First insights into plastic and microplastic occurrence in biotic and abiotic compartments, and snow from a high-mountain lake (Carnic Alps)

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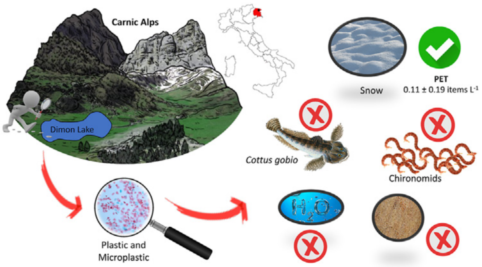
HIGHLIGHTS

- Plastic and microplastic were measured in a high-mountain-lake from Carnic Alps.
- Water, sediment, macroinvertebrates, fish and snow samples were analyzed.
- No microplastics (10–5000 μm) were detected in the biotic and abiotic samples.
- Microplastics were detected only in the snow: PET levels of 0.11 ± 0.19 items L^{-1}.
- No macro- and mesoplastics (>5000 μm) were found in this remote ecosystem.

GRAPHICAL ABSTRACT



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ABSTRACT

Plastic pollution has become a pervasive environmental problem on a global scale, from the ocean depths to the aquatic ecosystems of the Tibetan Plateau. To date, data on plastic and microplastic occurrence in pristine ecosystems like high-mountain lakes are lacking. In this study, plastic (>5000 μm) and microplastic (10–5000 μm) levels were measured in snow at the end of the winter season (April 2020), and in water, sediment, and biological samples collected monthly (June–October 2019) during the ice-free season from the Dimon Lake, a high-mountain lake in the Carnic Alps, northeast Italy. Biological samples consisted of chironomids (Diptera, Chironomidae; n = 150) and stomach contents of *Cottus gobio* (n = 40). Analysis of the water, sediment, and biological samples revealed the absence of plastic and microplastics larger than 10 μm, whereas the snow samples contained microplastics of *polyethylene terephthalate (PET) albeit* at very low levels (0.11 ± 0.19 L^{-1}). These results show that while the lake ecosystem could be considered unpolluted by microplastics, abundant snow precipitation in winter can trap microplastic particles that deposit on the ground. The very low levels of PET microparticles recorded in the snow samples suggest the need for further research to better understand the source of microplastic pollution in this environmental matrix.

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1. Introduction

An extraordinarily versatile material, plastic *has r*evolutionized the healthcare, the food packaging, the fashion and a host of other industries (Boucher and Friot, 2017). Since the 1950s the production and consumption of plastic products and containers have increased dramatically, surpassing all other materials and replacing most of them in the packaging sector. The negative impact from its growing use has become increasingly evident, starting with the consumption of non-renewable resources. Furthermore, plastic materials generate a huge amount of refuse. Most plastics are highly resistant to biodegradation, which is why they persist in the environment, where they shatter into smaller fractions and ultimately turn into microplastic particles (1 μm - 5 mm) as reported by Gigault et al. (2018). Their origin can be "primary", as in intentionally produced synthetic materials (i.e., raw materials for industry or abrasive components in chemicals and cosmetics) or "secondary" when derived from the fragmentation of plastics by physicochemical and atmospheric agents, mechanical erosion, and UV radiation or biological degradation (Singh and Sharma, 2008).

Global plastic production was an estimated 360 million tons in 2018, 62 million tons of which were produced in Europe (PlasticsEurope, 2019). Plastics are dispersed into the environment as mismanaged waste, polluting water, soil, and air (Windsor et al., 2019). Dispersion occurs largely through urban point source contamination and low efficiency of water waste management plants (Wagner and Lambert, 2018). Moreover, a portion of 32.9 million metric tons (Mt) of plastic waste enter the ocean every year. But because this amount accounts for only 10% of the plastic created, the question arises about the fate of the remaining 90% (PlasticsEurope, 2019). Natural forces such as storms or wind can contribute to the dispersion of plastic particles. Allen et al. (2019) suggested that the particles can reach remote ecosystems (e.g., the Pyrenees) by atmospheric transport: fibers up to 750 μm and fragments $\leq$300 μm have been identified in atmospheric deposition samples.

Studies investigating the presence of microplastics have shown that they are ubiquitous in terrestrial and aquatic environments, remote areas included. Artic Sea ice is a major sink and means of transport for microplastics (Peeken et al., 2018). Microplastics have also been detected in diverse areas, from ocean depths (Peng et al., 2018) to the rivers and lakes of the Tibetan Plateau (>4000 m a.s.l.) as reported by literature (Xiong et al., 2018; Jiang et al., 2019).

The Alps are the highest and most extensive mountain range system in Europe. Geologically, the Carnic Alps are among the most complex ranges since they rise in the region where the massive build-up of the Alps occurred millennia ago. They form part of the southern Alpine chain and host several high-altitude lakes. These remote ecosystems far from the main sources of industrial pollution can be used to assess the long-range transport of pollutants from the lowland (Moser et al., 2019). Also, they can act as a sink for certain pollutants such as trace elements and persistent organic pollutants (POPs) (Pastorino et al., 2020). To our best knowledge, no studies to date have investigated the occurrence of microplastics in high-mountain Alpine lakes.

Dimon Lake (northeast Italy, 1872 m a.s.l.) is considered a receptor of trace element contamination originating from the lowland and deposited via abundant annual precipitation (Pastorino et al., 2019a). The lake is covered by ice several months of the year; it is inhabited by bullhead (*Cottus gobio*) which feed almost exclusively on chironomid (Diptera, Chironomidae) larvae all year round (Pastorino et al., 2019b). In this study it was determined whether pollution by plastic and microplastics is impacting the trophic web in Dimon Lake. To do this, biotic and abiotic matrices were analyzed: preys (chironomids; Diptera, Chironomidae), predator (*C. gobio*), water, sediment, and the snow cover around the lake.

2. Material and methods

2.1. Study ecosystem

Dimon Lake (46°34′05.4″ N; 13°03′45.8″ E) is a typical high-mountain lake *sensu* Catalan et al. (2006) located in the municipality of Ligosullo (Udine Province, Friuli-Venezia Giulia Region) at 1872 m a.s.l. in the Carnic Alps (northeast Italy). The site is classified as a Site of Community Interest and Special Areas of Conservation (SCI/SAC-IT3320002 "Monti Dimon e Paularo"). The lake has a maximum depth of 4.27 m, measures 376 m in perimeter and 0.6 ha in surface area (Pastorino et al., 2019b), and it is covered by ice for several months during the winter. It provides the habitat for few but well-adapted species. Pastorino et al. (2020) recently reported the almost exclusive presence of chironomids (Diptera, Chironomidae) among macroinvertebrates and *C. gobio* among fish species. Physicochemical parameters indicate oligotrophic water (Pastorino et al., 2019a). Direct anthropogenic impact is limited to trekking and pasturing during summer.

2.2. Water and sediment sampling

Microplastics sampling from water from June to October 2019 was done by a plankton net (Apstein) with a circular opening (0.30 m in diameter, 0.90 m in length, and 50 μm in mesh size) hauled by a dinghy at low velocity (3 km h^{-1}) to investigate both the horizontal (surface) and vertical distribution (from the deepest part of the lake to the surface) and composition of microplastics (Prata et al., 2019). Time of sampling was set at 10 min covering the entire lake perimeter (376 m) by random transects. Approximately 750 mL of water sample (250 mL taken three times circling the lake) were collected each month. Sediment samples (1000 cm^2) were collected monthly ($n = 5$) using a Van Veen grab (250 cm^2 sampling surface) at four sites (250 cm^2 x 4) that differed in depth (0.5; 1.5; 3; 4.2 m). Samples were then frozen at −20 °C in plastic-free containers for storage and thawed prior to extraction of plastics and microplastics.

2.3. Biota sampling

Between June and October 2019, forty *Cottus gobio* (eight per month) were caught by electrofishing in the northeast area of the lake, near a rockfall that provides a refuge zone for the fish (Fig. 1). The fish were euthanized with an overdose of tricaine methanesulfonate-MS-222 (70 $mgkg^{-1}$) and transported refrigerated to the laboratory in plastic-free containers. They were measured for total length (TL; cm) and total weight (W; g) and carefully necropsied in the laboratory on a precleaned working surface where precautions were taken to avoid plastic contamination. Sex (F = female; M = male; ND = not determined), liver weight (LW; g), gonad weight (GW; g), hepatosomatic index (HSI), and gonadosomatic index (GSI) were determined for each specimen (Bertoli et al., 2019). The stomach from each fish was dissected and its contents identified by stereomicroscopy (Zeiss Stemi V8, Zeiss, Oberkochen, Germany) and optical microscopy (Olympus BX40, Olympus, Tokyo, Japan). The contents were weighed (SCW; g), placed in precleaned glass containers, and stored at −20 °C until analysis. Permission for fish sampling was obtained from the Ente Tutela Patrimonio Ittico del Friuli Venezia-Giulia (authorization 11/DIR/17/01/2017).

Macroinvertebrates were collected with a Surber net (mesh

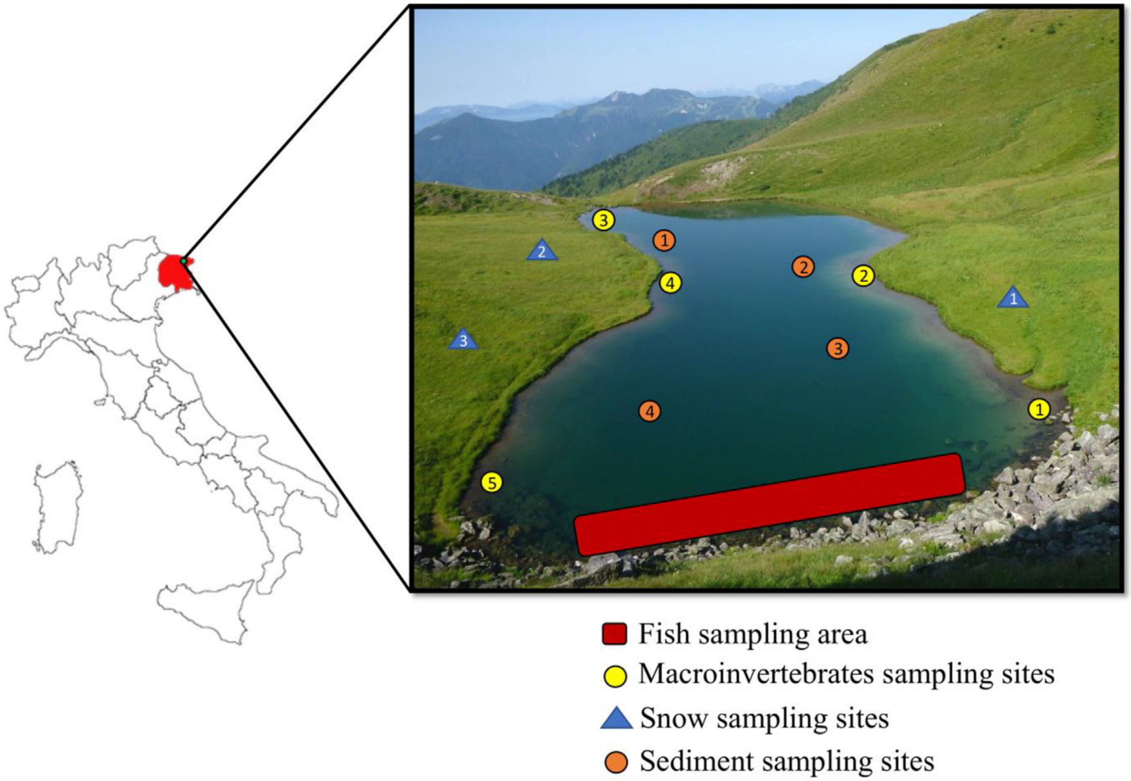


Fig. 1. Dimon Lake (46°34′05.4″ N; 13°03′45.8″ E), Friuli Venezia-Giulia Region, northeast Italy: fish sampling area (red rectangle) and sampling sites for macroinvertebrates (yellow circles) and snow (light blue triangles). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

250 μm) using a standardized method (Buffagni et al., 2014) at five sites in the littoral zone (Fig. 1). The samples were sorted in the field using steel forceps to select chironomids (Diptera, Chironomidae; n = 150; 30 per month) which were then placed in precleaned glass containers and stored at −20 °C until analysis.

2.4. Snow sampling

On 25 April 2020, surface snow (the first 10–15 cm) was sampled at three sites around the lakeside (Fig. 1). Snow from each site was placed in a precleaned glass bottle (750 mL), and four replicates (3 L) were obtained from each site. Samples (n = 12) were then transported on dry ice to the laboratory. To prevent potential contamination, personnel moved against the wind and took samples of the snow in front of them using their bare hands and a precleaned steel spoon (Bergmann et al., 2019). No footprints were seen at the site, indicating that it had not been contaminated by humans.

2.5. Laboratory analysis

Sediment samples were extracted three times by NaCl oversaturated pre-filtered solution as described elsewhere (Renzi et al., 2020) and the whole extract was recovered and filtered. Biological samples (chironomids and fish stomach contents) were pretreated by direct digestion of tissues with Creon enzyme (37 °C; TRIS-buffered pH) which quickly removes tissues without damaging plastic polymers for chemical identification (von Friesen et al., 2019). Snow samples were dissolved at room temperature in closed glass bottles without pretreatment. Water samples were filtered directly without digestion. The snow, water, liquid extracts from sediment samples and digested tissues were filtered through a filtering apparatus fitted with a paper fiber filter disk (pore diameter, 6 μm), stored in glass Petri dishes, dried overnight at 40 °C, and dyed with Rose-Bengal (4,5,6,7-tetrachloro-2′,4′,5′,7′-tetraiodofluorescein, Sigma-Aldrich, St. Louis, MO, USA) to target microparticles of possible interest on the filter (Ziajahromi et al., 2017), as it colors non-plastic particles such as natural fibers (cotton, cellulose), accelerates the sorting process, and reduces false positives. During laboratory analysis, air exposure was minimized to reduce potential airborne pollution while filtering the samples under a HEPA-filtered, laminar-flow fume hood. The filtered samples were sorted by stereomicroscopy at 10–80X (SMZ-800 N; software NIS-elements D, Nikon, Tokyo, Japan). The potential targets were then chemically analyzed by microscopy associated with Fourier transform infrared spectroscopy (μFT-IR; Nicolet iN10 MX, ThermoFischer Scientific, Waltham, MA, USA) equipped with an MCT-A detector (spectral range, 7.800–650 cm^{-1}) cooled with liquid nitrogen and operating in reflection mode. Identification was carried out by determining the spectral match (%) of the targeted items compared to the spectral libraries of normal and aged microplastics (OMNIC™ Picta™ software libraries, ThermoFisher Scientific) integrated with our own laboratory spectral libraries imposing a threshold for spectra back-recognition >80% of match; the limit of detection (LOD) was 10 μm of maximum particle size. Experimental blanks (negative controls) were analyzed to determine false-positives due to indoor plastic pollution from the laboratory by means of deionized pre-filtered (0.45 μm; n = 5; 100 mL) water as real samples. The data reported for the samples were not corrected according to the recovery in blanks. Recovered items were classified according to chemical type, shape, size, and color following criteria reported elsewhere (Galgani et al., 2014); shape and color categories were taken from Galgani et al., (2014); size classes of macroplastics, mesoplastics, and microplastics were taken from Alomar et al. (2016).

2.6. Statistical analysis

Descriptive statistics (mean and standard deviation, SD) were used to describe the basic features of microplastic concentration in the samples. Differences in microplastic concentration between the three snow sampling sites were assessed using the non-parametric Kruskal-Wallis test. Results were considered statistically significant at $p < 0.05$. Statistical analysis and graphics were performed using RStudio® version 1.1.463 (RStudio, Inc., Boston, MA, USA).

3. Results and discussion

The present study is the first to analyze plastic and microplastics in trophic web and snow samples from a high-mountain lake in the Carnic Alps.

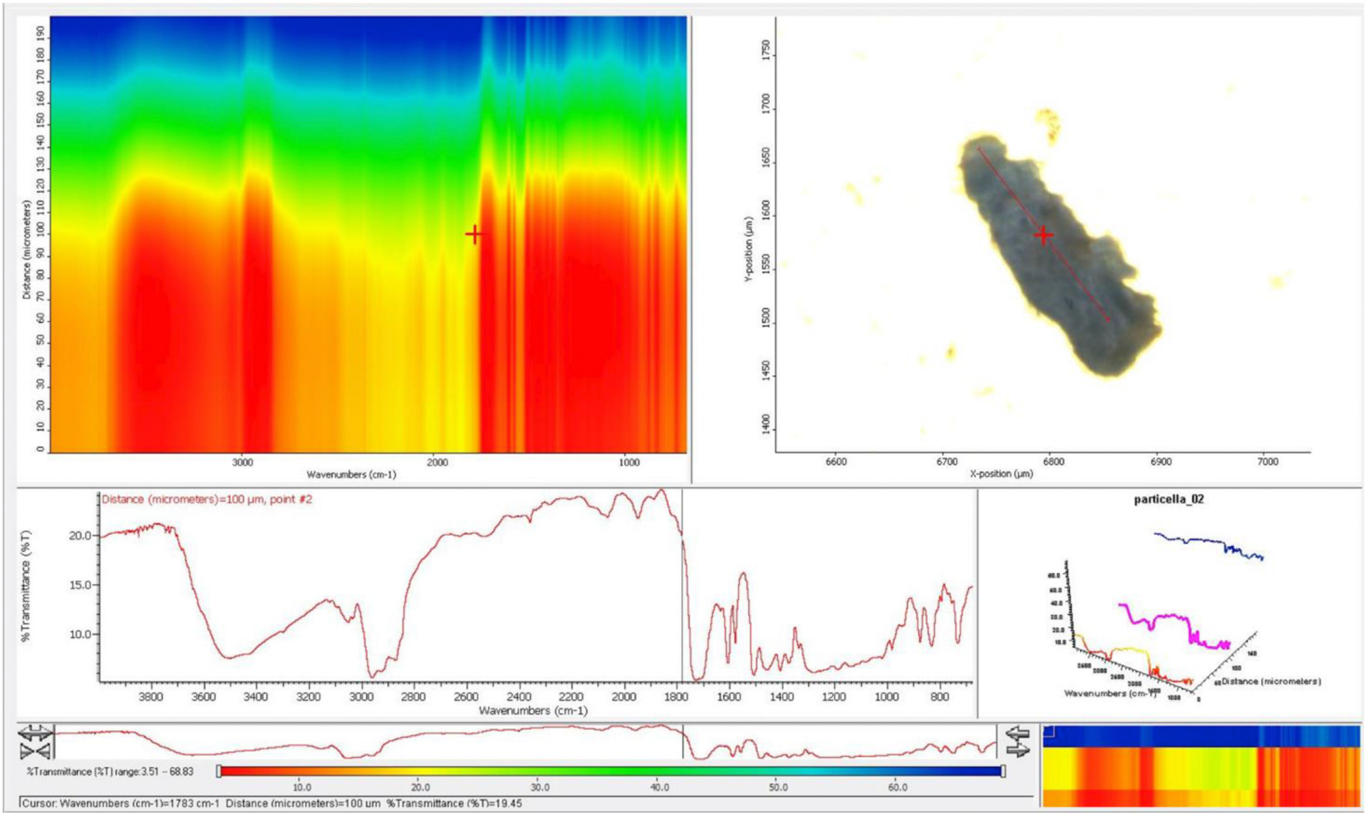
Table 1 presents the biometrical characteristics of the fish (*Cottus gobio*). Stomach content analysis disclosed the presence of Diptera Chironomidae. The weight of each stomach content is reported as wet weight (w.w.) in Table 1. The diet of the bullhead in Dimon Lake consists almost exclusively of chironomids (Pastorino et al., 2019b). Only chironomid (Diptera, Chironomidae) larvae were found in the fish stomachs due to the great abundance and almost exclusive presence of Chironomidae larvae in the macroinvertebrate assemblages of the lake (Pastorino et al., 2019a).

No macroplastic and mesoplastics were found in any of the matrices. Analysis of the matrices showed levels of detectable microplastic particles (at least 10 μm) lower than our limit of quantification in both the abiotic (water and sediment) and the biotic samples. We found one microparticle (one item in 3 L; 0.33 items L^{-1}) at site 3 (Fig. 2), with a significant difference between the three sites (Kruskal-Wallis test; $p < 0.05$). The snow samples were found to contain a microplastic particle of polyethylene terephthalate (PET) chemical type; the mean levels were 0.11 ± 0.19 items L^{-1} (Table 2). This particle was light blue colored; fragment shaped, and measured 75 × 220 μm. The frequency of records was very low: 8.3% (1/12) of the total snow samples. The presence of microplastics in remote areas has been attributed to the atmospheric transport of fibers or fragments from urban point source contamination (Dris et al., 2016; Klein and Fischer, 2019; Liu et al., 2019).

Polyethylene terephthalate, a pliable plastic made from synthetic organic polymers, is produced by the polymerization of substances typically derived from oil, gas, and coal (Ivleva et al., 2017). PET is now one of the most common thermoplastic resins

Table 1
Biometrical characteristics of *Cottus gobio* (n = 40) sampled from Dimon Lake. TL denotes total length; TW total weight; Sex (F female; M male; ND not determined); LW liver weight; GW gonad weight; HSI hepatosomatic index; GSI gonadosomatic index; SCW stomach content weight.

	TL (cm)	TW (g)	Sex	LW (g)	GW (g)	HSI	GSI	SCW (g)
Cottus gobio	10.65 ± 3.24	16.75 ± 12.72	M (38%); F (60%); ND (2%)	0.50 ± 0.49	0.97 ± 2.13	2.57 ± 1.28	3.35 ± 7.67	0.12 ± 0.16



Cursor: Wavenumbers (cm-1)=1783 cm-1 Distance (micrometers)=100 um %Transmittance (%T)=19.45

Fig. 2. Microparticle of microplastic recorded in snow samples by μFT-IR. Results are from reflection analyses performed on a possible target recorded by preliminary sorting. The image is a snapshot acquired with Thermo® software that highlights the target particle and associated chemical spectra recorded at three sampling points along the greatest length of the particle.

Table 2
Number of items collected from each replicate (0.750 mL) and total concentration of items (no. items L^{-1}) at the three snow sampling sites.

	Site 1	Site 2	Site 3
Replicate 1	0	0	0
Replicate 2	0	0	1
Replicate 3	0	0	0
Replicate 4	0	0	0
Total	**0**	**0**	**0.33**

produced in Europe, and it is widely used in beverage and food containers, tubes, bottles, containers, and labels. It is 100% recyclable and complies with consumer product safety standards for food, cosmetics, and drugs. By virtue of its crystalline transparency, light weight, high resistance, and long life, PET is the modern synthetic material; however, it can undergo fragmentation, hydrolysis, and photo-oxidation (Gewert et al., 2015).

Size and shape of particles are two characteristics that influence the interaction between microplastics and biota (Scherer et al., 2018). Generally, the members of the family Chironomidae are collector-gatherers that forage for particles in sediments. Although the presence of microplastics in the environment is relevant for this functional feeding group (FFG), few studies have investigated microplastic ingestion. One study reported that *Chironomus riparius* can ingest huge amounts of plastic litter (about 226 particles h^{-1}), indicating low selectivity of feeding and a high rate of ingested particles per hour (Scherer et al., 2018). Based on this observation, we assume that if microplastics were present in the sediment, we would have found them in the chironomids. Fish can also ingest microplastics by accident (mistaking them for prey) or via prey containing microplastics (Scherer et al., 2018).

Findings from this study indicate that snow was the only environmental compartment in which microplastics were detected. Sharing this observation, Bergmann et al. (2019) underlined the importance of the atmosphere as a source of microplastics in both Arctic and European snow. Snow precipitation is quite abundant during winter in the Carnic Alps, with a recorded mean snowfall of 7.6 cm during the winter of 2019 (ARPA FVG, 2019), and can bind atmospheric particles and pollutants, acting as a scavenger for microplastics that are then deposited on the ground (Zhao et al., 2015).

4. Conclusion

In this study, plastic and microplastics levels were measured for the time in several environmental matrices (snow samples included) collected during the ice-free season in a high-mountain Alpine lake. Findings suggest an absence of plastic pollution (>5000 μm, macro- and mesoplastics) in this ecosystem. No microplastics (10–5000 μm) were detected in the biotic and abiotic samples, indicating no significant pollution in this remote Alpine ecosystem. Nonetheless, the very low levels detected in the snow samples suggest the need for further sampling of this temporary matrix to better understand the more probable sources of microplastic pollution from long-range transport dynamics such as wind and snowfall rather than from local pollution sources.

CRediT authorship contribution statement

Paolo Pastorino: Investigation, Data curation, Methodology, Writing - original draft. **Elisabetta Pizzul:** Investigation, Methodology, Writing - review & editing. **Marco Bertoli:** Data curation, Writing - review & editing. **Serena Anselmi:** Investigation, Methodology. **Manuel Kušće:** Investigation, Methodology. **Vasco Menconi:** Investigation, Methodology. **Marino Prearo:** Methodology, Writing - review & editing. **Monia Renzi:** Conceptualization, Supervision, Writing - review & editing, All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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