Flash survey on SARS-CoV-2 variants in urban wastewater in Italy
24th Report
(Study period: August 7th to August 11th, 2023)

Edited by:

- Giuseppina La Rosa, Giusy Bonanno Ferraro, Pamela Mancini, Carolina Veneri, Claudia Del Giudice, Marcello Iaconelli, Luca Lucentini (National Center for Water Safety, Istituto Superiore di Sanità)
- David Brandtner (independent researcher)
- Mauro Grigioni, Mirko Rossi (National Center for Innovative Technologies in Public Health, Istituto Superiore di Sanità)
- Elisabetta Suffredini (Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità

Main findings:

- During the week of 7 August to 11 August, 2023, a total of 97 wastewater samples were collected from 15 Regions and 2 Autonomous Provinces (AP).
- Mutations characteristic of the Omicron variant were identified in 8 of these Regions/AP, while no sequencing data was obtained from the remaining areas.
- Analysis of the sequences obtained by Sanger sequencing showed that 58% of the positive samples had amino acid substitutions belonging to the Omicron XBB.1.5*/1.9* lineages, 25% to the Omicron XBB.1.16* lineage and 17% to the Omicron XBB.2.3* lineage.

Introduction

On March 17th, 2021, the European Union Commission issued Recommendation 2021/472, encouraging Member States to establish a systematic surveillance of SARS-CoV-2 and its variants in wastewater by October 1st, 2021. Responding to this recommendation, the Istituto Superiore di Sanità (ISS) initiated a series of "flash surveys". These surveys involve monthly sampling campaigns conducted at various locations across Italy over a short period of time. The primary objective of these flash surveys is to gather supplementary information on SARS-CoV-2 variants in the population, complementing data obtained through clinical surveillance. The aim of this report is to summarize the findings from the 24th national Flash Survey on SARS-CoV-2 variants in wastewater samples in Italy, which was conducted from August 7th to August 11th, 2023.

Methodology

During the period from 7 August to 11 August, 2023, the 24th national Flash Survey on SARS-CoV2 variants in wastewater samples was conducted in Italy. The survey involved the collection of 97 sewage samples from 93 wastewater treatment plants (WTPs) located across 15 Regions and 2 Autonomous Provinces. Information on the WTPs participating in the SARS-CoV-2 surveillance in urban wastewater in Italy can be found on the ISS website[1]. The samples collected during the survey were processed and the viral concentration was determined by laboratories within the SARI network using the protocol “Sorveglianza di SARS-CoV-2 in reflui urbani - Protocollo progetto SARI - rev.3”[2]. The purified RNAs extracted from the samples were then sent to ISS for variant detection.

For sequencing purposes, we used a long-nested PCR assay covering approximately 1330 base pairs and spanning from amino acid residues 34 to 475 of the spike protein (PCR ID 1033/1034). After amplification of the target sequences, Sanger sequencing was performed on individual samples.

For variant classification we adopted a lineage classification based on 'outbreak.info'[3] rather than specifying sublineages. This choice was made because there are many sublineages that evolve rapidly, often converging on specific amino acid substitutions. In some cases, the differences between sublineages can be as small as a single nucleotide mutation in our target region, making

[1] 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)

[2] DOI 10.5281/zenodo.5758724.

[3] https://outbreak.info/situation-reports, date: 17/11/2023

a reliable sublineage assignation, on the basis solely of mutations observed in the spike region, not feasible.

Results

Real Time qPCR

Out of the 97 samples analysed, a total of 73 (75.3%) tested positive for SARS-CoV-2 using the real-time RT-qPCR method employed for environmental surveillance (Table 1). The viral concentrations detected in these samples varied, ranging from 1.76E+00 to 1.59E+06 genome copies (g.c.) per liter of wastewater.

Sanger Sequencing

Table 1 provides a summary of the results obtained from the real-time PCR assays, long-nested PCR assays and sequencing methods. A total of 12 samples (12.4%) from 8 Regions/AP were successfully amplified using the long-nested PCR assay described above. Sanger sequencing confirmed that all the obtained sequences corresponded to the Omicron variant.
Analysis of wastewater samples revealed the presence of three SARS-CoV-2 lineages, as shown in Tables 1 and 2. Among these, the Omicron XBB.1.5*/XBB 1.9* lineages (which are not distinguishable in the sequenced region) were the most frequent, detected in 7 out 12 positive samples (58%). In addition, the Omicron XBB.1.16* was identified in 3 out of 12 positive samples (25%)and Omicron XBB.2.3* in 2 out of 12 positive samples (17%).
For ease of understanding, the mutations have been grouped into panels or "mutation packages" as follows:

- **Package A (assigned to the lineage Omicron XBB.1.5*/XBB.1.9*)** = V83A; G142D; DEL144; H146Q/H146K; Q183E; V213E; G252V; G339H; R346T; L368I; S371F; S373P; S375F; T376A; D405N; R408S; K417N; N440K; V445P; G446S; N460K
- **Package B (assigned to the lineage Omicron XBB.1.5*/XBB.1.9*)**[4] = Q52H; V83A; G142D; DEL144; H146Q; Q183E; V213E; G252V; G339H; R346T; L368I; S371F; S373P;S375F; T376A; D405N; R408S; K417N; N440K; V445P; G446S; F456L; N460K
- **Package C (assigned to the lineage Omicron XBB.1.16*)** = V83A, G142D, DEL144, H146Q, E180V, Q183E, V213E, G252V, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K
- **Package D (assigned to the lineage Omicron XBB.2.3*)** = V83A, G142D, DEL144, H146Q, Q183E, V213E, D253G, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K

Mutations of the Omicron XBB.1.5*/XBB.1.9* lineages were detected in 5 regions/A. P. (Emilia-Romagna, Liguria, Piemonte, Puglia and Sicilia). Key mutations of Omicron XBB.1.16* lineage

[4] Package B in combination with the 'Q52H' mutation may suggest the presence of the Omicron EG.5 sublineage. This last sublineage is characterised by the presence of the 'Q52H' mutation in approximately 92.7% and the 'F456L' mutation in about 96.1% of its sequences. The Omicron XBB.1.5* lineage and the Omicron XBB.1.9* lineage may also carry the 'Q52H' mutation at rates of 0.2% and 71.3% respectively, and the 'F456L' mutation at rates of 7.1% and 76.8% respectively (https://outbreak.info/situation-reports, date: 17/11/2023).

were observed in two Regions/A.P. (Abruzzo and Sicilia). Additionally, two Regions/P.A. (Lazio and P.A. Trento) were found to have key mutations of Omicron XBB.2.3* lineage.

Table 1. PCR and sequencing results

	Sample ID	Region/A.P.	City	WTP	RT-qPCR (c.g./L)	Mutations found by Sanger sequencing (long PCR ID_1034)	Sars-CoV-2 lineages (Sanger sequencing)
1	22005	Abruzzo	Pescara	Via Raiale	< LOD		
2	22006		Pescara	Villa Carmine	< LOD	Package C	Omicron XBB.1.16*
3	22007		Chieti	S. Martino	< LOD		
4	22008		L'Aquila	Pile	< LOD		
5	22084		Teramo	Villa Pavone	< LOD		
6	22100	Basilicata	Potenza	Tiera di Vaglio	6,74E+03		
7	22101		Matera	Pantano	3,23E+04		
8	21982	Emilia-Romagna	Ferrara	Ferrara - Linea 1	< LOD		
9	21983		Ferrara	Ferrara - Linea 2	< LOD		
10	22059		Bologna	Imola	1,59E+06		
11	22060		Modena	Naviglio	1,34E+06		
12	22061		Bologna	IDAR	1,26E+06		
13	22066		Ravenna - Forlì-Cesena	Ravenna	1,04E+06		
14	22067		Forlì-Cesena	Cesena	1,03E+06	Package B [a]	Omicron XBB.1.5*/XBB.1.9*
15	22068		Forlì-Cesena	Forlì	1,40E+06		
16	22069		Rimini - Forlì-Cesena	S. Giustina	9,78E+05		
88	22373		Piacenza	Borgoforte	1,42E+03		
89	22374		Parma	Parma	1,05E+04		
90	22375		Reggio Emilia	Mancasale	8,40E+03		
96	22072	Friuli-Venezia Giulia	Pordenone	Cordenons	1,10E+06		
97	22073		Udine	Udine	5,80E+05		
17	21790	Lazio	Roma	Civitavecchia Fiumaretta	2,50E+02		
18	21971		Latina	Aprilia (Via del Campo)	< LOD		
19	21972		Viterbo	Viterbo - Strada Bagni	< LOD		
20	21973		Roma	Anzio - Colle Cocchino	9,55E+02		

21	21974		Latina	Latina Loc Latina Est	5,38E+03		
22	21975		Roma	Pomezia - Via Cincinnato	1,70E+03		
23	21976		Roma	Velletri (LA CHIUSA-SORBO)	4,43E+03		
24	21977		Roma	Guidonia - Ponte Lucano	2,83E+03	Package D + F186I	Omicron XBB.2.3*
25	21989	Liguria	Savona	Savona	< LOD		
26	21990		Genova	Pegli	< LOD		
27	21991		Genova	Voltri	5,82E+03		
28	21992		Genova	Quinto	4,87E+04	Package B [a]	Omicron XBB.1.5*/XBB.1.9*
29	21993		Genova	Rapallo	2,76E+04		
30	21994		Genova	Sestri P	5,43E+03		
31	21995		Genova	Sturla	1,95E+04		
32	21996		Genova	Darsena	1,71E+04		
33	21997		Genova	Punta Vagno Genova	1,12E+04		
34	21998		Genova	Valpolcevera	4,20E+04		
35	21999		Imperia	Imperia	3,69E+03		
36	22000		Imperia	Sanremo - località Capo Verde	1,51E+04		
37	22001		Savona	Borghetto Santo Spirito	9,63E+03		
91	22454		Genova	Recco	8,08E+03		
95	22071		Savona	Borghetto Santo Spirito	4,96E+04		
38	21984	Lombardia	Brescia	Verziano	1,32E+04		
86	22698		Sondrio	Sondrio	< LOD		
39	22009	Marche	Pesaro-Urbino	Borgheria	2,70E+03		
40	22010		Pesaro-Urbino	Ponte Metauro	1,74E+03		
41	22011		Ancona	Zipa	1,76E+00		
42	22012		Ancona	Falconara	< LOD		
43	22002	Molise	Campobasso	Campobasso - San Pietro	< LOD		
44	22003		Campobasso	Termoli - località Porto	< LOD		
45	22004		Campobasso	Termoli - località Pantano Basso	3,08E+02		
46	22078	P.A. Bolzano	Bolzano	IDA Bolzano	2,07E+04		
47	22079		Bolzano	IDA Merano	1,86E+04		

48	22080		Bolzano	IDA Termeno	2,83E+04		
49	21942	P.A. Trento	Trento	Trento nord	7,21E+04		
50	21943		Trento	Trento sud	7,16E+04		
51	21944		Trento	Rovereto	3,37E+04	Package D	Omicron XBB.2.3*
52	21931	Piemonte	Torino	Castiglione Torinese	2,78E+03		
53	21932		Biella	Biella Nord	1,31E+03		
54	21933		Novara	Novara	< LOD		
55	21958		Alessandria	Alessandria	< LOD		
56	21959		Asti	Asti	8,68E+02		
57	21960		Cuneo	Cuneo	9,73E+02	Package B [a]	Omicron XBB.1.5*/XBB.1.9*
58	21967	Puglia	Taranto	Taranto Bellavista	3,93E+02		
59	21968		Taranto	Taranto Gennarini	9,64E+02	Package B + L455W [a]	Omicron XBB.1.5*/XBB.1.9*
60	21969		Bari	Bari Est	< LOD		
87	21970		Bari	Bari Ovest	1,31E+03		
61	21955	Sicilia	Trapani	Mazara del Vallo	5,54E+03		
62	21956		Trapani	Marsala	2,46E+03	Package B [a]	Omicron XBB.1.5*/XBB.1.9*
63	22013		Ragusa	Modica	1,45E+03		
64	22014		Ragusa	Vittoria	4,58E+03		
65	22015		Ragusa	Ragusa	5,15E+03	Package B [a]	Omicron XBB.1.5*/XBB.1.9*
66	22016		Caltanissetta	Gela Macchitella	8,83E+03		
67	22017		Messina	Mili Marina	7,20E+03	Package A	Omicron XBB.1.5*/XBB.1.9*
68	22020		Agrigento	Agrigento	2,00E+04	Package C	Omicron XBB.1.16*
69	22021		Enna	Enna	8,59E+03		
70	22022		Caltanissetta	Caltanissetta e San Cataldo	1,20E+04		
71	22023		Palermo	Acqua dei Corsari	1,29E+04		
72	22024		Palermo	Fondo Verde	6,75E+03		
92	22396		Catania	Pantano d'Arci	9,09E+03		
93	22398		Siracusa	Siracusa	9,25E+03		
94	22397		Catania	Giarre	7,83E+03	Package C	Omicron XBB.1.16*
73	22296	Umbria	Perugia	Perugia - Pian della Genna	6,03E+04		

74	22057	Valle d'Aosta	Aosta	La Salle	2,21E+03
75	22058		Aosta	Brissogne	8,00E+02
76	21791	Veneto	Padova	Padova Ca' Nordio - centro storico	< LOD
77	21792		Padova	Padova Ca' Nordio - zip	< LOD
78	21793		Padova	Padova Guizza	< LOD
79	21794		Padova	Abano Terme	< LOD
80	21952		Treviso	Treviso	< LOD
81	21953		Venezia	Venezia Fusina	4,23E+03
82	21954		Vicenza	Vicenza Casale	< LOD
83	21979		Verona	Verona_collettore 1M	1,13E+04
84	21980		Verona	Verona_collettore 3M	6,33E+03
85	21981		Verona	Verona_collettore 8M	2,27E+04

[a] Package B in combination with the 'Q52H' and 'F456L' mutations may suggest the presence of the Omicron EG.5 sublineage. This last sublineage is characterised by the presence of the 'Q52H' mutation in approximately 92.7% and the 'F456L' mutation in about 96.1% of its sequences. The Omicron XBB.1.5* lineage and the Omicron XBB.1.9*lineage may also carry the ‘Q52H’ mutation at rates of 0.2% and 71.3% respectively, and the ‘F456L’ mutation at rates of 7.1% and 76.8% respectively (https://outbreak.info/situation-reports, date: 17/11/2023).

Table 2. Sanger sequencing results

ID SAMPLES	Q52H	V83A	G142D	DEL 144	H146Q/H146K	E180V	Q183E	V213E	G252V	D253G	G339H	R346T	L368I	S371F	S373P	S375F	T376A	D405N	R408S	K417N	N440K	V445P	G446S	F456L	N460K	VARIANTS
67		■	■	■	■		■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■		■	**Package A (Omicron XBB.1.5*/XBB.1.9*)**
14, 28, 57, 59, 62, 65	■	■	■	■	■		■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	**Package B (Omicron XBB.1.5*/XBB.1.9*)**
2, 68, 94		■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■		■	**Package C (Omicron XBB.1.16)**
24, 51		■	■	■	■		■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	**Package D (Omicron XBB.2.3*)**

Limitations of the study

The geographic and population coverage of this flash survey was not comprehensive, as it encompassed 17 out of 21 of the Italian regions/Autonomous Provinces.
It is important to note that the molecular analytical methods used for complex environmental matrices, such as wastewater, can be challenged by factors such as low viral concentrations, insufficient analyte recovery, and/or inhibition of PCR amplification. Consequently, both the detection/quantification and PCR amplification for sequencing may yield false negatives, making it challenging to achieve molecular characterization and variant detection for all samples.
Partial sequencing of the spike region does not provide conclusive results for sublineage assignment. Our decision to adopt a broader lineage classification from 'outbreak.info' for variant classification, rather than specifying sublineage assignments, was influenced by the rapid evolution of numerous sublineages, often with minor differences, that hampered the reliable assignation to sublineages based solely on mutations observed in the spike region.

Conclusions and final considerations

This report is part of a monthly series focusing on SARS-CoV-2 and its variants in wastewater samples in Italy, in accordance with the EU Commission Recommendation 2021/472. The primary objective is to provide additional information on SARS-CoV-2 variants in the population, complementing data obtained through clinical surveillance. The results of this survey indicate that the Omicron variant is the sole of SARS-CoV-2 variant in Italy, with the Omicron XBB.1.5*/XBB.1.9* lineages being prevalent. However, mutations characteristic of Omicron XBB.1.16* and Omicron XBB.2.3* were also detected.

The sequencing of SARS-CoV-2 in wastewater samples provides valuable additional information alongside the sequencing of clinical cases. This approach provides a more complete and accurate understanding of the circulating variants in the country, contributing to a better characterization of the spread and evolution of this virus.

Acknowledgements

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, Vicdalia Aniela Acciari (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, 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);
- **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, Giulia Salmoiraghi (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, Elena Ballarini, Annalisa Grucci (ARPAM);
- **Molise:** Michele Colitti (Regione Molise); Carmen Montanaro (ASReM); Giuseppe Aprea, Silvia Scattolini, 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 (Dipartimento di Scienze della Vita e Biologia dei Sistemi), 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");
- **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; Andrea Anselmo, 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).