Surveillance of SARS-CoV-2 in urban wastewater in Italy
2nd Report
(Study period: 01 - 30 April 2022)

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Main findings:

- Report on SARS-CoV-2 surveillance in urban wastewaters in Italy, April 2022.
- As on 30 April 2022, 20/21 Regions/Autonomous Provinces produce data within the environmental surveillance program and the environmental network includes a total of 166 wastewater treatment plants throughout Italy.
- A total of 754 wastewater samples were collected during April 2022, 708 of which were analysed for SARS-CoV-2 RNA by real time PCR. Overall, 92.5% of the tested samples showed the presence of SARS-CoV-2 RNA, average concentrations ranging from 3,48E+02 to 4,22E+05 c.g./L wastewater.
- The national trend of SARS-CoV-2 concentrations in wastewater, represented using Quiver graphs, showed a gradual decrease of viral concentrations over the entire month

Introduction

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 national wastewater surveillance systems aimed at the collection of data on SARS-CoV-2 and its variants[1]. 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 (Sorveglianza Ambientale SARS-CoV-2 in Reflui in Italia) project were transformed into a surveillance system, coordinated by Istituto Superiore di Sanità (ISS).

Aim

The aim of this report is to summarize the results of the environmental surveillance of SARS-CoV-2 obtained during April 2022.

Enrolled Regions/Autonomous provinces

In April 2022, 20 of the 21 Italian Regions/A.P. produced SARS-CoV-2 concentration data within the environmental surveillance program. The Region of Sardinia has not yet provided an operational plan to activate the surveillance.

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

1 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)

inhabitants. Urban centres with a population between 50k and 150k inhabitants were also included to improve both the population and territorial coverage. This resulted in the inclusion of a total of **167 WTPs** (Figure 1 and Table 1) within the environmental surveillance network serving a total of 31.734.984 population equivalent[2]. For details on the network see the 1° Report on the Surveillance of SARS-CoV-2 in urban wastewater in Italy (Study period: 01 October 2021 - 31 March 2022). The WTPs serving urban centres with more than 150k inhabitants are monitored twice per week as per Rec. 2021/472, while WTPs collecting wastewaters from centres with a population between 50k and 150k are monitored once per week.

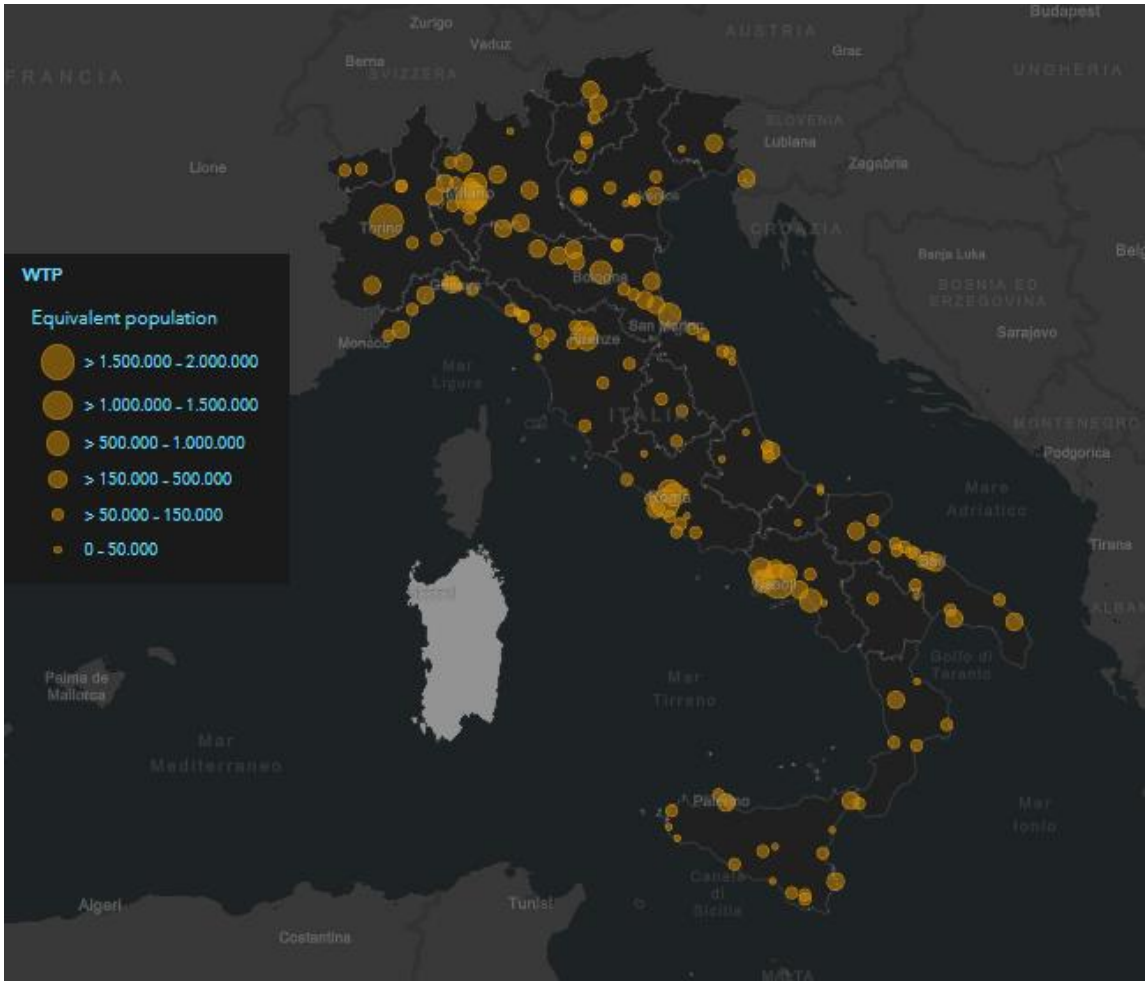


Figure 1. WTPs included in the environmental surveillance network

Table 1. Sampling sites and characteristics of the WTPs studied

[2] 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)

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)

During April 2022, **754 wastewater samples** were collected, as follow (Figures 1 and 2):
Week 13 (limited to 01.04.2022 - 03.04.2022): 3 samples
Week 14 (04.04.2022 - 10.04.2022): 213 samples
Week 15 (11.04.2022 - 17.04.2022): 195 samples
Week 16 (18.04.2022 - 24.04.2022): 173 samples
Week 17 (limited to 25.04.2022 - 30.04.2022): 170 samples

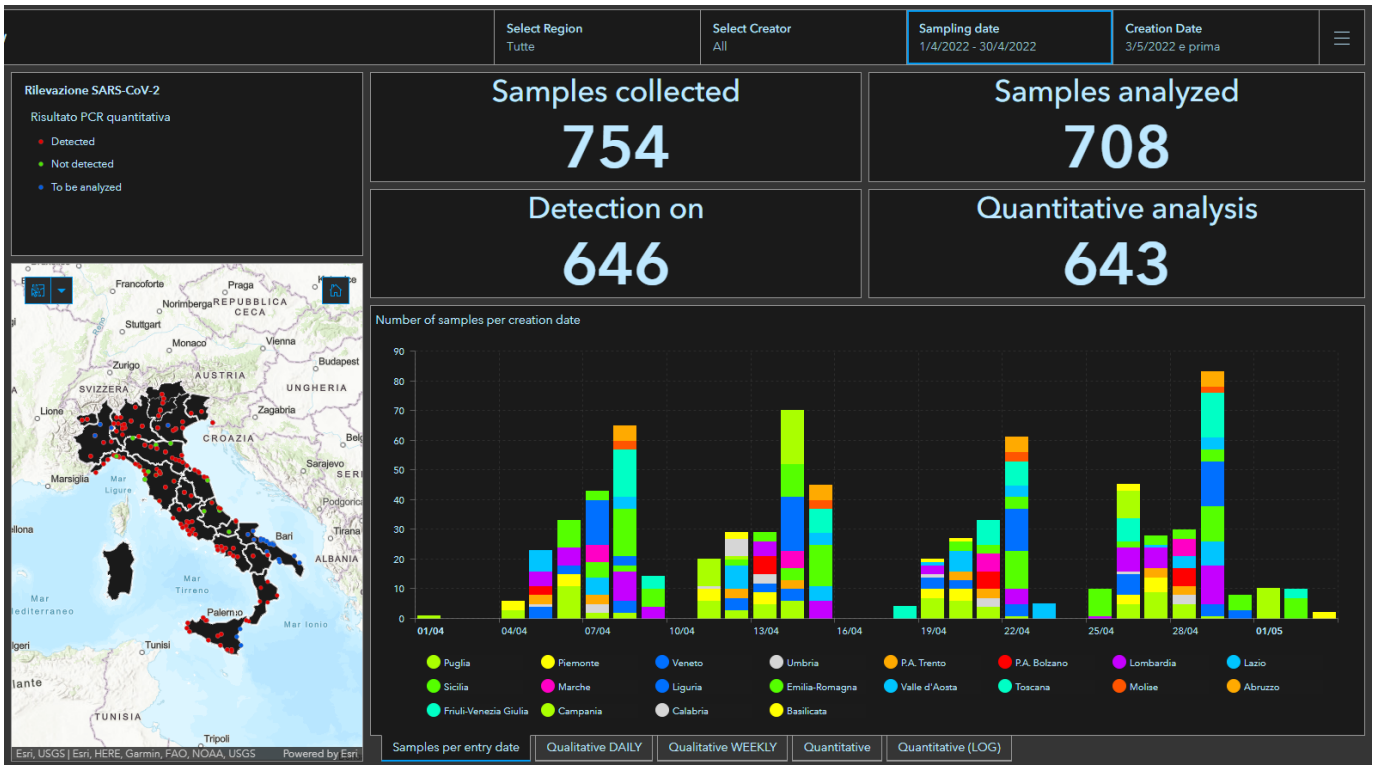


Figure 1. Total samples collected and analysed during April 2022 by the 20 Regions/A.P.

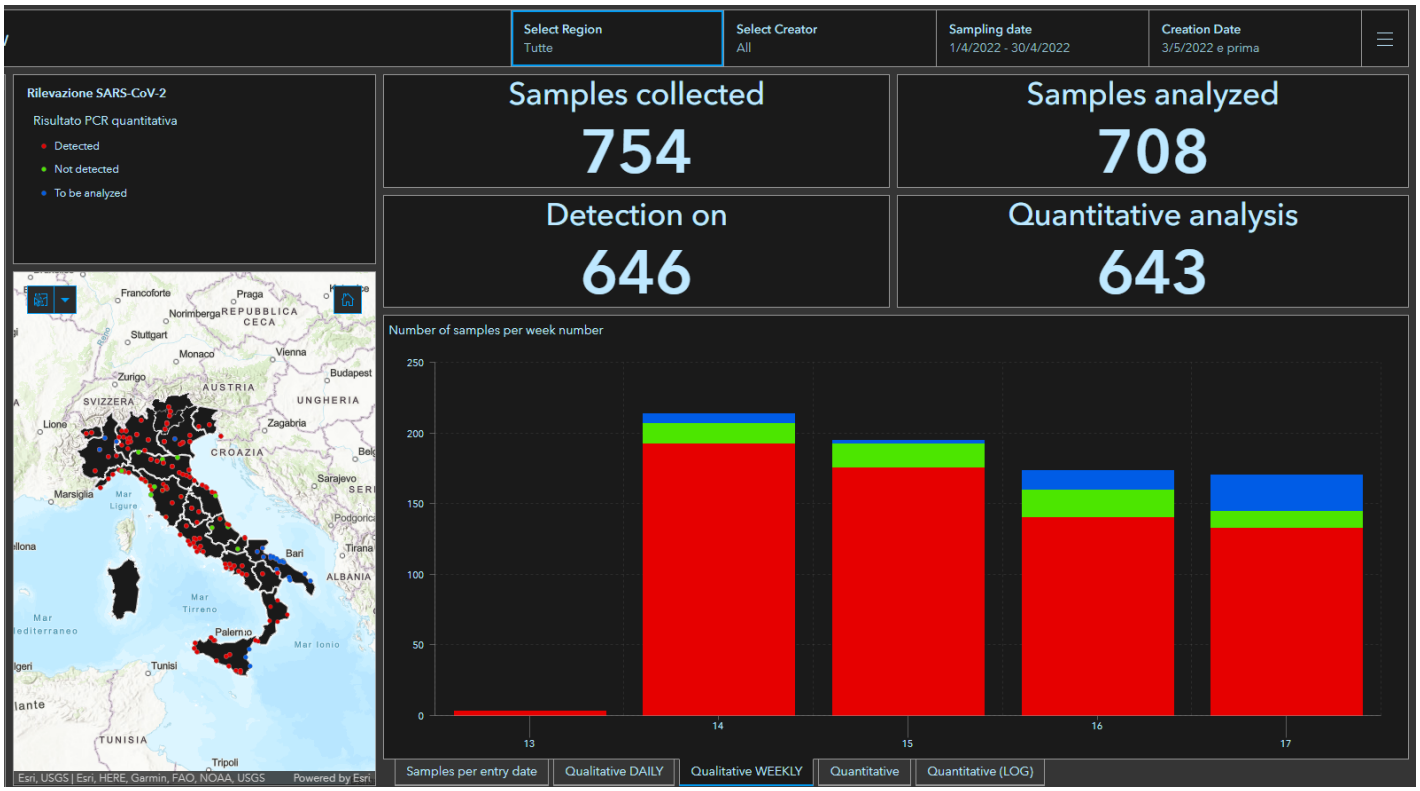


Figure 2. Samples collected and analysed by week.
Week 13 (limited to 01.04.2022 - 03.04.2022); Week 14 (04.04.2022 - 10.04.2022); Week 15 (11.04.2022 - 17.04.2022); Week 16 (18.04.2022 - 24.04.2022); Week 17 (limited to 25.04.2022 - 30.04.2022). Red = positive sample; green = negative sample; blue = not yet tested.

Results

The data on SARS-CoV-2 concentrations in wastewater were produced by the SARI network laboratories (see Acknowledgement section). Table 2 summarises the number of samples collected in April by each Region/A.P. and quantitative results expressed as c.g./L wastewater. SARS-CoV-2 RNA was detected in 92.1% of the tested samples (693/698), prevalence ranging from 52.4% to 100%. Average concentrations ranged from 3,48E+02 to 4,22E+05 c.g./L wastewater.

Table 2. Quantitative results obtained during April 2022

Region/A.P.	**N° of WTP**	**Collected Samples**	**Analysed samples**	**Positive samples (SARS-CoV-2 quantified)**	**Concentration range in positive samples** (c.g./L wastewater)	**Average Concentration** (c.g./L wastewater)
Abruzzo	5	20	10	10 (100%)	4,35E+01 - 1,08E+03	3,48E+02
Basilicata	2	8	8	8 (100%)	1,60E+03 - 7,53E+04	2,62E+04
Calabria[a]	6	6	6	6 (100%)	5,83E+02 - 2,59E+03	1,74E+03
Campania	10	47	47	46 (97.8%)	3,85E+03 - 2,90E+05	6,84E+04
Emilia Romagna	14	75	75	64 (85.3%)	2,60E+03 - 2,12E+06	1,79E+05
Friuli Venezia Giulia	3	13	13	13 (100%)	2,78E+04 - 2,04E+05	1,21E+05
Lazio	13	52	52	50 (96.1%)	2,50E+01 - 4,60E+05	7,63E+04
Liguria	15	68	68	66 (97.1%)	1,13E+04 - 3,12E+05	7,40E+04
Lombardia	15	73	73	69 (94.5%)	1,09E+03 - 7,30E+06	4,22E+05
Marche	6	24	24	22 (91.6%)	1,74E+03 - 6,49E+04	2,96E+04
Molise	3	11	11	7 (63.6%)	7,50E+01 - 1,95E+03	3,86E+02
Piemonte	7	30	24	24 (100%)	5,43E+02 - 1,72E+04	5,94E+03
Puglia	16	77	45	45 (100%)	1,30E+03 - 4,85E+04	9,89E+03
Sicilia	17	68	61	61 (100%)	2,29E+03 - 9,67E+04	3,10E+04
Toscana	13	61	61	32 (52.4%)	1,38E+03 - 1,03E+05	1,78E+04
Umbria	3	16	16	16 (100%)	9,93E+04 - 3,69E+05	1,70E+05
Valle d'Aosta	2	16	16	16 (100%)	2,16E+03 - 4,43E+05	5,80E+04
Veneto	10	44	43	43 (100%)	3,78E+03 - 3,12E+05	5,88E+04
A.P. Bolzano	3	21	21	21 (100%)	7,00E+03 - 7,48E+04	3,15E+04
A.P. Trento	3	24	24	24 (100%)	2,67E+04 - 3,46E+05	1,76E+05
Total	**166**	**754**	**698**	**643 (92.1%)**	7,50E+01- 7,30E+06	7,79E+04

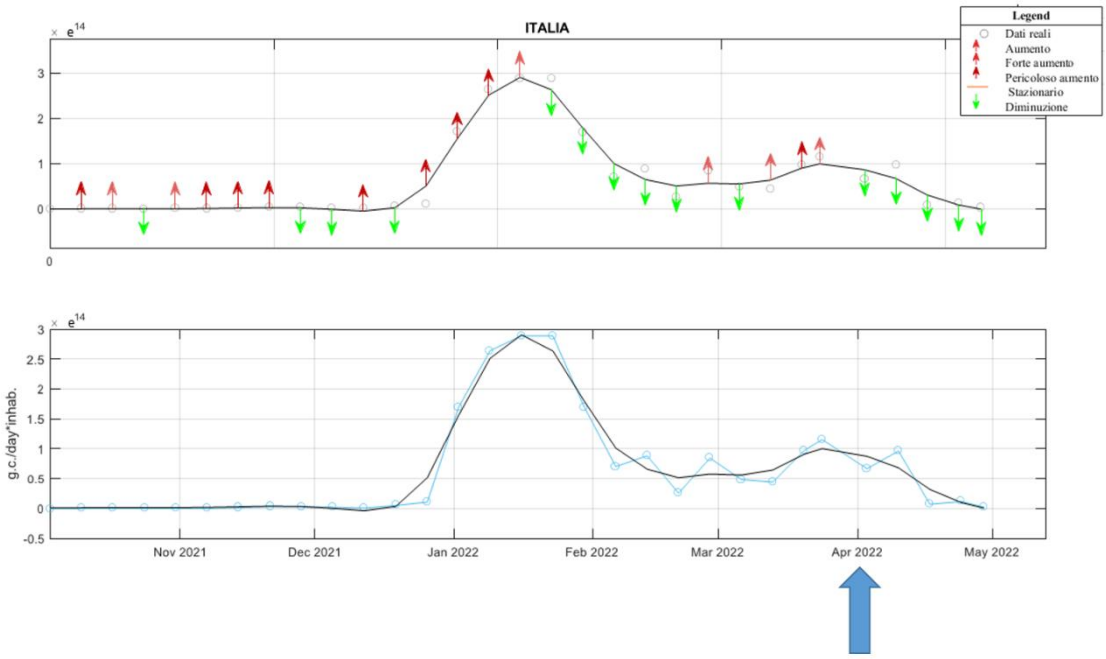
[a] only one week of sampling during April 2022

Weekly changes are shown in Table 3. Variation compared to the previous week are shown with arrows (red= increase, green = decrease, black = stationary). Data from the last nine weeks of surveillance are shown in the table.

Table 3. Weekly changes. The last nine week of surveillance are represented

Region/A.P.	week								
	28.02 – 06.03	07.03 – 13.03	14.03 – 20.03	21.03 – 27.03	28.03 – 03.04	04.04 - 10.04	11.04 - 17.04	18.04 - 24.04	25.04 - 01.05
Abruzzo	↘	↗	↗	↗	↗	↘	↘	↘	↗
Basilicata	↘	↗	↗	↗	↗	↘	↘	↘	↗
Campania	↗	↗	↗	↗	↗	↗	↗	↘	↘
Emilia-Romagna	↘	↗	↗	↗	↗	↘	↗	↗	↘
Friuli-Venezia Giulia	↘	↗	↗	↗	↗	↗	↘	↘	↘
Lazio	↗	↗	↗	↘	↘	↗	↗	↗	↘
Liguria	↗	↗	↗	↗	↗	↗	↗	↘	↗
Lombardia	↘	↗	↗	↗	↘	↘	↘	↘	↘
Marche	↗	↗	↘	↗	↗	↗	↗	↗	↘
Molise	↘	↘	↗	↗	↗	↗	↘	↘	↗
A.P. Bolzano	↘	↗	↗	↗	↘	↘	↔	↘	↘
A.P. Trento	↘	↗	↗	↗	↘	↘	↘	↘	↗
Piemonte	↗	↗	↗	↗	↗	↗	↘	↘	↘
Puglia	↗	↗	↘	↘	↔	↗	↘	↘	↘
Sicilia	↗	↗	↗	↗	↘	↘	↗	↗	↗
Toscana	↗	↗	↗	↗	↗	↘	↘	↘	↘
Umbria	↗	↗	↗	↗	↘	↘	↘	↘	↘
Valle d'Aosta	↗	↗	↗	↗	↗	↗	↗	↘	↘
Veneto	↗	↗	↗	↗	↘	↘	↘	↘	↘
Italy	↘	↗	↗	↗	↘	↘	↘	↘	↘

Quantitative data were used to elaborate the Quiver graphs, as described previously[3]. Figure 3 represents the global data obtained in Italy over the seven months of surveillance (October 2021-April 2022), including results obtained from over 4000 measurements. Since the beginning of April concentrations decreased gradually over the entire month, reaching concentrations found in December 2021, before the Omicron wave.



Quiver graphs for each Region/A.P. (with the exception of the Region of Calabria for which data was insufficient for graphical representation) are shown in Appendix 1.

Limitations of the study

- The geographical and population coverage of the surveillance network is still incomplete, as 20 of the 21 Italian Regions/A.P. are actively reporting data to the surveillance system.
- Trend analysis for Regions in which surveillance has been only recently activated (e.g. Region of Calabria) should be taken with caution due to the limited time series.
- Caution should be used in the interpretation of the most recent data, as trend analysis may be affected by missing data. At the time of drafting of this report 7.4% of the samples collected in the period under observation was still under analysis.
- 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

[3] Surveillance of SARS-CoV-2 in urban wastewater in Italy 1° Report (Study period: 01 October 2021 - 31 March 2022. 8e5e2edb-bae0-f1b0-ee6e-08255c76484f (iss.it)

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.

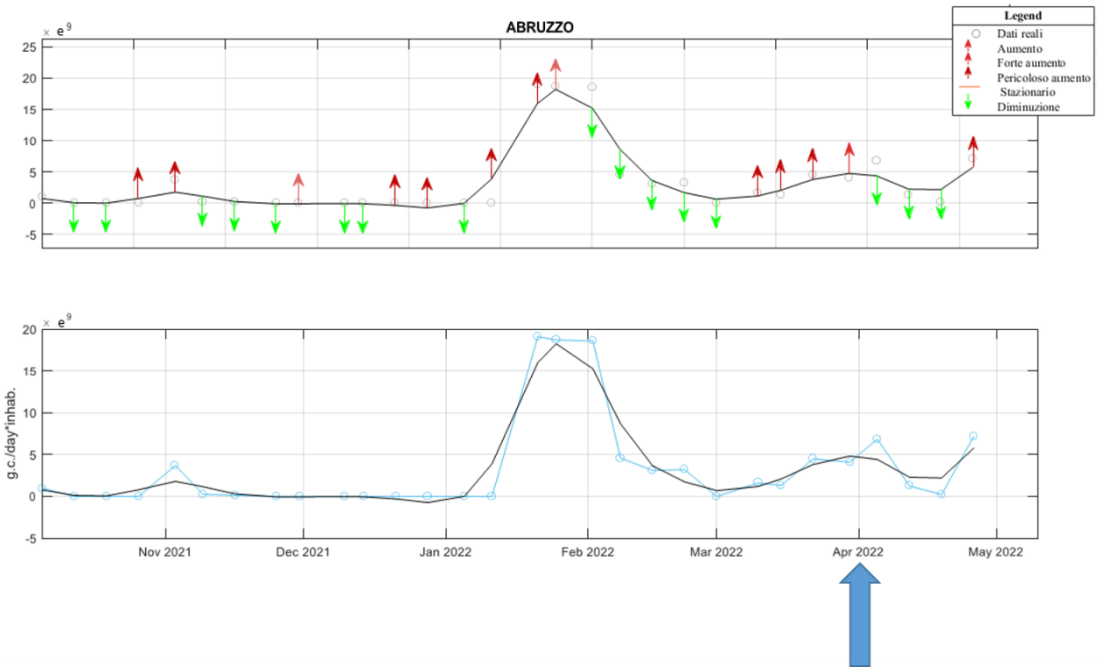
Conclusions and final considerations

The observed SARS-CoV-2 loads in sewage decreased gradually since the beginning of April, with concentrations reaching those found in December 2021, before the Omicron wave.

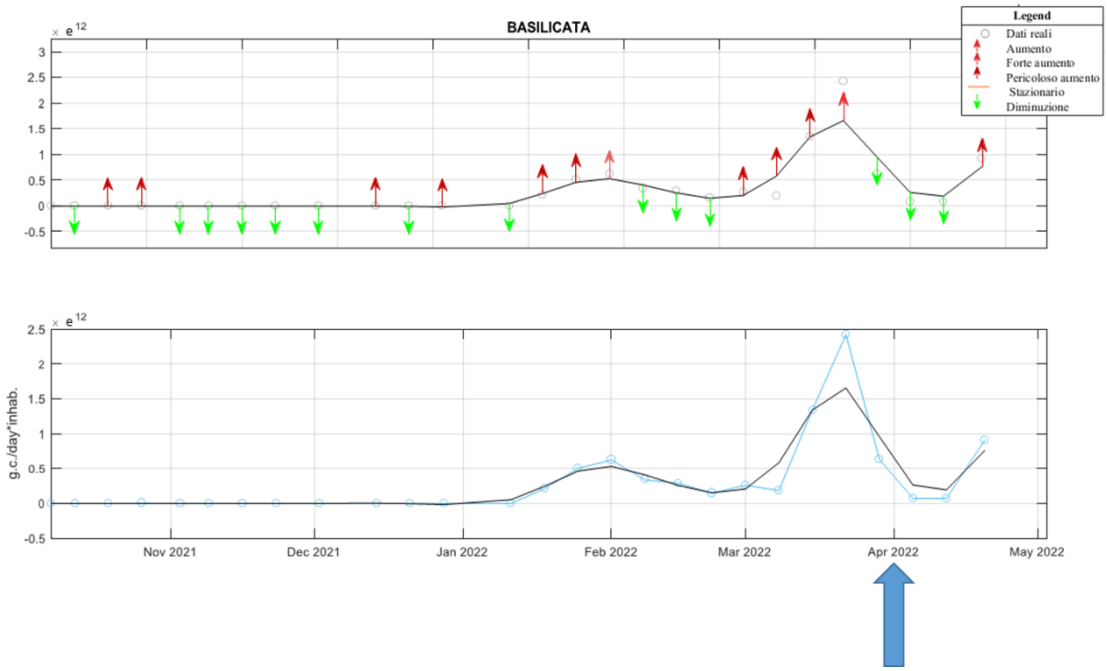
Appendix 1: Quiver graphs for Regions and Autonomous Provinces.

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

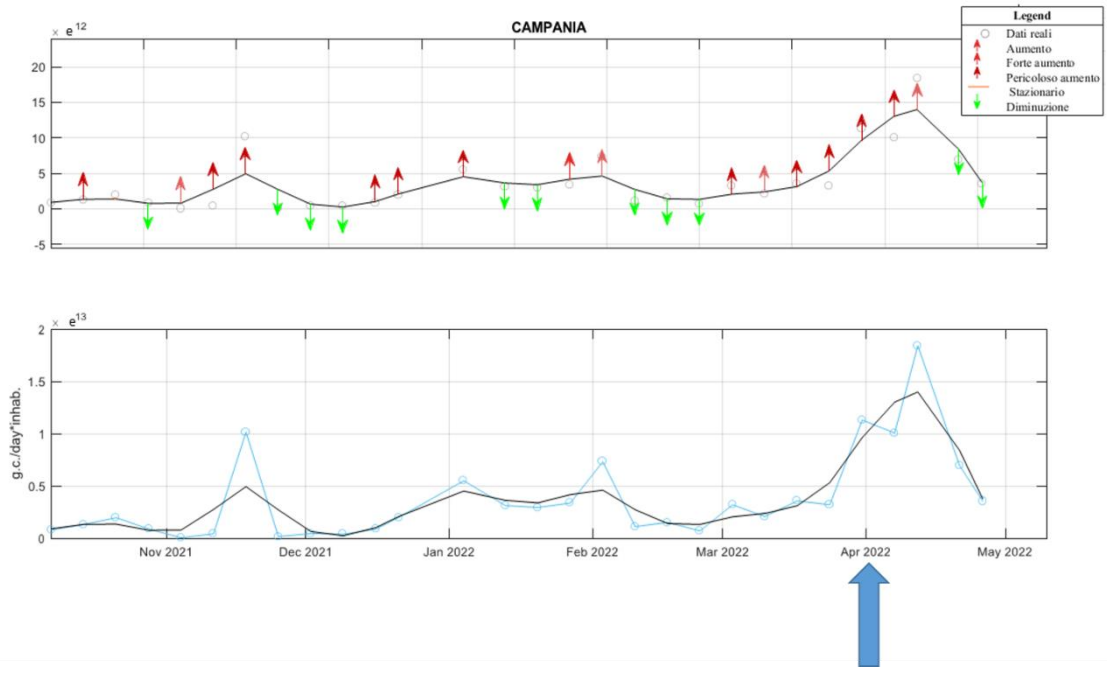
ABRUZZO



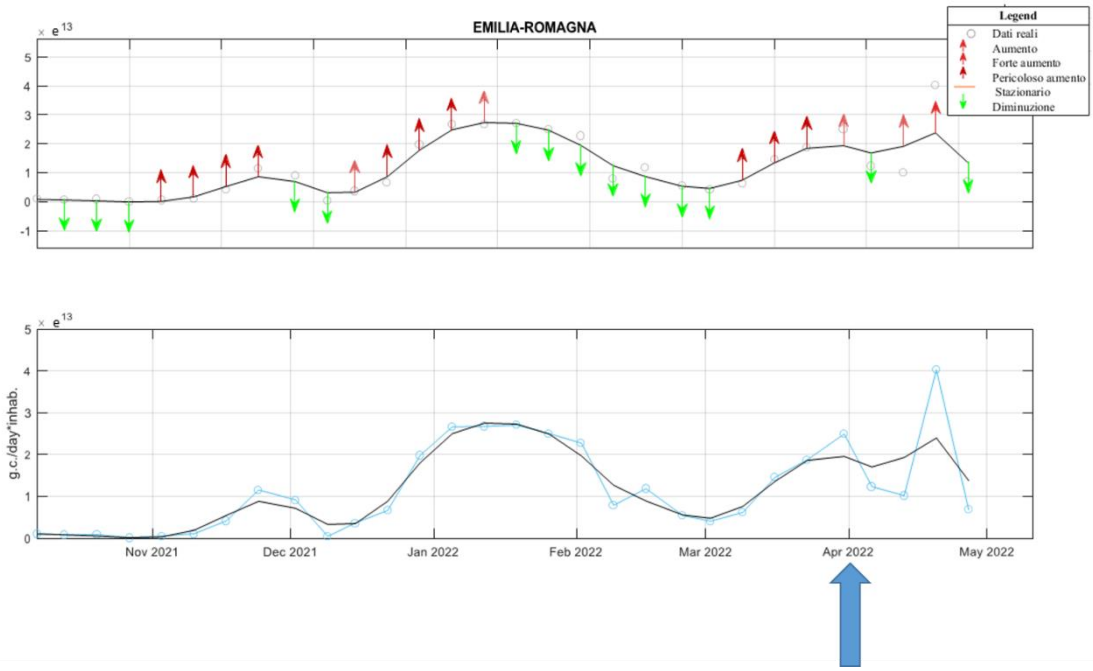
BASILICATA



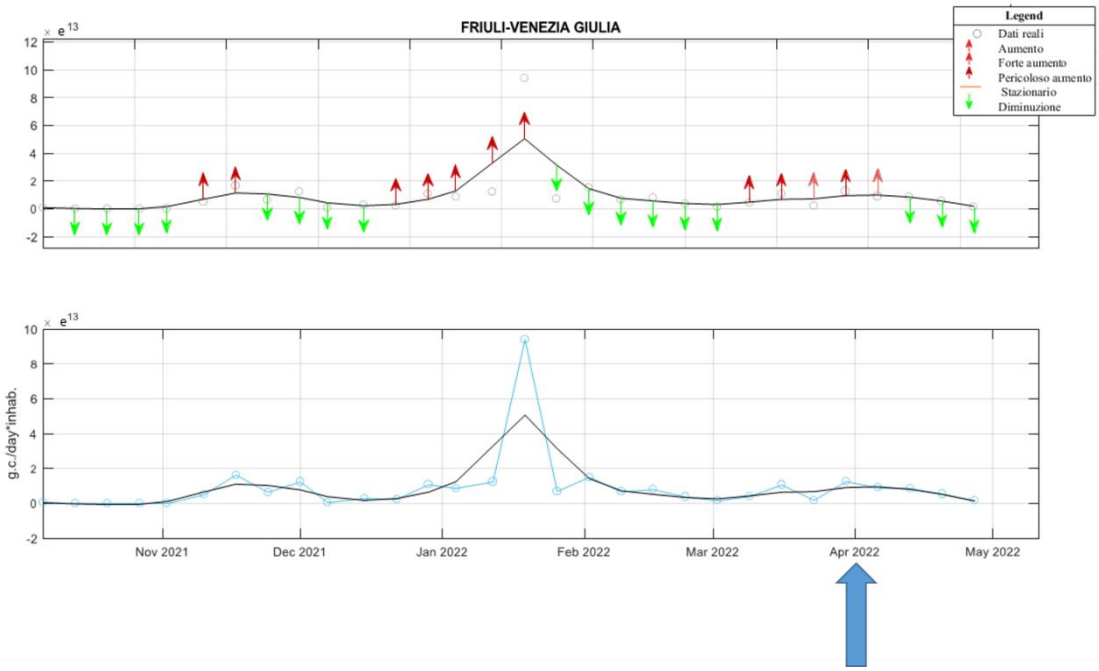
CAMPANIA



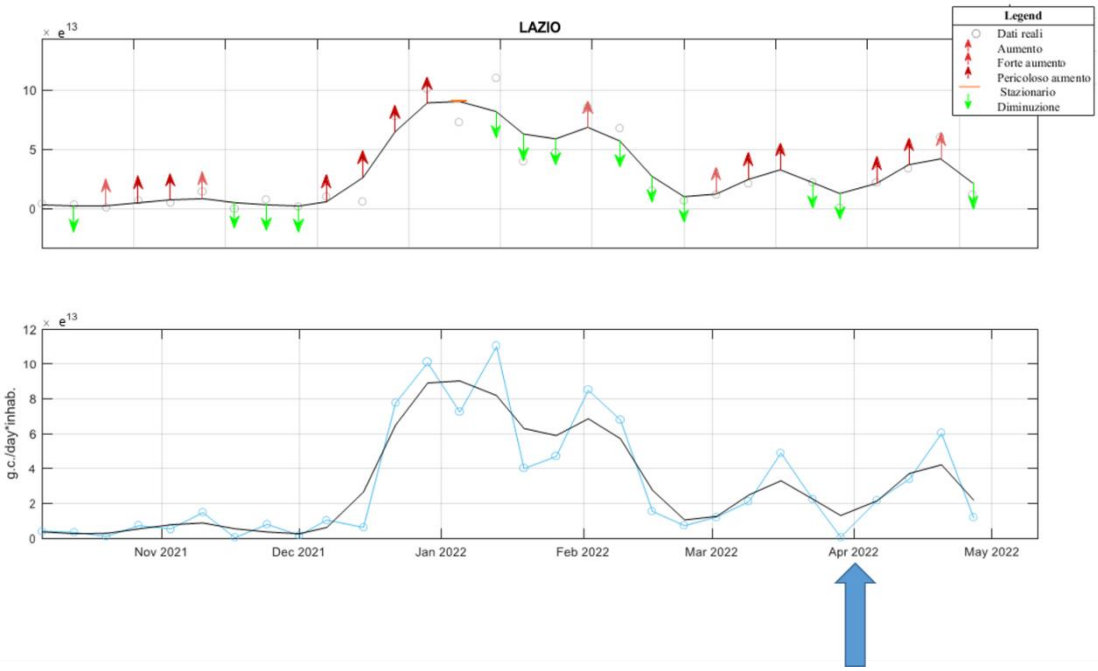
EMILIA-ROMAGNA



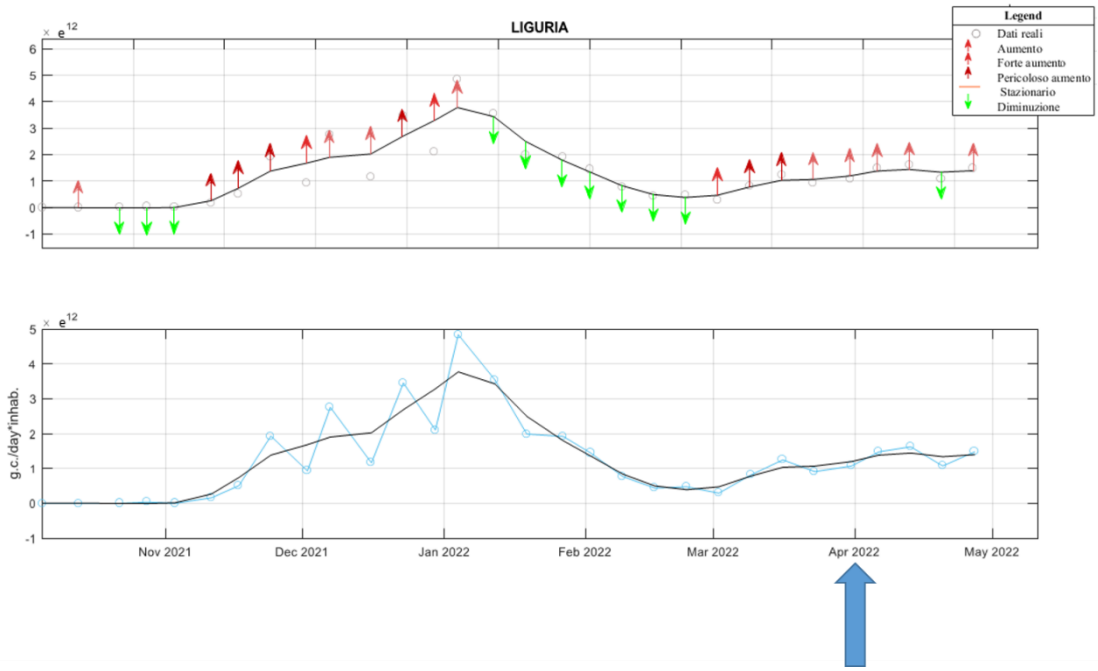
FRIULI-VENEZIA GIULIA



LAZIO



LIGURIA



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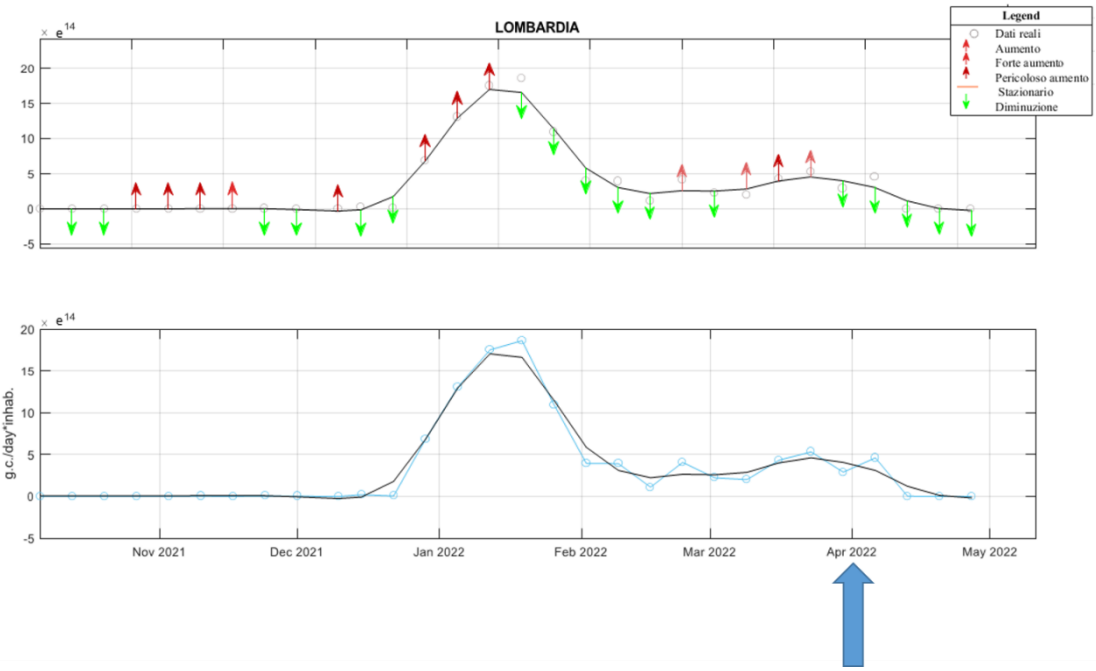

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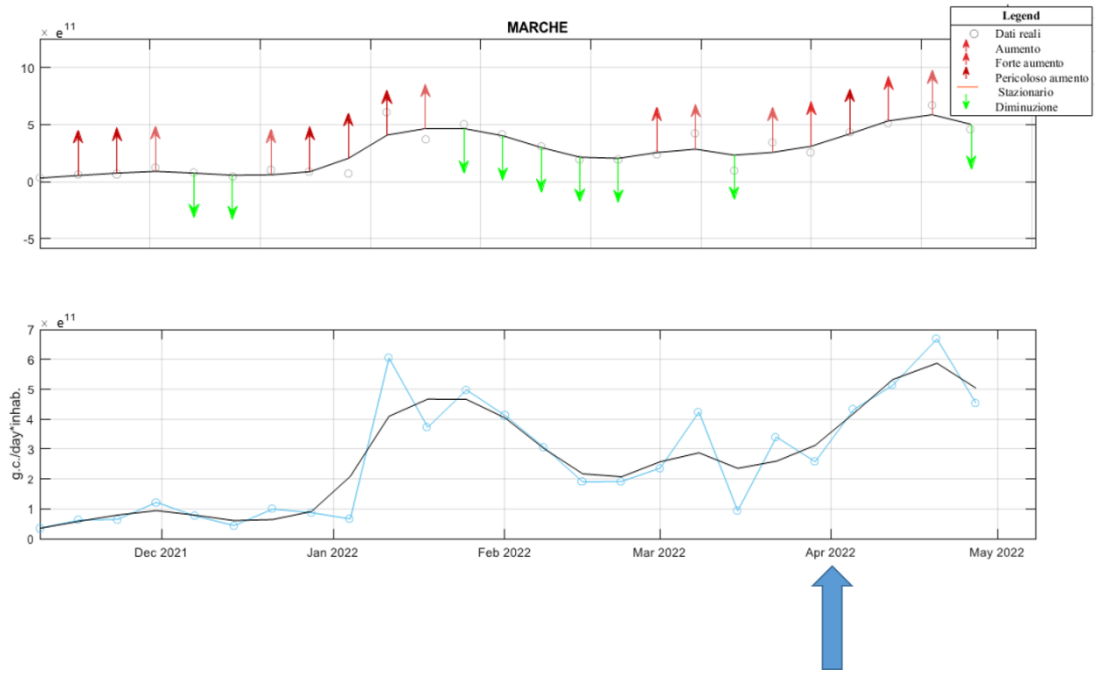

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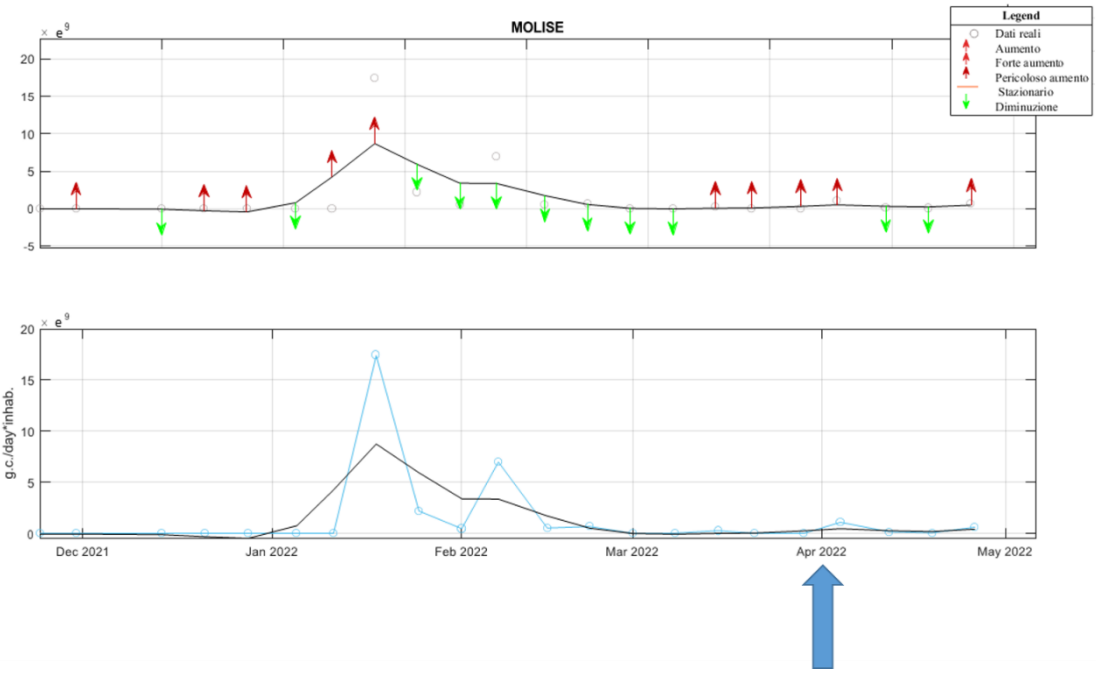

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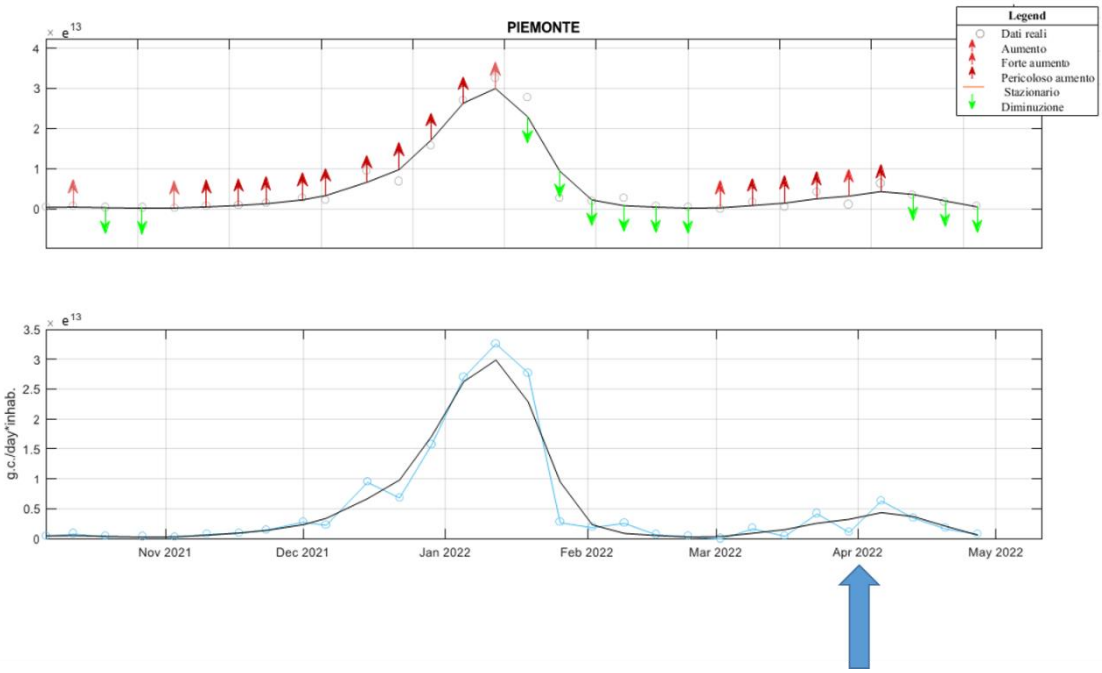

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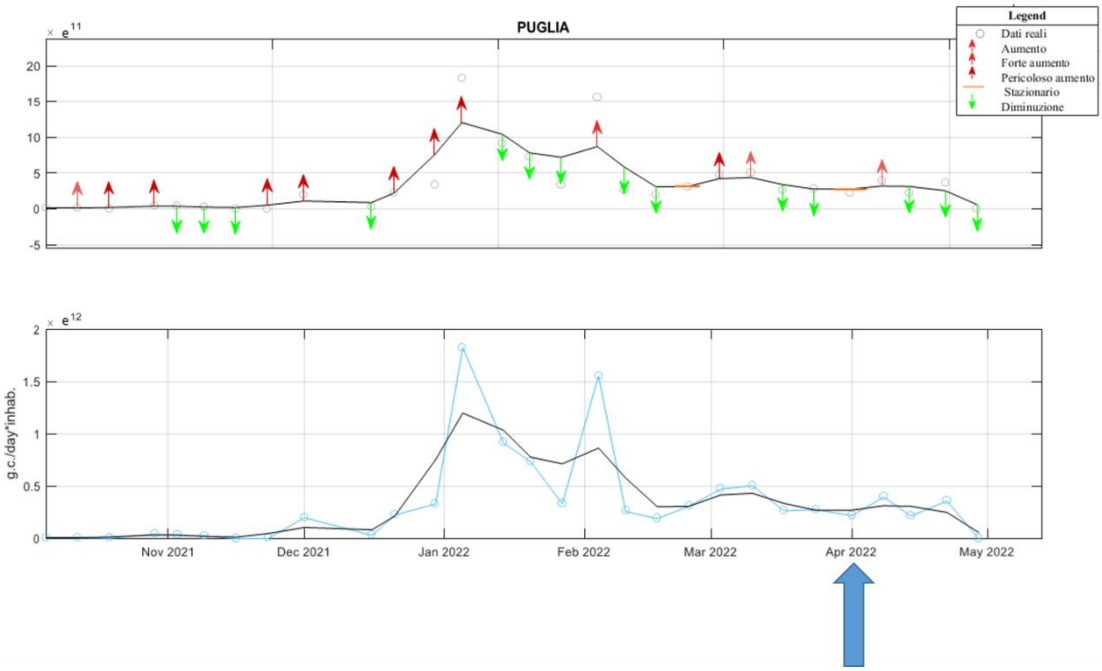

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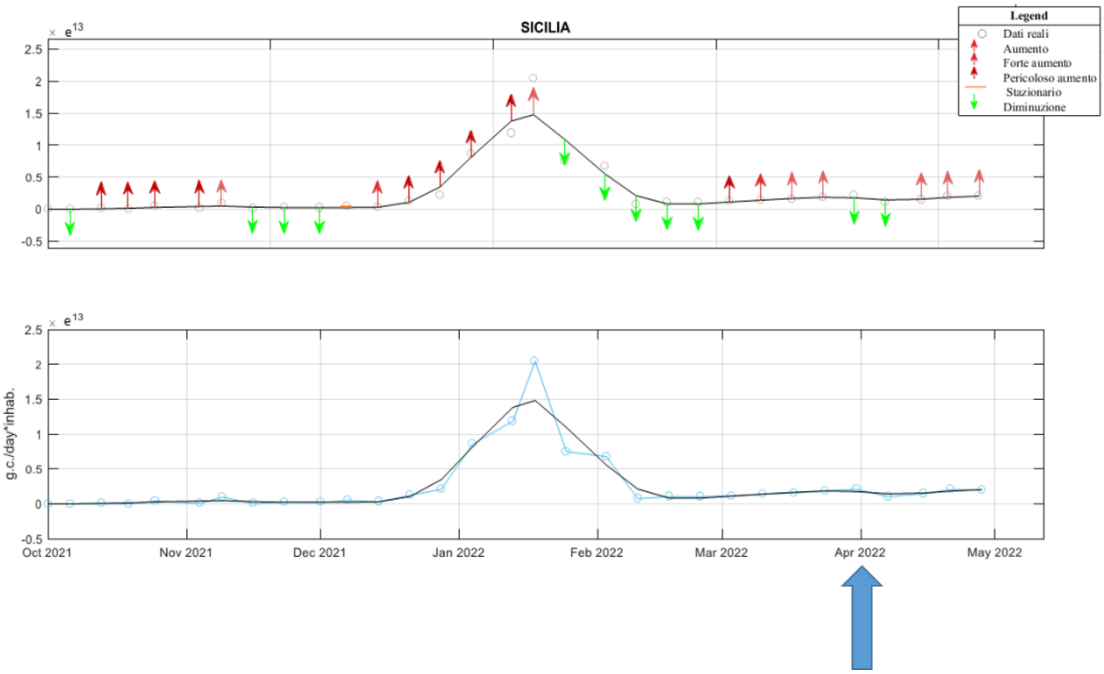

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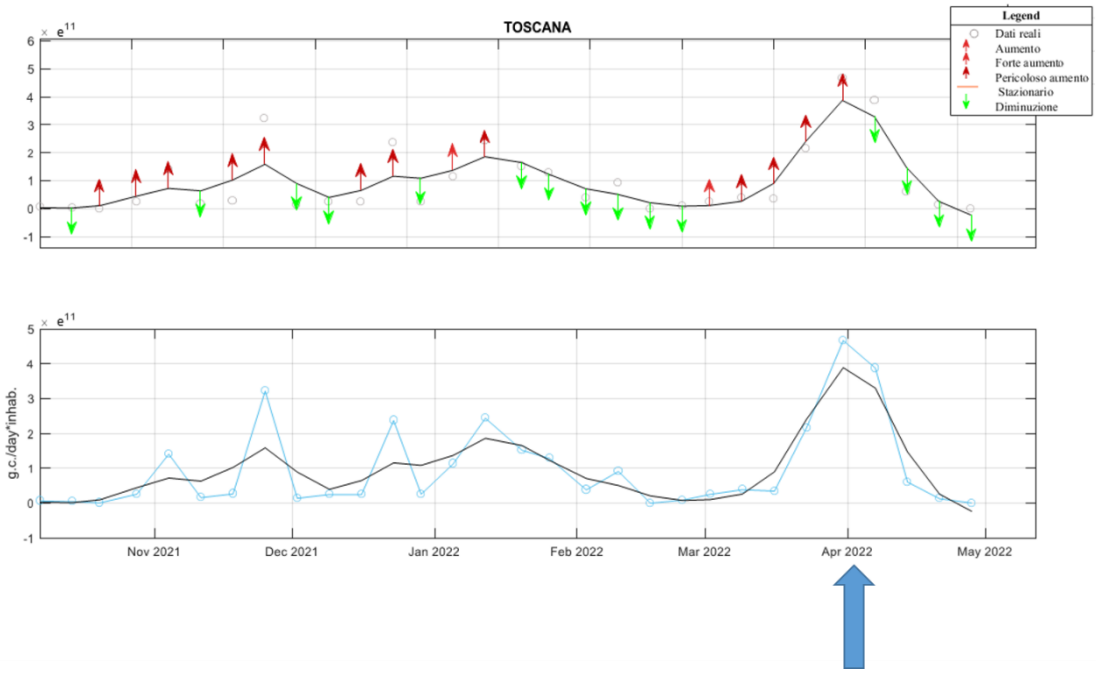

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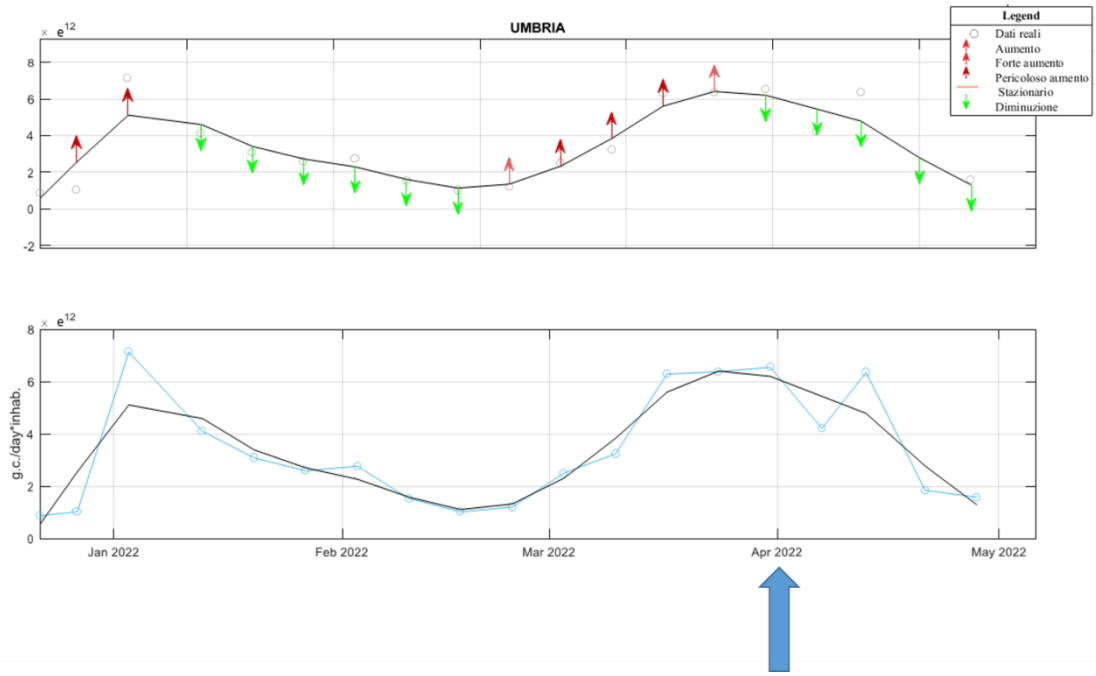

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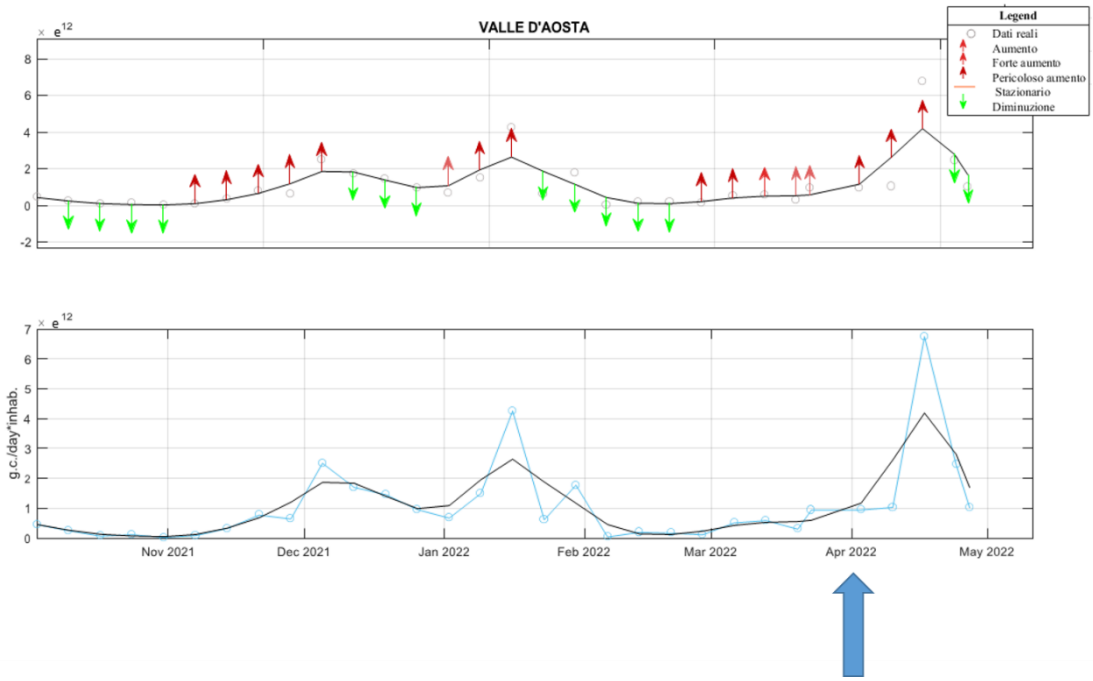

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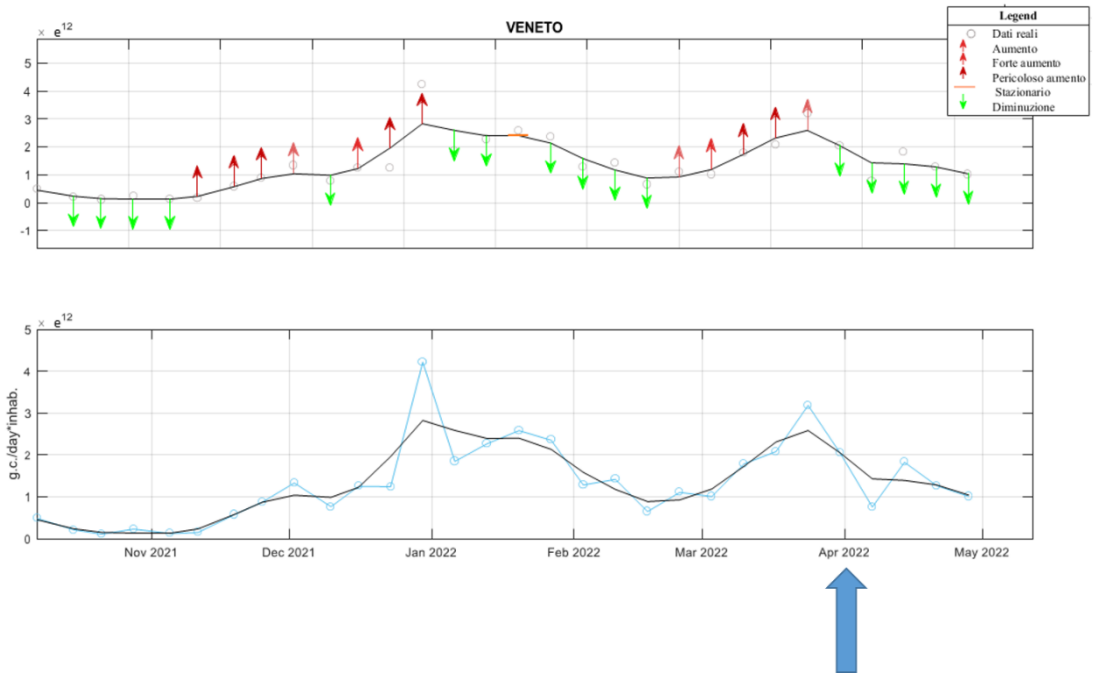

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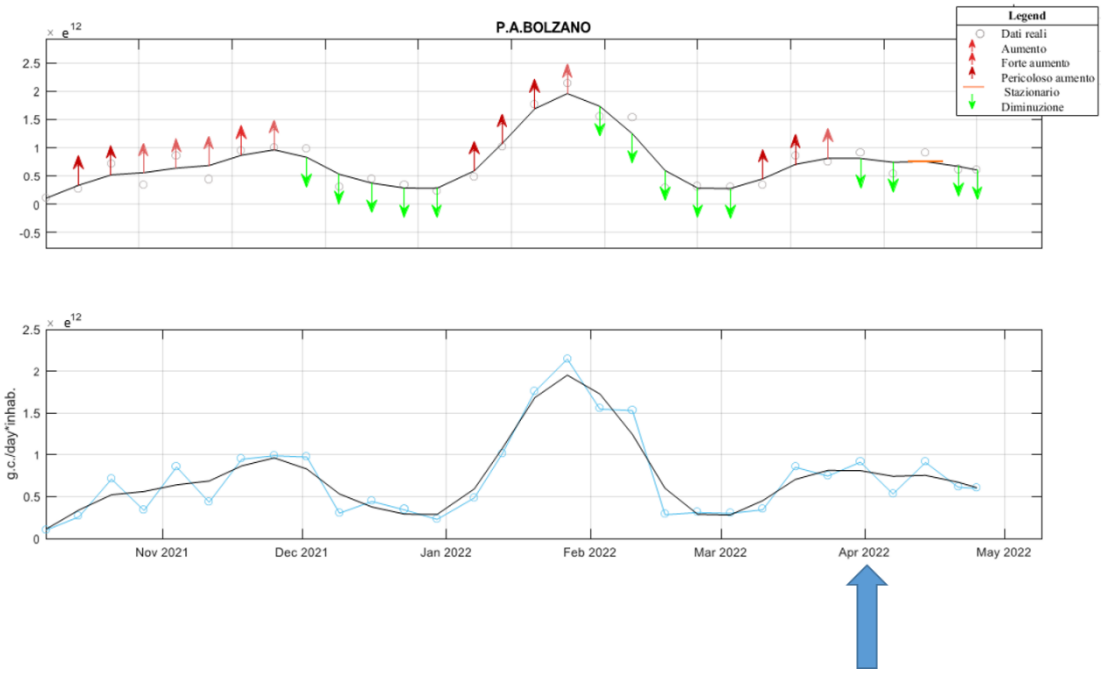

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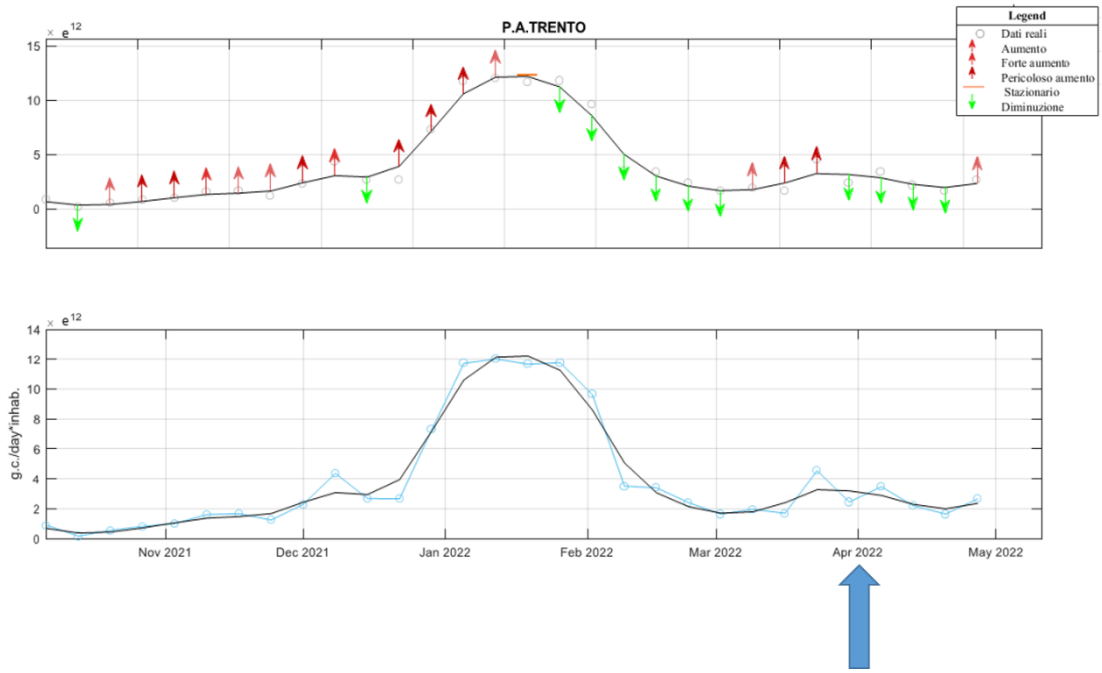

Acknowledgements

We wish to acknowledge the financial support on the Italian Government (Decree of the Ministry of Health 30.10.2021, GU Serie Generale n.294 del 11-12-2021), of Ministry of Health (program CCM 2020, project "Epidemiologia delle acque reflue: implementazione del sistema di sorveglianza per l'identificazione precoce di agenti patogeni, con particolare riferimento al SARS-CoV-2" [Wastewater based epidemiology: implementation of a surveillance system for the early detection of pathogens, and specifically SARS-CoV-2]) and of EU Commission (Gran Agreement 060701/2021/864481/SUB/ENV.C2).
We wish to thank Dr. Bernd Manfred Gawlik, European Commission's Joint Research Centre, for his coordination on building the EU Sewage Sentinel System for SARS-CoV-2 and its variants.
We also thank Lidia Orlandi, Claudia Del Giudice, Simona Di Pasquale (ISS) for technical and logistical support and Dr. Giuseppe Bortone Director of the Arpae, Emilia-Romagna for the support to the SARS-CoV-2 environmental surveillance in Italy.

We thank all of the members of the SARI network ("Sorveglianza Ambientale di SARS-CoV-2 attraverso i Reflui urbani in Italia") for the cooperation in sample collection and processing, data gathering and management, organization and logistic support. The SARI network includes:

- **Abruzzo:** Giuseppe Bucciarelli, Paolo Torlontano (Regione Abruzzo); Giuseppe Aprea, Silvia Scattolini, Ilaria Rosa, Daniela D'Angelantonio, Giacomo Migliorati (Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale");
- **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");
- **Emilia Romagna:** Paola Angelini, Lisa Gentili (Regione Emilia – Romagna); Laura De Lellis, Daniele Nasci (HERATech); Giovanni Alborali; Nicoletta Formenti, Flavia Guarneri (Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna); Nadia Fontani, Giulia Nani, Franca Palumbo, Gianluca Borlone, Marco Guercio (IREN);
- **Friuli Venezia Giulia:** Marika Mariuz, Gabriella Trani (Direzione Centrale Salute FVG); Anna Pariani (LABORATORIO HERAtech di Sasso Marconi –BO);
- **Lazio:** Carla Ancona (DEPLAZIO - Dipartimento di Epidemiologia del Servizio Sanitario Regionale - Regione Lazio); Alessandra Barca, Flavia Serio (Regione Lazio); Doriana Antonella Giorgi, Irene Ferrante, Monica Monfrinotti, Silvia Riosa, Valeria Capparuccini (ARPA Lazio - Agenzia Regionale per la Protezione Ambientale del Lazio); Maria Teresa Scicluna, Antonella Cersini (IZSLT - Istituto Zooprofilattico Sperimentale del Lazio e della Toscana); Mariaconcetta Arizzi, Giancarlo Cecchini, Claudio Ottaviano (Acea Elabori);
- **Liguria:** Elena Nicosia (Regione Liguria settore tutela della salute negli ambienti di vita e di lavoro); Nadia Fontani, Giulia Nani, Franca Palumbo, Gianluca Borlone, Marco Guercio (Iren); Elena Grasselli; Giorgia Allaria, Lorenzo Dondero, Francesca Rispo (UNIGE - DISTAV); Alberto Izzotti (UNIGE – DIMES); Rosa Maria Bertolotto, Elena Nicosia, Stefano Rosatto, Marta Bellisomi, Irene Tomesani (ARPAL); Micaela Tiso (MICAMO srl);
- **Lombardia:** Emanuela Ammoni, Danilo Cereda (Regione Lombardia); Marina Nadia Losio, Barbara Bertasi (IZSLER - Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia); Desdemona Oliva, Maria Giovanna Guiso, Fabio Ferrari, Maria Mundo ed Antonino Martines (CAP Holding); Sara Castiglioni, Silvia Schiarea, Ettore Zuccato (Istituto Mario Negri IRCCS); Manuela Antonelli, Arianna Azzellino, Francesca Malpei, Andrea Turolla (POLIMI); Sandro Binda,

Pellegrinelli Laura, Valeria Primache (Università degli Studi di Milano, Dipartimento di Scienze Biomediche per la Salute), Clementina Cocuzza, Andrea Franzetti, Rosario Musumeci e Marianna Martinelli (Università di Milano-Bicocca); Giorgio Bertanza (Università di Brescia), Maria Luisa Callegari (Università Cattolica del Sacro Cuore);

- **Marche:** Luigi Bolognini, Fabio Filippetti (Regione Marche); Marta Paniccia', Francesca Ciuti, Sara Briscolini (IZSUM - Istituto Zooprofilattico Sperimentale Umbria Marche); Silvia Magi (ARPAM);
- **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);
- **Piemonte:** Bartolomeo Griglio, Renza Berruti, Mauro Cravero, Angela Costa (Regione Piemonte); Manila Bianchi, Lucia Decastelli; Angelo Romano; Clara Tramuta (IZSTO - Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta SC Sicurezza e Qualità degli Alimenti); Elisabetta Carraro, Cristina Pignata (Dipartimento di Scienze della Sanità Pubblica e Pediatriche, Università di Torino), Silvia Bonetta, Lisa Richiardi (Dipartimento di Scienze della Sanità Pubblica e Pediatriche, Università di Torino);
- **Puglia:** Giuseppe Di Vittorio, Onofrio Mongelli (Regione Puglia); Osvalda De Giglio, Francesca Apollonio, Francesco Triggiano, Maria Teresa Montagna (Università degli Studi di Bari Aldo Moro - Dipartimento Interdisciplinare di Medicina); Nicola Ungaro (ARPA Puglia);
- **Sicilia:** Mario Palermo (Regione Sicilia); Carmelo Massimo Maida, Walter Mazzucco (Università degli Studi di Palermo-Dipartimento PROMISE - sezione di Igiene); Simona De Grazia, Giovanni Giammanco (Centro di Riferimento Regionale per la Sorveglianza delle Paralisi Flaccide Acute (PFA) e ambientale della circolazione di poliovirus in Sicilia - AOUP Palermo); Giuseppa Purpari (IZS - Istituto Zooprofilattico Sperimentale della Sicilia); Margherita Ferrante; Antonella Agodi, Martina Barchitta (Università degli Studi di Catania - Dipartimento "G. F. Ingrassia");
- **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).