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**Surveillance of SARS-CoV-2 in urban wastewater in Italy**  
**3<sup>rd</sup> Report**  
**(Study period: 01 - 31 May 2022)**

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### Main findings:

- Report on SARS-CoV-2 surveillance in urban wastewaters in Italy, May 2022.
- As on 31 May 2022, 20/21 Regions/Autonomous Provinces produce data within the environmental surveillance program and the environmental network includes a total of 167 wastewater treatment plants throughout Italy.
- A total of 926 wastewater samples were collected during April 2022, 898 of which were analysed for SARS-CoV-2 RNA by real time PCR. Overall, 91.5% (822/898) of the tested samples could be quantified, average concentrations ranging from 1,31E+02 to 1,42E+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, confirming the trend already shown in April 2022.

## Introduction

On 17<sup>th</sup> 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<sup>1</sup>. 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 during May 2022.

### Enrolled Regions/Autonomous provinces

In May 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.

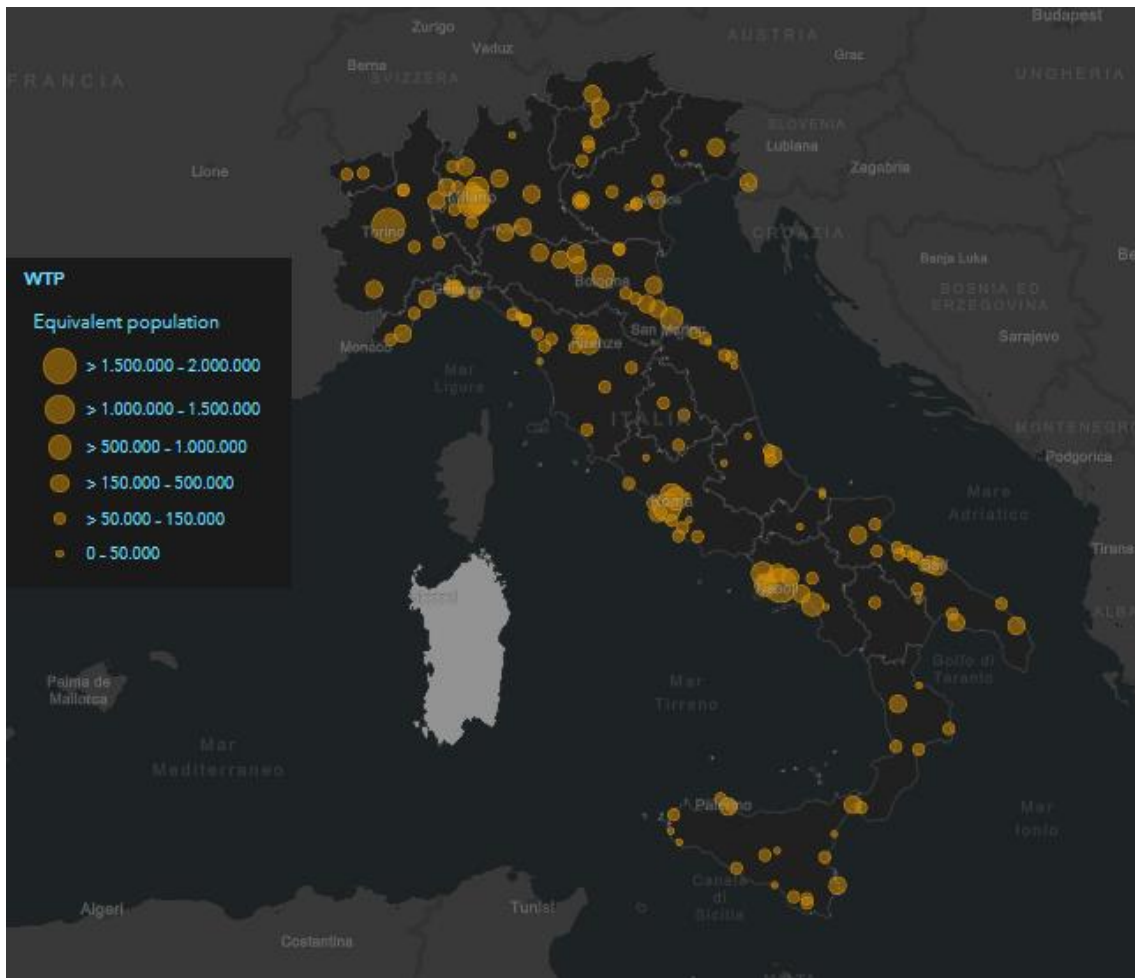
### Sampling sites and frequency

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 inhabitants. Urban centres with a population between 50k and 150k inhabitants were also included

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<sup>1</sup> 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>)

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<sup>2</sup>. 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

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<sup>2</sup> 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)

**Table 1.** Sampling sites and characteristics of the WTPs studied

Region /A. P.	Metropolitan City	WTP	Population equivalent <sup>#</sup>
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
Lombardia	Genova	Punta Vagno Genova	75.000
	Genova	Valpolcevera	157.650
	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

‡ 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 May 2022, **926 wastewater samples** were collected, as follow (Figures 2 and 3):

Week 17 (limited to 01.05.2022): 2 samples

Week 18 (02.05.2022 - 08.05.2022): 210 samples

Week 19 (09.05.2022 - 15.05.2022): 211 samples

Week 20 (16.05.2022 - 22.05.2022): 215 samples

Week 21 (23.05.2022 - 29.05.2022): 209 samples

Week 22 (limited to 30.05.2022 - 31.05.2022): 79 samples



Figure 2. Total samples collected and analysed during May 2022 by the 20 Regions/A.P.

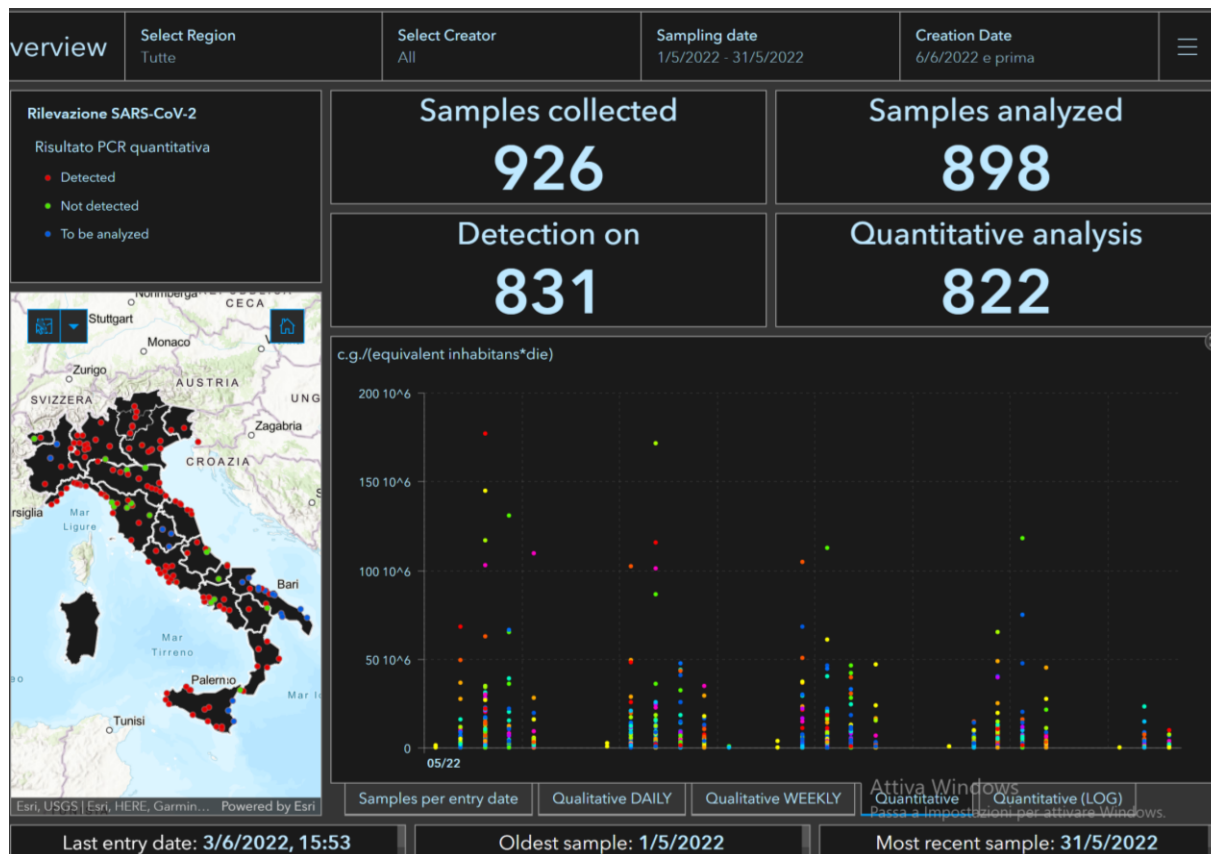


Figure 3. Samples collected and analysed by week during May 2022. Week 17 (limited to 01.05.2022); Week 18 (02.05.2022 - 08.05.2022); Week 19 (09.05.2022 - 15.05.2022); Week 20 (16.05.2022 - 22.05.2022); Week 21 (23.05.2022 - 29.05.2022), Week 22 (limited to 30.05.2022 - 31.05.2022). Red = positive sample; 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). Raw quantitative data, expressed as c.g./ (equivalent inhabitants\*die) are shown in Figure 4.



**Figure 4.** Raw quantitative data obtained in all WTPs under analysis during May 2022. Each dot represent a concentration value, expressed as c.g./ (equivalent inhabitants\*die)

Table 2 summarises the number of samples collected in May by each Region/A.P. and quantitative results expressed as c.g./L wastewater. SARS-CoV-2 RNA was detected in 91.5% (822/898) of the tested samples prevalence ranging from 62.5% to 100%. Average concentrations ranged from 1,31E+02 to 1,42E+05 c.g./L wastewater, lower than those documented in the previous month.

**Table 2.** Quantitative results obtained during April 2022

Region/A.P.	N° of WTP	Collect ed 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	25	25	16 (100%)	7,50E+01 - 4,25E+02	2,18E+02
Basilicata	2	8	8	5 (62.5%)	1,13E+03 - 1,60E+04	7,68E+03
Calabria	6	27	27	27 (100%)	1,93E+01 - 3,80E+02	1,31E+02
Campania	10	70	70	68 (97.1%)	1,25E+03 - 1,31E+05	2,46E+04
Emilia Romagna	14	84	84	71 (84.5%)	1,10E+03 - 1,19E+06	1,15E+05

Friuli Venezia Giulia	3	16	16	16 (100%)	2,73E+03 - 8,95E+05	1,36E+05
Lazio	13	59	59	58 (98.3%)	2,50E+01 - 4,60E+05	5,22E+04
Liguria	16	66	66	66 (100%)	6,91E+03 - 2,18E+05	6,89E+04
Lombardia	15	105	105	90 (85.7%)	7,63E+02 - 3,40E+05	2,95E+04
Marche	6	24	24	23 (95.8%)	1,30E+03 - 2,56E+04	9,42E+03
Molise	3	15	15	9 (60%)	7,50E+01 - 2,86E+04	3,77E+03
Piemonte	7	33	30	30 (100%)	7,50E+02 - 1,52E+04	5,44E+03
Puglia	16	90	72	72 (100%)	3,09E+02 - 2,36E+04	5,35E+03
Sicilia	17	93	89	88 (98.8%)	1,15E+03 - 2,83E+05	3,89E+04
Toscana	13	70	70	49 (70%)	1,79E+03 - 7,74E+04	1,89E+04
Umbria	3	19	16	16 (100%)	7,26E+04 - 2,43E+05	1,42E+05
Valle d'Aosta	2	17	17	15 (88.2%)	5,72E+02 - 2,46E+04	6,70E+03
Veneto	10	51	51	49 (96%)	8,00E+02 - 1,51E+05	3,23E+04
A.P. Bolzano	3	27	27	27 (100%)	5,99E+03 - 4,13E+04	1,96E+04
A.P. Trento	3	27	27	27 (100%)	2,29E+01 - 3,59E+05	1,05E+05
<b>Total</b>	<b>167</b>	<b>926</b>	<b>898 (97.0%)</b>	<b>822 (91.5%)</b>	<b>2,29E+01 -1,19E+06</b>	<b>4,11E+04</b>

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. Variation compared to the previous week are shown with arrows (red= increase, green = decrease, black = stationary).The last three months of surveillance are represented.

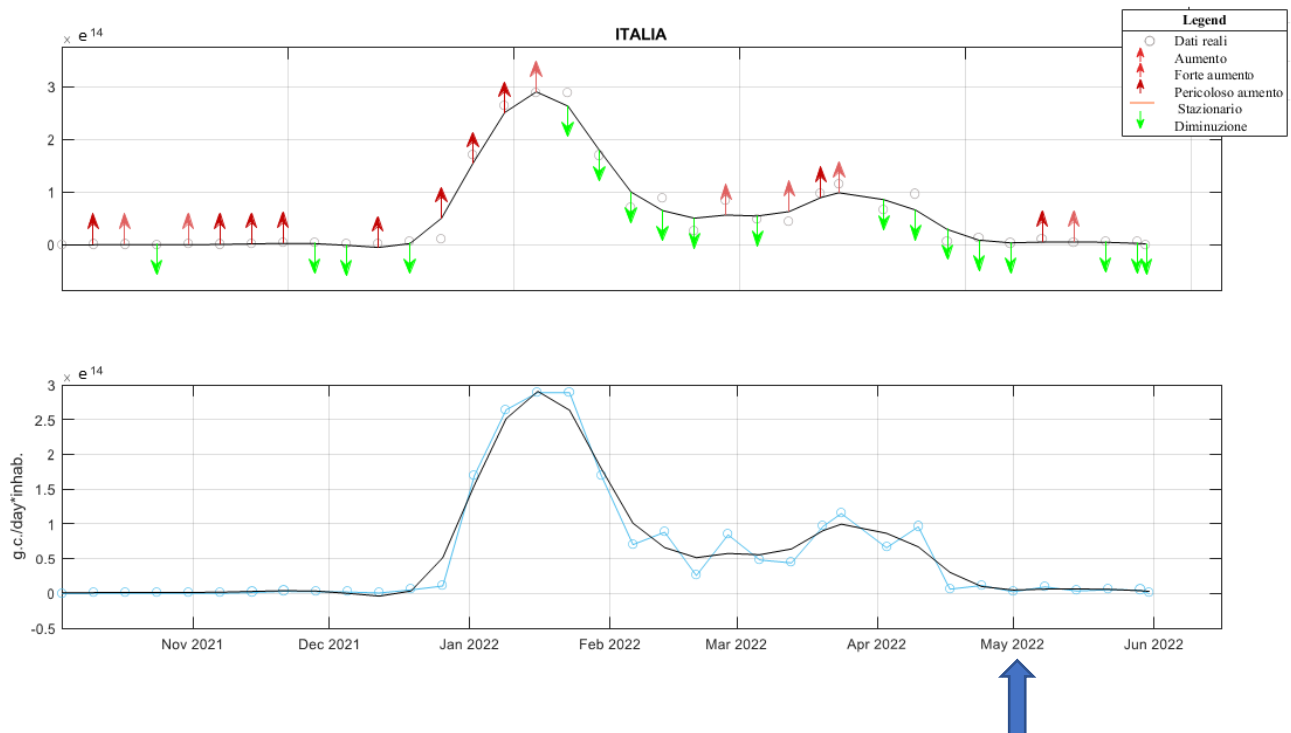
Region/A.P.	week													
	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Abruzzo	↓	↗	↗	↗	↗	↓	↓	↓	↗	↗	↗	↓	↓	↓
Basilicata	↓	↗	↗	↗	↗	↓	↓	↓	↗	↗	↓	↓	↓	↗
Campania	↗	↗	↗	↗	↗	↗	↗	↓	↓	↓	↓	↓	↓	↓
Calabria	↓	↗	↗	↗	↓	↓	↓	↓	↓	↓	↓	↓	n.a.	n.a.
Emilia-Romagna	↓	↗	↗	↗	↗	↓	↗	↗	↓	↓	↗	↓	↓	↓
Friuli-Venezia Giulia	↓	↗	↗	↗	↗	↗	↓	↓	↓	↗	↗	↔	↓	n.a.
Lazio	↗	↗	↗	↓	↓	↗	↗	↗	↗	↗	↓	↓	↓	↓
Liguria	↗	↗	↗	↗	↗	↗	↗	↓	↗	↗	↓	↓	↓	n.a.
Lombardia	↓	↗	↗	↗	↓	↓	↓	↓	↓	↓	↓	↗	↓	↓
Marche	↗	↗	↓	↗	↗	↗	↗	↗	↓	↓	↓	↓	↓	↓
Molise	↓	↓	↗	↗	↗	↗	↓	↓	↗	↗	↗	↗	↓	↓
A.P. Bolzano	↓	↗	↗	↗	↓	↓	↔	↓	↓	↔	↓	↓	↓	↓
A.P. Trento	↓	↗	↗	↗	↓	↓	↓	↓	↔	↓	↗	↓	↓	↗
Piemonte	↗	↗	↗	↗	↗	↗	↓	↓	↔	↗	↗	↓	↓	↓
Puglia	↗	↗	↓	↓	↓	↗	↗	↗	↗	↓	↓	↓	↓	↓
Sicilia	↗	↗	↗	↗	↓	↓	↔	↗	↗	↗	↓	↓	↓	↓
Toscana	↗	↗	↗	↗	↗	↓	↓	↓	↓	↗	↗	↓	↓	↓
Umbria	↗	↗	↗	↗	↓	↓	↓	↓	↓	↓	↓	↗	↓	↓
Valle d'Aosta	↗	↗	↗	↗	↗	↗	↗	↓	↓	↓	↓	↓	↓	↓
Veneto	↗	↗	↗	↗	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
Italy	↓	↗	↗	↗	↓	↓	↓	↓	↓	↗	↗	↓	↓	↓

n.a. not available

Week 9=28.02.2022 - 06.03.2022; week 22=30.05.2022 - 05.06.2022 (limited to 30-31 May)

Quantitative data were used to elaborate the Quiver graphs, as described previously<sup>3</sup>. Figure 5 represents the global data obtained in Italy over the eight months of surveillance (October 2021-May 2022), including results obtained from over 5700 measurements. Since the beginning of May concentrations decreased gradually over the entire month, reaching concentrations found in December 2021, before the Omicron wave.

Quiver graphs for each Region/A.P. are shown in Appendix 1.



**Figure 5.** Quiver graph representing SARS-CoV-2 trends in wastewater in Italy in the period 1 October 2021 - 31 May 2022

<sup>3</sup> 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 \(iss.it\)](https://www.iss.it/8e5e2edb-bae0-f1b0-ee6e-08255c76484f))

## 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 6% 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 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 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 May, 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%

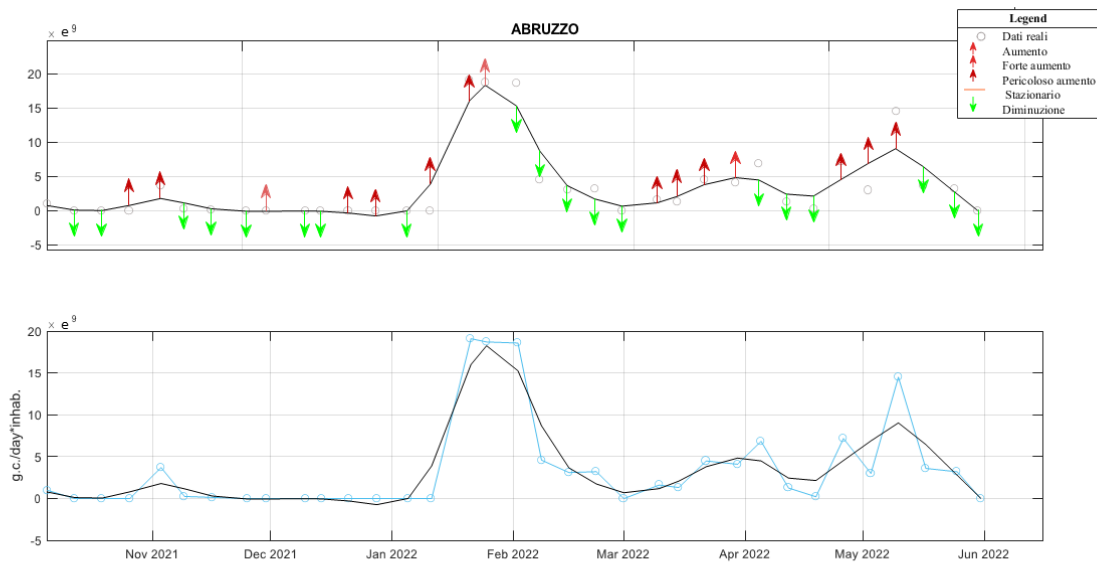
Strong Increase = 20%-30%

Dangerous Increase = >30%

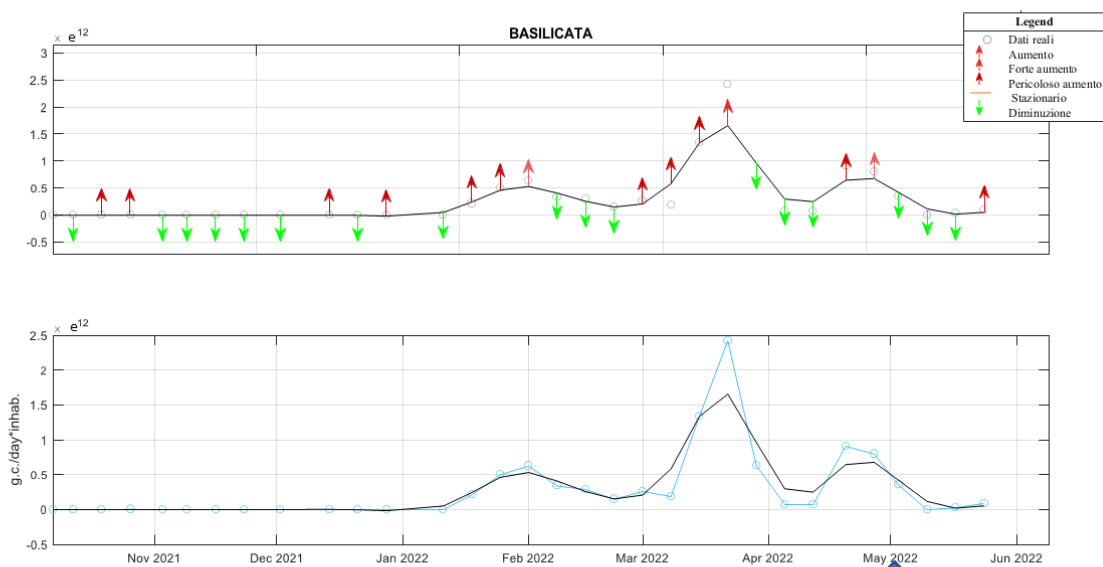
Stationary = 0-2%

Decrease = reduction of the concentration

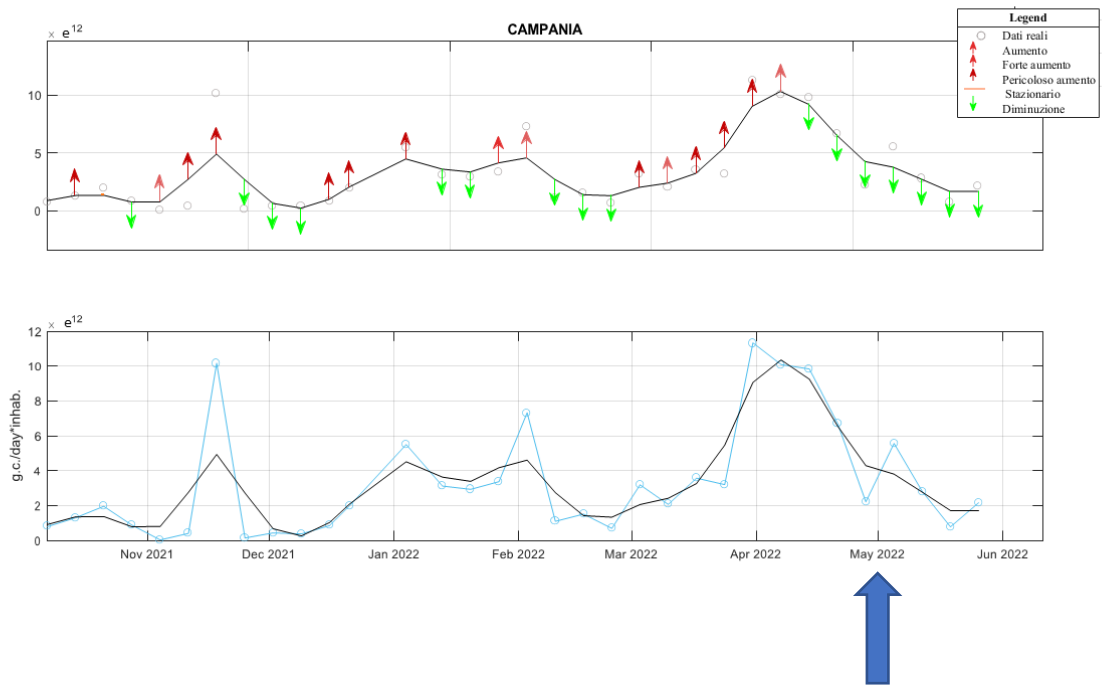
### ABRUZZO



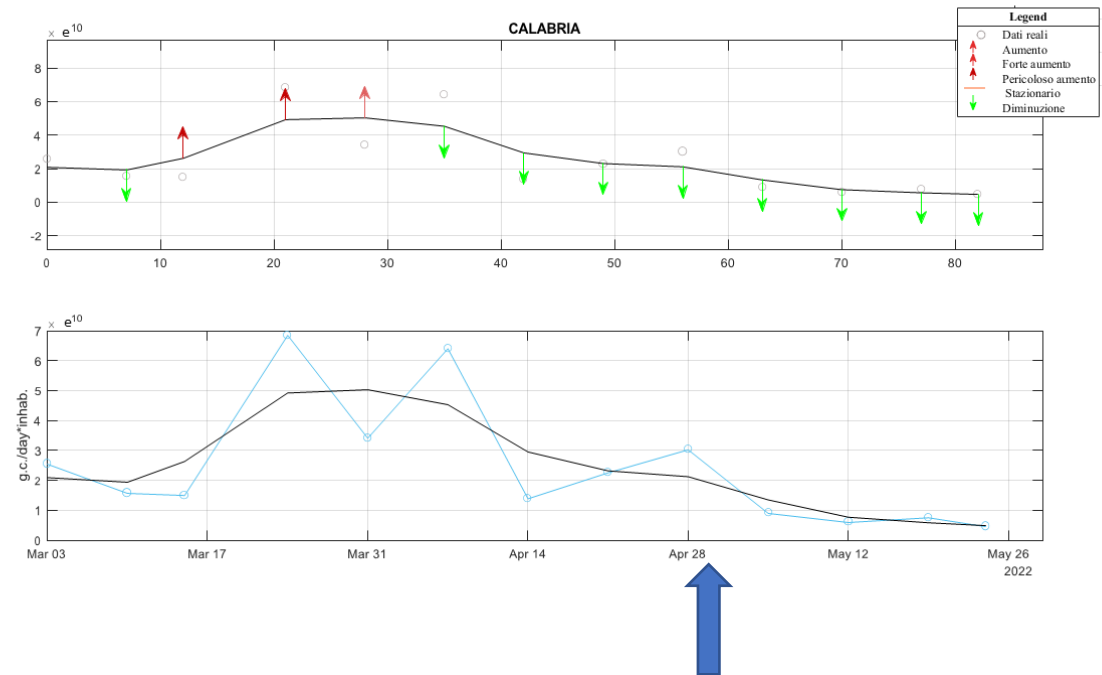
### BASILICATA



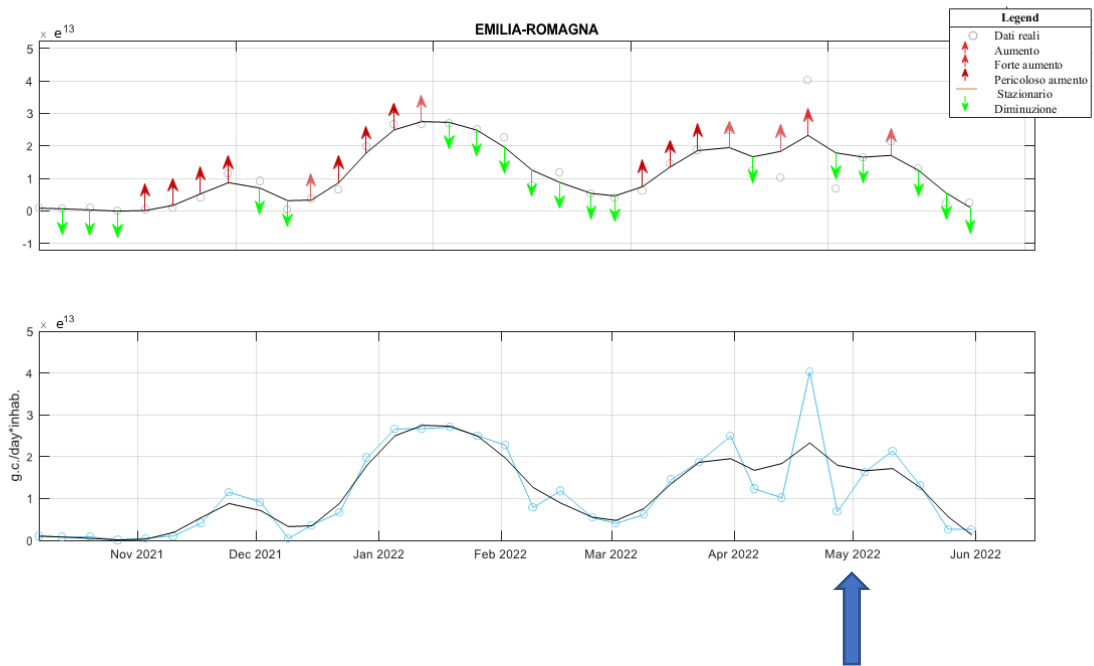
# CAMPANIA



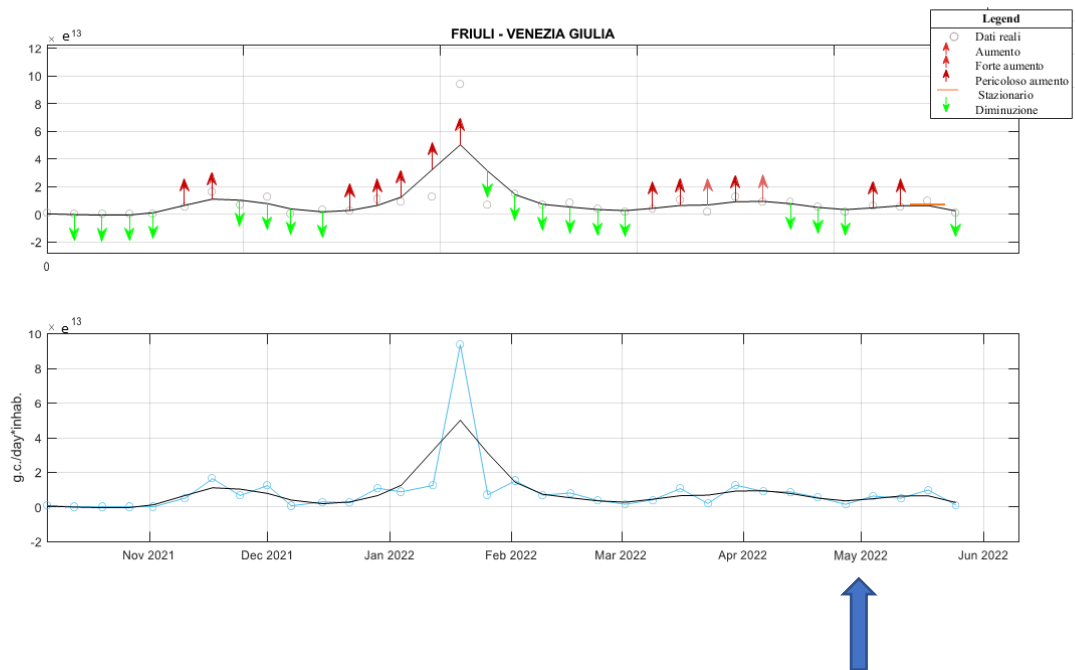
# CALABRIA



# EMILIA-ROMAGNA

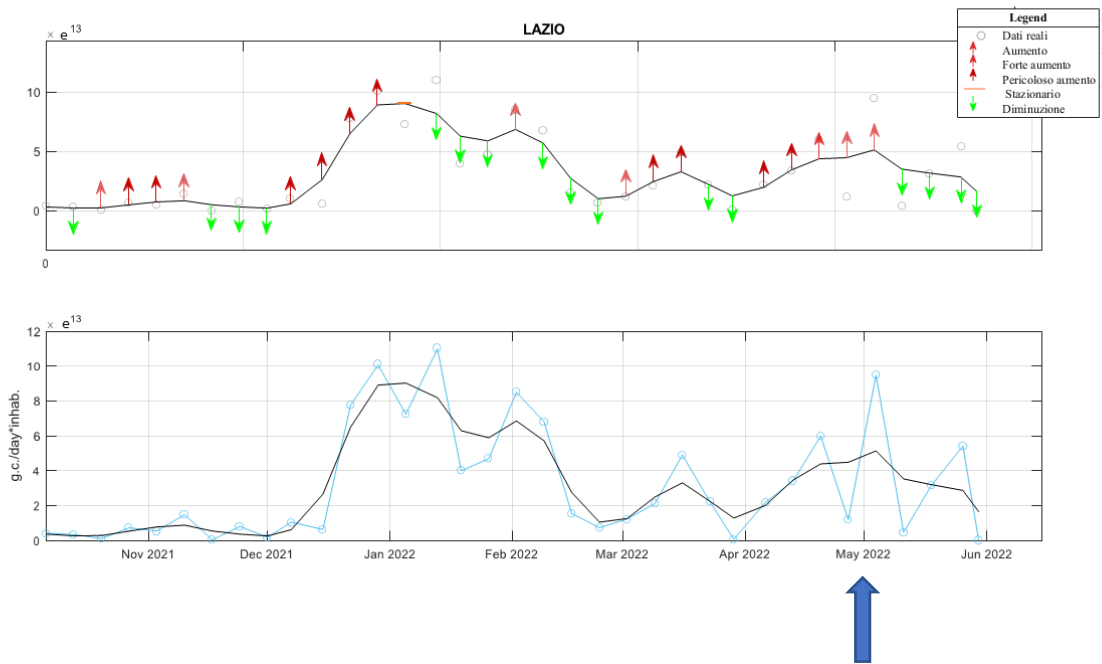


# FRIULI-VENEZIA GIULIA

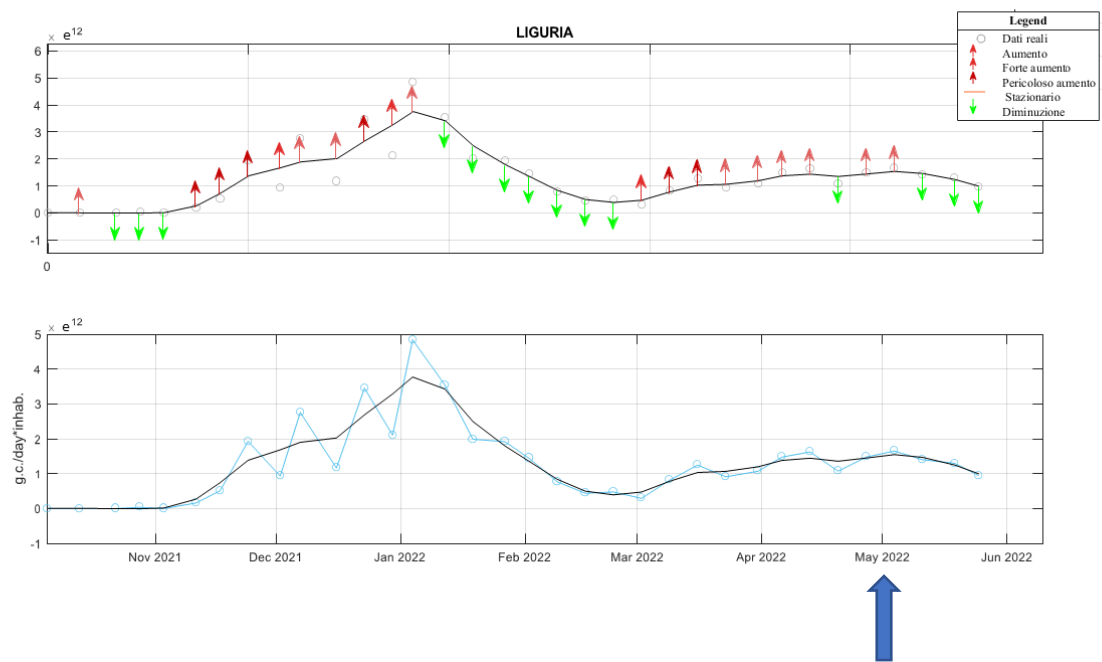




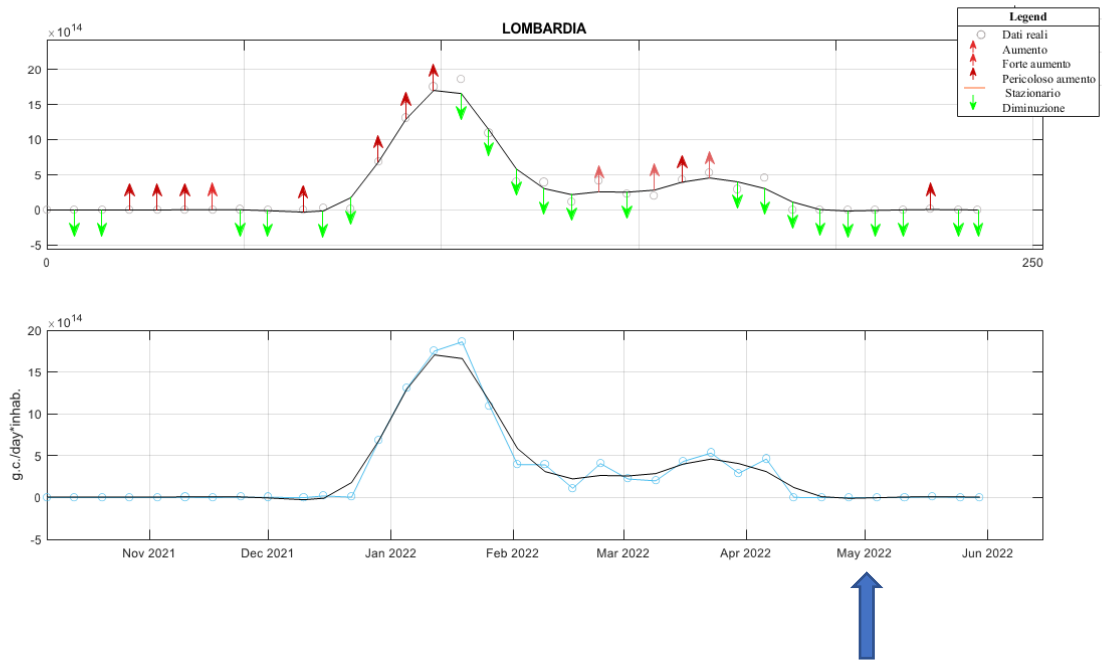
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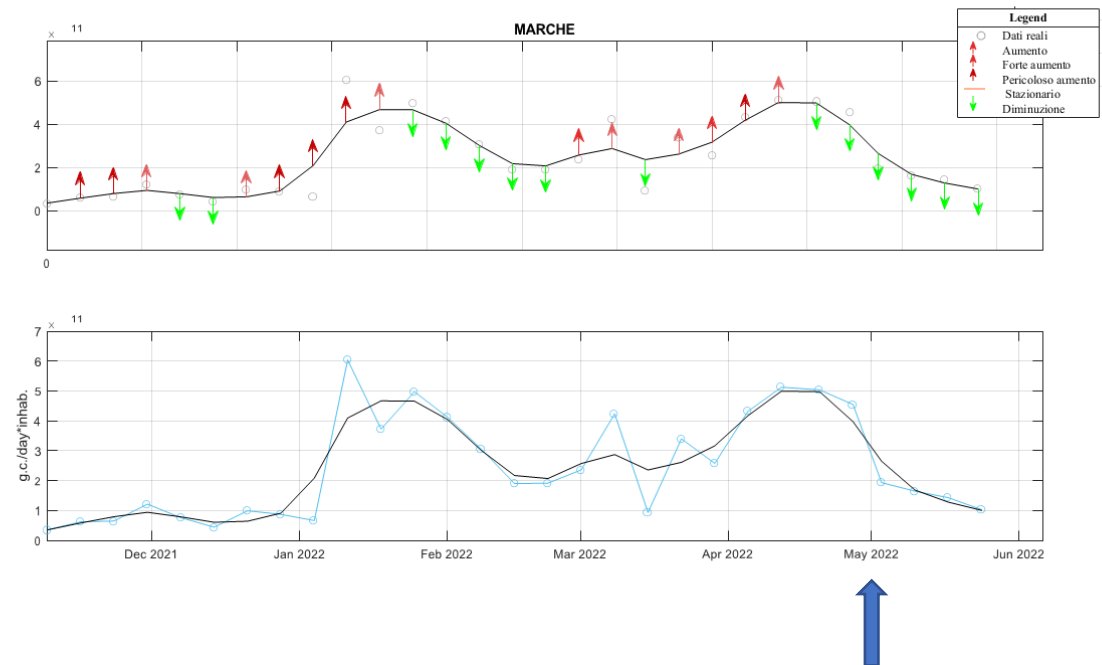
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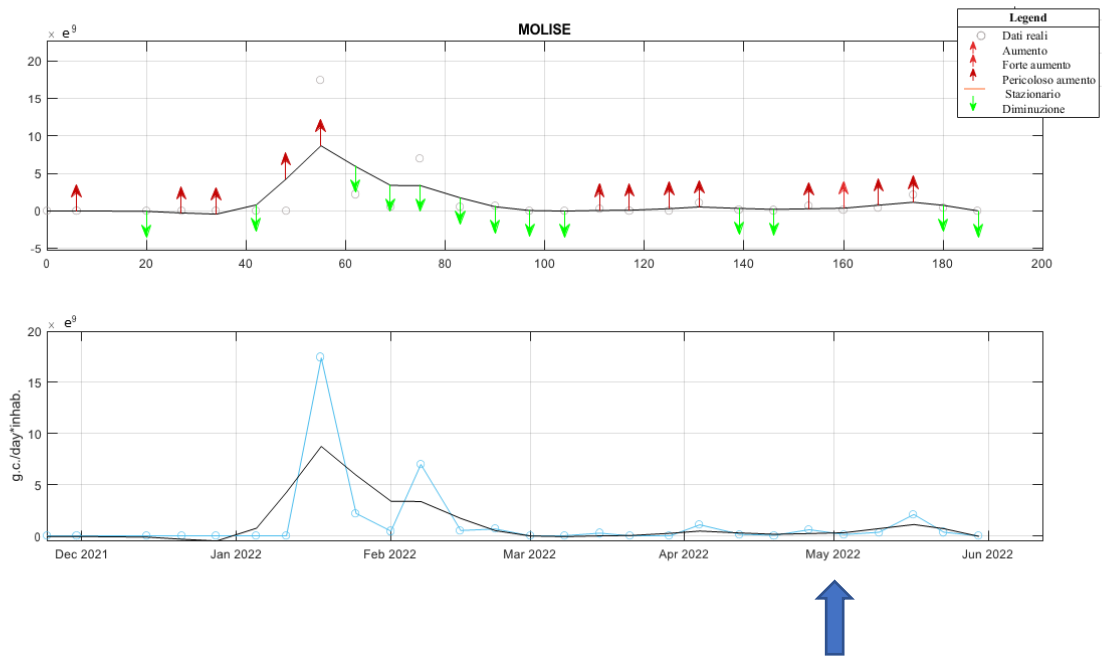
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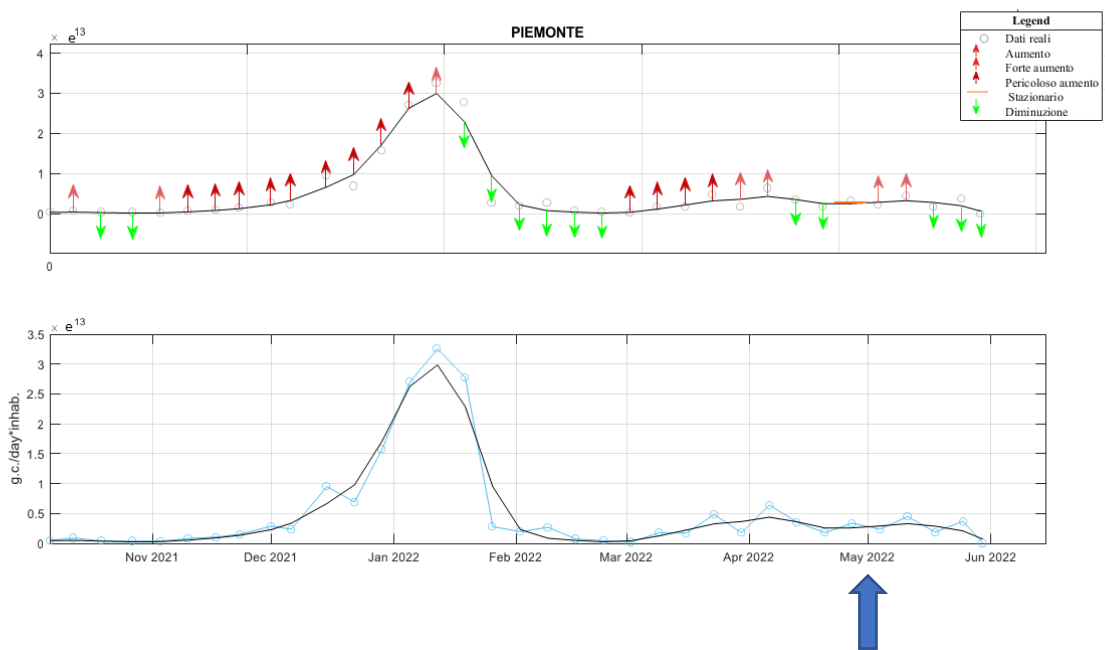
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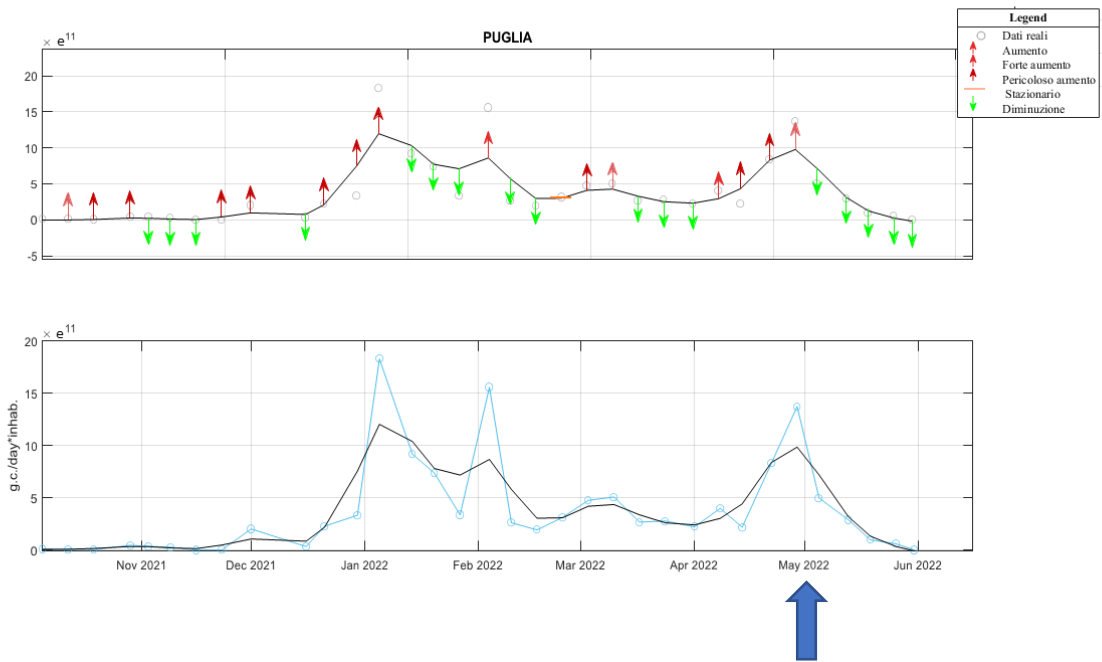
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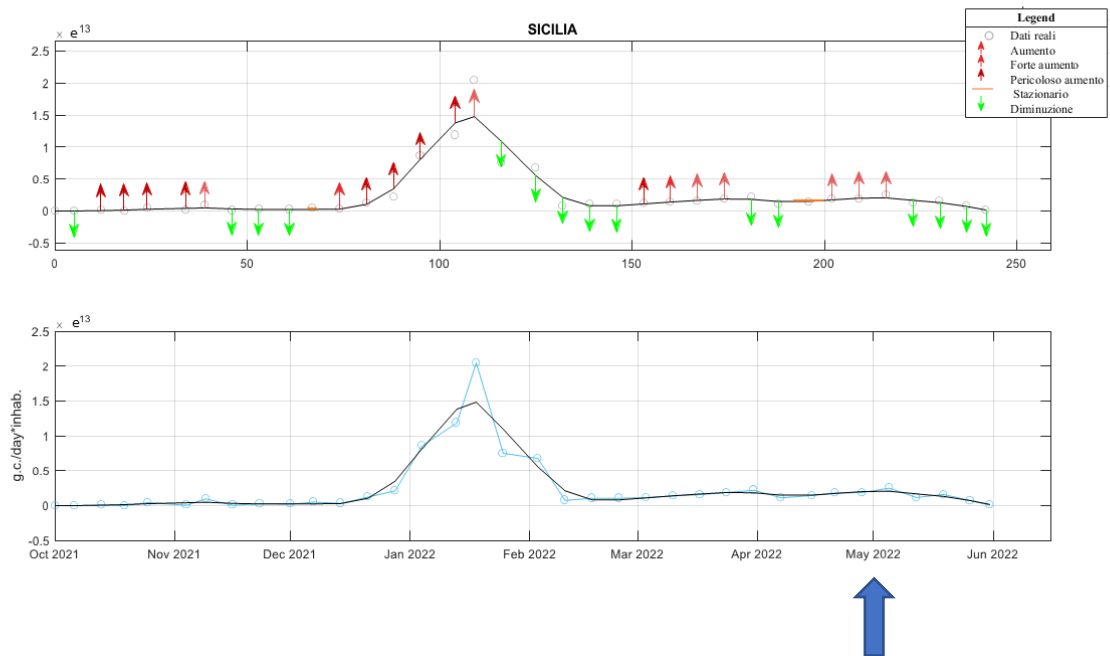
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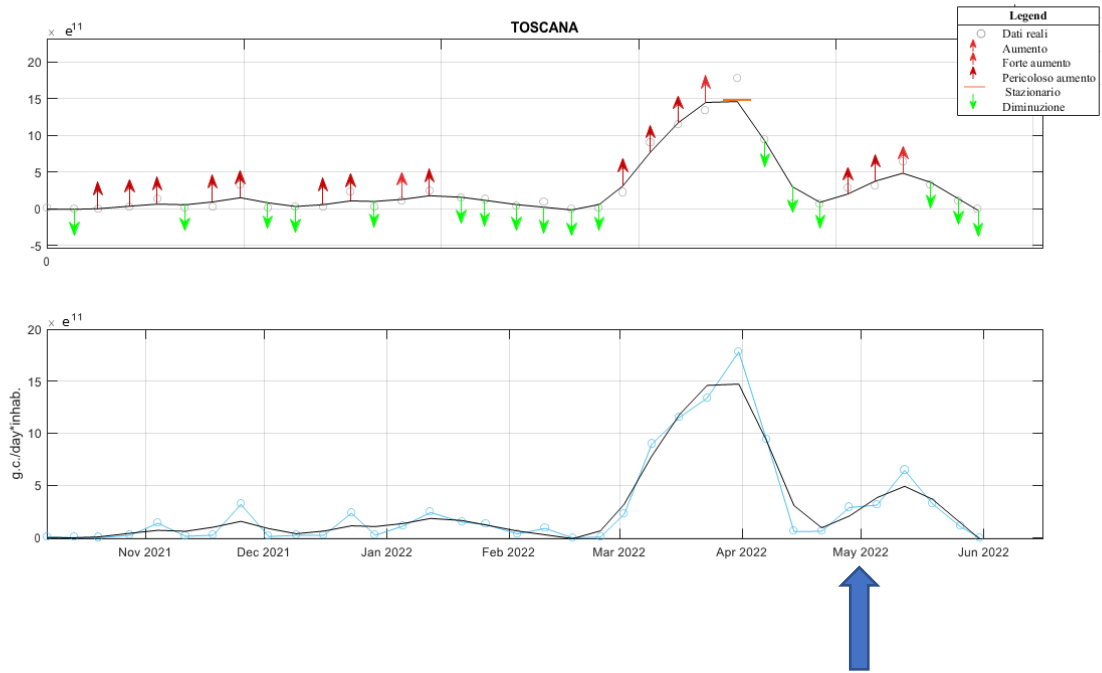
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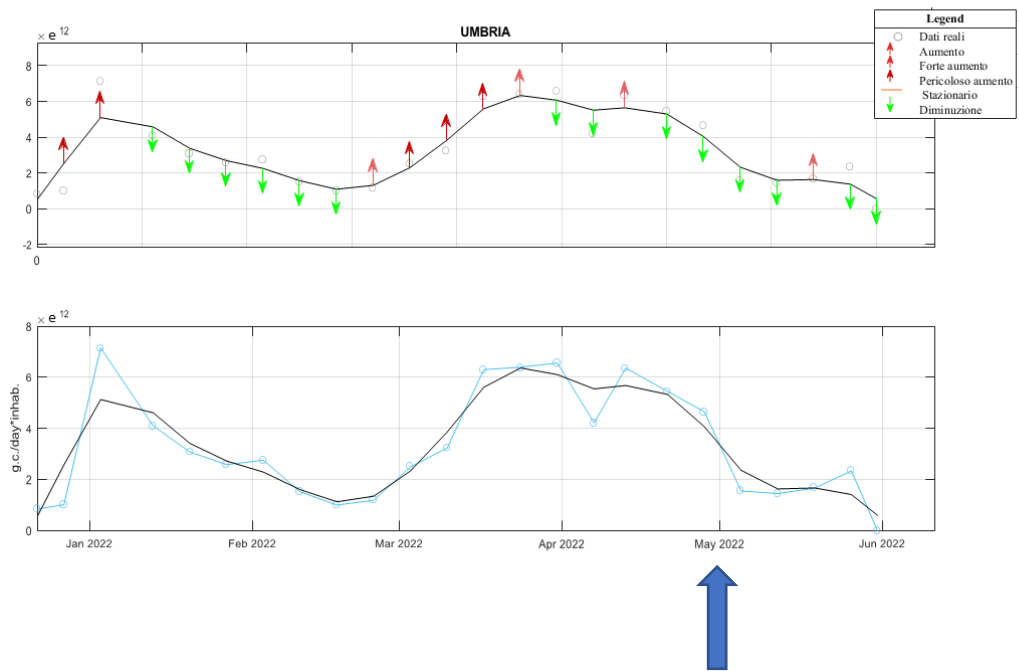
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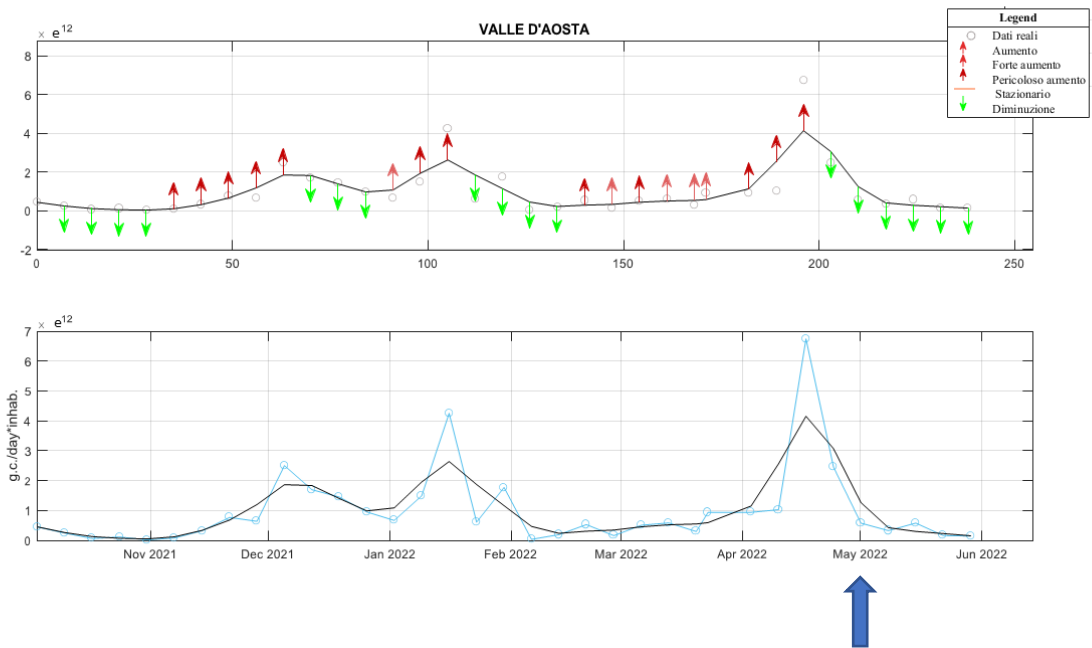
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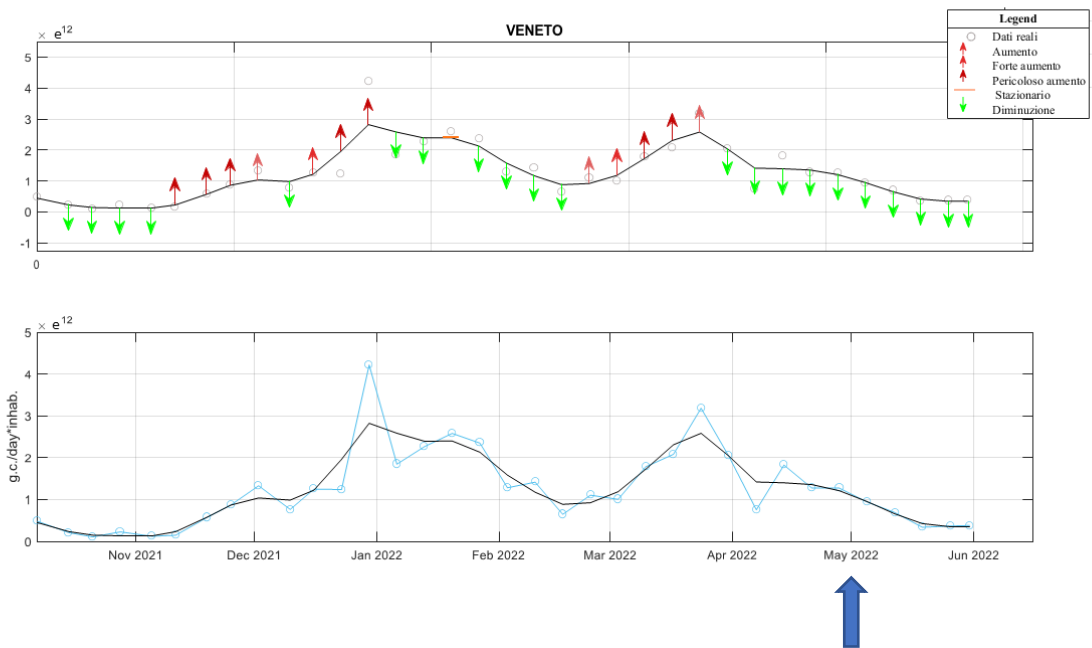
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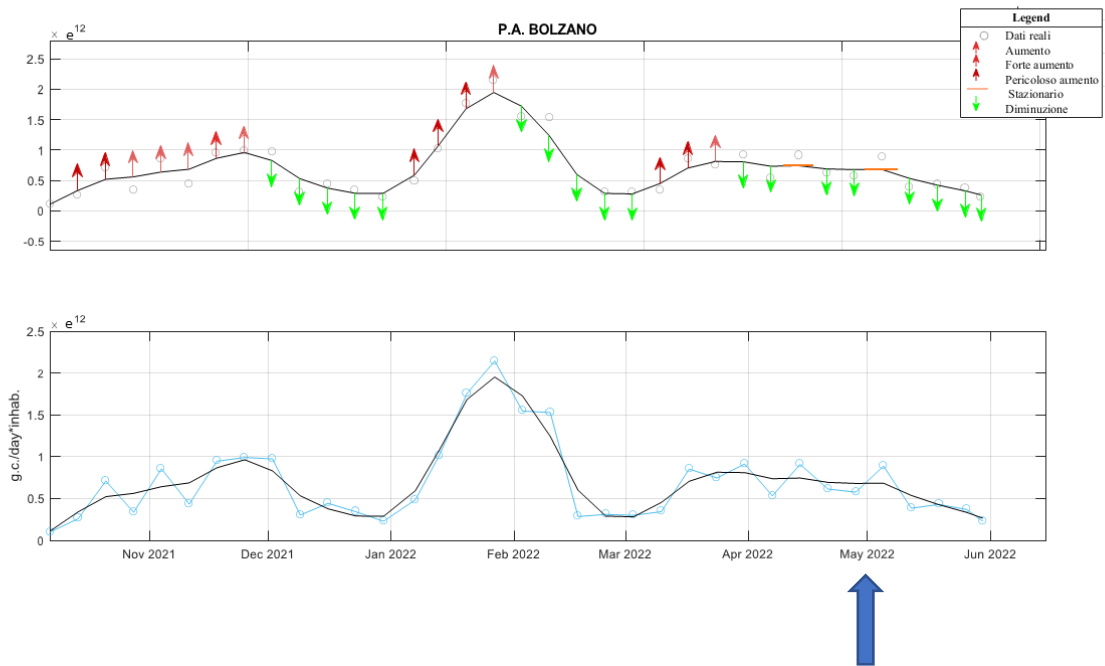
# VALLE D'AOSTA



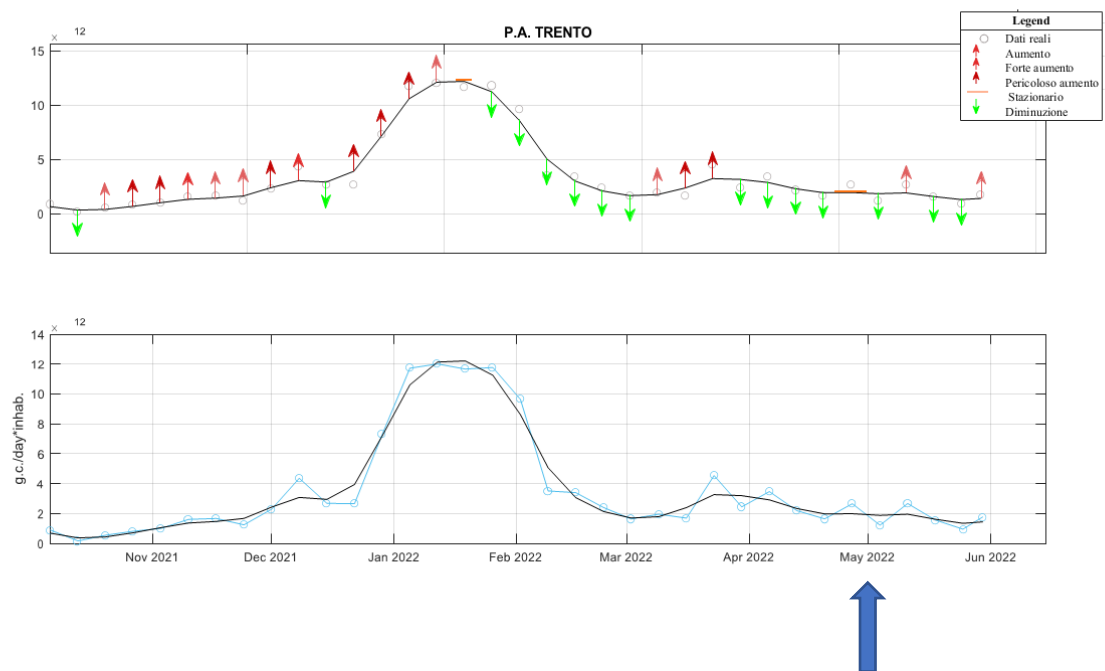
# VENETO



## P.A. BOLZANO



## P.A. TRENTO



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