Surveillance of SARS-CoV-2 in urban wastewater in Italy
6th Report
Update to September 30, 2022

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Main findings:

- As of September 30, 2022, a total of **9.374** wastewater samples were collected throughout Italy in the framework of the SARS-CoV-2 environmental surveillance (EU Commission Recommendation 2021/472)
- Overall, **9.368** wastewater samples were analysed and **8.315** (88.8%) were positive for SARS-CoV-2 RNA.
- The national trend of SARS-CoV-2 concentrations in wastewater showed four different "waves" during the one-year surveillance, associated with the emergence and spread of the different Omicron sublineages (BA.1, BA.2, and BA.4/5).

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]. The implementation of the Recommendation was granted by the Decree Law n. 73 of 25.05.2021, art. 34 and coordination of the activities was assigned to Istituto Superiore di Sanità (ISS).
As on September 2022, 20 of the 21 Italian Regions/A.P. provide SARS-CoV-2 concentration data within the environmental surveillance program. Details on enrolled Regions/Autonomous provinces, sampling sites, sampling frequencies and methods can be found in the 1° Report on Surveillance of SARS-CoV-2 in urban wastewater in Italy (https://www.iss.it/en/cov19-acque-reflue).

The aim of this report is to update the results of the environmental surveillance of SARS-CoV-2 up to September 30, 2022, one year after the official launch of environmental surveillance in Italy.
For the purpose of finalizing the trend analysis related to the 12 months of surveillance, a quality check was performed on the database, and outlier data related to viral loads or treatment plants flow rates were amended where needed. This resulted, for some Regions/P.A., in slight changes in the representation of data by Quiver graphs compared to previously published bulletins.

Results

The data on SARS-CoV-2 concentrations in wastewater are produced by the SARI network laboratories (see Acknowledgement section). Results on SARS-CoV-2 detection obtained since October 2021 are shown in Table 1.

Table 1. Results obtained in 20 Regions/Autonomous Provinces and as on September 30, 2022

Region/A.P.	N° of WTP	Collected Samples	Analysed samples	Positive samples	% of positive samples
Abruzzo	5	**258**	**258**	**105**	**40.7%**
Basilicata	2	**97**	**97**	**77**	**79.4%**
Calabria	6	**202**	**202**	**200**	**99.0%**
Campania	10	**785**	**785**	**687**	**87.5%**
Emilia Romagna	14	**857**	**852**	**723**	**84.9%**
Friuli Venezia Giulia	3	**166**	**166**	**153**	**92.2%**
Lazio	13	**620**	**620**	**587**	**94.7%**
Liguria	16	**926**	**926**	**804**	**86.8%**
Lombardia	15	**978**	**978**	**912**	**93.3%**

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)

Marche	6	**277**	**277**	**265**	**95.7%**
Molise	3	**130**	**130**	**66**	**50.8%**
Piemonte	7	**353**	**353**	**340**	**96.3%**
Puglia	16	**784**	**784**	**731**	**93.2%**
Sicilia	17	**855**	**855**	**814**	**95.2%**
Toscana	13	**561**	**561**	**357**	**63.6%**
Umbria	3	**157**	**157**	**157**	**100.0%**
Valle d'Aosta	2	**204**	**204**	**182**	**89.2%**
Veneto	10	**572**	**572**	**565**	**98.8%**
A.P. Bolzano	3	**280**	**280**	**280**	**100.0%**
A.P. Trento	3	**312**	**311**	**310**	**99.7%**
Total	**167**	**9.374**	**9.368**	**8.315**	**88.8%**

Data reported since October 2021, extrapolated by the GIS database on October 21, 2022, at 18:00 PM.

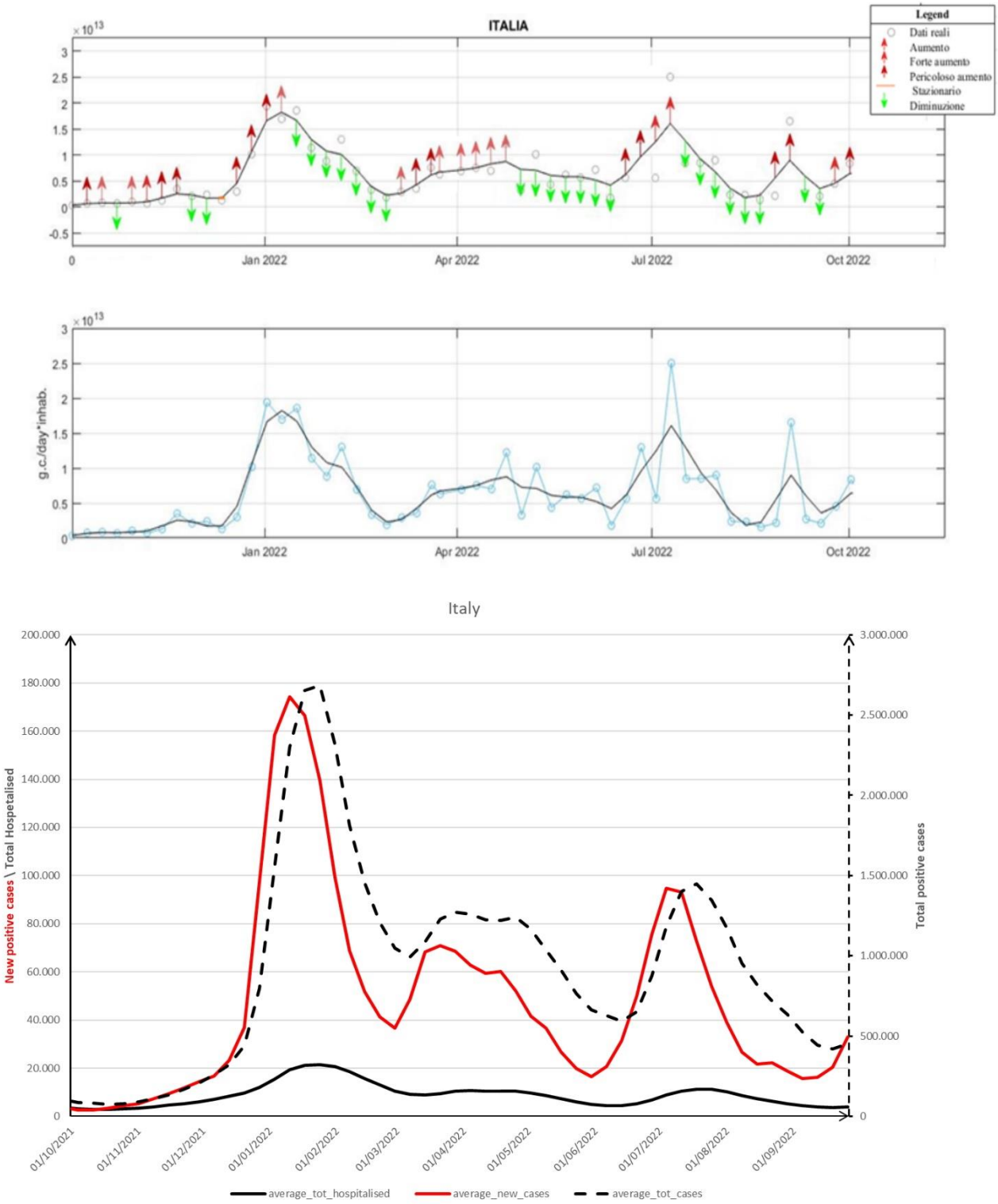
Weekly changes in term of SARS-CoV-2 concentrations are shown in Table 2. Variation compared to the previous week are shown with arrows (red= increase, green = decrease, black = stationary). Data from the last 14 weeks of surveillance are shown in the table.

Table 2. Weekly changes. Variations compared to the previous week are shown with arrows (red= increase, green = decrease, black = stationary).The last 14 weeks of surveillance are represented.

Region/A.P.	week													
	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Abruzzo	↘	↘	↗	↗	↗	↘	↘	↘	↘	↗	↗	↗	↘	↗
Basilicata	↗	↘	↘	↘	↘	↘	↘	×	↘	↘	↔	↗	↗	↗
Calabria	↗	↘	↘	↘	↘	↘	↗	↔	↘	↘	↘	↘	↗	↗
Campania	↗	↗	↘	↘	↘	↘	×	×	↗	↘	↘	↘	↔	↗
Emilia-Romagna	↗	↗	↘	↘	↘	↘	↘	↘	↘	↗	↗	↗	↘	↔
Friuli-Venezia Giulia	↘	↘	↗	↗	↘	↘	↘	↘	↘	↗	↗	↗	↗	↘
Lazio	↘	↘	↘	↘	↘	↗	↘	↘	↘	↗	↗	↗	↗	↗
Liguria	↗	↗	↗	↗	↗	↘	↘	↘	↘	↘	↘	↗	↗	↗
Lombardia	↗	↗	↗	↗	↗	↘	↘	↗	↗	↗	↘	↘	↗	↗
Marche	↗	↗	↗	↘	↘	↘	↗	↘	↘	↘	↘	↗	↗	↗
Molise	↗	↘	↘	↘	↗	↗	↗	↘	↘	↘	↘	↗	↘	↘
Piemonte	↗	↗	↗	↘	↘	↘	↘	↘	↗	↗	↗	↘	↘	↗
Puglia	↗	↗	↘	↘	↘	↘	↘	↘	↗	↗	↘	↗	↗	↗
Sicilia	↗	↗	↗	↘	↘	↘	↘	↘	↗	↗	↘	↘	↗	↗
Toscana	↗	↘	↘	↘	↘	↗	↗	↘	↗	↗	↗	↘	↘	↗
Umbria	↗	↗	↘	↘	↘	↘	↘	↘	↗	↗	↘	↘	↗	↗
Valle d'Aosta	↗	↗	↗	↗	↔	↗	↘	↘	↘	↘	↘	↘	↘	↘
Veneto	↗	↗	↗	↘	↘	↔	↘	↘	↘	↘	↘	↗	↗	↗
A.P. Bolzano	↗	↗	↗	↘	↘	↘	↘	↘	↘	↗	↗	↗	↗	↗
A.P. Trento	↗	↘	↘	↘	↘	↘	↘	↘	↗	↗	↗	↗	↗	↗
Italy	↗	↗	↘	↘	↘	↘	↘	↘	↗	↗	↘	↘	↗	↗

Week 26= data as on 03.07.2022;
Week 39= data as on 02.10.2022 (limited to 30.09.2022).

Quantitative data were used to elaborate the Quiver graphs, as described previously[2]. Figure 1 represents the global data obtained in Italy during one-year of surveillance (01/10/2021-30/09/2022).



[2] 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)

Figure 1. Quiver graph representing SARS-CoV-2 trends in wastewater in Italy in the period 1 October 2021 – 30 September 2022. Increase = 2%-20%; strong Increase = 20%-30%; dangerous Increase = >30%; stationary = 0-2%.

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

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

The national Quiver graph shows 4 succeeding "waves" (increases in SARS-CoV-2 concentrations) over the time period analysed. SARS-CoV-2 concentrations remained low but gradually increased from the beginning of October until mid-December; the same trend can be observed in new positive cases and total cases. A sharp increase in the concentration of SARS-CoV-2 in wastewater was observed in the last week of December, which continued until mid-January, when the concentration of SARS-CoV-2 tripled from early October. The same increase, due to the surge associated with the Omicron variant, was documented in the number of new positive and total cases. In addition, an increase in hospitalizations has also been found, since January 2022. Thereafter, SARS-CoV-2 concentrations decreased gradually until the end of February, when a new, smaller increase than the previous one was detected, with a peak at the end of March (SARS-CoV-2 concentrations doubled from the beginning of October). In fact, a new but less significant surge was documented in the epidemiologic curve in March 2022, along with the recording of a new increase in infections, probably in association with the Omicron BA.2 sublineage. Finally, the Quiver graph shows 2 more increases in SARS-CoV-2 concentrations in July 2022, corresponding to the first wave of the Omicron BA.4/BA.5 variant and in early September 2022, likely related to the relaxation of containment measures and the emergence of new sublineages of the Omicron variant.

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.
- Caution should be used in the interpretation of the most recent data, as trend analysis may be affected by missing data.
- 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 1), 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

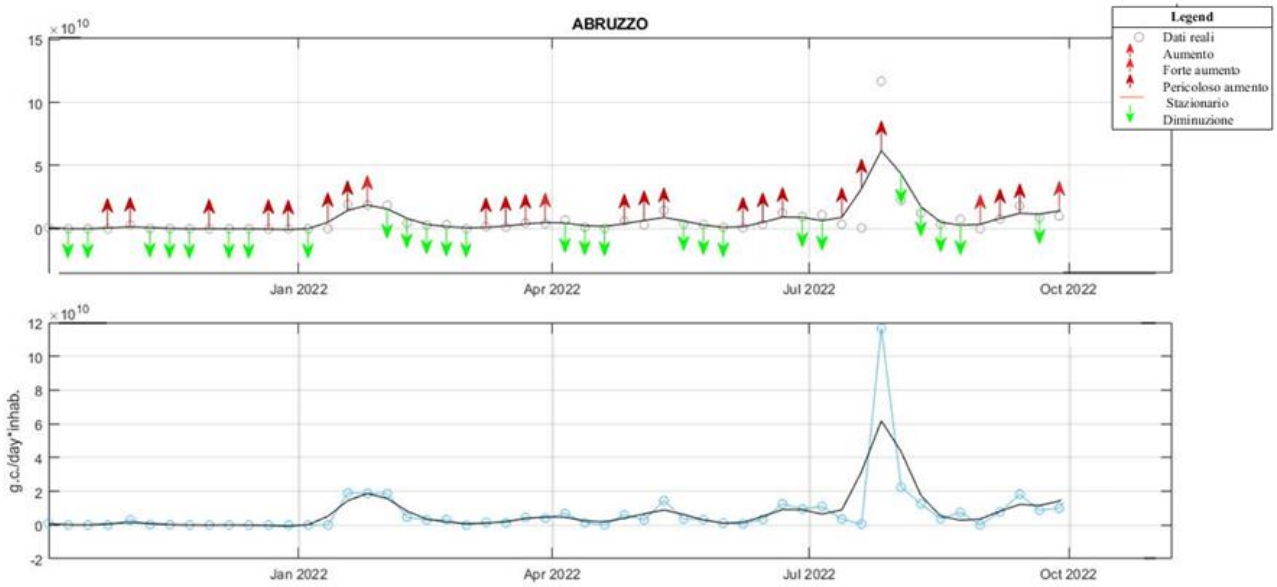
Since 1st October 2021, the environmental surveillance of SARS-CoV-2 began in Italy according to the EU Commission Recommendation 2021/472. During the first months, the environmental surveillance network was structured and, while still incomplete due to lack of data for the Region of Sardinia, the network reached its final structure in March 2022. Over the 12 months between 01.10.2021 and 30.09.2022, a progressive and significant increase in the number of Regions/A.P. involved in the surveillance and in the number of sampling points included in the surveillance has been registered. Moreover, an improvement in the analytical capacity of the designated laboratories and in the timing of data transmission has been shown.

Environmental data at national level showed upward and downward trends of SARS-CoV-2 concentrations in wastewater in the 12 months of surveillance, reflecting the trends observed in clinical cases in most Regions/P.A. These results confirm that environmental surveillance can successfully complement the tools used for the integrated surveillance of COVID-19. The development of a new Rt-wastewater indicator, currently under finalization, will provide an additional tool to use environmental surveillance data to support public health decisions.

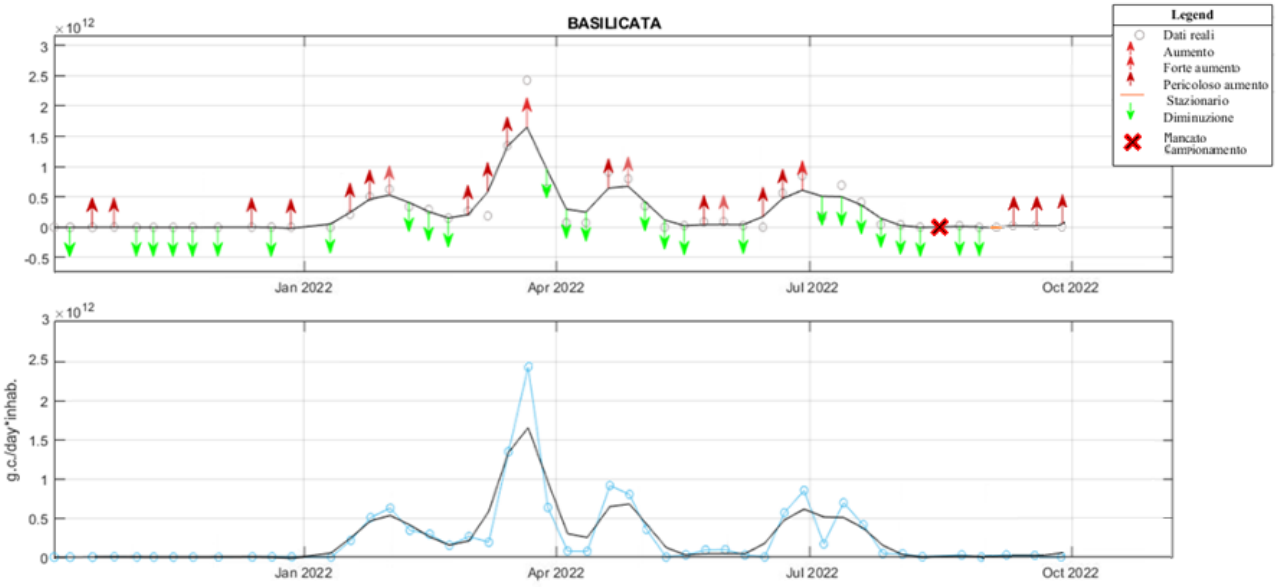
Appendix 1: Quiver graphs for Regions and Autonomous Provinces

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

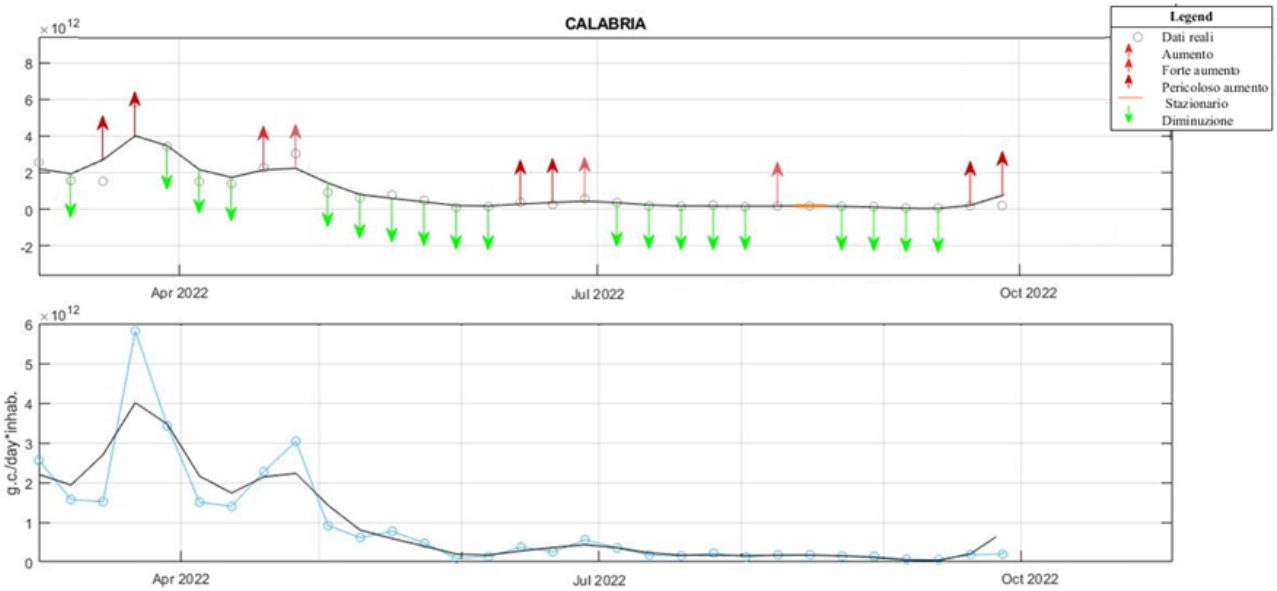
ABRUZZO



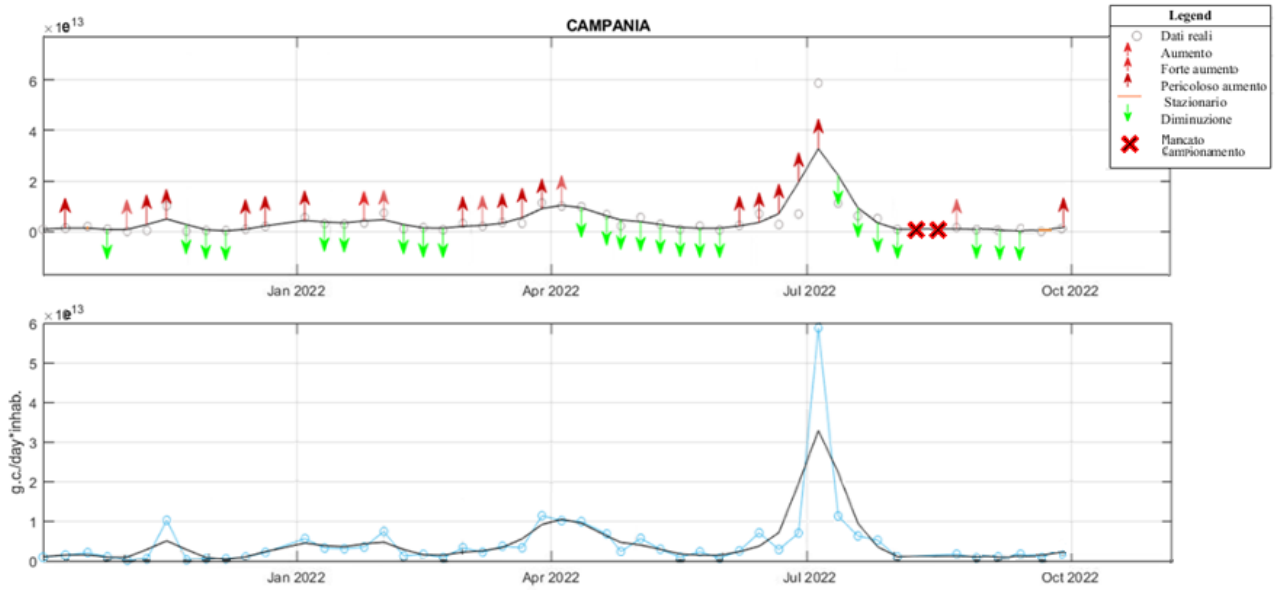
BASILICATA



CALABRIA



CAMPANIA



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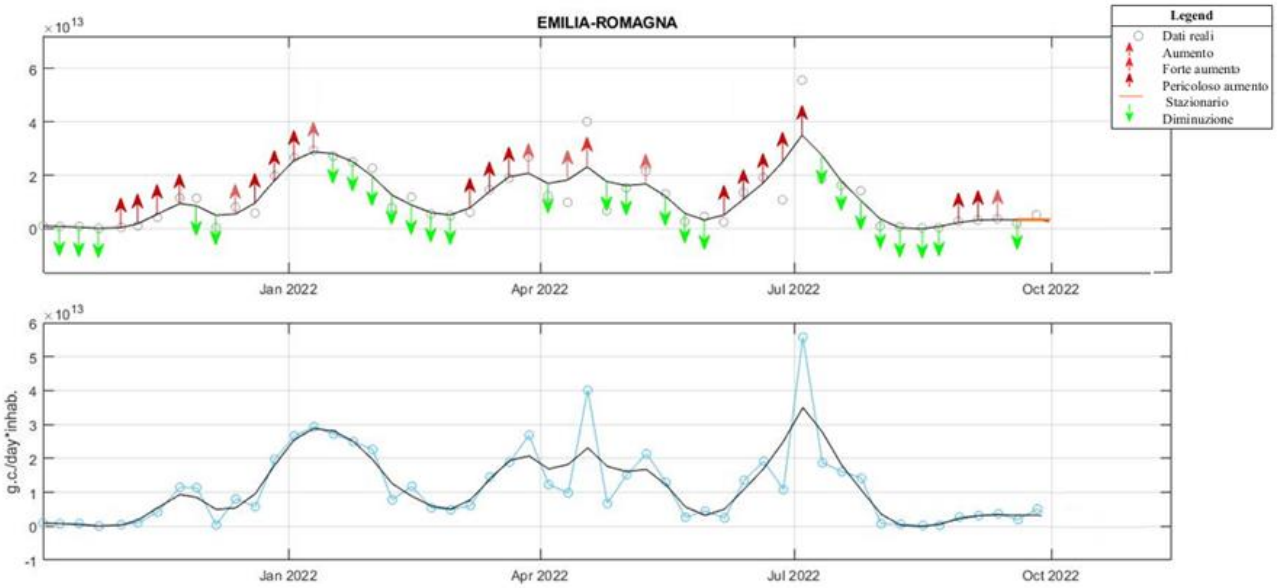
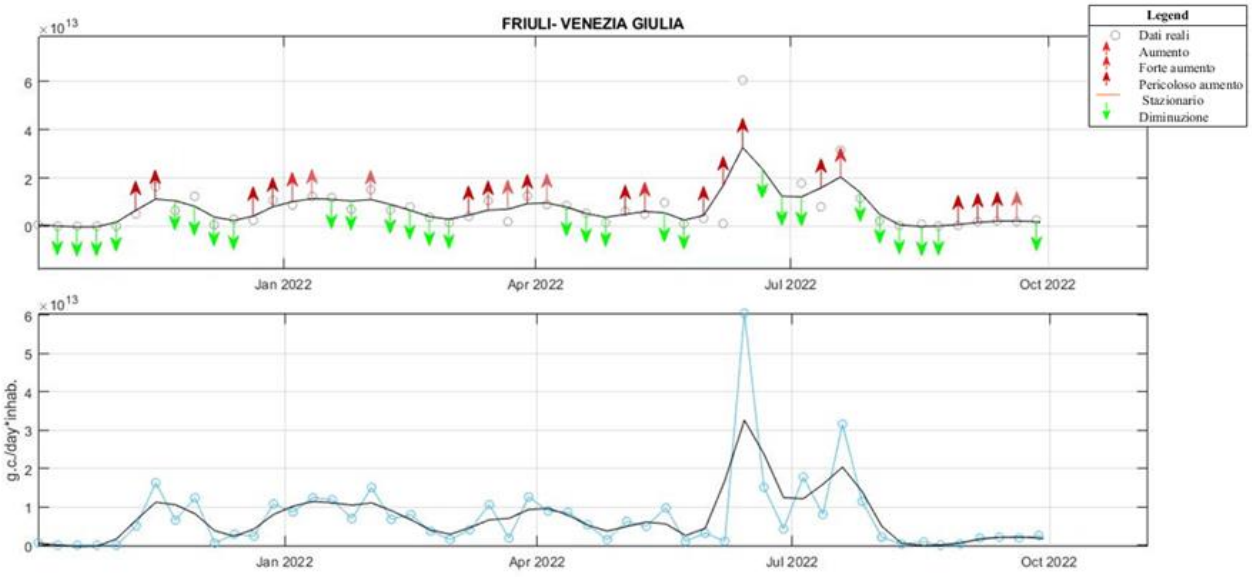


FRIULI-VENEZIA GIULIA



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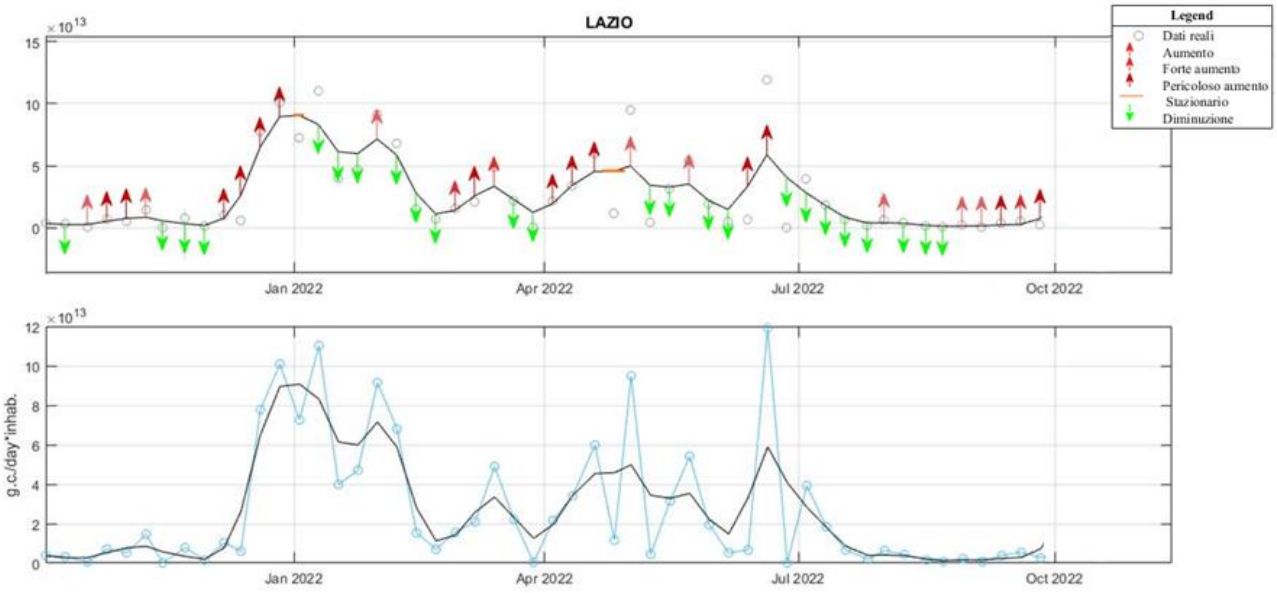
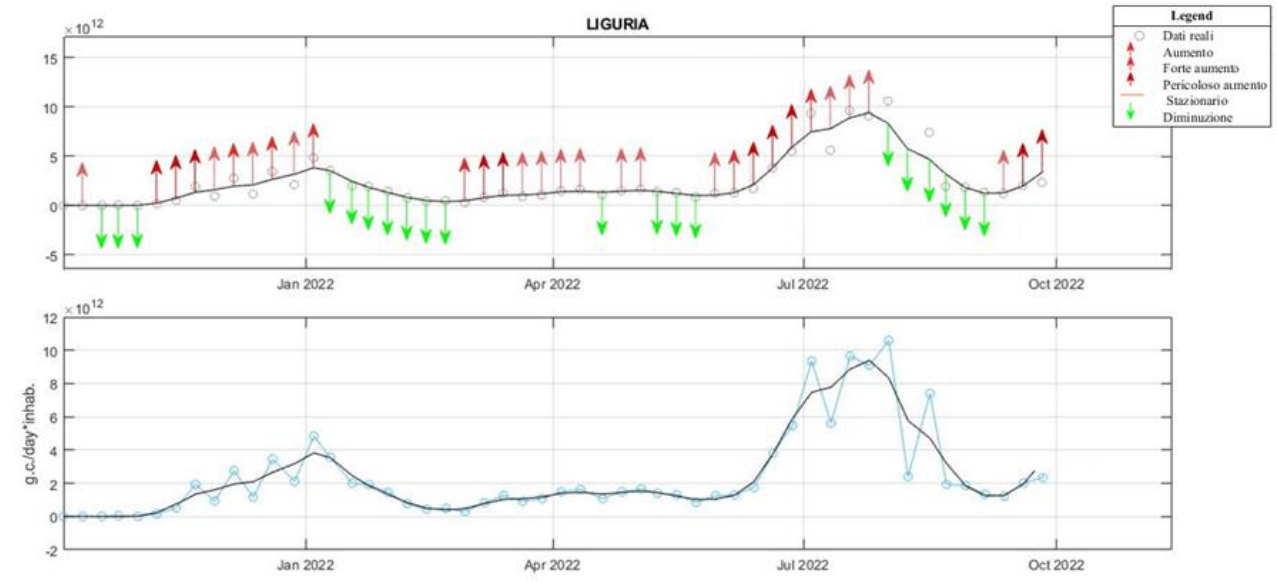
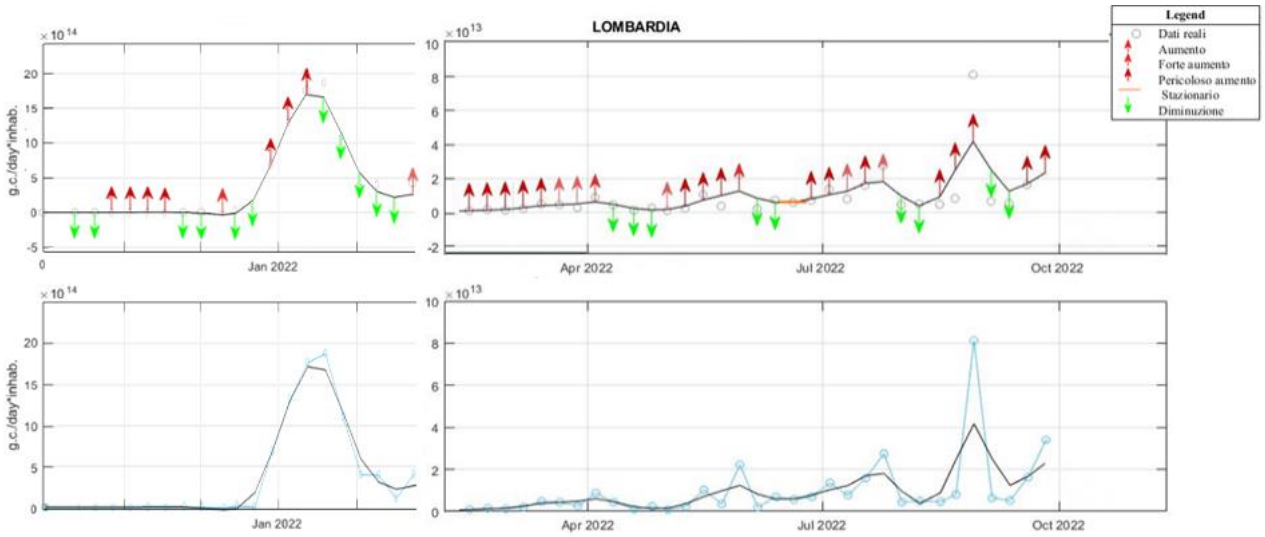


LIGURIA

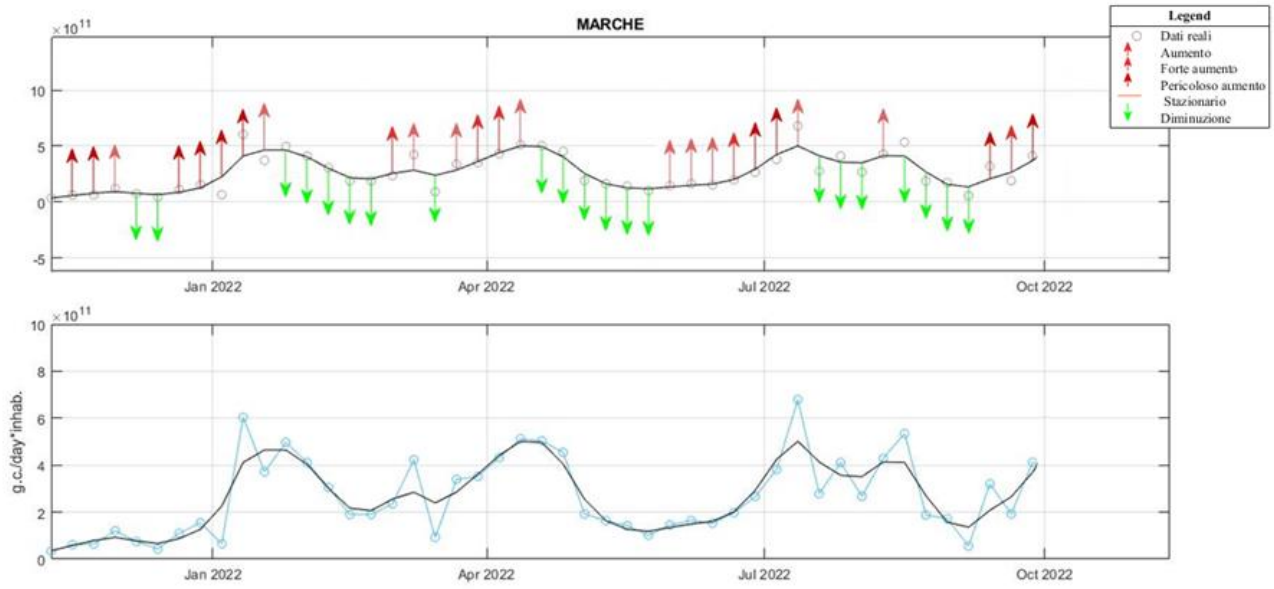


LOMBARDIA



Since March 2022, the Region of Lombardy expanded the sampling plan including nine additional WTPs and some of the laboratories modified the analytical procedures (target gene for viral quantification) to improve data harmonization at regional and national level. To avoid a retrospective modification of the trends calculated for the Region, the analysis was divided in two periods, namely: 01.10.2022 – 28.02.2022 (surveillance on 6 WTPs using two different real-time PCR protocols; target regions ORF1 and N), and 01.03.2022 – 30.09.2022 (surveillance on 15 WTPs using the national SARI protocol rev.3; target region ORF1). Two different scales are shown in the y-axis for the two periods.

MARCHE



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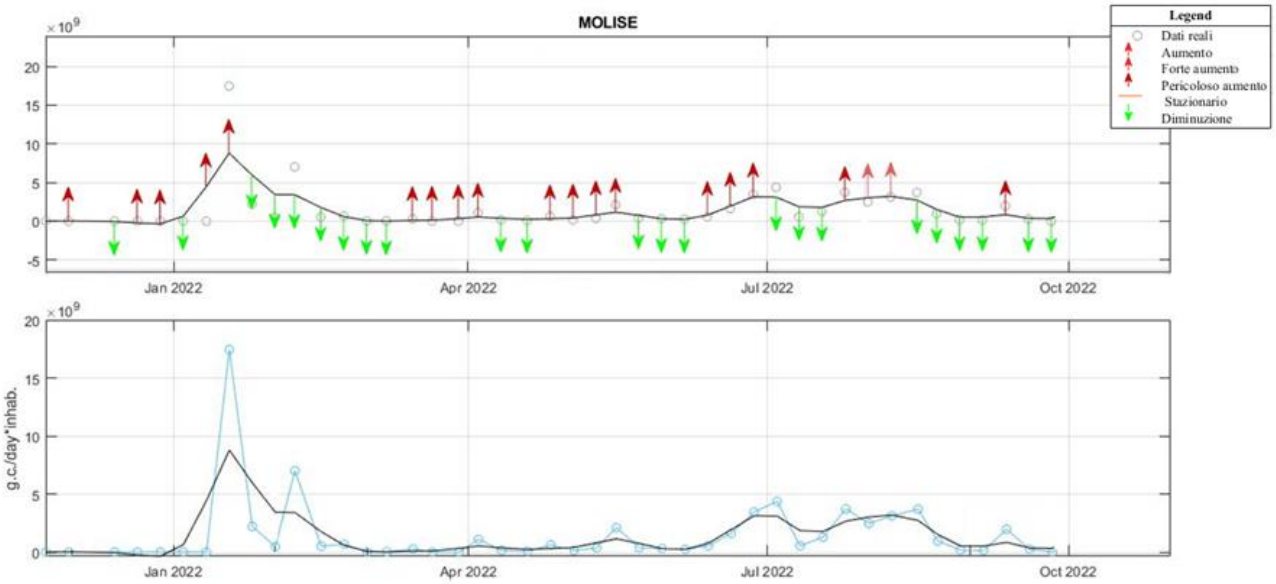
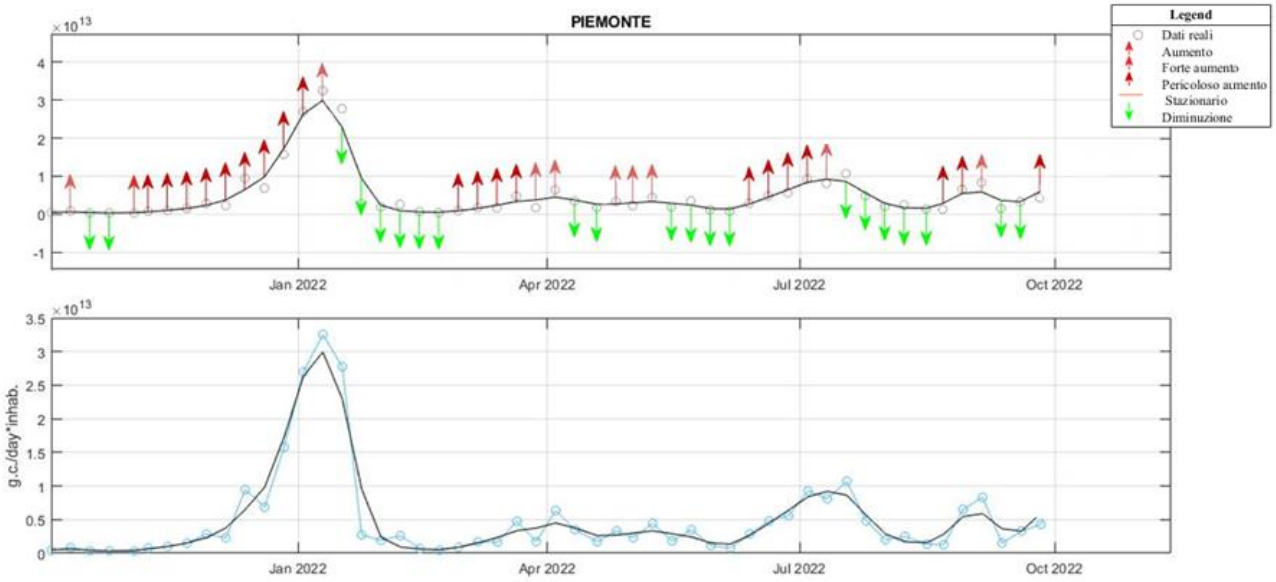


PIEMONTE



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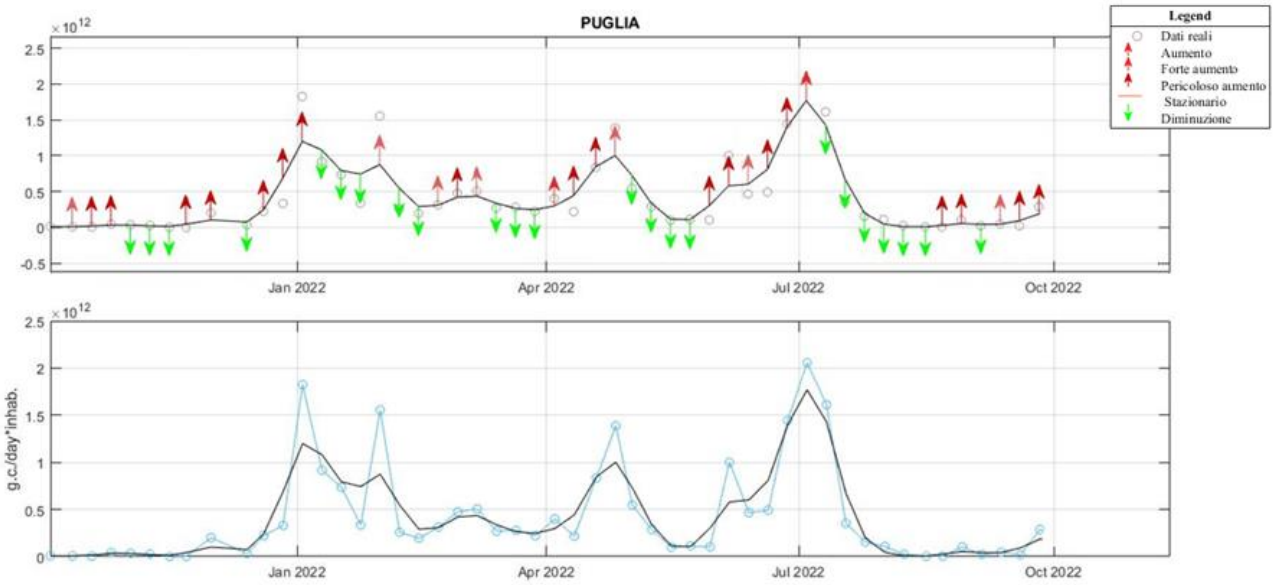

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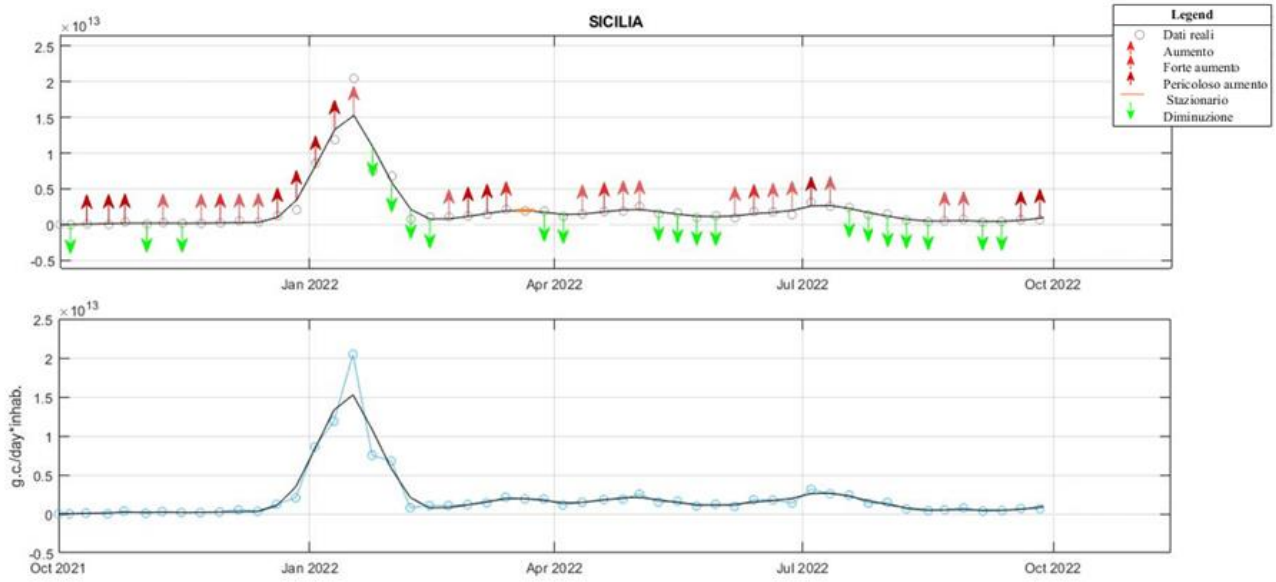

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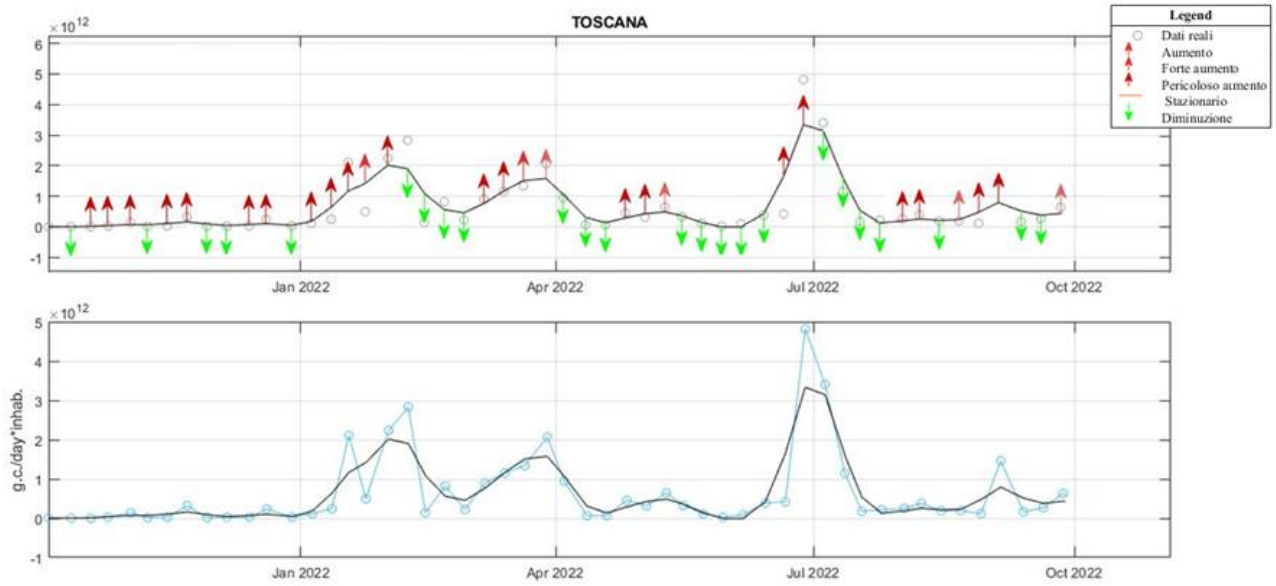

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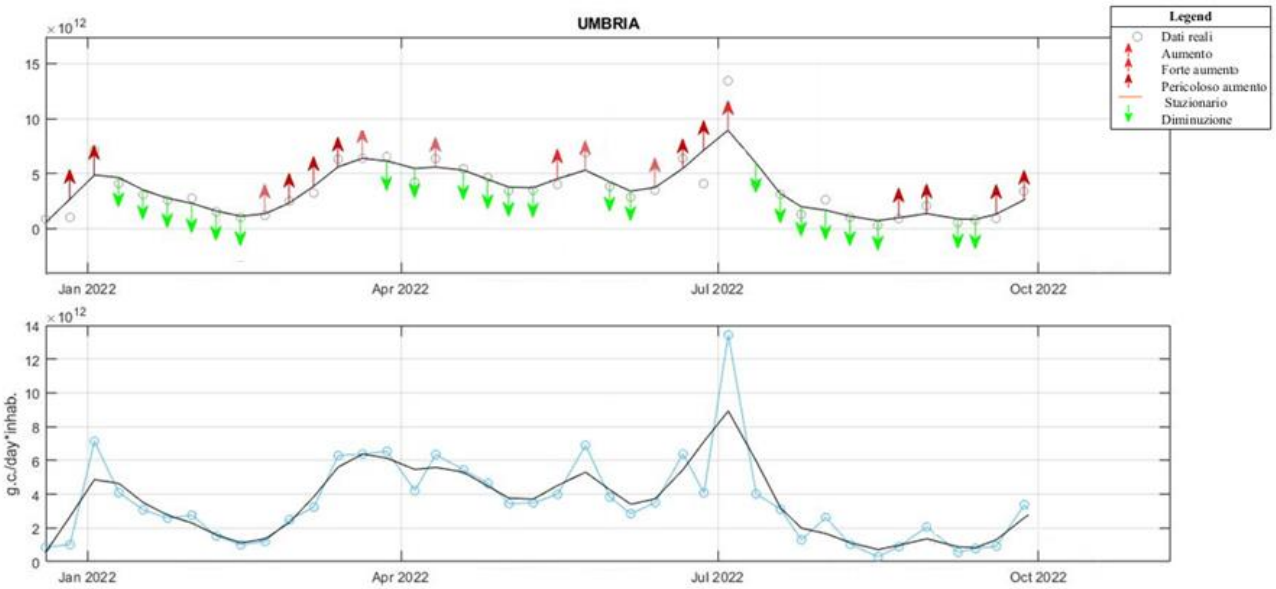
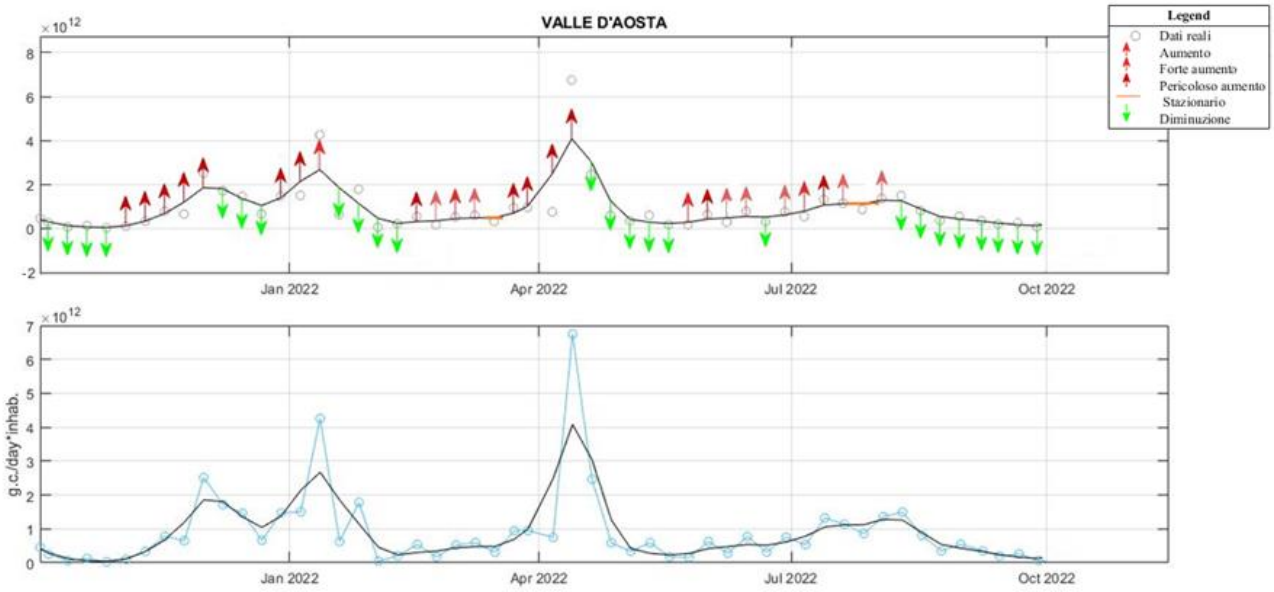


VALLE D'AOSTA



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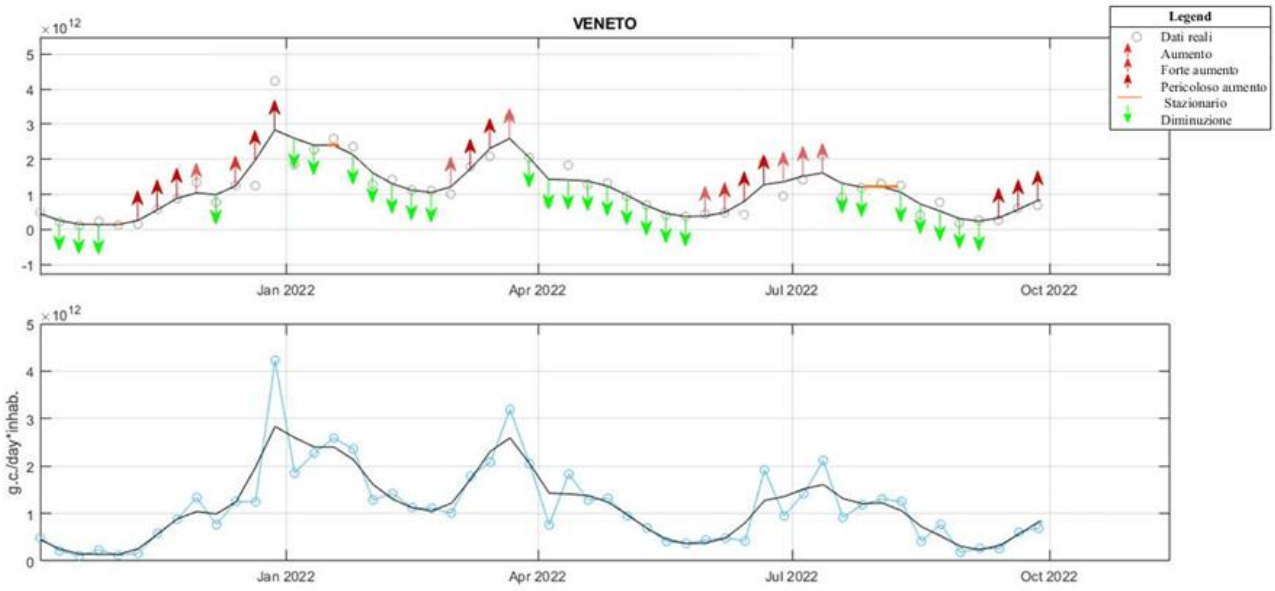
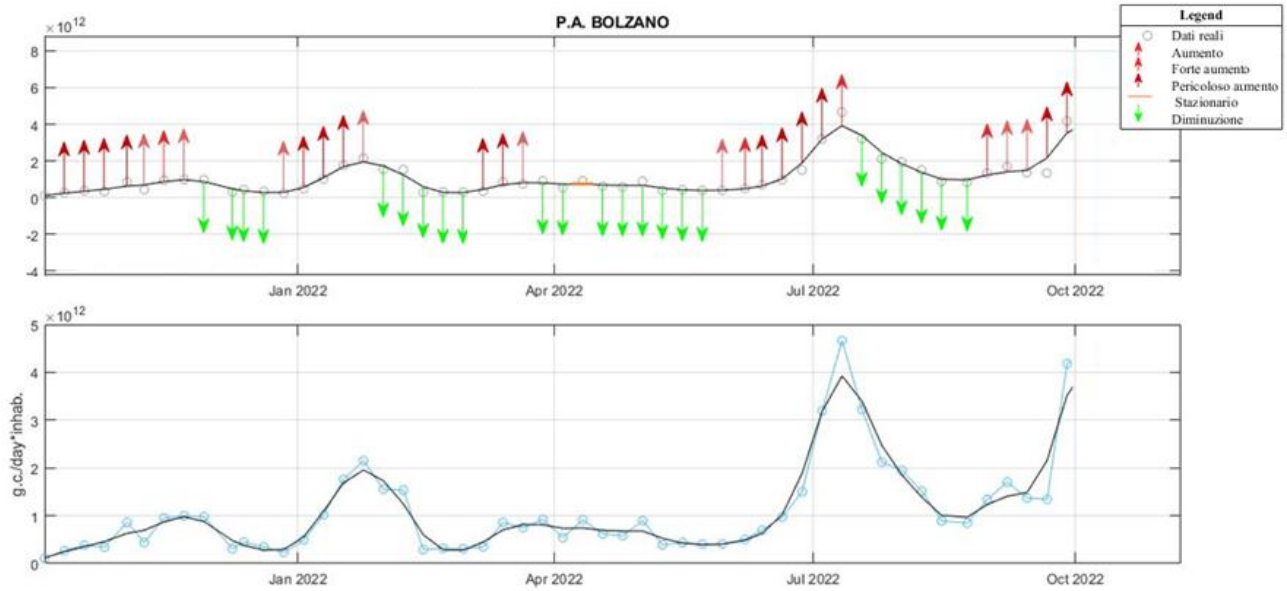
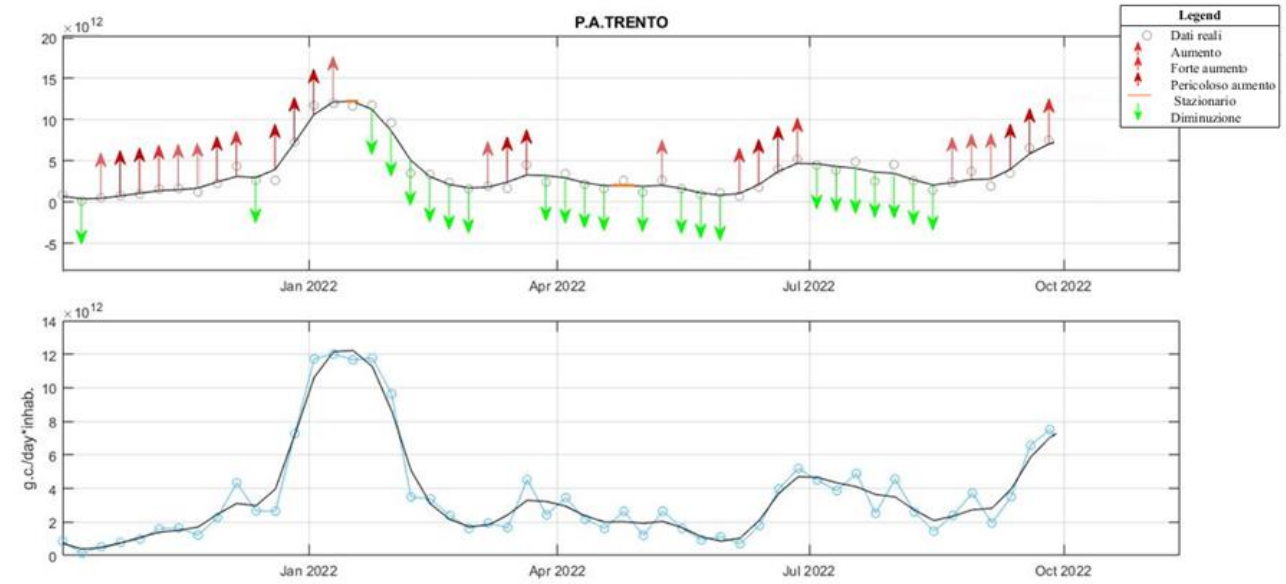


P.A. BOLZANO



P.A. TRENTO



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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, Michelangelo Iannone, Filomena Casaburi, Giorgia Bulotta, Emanuela Barillari, Melania Dragone, Iolanda Sacco, Carmine Tomaino, Cristina Felicetta, Adelaide Calabria, Ottavia Varcasia, Francesca Stefanizzi, Concetta Vizza (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);
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