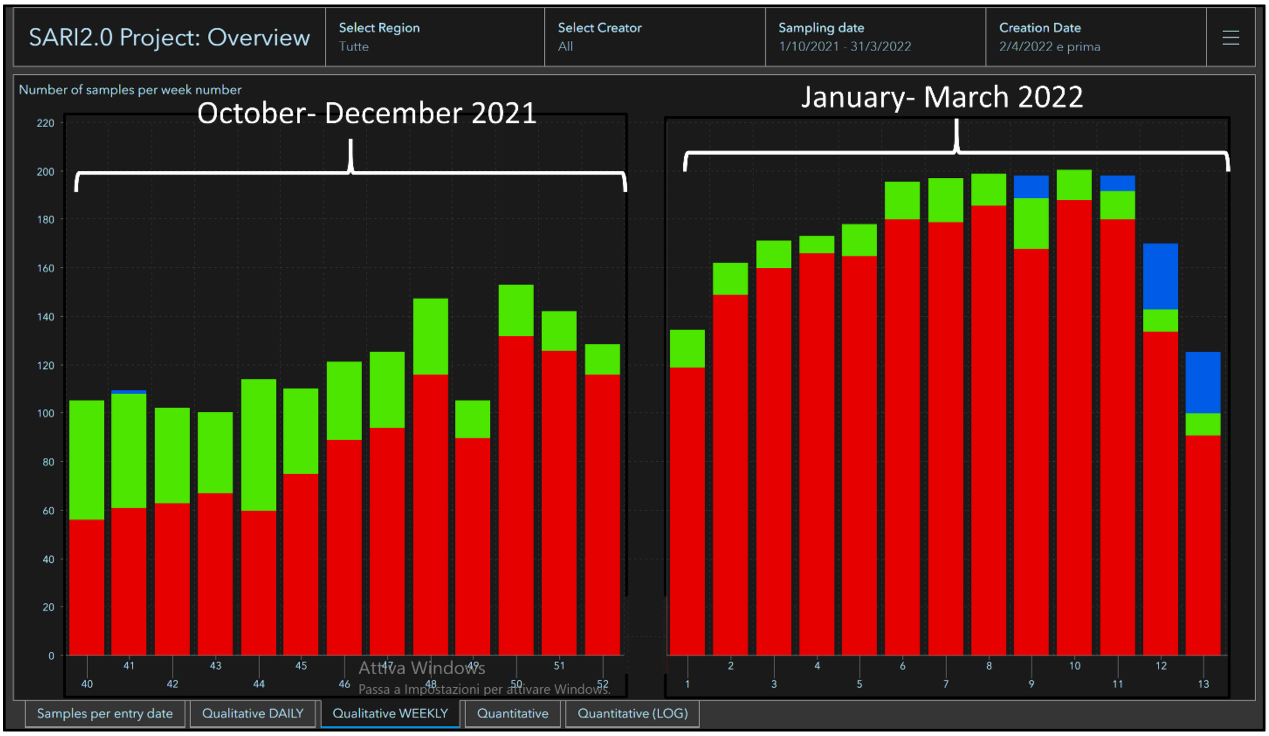
The percentage of positive samples (% pos) is calculated on the number of analysed samples.

[a] 69 analyzed samples; [b] 11 analyzed samples; [c] 52 analyzed samples; [d] 24 analyzed samples; [e] 56 analyzed samples; [f] 87 samples; [g] 55 analyzed samples.

† One incomplete sample was not included in the calculations

Figures 3 shows the results of SARS-CoV-2 detection (presence/absence) based on the sampling week. Green and red colours represent negative and positive samples, respectively. The samples represented in blue are still to be analysed.
As shown in Figure 3 and Table 2, the number of collected samples gradually increased since the beginning of the surveillance, doubling in 6 months (from 419 in October 2021 to 850 in March 2022), as a consequence of the progressive sampling points activation envisioned by the surveillance plan.
Figure 3 and Table 2 also clearly show an increase in the proportion of positive samples in the first quarter January-March 2022 compared to the last quarter 2021, as consequence of the Omicron wave.

Figure 3. Presence/absence results by week of sampling

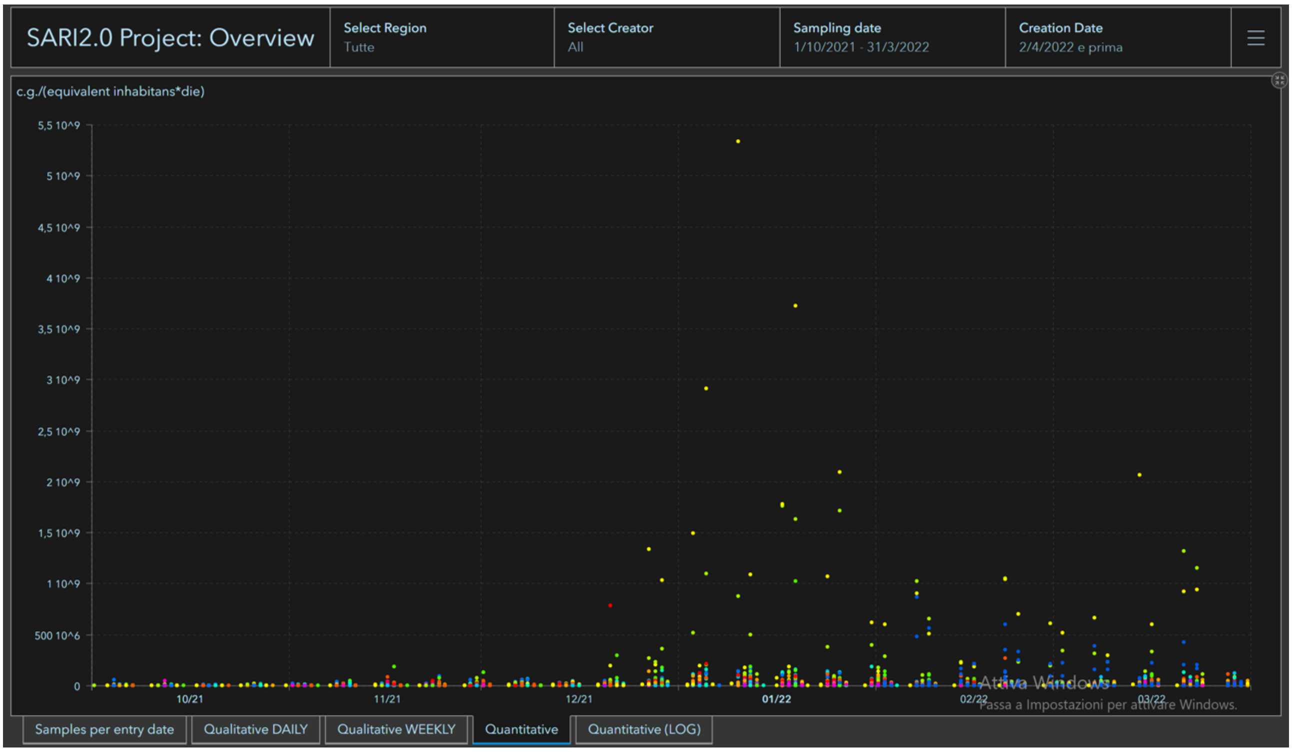


Red = positive sample; green = negative sample; blue = not yet tested.
2021: week 40 = 04.10.2021 - 10.10.2021; week 52 = 27.12.2021 - 02.01.2022.
2022: week 1 = 03.01.2022 - 09.01.2022; week 13 = 28.03.2022 - 03.04.2022.

Quantitative data are represented as genome copies (c.g) of SARS-CoV-2 per inhabitant in 24 hours (Figure 4). In the graph, different colours represent different Regions/A.P. The highest concentrations were observed in January 2022, with viral loads increasing since mid-December 2021 and then decreasing in February 2022. In December 2021 the Omicron variant spread quickly in Italy, as demonstrated by an *ad hoc* survey on urban wastewaters (Study period: 05 December – 25 December 2021)[14]. Subsequently to this Omicron wave, a second smaller increase of SARS-CoV-2 concentrations in wastewaters was recorded in February-March 2022.

[14] La Rosa, Giuseppina; Bonanno Ferraro, Giusy; Mancini, Pamela; Veneri, Carolina; Iaconelli, Marcello; Lucentini, Luca; Bonadonna, Lucia; Brandtner, David; Grigioni, Mauro; Rossi, Mirko; Suffredini, Elisabetta. Ad hoc survey on B.1.1.159 (Omicron) variant on SARS-CoV-2 in urban wastewater in Italy (Study period: 05 December – 25 December 2021). DIO: 10.5281/zenodo.5985319.

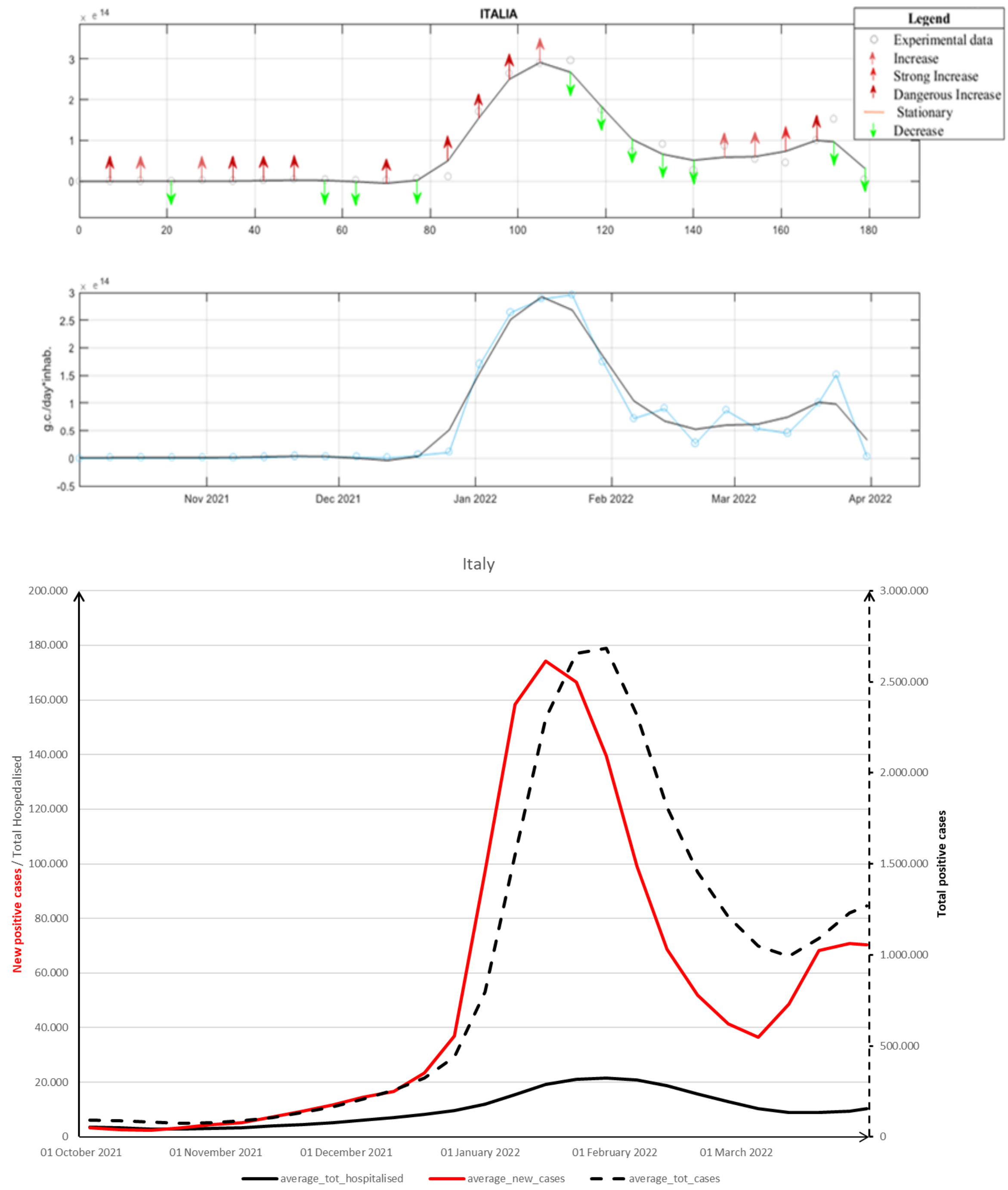
Figure 4. SARS-CoV-2 quantitative data expressed as genome copies/(die*equivalent inhabitant)



Different colours represent different Regions/A.P.

Quantitative data were used to elaborate the Quiver graphs, as described in the Methodology section. Figure 5 represents the global data obtained in Italy over the six months of surveillance, including results obtained from over 3000 measurements. For the sake of comparison, the graph showing total and new (daily) positive cases and hospitalized patients taken from the Coronavirus maps of the Civil Protection Department of the Italian Government (https://mappe.protezionecivile.gov.it/en/emergencies-maps/coronavirus/coronavirus-situation-desktop) is displayed under the Quiver. The Quiver graph shows that SARS-CoV-2 concentrations were low but gradually increasing from the beginning of October until mid-December; the same trend can be observed in the new and total positive cases, as shown in the graph below. In the last week of December a sharp increase of SARS-CoV-2 concentration in wastewater was observed, which continued until the middle of January when SARS-CoV-2 concentration tripled compared to early October. Due to the Omicron wave, the same increase was documented in the number of new and total positive cases. Besides, an increment in the hospitalizations was also documented, since January 2022. Later on, SARS-CoV-2 concentrations decreased gradually until the end of February when a new increase was documented (moderate compared to the previous one), peaking at the end of March when SARS-CoV-2 concentrations were doubled compared to early October. Indeed, a new but less significant wave was reported in the epidemiological curve in March 2022, alongside the registration of a new increase in infections, possibly associated with the Omicron BA.2 variant. Finally, the Quiver graph shows a decrease in SARS-CoV-2 concentrations in the last week of March. However, trends of the last two weeks should be considered with caution, since data from some Regions were missing at the moment of this report drafting, due to incomplete analysis on the collected samples. To summarize, environmental data mirrored clinical data, with two waves observed over the six–month period: a larger one with a peak in January and a smaller one with a peak in March.

Figure 5. Quiver graph representing SARS-CoV-2 trends in wastewater in Italy in the period 01 October 2021-31 March 2022 (top) and trends in COVID-19 cases (bottom)



New positive cases (red graph); total positive cases (dashed black line); hospidalized patients (black line).
Quiver graph: increase = 2%-20%; strong Increase = 20%-30%; dangerous Increase = >30%; stationary = 0-2%.

The data for the individual Regions/A.P. are reported in the Appendices of the present report. Most of the Quivers of the single Regions/A.P. display trends similar to the national graph, with environmental data mirroring clinical data; in some cases, however, the correspondence between environmental and clinical data is not not clearly evident (e.g. unseen or non-proportional increases or delay of the increase).

Limitations of the study

- The geographical and population coverage of the surveillance network is still incomplete, as 19 of the 21 Italian Regions/A.P. are actively reporting data to the surveillance system. Furthermore, in some Regions, the activation of all planned sampling points is not yet completed, leading to a non-uniform coverage also within the regional territory.
- According to EU Rec. 2021/472 and the national protocol adopted for SARS-CoV-2 analysis in wastewaters, analytical results should be uploaded to the SARI 2.0 databases within 48 hours after sample collection. According to available data, laboratories of the surveillance network comply with this time limit in most cases. However, different technical issues (e.g. the need to repeat the analysis to reach the quality assurance criteria, delays in samples collection/shipment, unexpected personnel shortage, delays in data validation or uploading, etc.) may hamper the timely update of results. Therefore, data within the last two weeks of observation should always be taken with caution, as they might be not completely consolidated yet.
- Molecular analytical methods applied to complex environmental matrices like wastewaters may be hampered by low viral concentration, poor recovery of the analyte, and/or inhibition of PCR amplification. Therefore, both the detection and quantification of SARS-CoV-2 in wastewaters may be affected by false negative results and/or by underestimation. According to collected data (Table 2), samples positivity rate varied significantly among Regions/A.P. and may conceal variability of detection performance. Besides this, analytical problems issues may sporadically arise depending on specific climatic/meteorological conditions or due to the characteristics of some samples or sampling points, leading to outlier results and, in turn, to trend alterations.
- Sewage networks are highly diverse (e.g. linear development, daily flow, ramification complexity, the ratio of urban to industrial waters, single/large vs. multiple/small WTPs, etc.) and the effect of such diversity on the representativeness of the different sampling points and on virus detectability is unknown.

Conclusions and final considerations

The environmental surveillance of SARS-CoV-2 started in Italy on October 2021 as per EU Recommendation 2021/472. During the first six months of surveillance, a significant effort has been made to build the environmental network, which - although still incomplete - is currently fully operational. During the semester from October 2021 to March 2022, there was a gradual increase in both the number of Regions involved and of sampling points, as well as an improvement of analytical expertise of the laboratories, and of timing of data reporting.
Nationwide environmental data tracked increasing and decreasing trends at community level. The observed SARS-CoV-2 loads in sewage mirrored trends observed in clinical cases in most of the Regions/A.P., confirming that environmental surveillance can effectively describe both trends and changes in viral circulation in the population.
Concerning some Regions/A.P., there are still issues to be investigated and solved to facilitate the detection and spatiotemporal monitoring of SARS-CoV-2 virus dynamics in the environment. In conclusion, data analysis related to the first six months of surveillance confirms that the environmental surveillance approach can be successfully integrated into the tools used for COVID-19 surveillance. However, considering the limitations outlined above, further

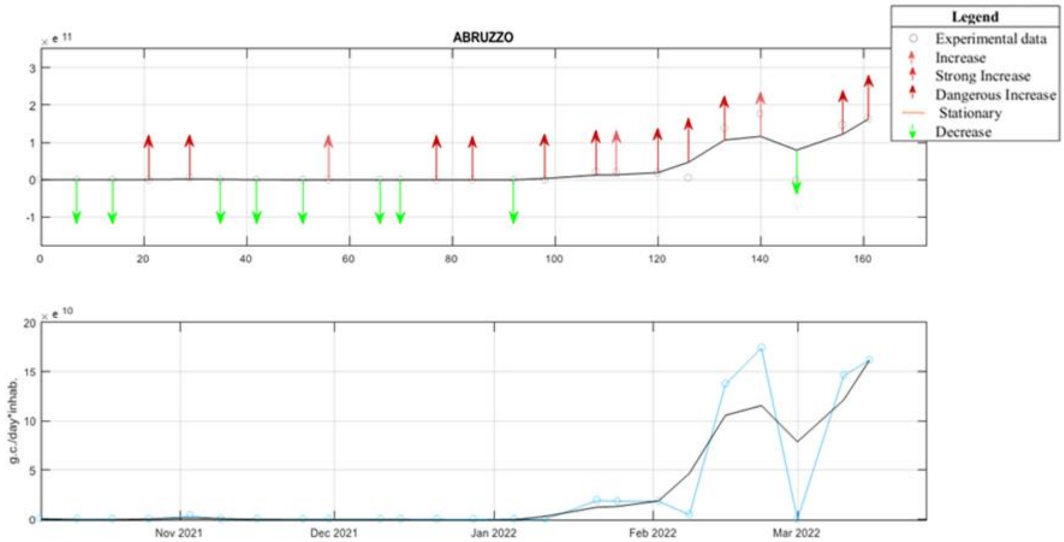
optimization and improvement is needed for environmental surveillance to be used as a support tool for public health decision-making processes, to help target COVID-19 responses and interventions. Research on data analysis is currently under development to define the possible early indication (e.g. 4–7 days based on international studies) of increasing/decreasing trends at community levels by changes of viral concentration in wastewater, to assists timely decisions on public health surveillance strategies.

Noticeably, the national environmental network developed for SARS-CoV-2 monitoring in wastewater is also ready for surveillance applications beyond COVID-19, supporting monitoring and collecting data on a broad range of biological and chemical markers of human health.

Appendix 1: Quiver graphs for Regions and Autonomous Provinces

Legend (relative variation compared to previous week):
Increase = 2%-20%
Strong Increase = 20%-30%
Dangerous Increase = >30%
Stationary = 0-2%
Decrease = reduction of the concentration

ABRUZZO



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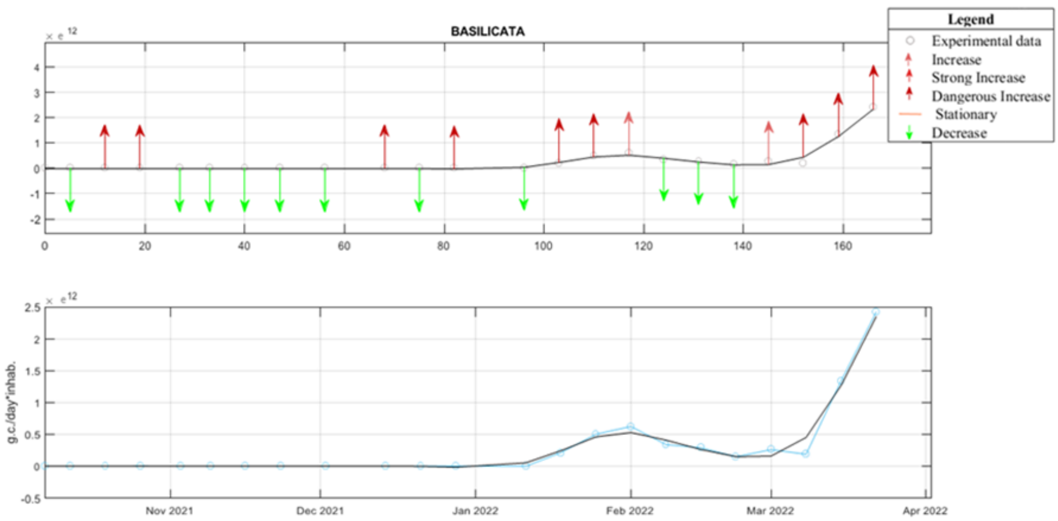

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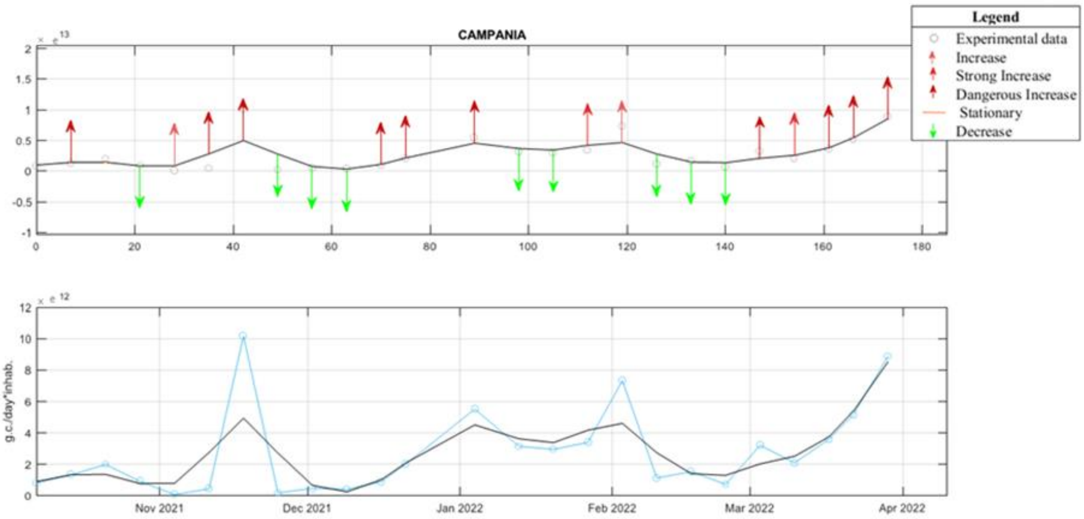

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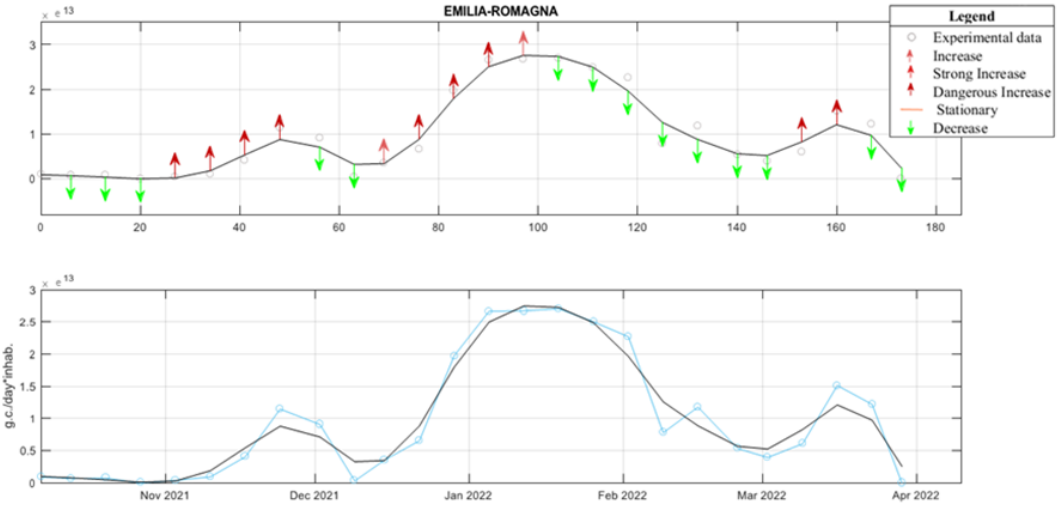

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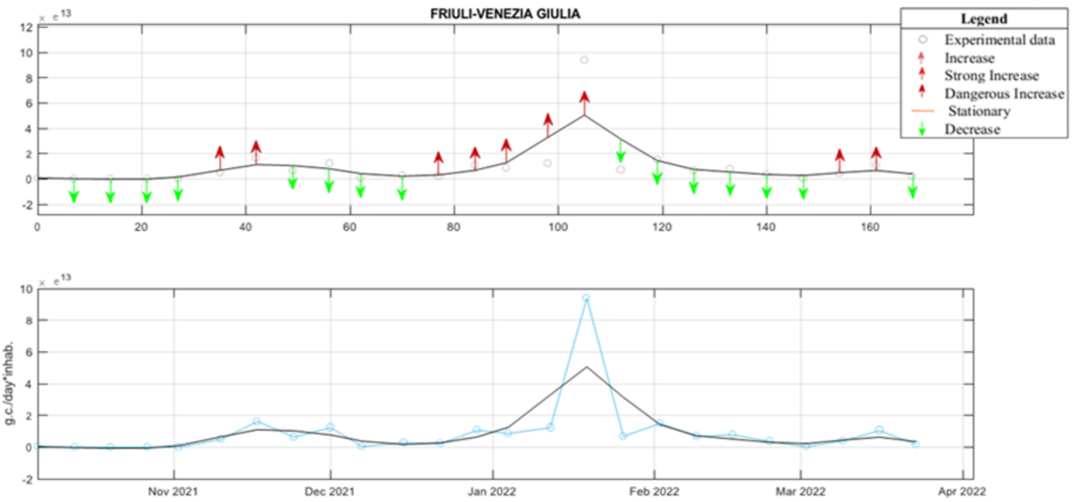

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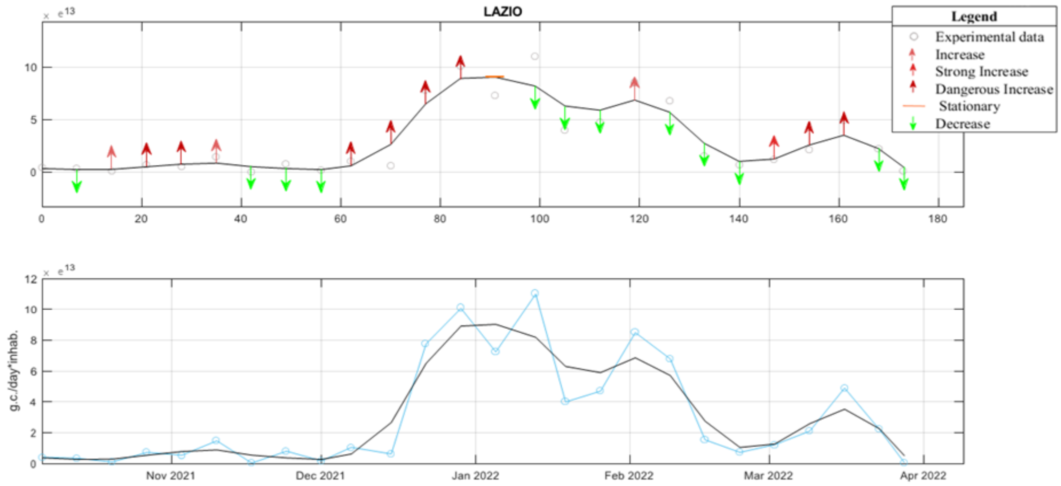

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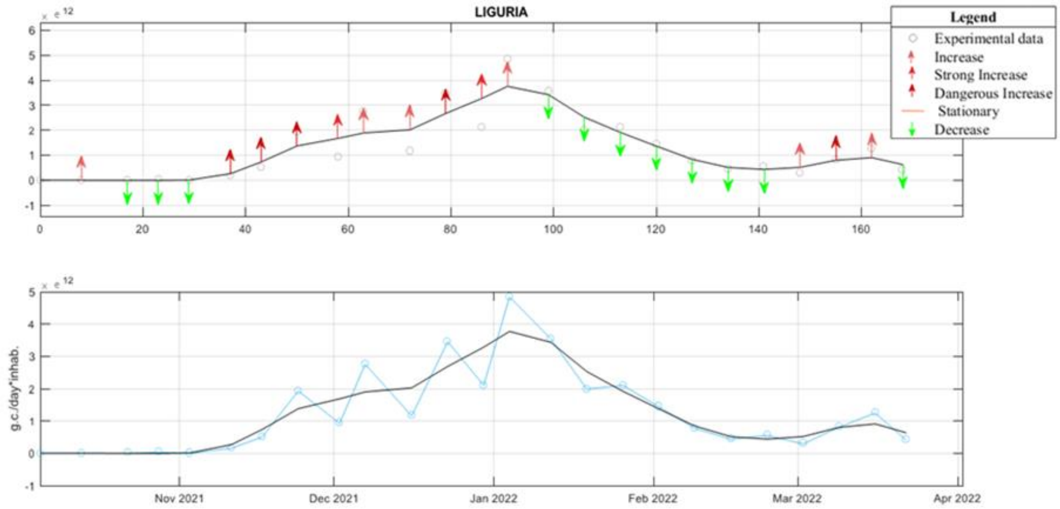

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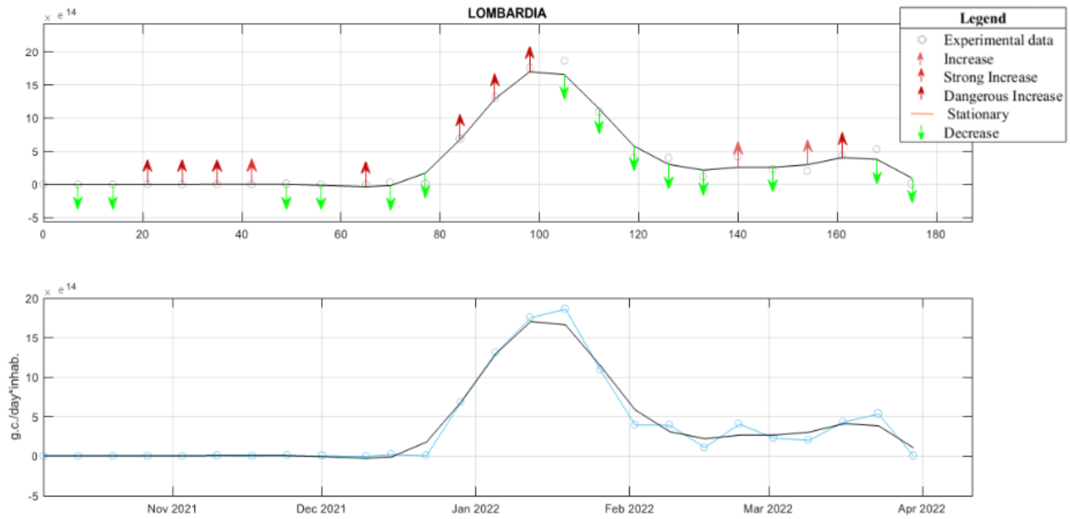

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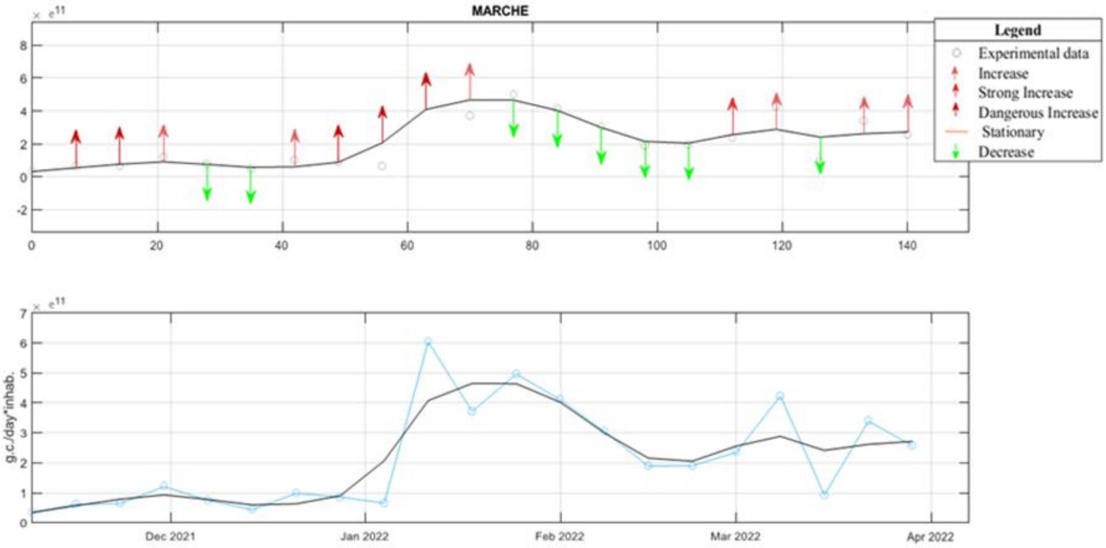

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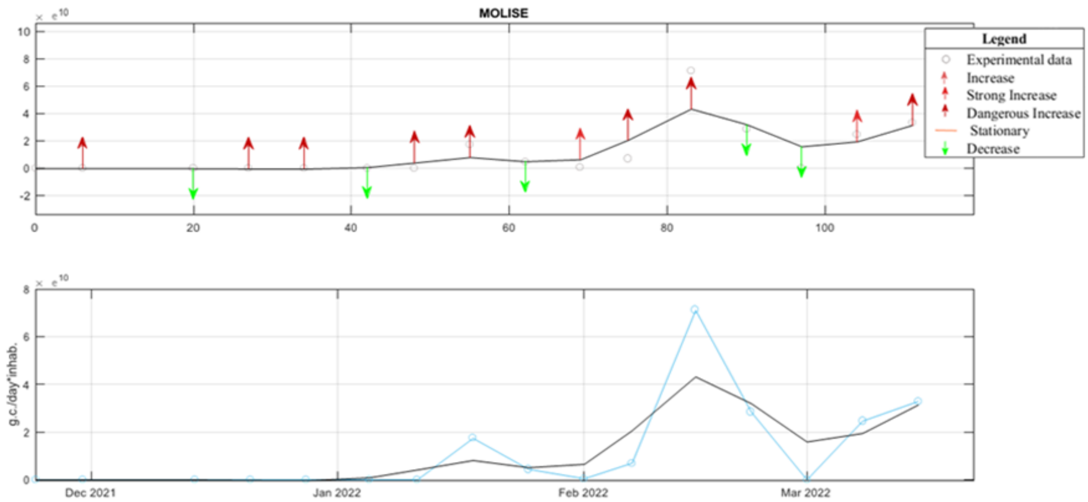

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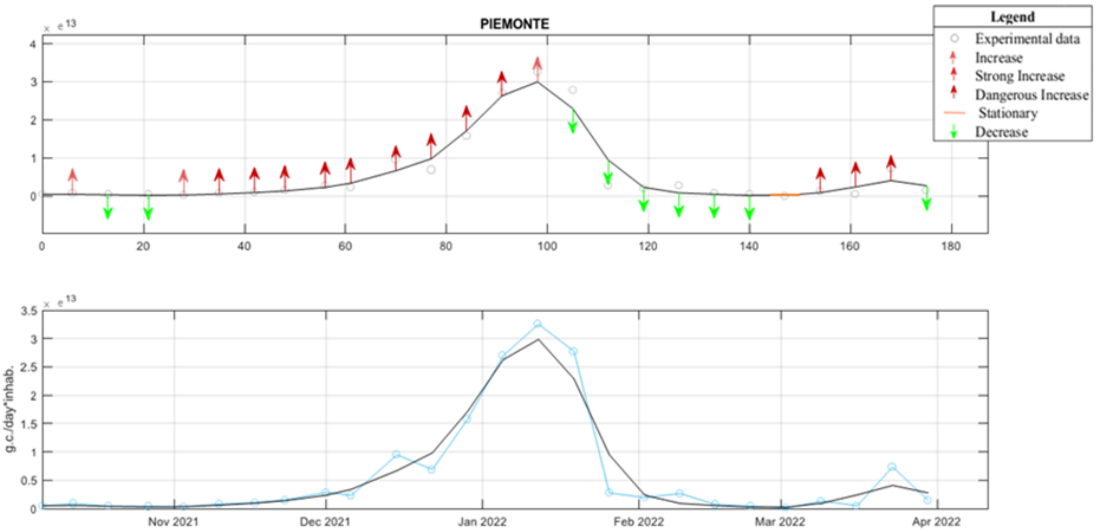

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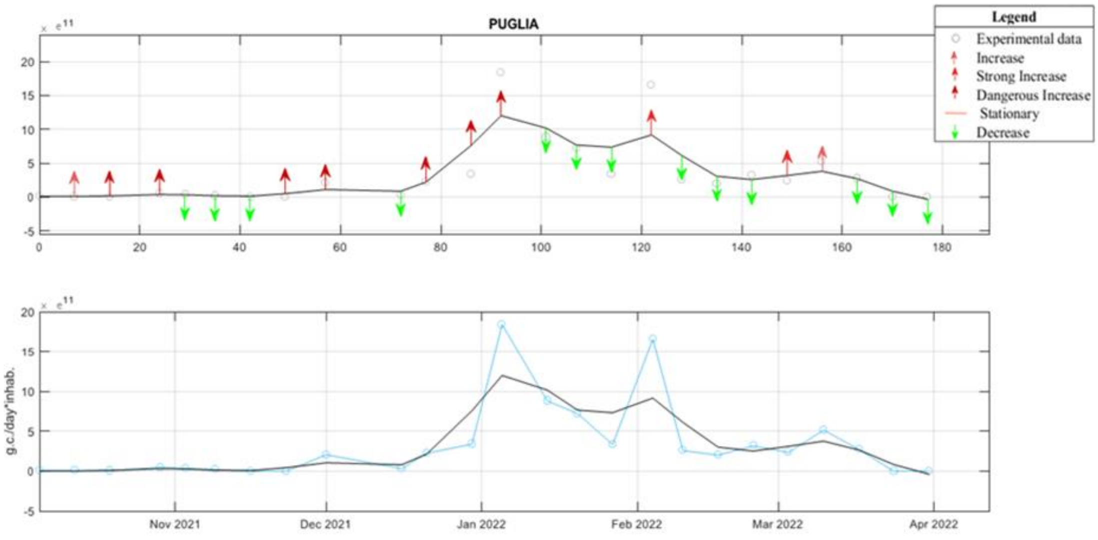

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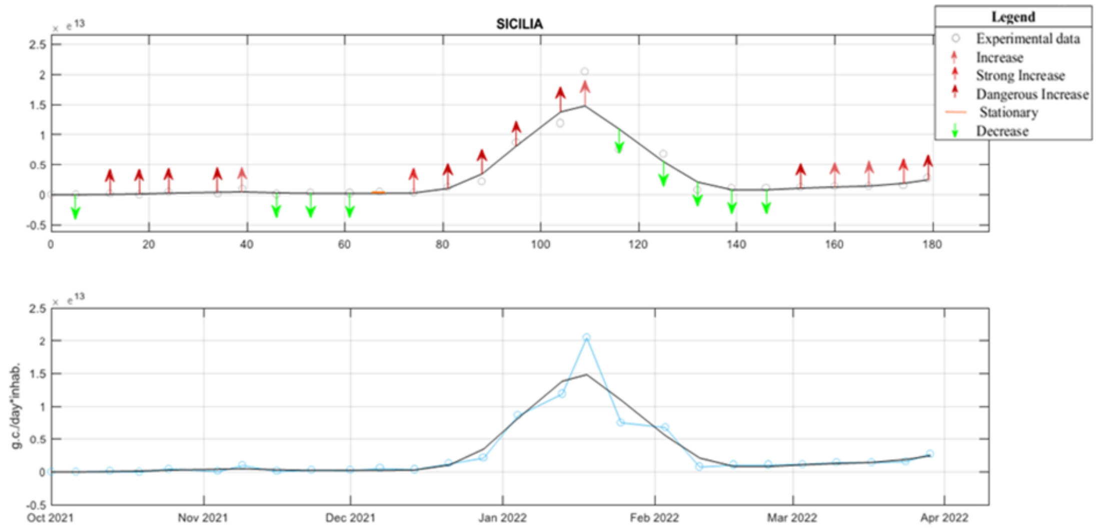

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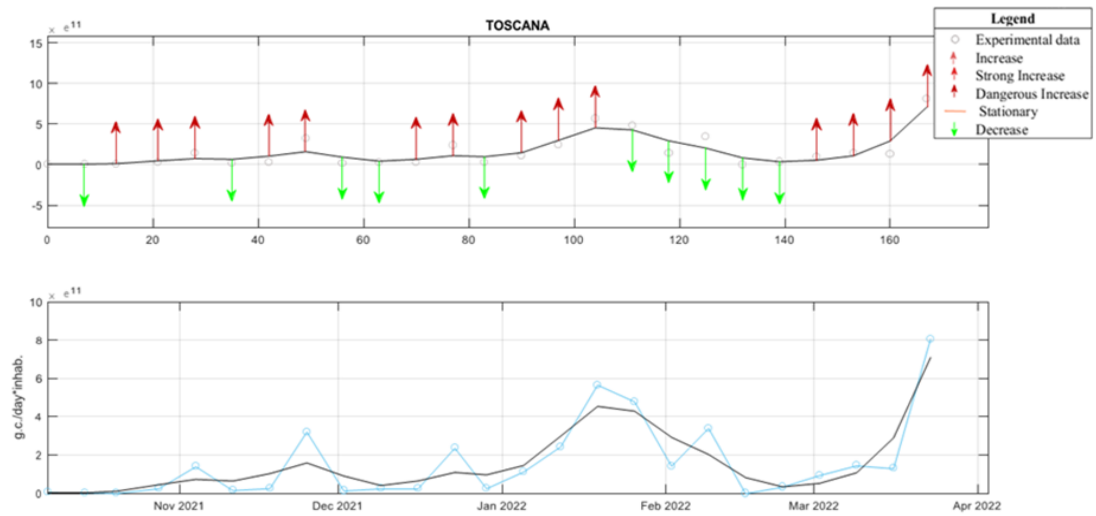

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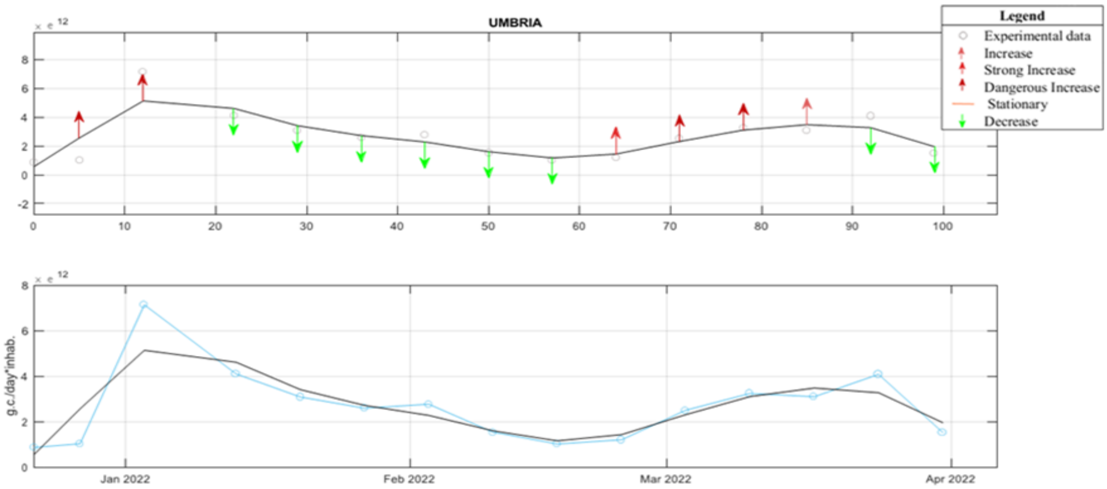

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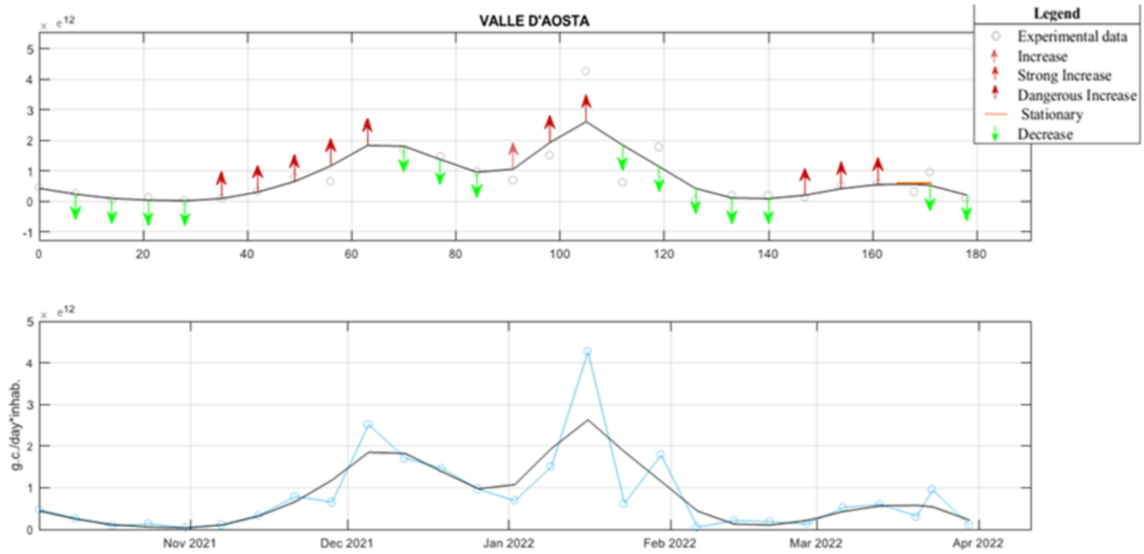

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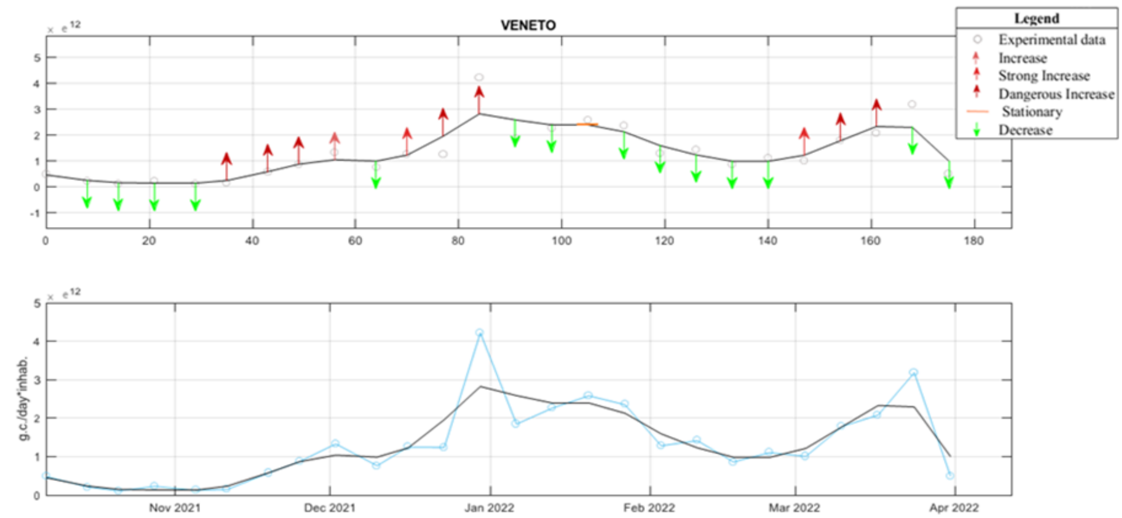
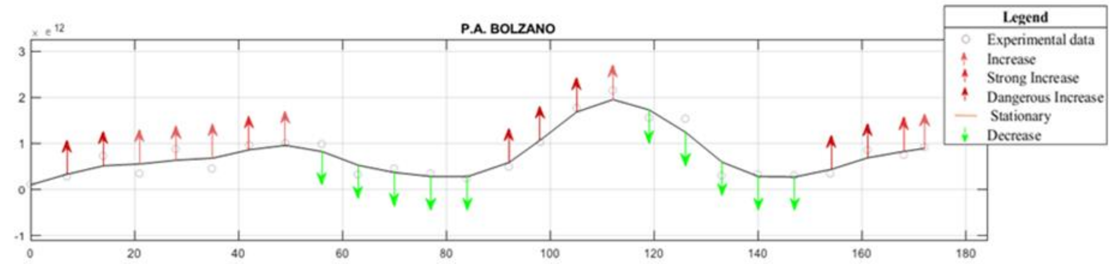
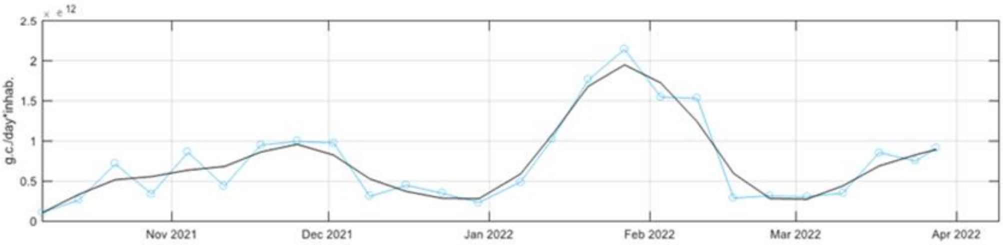
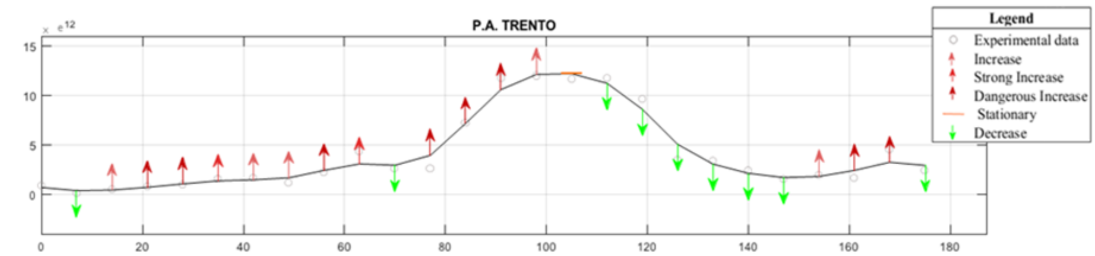


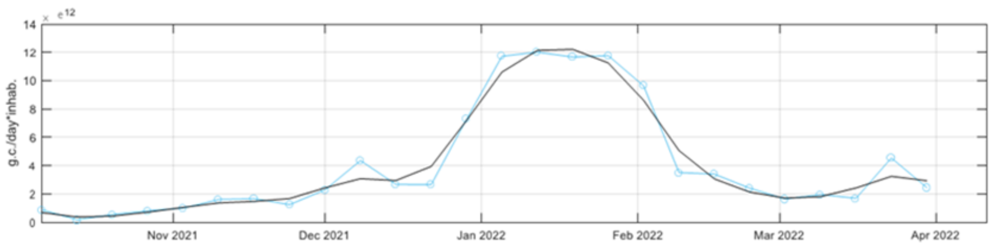
P.A. BOLZANO





P.A. TRENTO





Appendix 2: Summary of weekly relative changes in SARS-CoV-2 concentrations in wastewaters in the last 5 weeks of the surveillance

Region/A.P.	week				
	28.02 – 06.03	07.03 – 13.03	14.03 – 20.03	21.03 – 27.03	28.03 – 03.04
Abruzzo	↘	↗	↗	undetermined	undetermined
Basilicata	↗	↗	↗	↗	undetermined
Campania	↗	↗	↗	↗	↗
Emilia-Romagna	↘	↗	↗	↘	↘
Friuli-Venezia Giulia	↘	↗	↗	↘	undetermined
Lazio	↗	↗	↗	↘	↘
Liguria	↗	↗	↗	↘	undetermined
Lombardia	↘	↗	↗	↘	↘
Marche	↗	↗	↘	↗	↗
Molise	↘	↗	↗	undetermined	undetermined
A.P. Bolzano	↘	↗	↗	↗	↗
A.P. Trento	↘	↗	↗	↗	↘
Piemonte	↔	↗	↗	↗	↘
Puglia	↗	↗	↘	↘	↘
Sicilia	↗	↗	↗	↗	↗
Toscana	↗	↗	↗	↗	undetermined
Umbria	↗	↗	↗	↘	↘
Valle d'Aosta	↗	↗	↔	↘	↘
Veneto	↗	↗	↗	↘	↘
Italy	↗	↗	↗	↘	↘

Undetermined: weekly relative changes were not calculated for Regions/A.P. whose data for the corresponding surveillance period were unavailable in the SARI 2.0 database at the date of data extraction (01.04.2022).

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- **Basilicata:** Michele La Bianca (Regione Basilicata); Rosa Anna Cifarelli, Achille Palma, Giovanna La Vecchia e Giuseppe Lauria (Agenzia Regionale per la Protezione dell'Ambiente Basilicata – ARPAB); Rosanna Brienza e Patrizia Montenegro (Acquedotto Lucano-AQL);
- **Calabria:** Eduardo Malacaria (Regione Calabria), Giuseppe Folino (Arpacal);
- **Campania:** Angelo D'Argenzio (Regione Campania); Luigi Cossentino, Renato Olivares (Arpac - Agenzia Regionale per la Protezione Ambientale in Campania); Antonio Pizzolante, Giovanna Fusco (Istituto Zooprofilattico Sperimentale del Mezzogiorno); Alessandra Tosco, Amalia Porta (Università degli Studi di Salerno); Francesca Pennino, Triassi Maria (Università degli Studi di Napoli "Federico II");
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- **Molise:** Michele Colitti (Regione Molise); Carmen Montanaro (ASReM); Giuseppe Aprea, Silvia Scattolini, Ilaria Rosa, Daniela D'Angelantonio, Giacomo Migliorati (Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale"); Maria Grazia Cerroni (Arpa Molise);

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- **Toscana:** Piergiuseppe Cala' (Regione Toscana); Annalaura Carducci, Marco Verani, Ileana Federigi, Giulia Lauretani, Sara Muzio (Laboratorio di Igiene e Virologia Ambientale - Dipartimento di Biologia Università di Pisa); Matteo Ramazzotti, Alberto Antonelli (SOD microbiologia e virologia, azienda ospedaliera universitaria Careggi, Firenze);
- **Umbria:** Giovanni Santoro (Regione Umbria), Ermanno Federici, Maya Petricciuolo, Sofia Barigelli (Laboratorio Microbiologia Applicata e Ambientale, DCBB Università di Perugia);
- **Valle D'Aosta:** Mauro Ruffier (Regione Valle d'Aosta); Francesca Borney, Eric Grange, Florida Damasco (Laboratorio chimico biologico microbiologico Arpa Valle d'Aosta);
- **Veneto:** Francesca Russo, Gisella Pitter, Vanessa Groppi (Regione Veneto); Franco Rigoli, Marco Zampini (ARPAV - Agenzia Regionale per la Prevenzione e Protezione Ambientale del Veneto); Tatjana Baldovin, Irene Amoruso (Università di Padova);
- **P.A. Bolzano:** Lorella Zago (P.A. Bolzano); Alberta Stenico, Anna-Maria Prast (A.P.P.A. Agenzia provinciale per l'ambiente e la tutela del clima, Laboratorio biologico)
- **P.A. Trento:** Francesco Pizzo; Alessandra Schiavuzzi, Elena Mengon (P. A. Trento) (P.A. Trento); Maria Cadonna, Mattia Postinghel (ADEP SGI PAT), Francesca Cutrupi, Paola Foladori, Serena Manara (UNITN – Università di Trento).