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Passive biomonitoring of airborne microplastics using lichens: A comparison between urban, natural and protected environments

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ABSTRACT

Currently, natural and urban ecosystems are affected by different types of atmospheric deposition, which can compromise the balance of the environment. Plastic pollution represents one of the major threats for biota, including lichens. Epiphytic lichens have value as bioindicators of environmental pollution, climate change, and anthropic impacts. In this study, we aim to investigate the lichen bioaccumulation of airborne microplastics along an anthropogenic pollution gradient. We sampled lichens from the Genera *Cladonia* and *Xanthoria* to highlight the effectiveness of lichens as tools for passive biomonitoring of microplastics. We chose three sites, a "natural site" in Altipiani di Arcinazzo, a "protected site" in Castelporziano Presidential estate and an "urban site" in the centre of Rome. Overall, we sampled 90 lichens, observed for external plastic entrapment, melt in oxygen peroxide and analysed for plastic entrapment. To validate the method, we calculated recovery rates of microplastics in lichen. Particularly, 253 MPs particles were detected across the 90 lichen samples: 97 % were fibers, and 3 % were fragments. A gradient in the number of microplastic fibers across the sites emerged, with increasing accumulation of microplastics from the natural site (n = 58) to the urban site (n = 116), with a direct relationship between the length and abundance of airborne microplastic fibers. Moreover, we detected the first evidences of airborne mesoplastics entrapped by lichens. On average, the natural site experienced the shortest fibre length and the centre of Rome the longest. No differences in microplastics accumulation emerged from the two genera. Our results indicated that lichens can effectively be used for passive biomonitoring of microplastic deposition. In this scenario, the role of lichens in entrapping microplastics and protecting pristine areas must be investigated. Furthermore, considering the impact that airborne microplastics can have on human health and the effectiveness of lichens as airborne microplastic bioindicators, their use is encouraged.

1. Introduction

Plastic, with 150 million tons of annual production, is considered one of the major environmental persistent pollutants (Adeniran et al., 2022; Williams and Rangel-Buitrago, 2022; Rajmohan et al., 2019). It is estimated that between 19 and 23 million tonnes of plastic enter aquatic habitats annually (Gondal et al., 2022) and, on average, soils are contaminated with ca. 150 kg ha^{-1} plastic debris (Qi et al., 2020). The plastic pollution crisis derives both from excessive production and, at the same time, from insufficient and negligent recycling and disposal projects (Ellis et al., 2021). Since 1950, only 9 % of plastic produced has been recycled in the right way (d'Ambrières, 2019), while 76 % was usually dumped as waste (Hataway, 2017). The problem is sharpened by plastic fragmentation into smaller pieces due to physical, chemical, mechanical, and biological forces acting on them, especially in the water matrix (Gallitelli et al., 2022; Habibi et al., 2022). Microplastics (MPs) (1 μm to 5 mm) (Da Costa Filho et al., 2021) and nanoplastics (NPs) (<1 μm) (Jakubowicz et al., 2021) are described as emerging persistent contaminants, widely underrated in terms of abundance and accumulation in biotic and abiotic matrices (Cesarini et al., 2023; He et al., 2021; Sendra et al., 2021; de Souza Machado et al., 2018). Many studies evinced the ubiquity of MPs in marine, freshwater and transitional

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ecosystems, but also in estuaries, cultivated soils and numerous terrestrial habitats (Cera et al., 2022; Chen et al., 2020). Furthermore, contaminated foods were widely documented including honey, sugar, beer, and animal-derived foods, which indicates largely diffused plastic pollution in terrestrial environments (Wen et al., 2022; Malizia and Monmany-Garzia, 2019). Unfortunately, humans can uptake plastics not only from ingested food, but also from airborne particulate (Amato-Lourenço et al., 2021). The annual consumption of MPs per person was assessed in about 46,000 particles depending on age and sex; However, these data can increase up to 100,000 when inhalation is considered (He et al., 2021).

In the last decade, studies on microplastic pollution mainly focused on the sea, followed by biota, freshwater and soil; air pollution is largely underrated and only a few studies have been conducted on this topic (Kim et al., 2021). The most important drivers of MPs in the air are due to meteorological conditions and human activities that mostly influence MPs deposition (Paramitadevi et al., 2023; Habibi et al., 2022; Pandey et al., 2022) There are many sources of aerial plastics: road traffic emissions (estimated as 7 million kg year $^{-1}$) (Evangeliou et al., 2020), sea spray (Harb et al., 2023), waste incineration, building materials, synthetic fabrics (Amato-Lourenço et al., 2020), but also public fountains, which can spray and release MPs into the air (Sridharan et al., 2021) (Sridharan et al., 2020). Dris et al. (2016) also highlighted the importance of atmospheric fallout as a source of MPs, pointing out that the more urbanized the site, the higher the MPs atmospheric fallout, and the related effects on biota and human health. It's estimated that almost 1 fibre out of 3 in atmospheric fallout contains petrochemicals (Dris et al., 2016). Furthermore, few research highlighted that MPs can be inhaled posing a risk to human health (Habibi et al., 2022; Chen et al., 2020). Liu et al. (2019) demonstrated that indoor and outdoor dust can be an important vector of MPs, affecting human health (Kaushik et al., 2024; Uddin et al., 2023). Indeed, long-term exposure can cause asthma, pneumothorax, alveolitis, chronic bronchitis and pneumonia; besides, suspended MPs can be inhaled and deposited. MPs can be ingested through hand-to-mouth contact (Zhang et al., 2020). Despite their importance, data on the size, distribution, and shape of MPs in the atmosphere are lacking (Wright et al., 2021).

Similar to the monitoring of other non-plastic important pollutants, like particulate matter (Kousehlar and Widom, 2020), metal pollution (Dörter et al., 2020), polycyclic aromatic hydrocarbons (PAHs) (Boonpeng et al., 2023) and Persistent Organic Pollutants (Massimi et al., 2021), lichens can be used also for detecting MPs air pollution (Jafarova et al., 2023; Jafarova et al., 2022). Lichens are excellent biomonitors as they have a high surface area to volume ratio, simple anatomy (lack of root system) (Frati & Brunialti, 2023), capture and retain mineral elements via physical entrapment (Boonpeng et al., 2023), are easy to sample (Cecconi et al., 2019) and occur in areas with variable human impact (Loppi et al., 2021). Furthermore, their cost-effectiveness with respect to instrumental monitoring represents a fundamental advantage in the use of lichens as bioindicators (Contardo et al., 2020). However, despite their use in biomonitoring of contaminants, their use as a tool to detect MPs in the environment, in particular in air, is poorly investigated. So far, only three studies have been carried out using lichens as bioaccumulators of airborne MPs (Jafarova et al., 2023; Jafarova et al., 2022; Loppi et al., 2021).

This research aims to investigate the lichen bioaccumulation of airborne MPs along an anthropogenic pollution gradient and to highlight the effectiveness of lichens as a tool for monitoring MPs in the environment. Considering three different sites, we hypothesize the increasing entrapment by lichens of MPs from the natural to the urban site, with the protected site showing the lower MPs accumulation. We also hypothesise that MPs length, where particles are present, result lower in natural sites with respect to the urban one. To the best of our knowledge, this is the first paper exploring the possibility of using lichens through passive biomonitoring as biomonitors for airborne MPs concentrations considering both urban, natural and protected sites. This study could pose an important step to the knowledge of lichen bioaccumulation processes and plastic airborne pollution as it could raise awareness about potential threats to human health by plastics. Therefore, to monitor plastic pollution and tackle it in the future, it is necessary to identify targeted strategies for the reduction of plastic release in the air.

2. Materials and methods

2.1. Study area

The experimental protocol provided for the identification of sampling sites, the selection of trees with epiphytic lichen assemblages, and the removal of 30 lichens for each site. Sampling sites were chosen in order to include areas showing different urbanization levels and anthropogenic impacts. The study area is the Latium region, in Italy. We selected three stations showing different pollution situations and different altitudes (Fig. 1). The evaluation of the potential anthropic pollution sources was estimated considering some criteria such as the presence of industrial activities, traffic flow, precipitation rate and air pollution indicators like the airborne particulate. The urbanization of each site was evaluated according to the inhabitant's density, the green areas' density and the motorization rates, collected from national databases (National Institute of Statistics and Italian Automobile Club).

The first site is located in Altipiani di Arcinazzo (41°50′40″N, 13°12′18″E; 853 m a.s.l.), described as the "Natural site", a mountain locality in Lazio, south of the Sirente-Velino Natural Park, straddling the province of Frosinone and the province of Rome, characterized by the presence of a typically alpine-looking plateau. The study site is a hill system characterized by cattle bred in the wild, temporary ponds and away about 1000 m from the nearest urban centre and 65 km from the centre of Rome. The study area doesn't show important atmospheric pollution sources (industries, agricultural sector), while transportation represents the only direct pollution source.

The second site is the Castelporziano Presidential Estate (41°45′13″N, 12°25′23″E; 70 m a.s.l.), described as the "Protected site", a natural area which obtained State Nature Reserve status in 1999, covering over 6000 ha and extending south–southwest of Rome towards the Tyrrhenian Sea. Castelporziano has most of the typical ecosystems characterizing the Mediterranean biogeographical region: proceeding from the sea towards the hinterland, there is a large area of pristine beach, sand dunes with pioneer and colonizing plants, ancient dunes consolidated with wetlands behind the dunes and Mediterranean maquis areas. There are also vast areas of lowland forests. The study site is 21 km away from the centre of Rome. In the study area, pollution from industries, agricultural activities and transport impacts are negligible. On the other side, outside the boundaries, the site is surrounded by the south side of the city of Rome, characterized by high population density and industrial activities. Transport and industrial activities represent the main source of pollution from the surrounding infrastructures.

The third site is the Roma Tre University (41°51′17″N, 12°28′11″E; 11 m a.s.l.), described as the "Urban site", located in a highly urbanized area in the centre of Rome. This site is characterized by a small park and a mosaic of vegetational assemblages surrounded by numerous human activities like buildings, shops, roads and train stations, with a high traffic flow. Here, lichens were sampled on trees living in an urban park.

Altipiani di Arcinazzo, the natural site, shows an average good air quality (PM10 < 20 μg/m^3) in the sampling date. The area, considering a buffer zone of 5 km around the city centre is characterized by a 40 % of mixed forest (Code 313), 30 % of natural grasslands (Code 321), 10 % sparsely vegetated area (Code 333), 10 % non-irrigated arable land (Code 211) and 5 % of discontinuous urban fabric (Code 112) and 5 % of green urban areas (Code 141).

Castelporziano, the protected site, shows discrete air quality (20 $<$ PM10 $<$ 27 μg/m3) in the sampling date. The area, considering a buffer zone of 5 km around the city centre is characterized by 25 % of non-

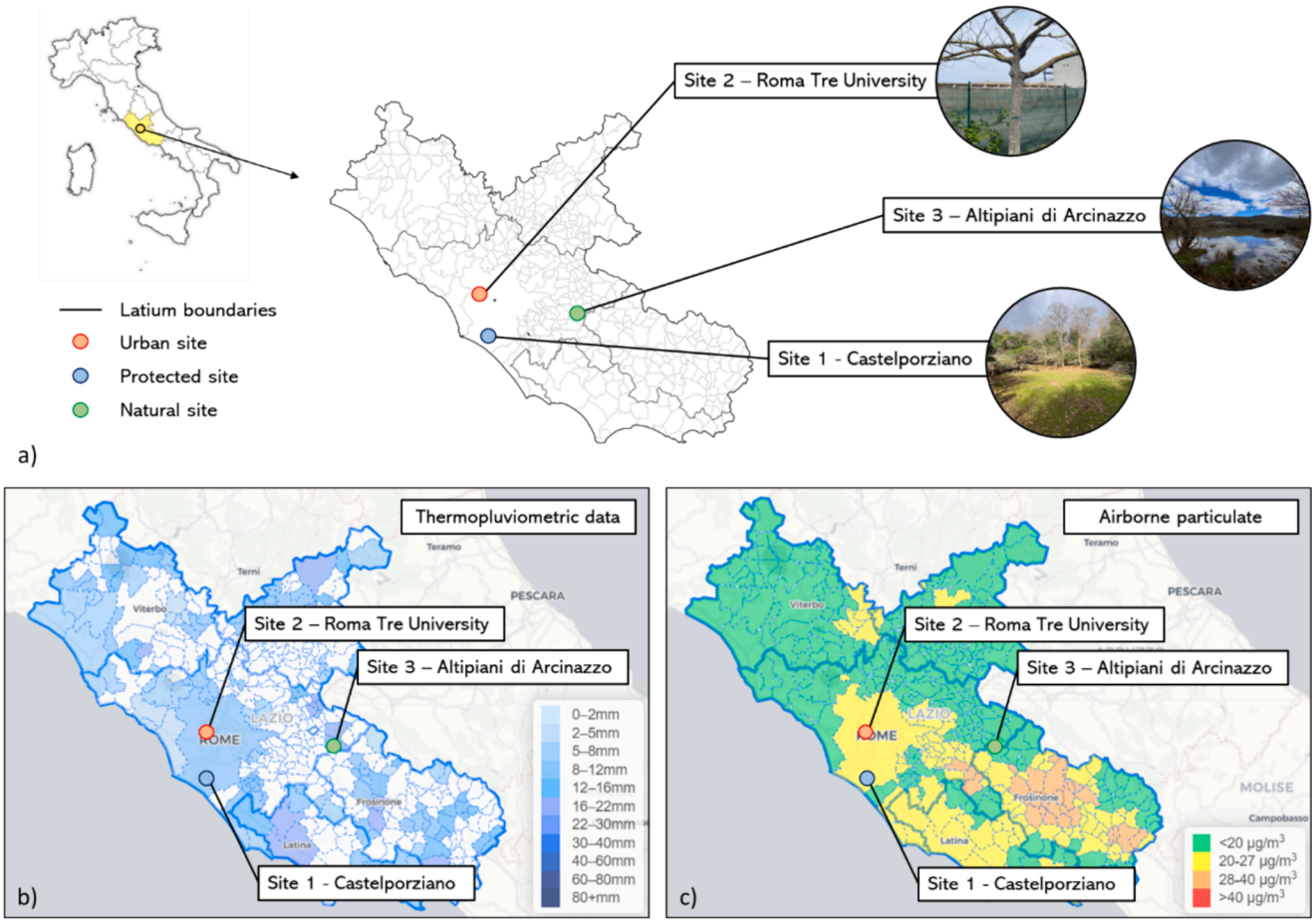

Fig. 1. Map showing the location of the study area, the location of sampling sites and a photo of the sites (a); Map showing thermopluviometric data (b) and airborne particulate data (PM10) (c) of the study sites in the sampling dates (www.dati.lazio.it).

irrigated arable lands (Code 211), 25 % of mixed forest (Code 313), 20 % of discontinuous urban fabric (Code 112), 15 % of coniferous forest (Code 312), 5 % of land principally occupied by agriculture (Code 243), 5 % of complex cultivation patterns (Code 242) and 5 % of industrial or commercial units (Code 121).

Rome, the urban site, shows poor air quality (28 < PM10 < 40 µg/m3) in the sampling date. The area, considering a buffer zone of 5 km around the city centre is characterized by a 35 % discontinuous urban fabric (Code 112), 30 % continuous urban fabric (Code 111), 15 % green urban areas (Code 141), 15 % complex cultivation patterns (Code 242) and 5 % industrial or commercial units (Fig. 1).

2.2. Experimental design

The monitoring campaign was performed on 19 April 2023, during the spring season. For the study, we selected two epiphytic lichens belonging to the genera *Cladonia* and *Xanthoria*. These two lichens are very common genera with wide distribution, living on tree barks. Lichens of the genus *Cladonia* are foliose lichens widely distributed in the Italian peninsula, easy to recognize and sample (De La Cruz et al., 2018). Lichens of genus *Cladonia* are largely studied for their acetone extracts and their antitumoral, anticoagulant, antithrombotic, and immunomodulating properties (Kosanić et al., 2018). Lichens of the genus *Xanthoria* are foliose lichens growing on barks and rocks, colonizing almost all habitats from the sea to mountain ecosystems (Lorenz et al., 2023). They grow both in natural and anthropized environments and result as one of the most widespread biomonitors in the study area, being tolerant to air pollutants and absent only in very highly polluted situations (Vitali et al., 2019). These two lichen genera have been yet used in literature for monitoring airborne pollution (Mohamed et al., 2023; Jafarova et al., 2022). Lichens were both collected from the same tree trunks located at each sampling site using metal tweezers. To sample lichens on the tree's bark, a standardised plot area of 10 cm x 10 cm was used following Loppi et al. (2006). Lichens were harvested from all cardinal exposures. Latex gloves were used to detach the thallus of the lichen from the substrate at a height of 1.5–2.0 m to avoid contamination by soil particles and to gain data from lichens present at "breath height" by humans. At each sampling site, 15 individuals of *Cladonia* and 15 individuals of *Xanthoria* were collected, with a minimum distance of 50 m between each tree to avoid contamination. In the end, 50 ml sterile falcon was used to store samples, which were brought to the laboratory for analysis.

2.2.1. Materials, reagents and instruments

Field:

- Metallic tweezers
- Powder free latex gloves (Clinilab®)
- Self-standing tube 50 ml sterile falcon, screw cap (CLEARLine®)
- Petri dishes 60x15 mm made with soda-lime glass
- Glass microfibers filter papers (Lab Logistic Group GmbH, VWRI516-08882, binder free, 0.7 µm, 47 mm, 100, 516–0882)

Laboratory:

- Hydrogen peroxide solution 30 % (CARLO ERBA reagents S.A.S., CAS n° 7722–84-1)
- Distilled water (H_2O)
- Stereomicroscope (Nikon C-LEDS with 4.0x objective)

- Plastic jar (Ø = 3 cm)
- Petri dishes 60x15 mm made with soda-lime glass
- Glass microfibers filter papers (Lab Logistic Group GmbH, VWRI516-08882, binder free, 0.7 μm, 47 mm, 100, 516–0882
- Vacuum pump with valve and vacuum gauge (LBX V10 series, 18 l/min, −670 mmHg)
- Analytical balance (Gibertini elettronica S.R.l.), model E42B 120 g, 0.1 mg
- Micro-FTIR: Nicolet iN10 infrared microscope (Thermo Fisher) with a Mercury-Cadmium-Telluride (MCT-A)

2.2.2. Microplastic analysis

In the laboratory, to compare the microplastic external uptake ability of lichens with the internal one, preliminary analyses were performed using metallic tweezers and latex gloves to separate the vegetal substrates from plastic fragments. All thalli were carefully cleaned from fragments of tree bark and other lichens using powder free gloves and metal tweezers. Then, lichens were stored in petri dishes 60x15 mm made with soda-lime glass and observation of the lichen samples was performed under a stereomicroscope (Nikon C-LEDS with 4.0x objective) to identify MPs entrapped by lichens. Data on lichen weight and length, the number of plastic fibres detected, fiber colour and length were collected.

The digestion method used in this research were adopted by Jafarova et al. (2022), Loppi et al. (2021), and Windsor et al. (2019). After manual separation, lichen material was dried out at room temperature until reaching constant mass. Each lichen sample was individually digested using a wet peroxide oxidation method to degrade the organic matter without impact on MPs. For the digestions, hydrogen peroxide solution of 30 % was used following literature (Prata et al., 2019, see Fig. S1). The digestion was performed following the Vegetation I.C.P. Monitoring manual (Vegetation, 2023), adapting H_2O_2 concentrations. Samples were individually inserted into a plastic jar (Ø = 3 cm), filled with 1 ml oxygen peroxide 30 % to degrade organic matter and left at room temperature for five minutes. The digest is subsequently stored at 45° C. However, lichens appear to be more difficult to digest because of their robustness (Roblin and Aherne, 2020). To boost lichen degradation, we adapted and modified the protocol by Gallitelli et al. (2021): after two weeks each sample was refilled with 2 ml oxygen peroxide 30 % when the reaction slows down and the procedure was repeated two weeks later, for a total of 5 ml oxygen peroxide 30 % for each jar/lichen sample. Lichens were stored for one month at 45° C. At least, three aliquots will be required. Degraded samples were then vacuum filtered onto glass microfibers filter papers (Lab Logistic Group GmbH, VWRI516-08882, binder free, 0.7 μm, 47 mm, 100, 516–0882), and the filters were placed into glass Petri dishes for storage. Filter papers are visually analysed for the presence of microfibres using a stereomicroscope. Identification of MPs fibers was performed following a five criteria visual identification method modified from Norén (2007) and Windsor et al. (2018). All plastic fibers and fragments were measured in length using the open-source image processing software ImageJ. Data about the number of plastic fibers, fiber colour and length were collected (Fig. 2). The chemical features of MPs within the lichens were investigated by micro-Fourier-transform infrared spectroscopy (micro-FTIR) which combines an infrared spectrometer with a microscope equipped with high-resolution 1/3 in. color digital camera with 1024 × 768 XGA for the collection of visual images of the sample surface (Fig. S4). Infrared spectroscopy is one of the most widely used techniques to identify polymer types and thus to detect microplastics (Andrade et al., 2020). The instrument used was a Nicolet iN10 infrared microscope (Thermo Fisher) with a Mercury-Cadmium-Telluride (MCT-A) nitrogen-cooled detector and motorized stage (Fig. S5). Measurements were carried out in the Attenuated Total Reflection (ATR) mode using an accessory with a germanium micro-tip of 350 μm diameter. The crystal,

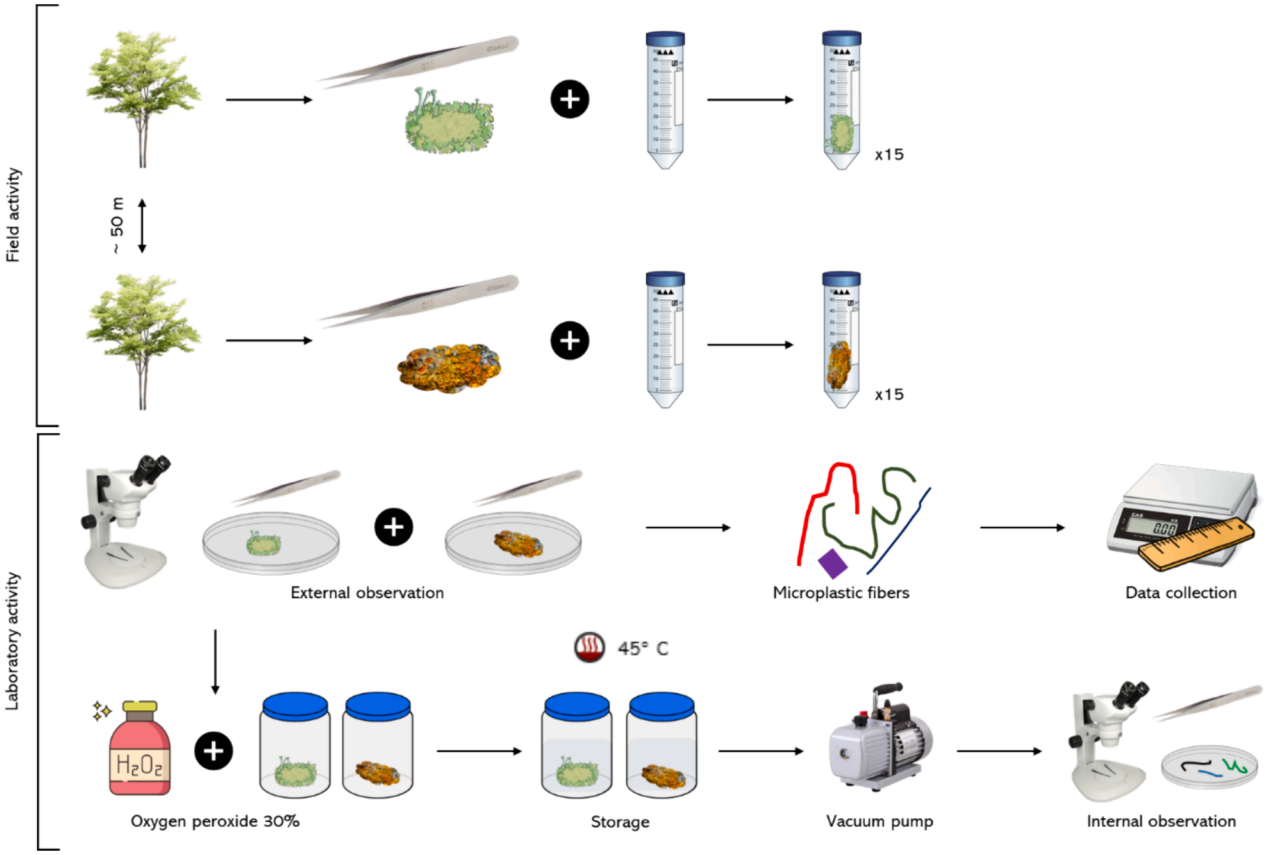

Fig. 2. Field activity and laboratory activity for the external and internal analysis of MPs in lichens for each site.

in contact with the sample with the application of minimal pressure (comparable to about 30 g), enabled the acquisition of clear spectra for all samples analysed. (Vianello et al., 2018). Spectra were recorded within the 4000 to 650 cm^{-1} range with a resolution of 8 cm^{-1} and 64 scans, using an aperture of 100 x 100 μm Data acquisition was carried out with the OMNIC SPECTA software provided by Thermo Fisher Scientific. Interferograms were averaged per spectrum and apodized using a Blackman-Harris correction. The background spectrum was collected on air. Spectra were also treated for atmospheric compensation to eliminate water vapour and CO2 contributions. To identify the composition of microplastics, the spectra obtained were matched with libraries provided by Thermo Fischer. All spectral matches above 70 % were considered as MPs.

2.3. Microplastic recoveries

In the literature there were many studies considering MP detection in other biological matrices (e.g., molluscs, fishes, mosses, etc) which validate the method used (Way et al., 2022). Considering the crucial importance of the method validation and the novelty of the degradation method proposed here, we decided to perform recoveries to test the effectiveness of our method to degrade a specific biologicalmatrix (i.e., lichens).

In order to perform method validation and recovery experiments, plastic particles of approximately 1——3 mm in size were produced by cutting plastic products made of polypropylene (PP), polyethylene terephthalate (PET), polystyrene (PS) and expanded polystyrene (EPS). The use of PP, PET, PS and EPS compounds reflects the massive production at global scale of this plastic polymer (Way et al., 2022). To determine the average recovery rate (positive control) of the protocol, overall, 12 lichen samples were spiked with 120 MPs particles. In particular, to test the recovery rate of each polymer, 1 lichen and 10 weighted MPs particles were stored together in a single plastic jar, providing three replicates (following Munno et al., 2018; Catarino et al., 2017). The particles were exposed to a 30 % solution of H_2O_2 (5 ml) and stored at 50° C for seven days (following Hagelskjær et al., 2023). Oxygen peroxide (H_2O_2) was tested for its capacity to dissolve organic matter of lichens and MP particles. The MP particles were then filtered onto glass microfibers filter papers (Lab Logistic Group GmbH, VWRI516-08882, binder free, 0.7 μm, 47 mm, 100, 516–0882), and the filters were placed into glass Petri dishes for storage and MPs were manually counted. MPs, stored in Petri dishes, were then dried in a laboratory stove at 60° C for 24 h (following Munno et al., 2018) and then weighted (Fig. S2). The final weight was compared with the starting weight of the MP particles, according to Yu et al. (2019).

Recovery rates were calculated following two different approaches.

Firstly, the recovery rate referring to the number of MPs (RR_N) was calculated considering the difference between the final (MPs recovered) and the initial (MPs spiked) number of particles on plastic jars, following Munno et al. (2018) and Catarino et al. (2017), referring to the formula:

(1) RR_N (%) = (No. of MPs recovered/No. of MPs spiked) x 100.

Then, the recovery rate referring to the weights of MPs (RR_W) was calculated considering the weights (mg) calculated before (MPs spiked) and after (MPs recovered) the treatment, following the formula from Shim et al. (2016):

(2) RR_W (%) = (MPs recovered(mg)/MPs spiked(mg)) x 100.

Given that the bleaching of MPs has a negative effect on recovery rates and method validation, colour changes were also noted according to Miller et al. (2017).

2.4. Sample quality assurance and control (QA/QC)

To avoid atmospheric contamination of our samples, we used a quality assurance/quality control (QA/QC) approach as suggested by literature (Ziajahromi and Leusch, 2022; Gallitelli et al., 2020). First of all, we prevented polluting our samples by using nitrile gloves, cotton clothes, and sterilized tweezers (Gallitelli et al., 2020). In particular, we used sampling tools cleaned with distilled water and plastic-free containers (e.g., glass bottles and aluminium pots). In the field and laboratory, we performed blanks to evaluate if potential contamination was present during sample processing. To do that, we prepared negative controls for both field and laboratory activities to assess the MP contamination of our samples. In the field, we performed two control blanks per station, while in the laboratory three blanks for external contamination. Then, about reagents and materials, we performed three blanks for distilled water, hydrogen peroxide, and falcon tubes. When fibers were found in our control blanks, we subtracted those from our results. We found three fibers of cellulose in the field blanks, and two cellulose fibers from reagents (one for peroxide hydrogen and one for distilled water). Considering that cellulose is not plastic but is an anthropogenic material (Finnegan et al., 2022), we have not considered it in our results. As we found no other MPs in the controls, zero contamination was found.

2.5. Data analysis

Data normality was verified with the Shapiro-wilk test. Significant differences concerning recovery rates were evaluated by a Kruskal-Wallis test. Significant differences in the accumulation of plastics between sampling sites were evaluated by one-way ANOVA. Significant differences between fibers length across the sampling sites were calculated by one-way ANOVA; *t*-test highlights the differences between sites. *T*-test highlights the differences in accumulation between the two genera. Significant differences between lichen size and the number of plastic items found were calculated by one-way ANOVA. Bivariate linear analysis was used to evaluate the correlation between the number of plastic items and population density in sampling sites and between lichen length and MPs fibers length A probability of 0.05 was chosen as the level of significance for all statistical tests, and all statistical analyses were carried out using the open-source software R and PAST (Hammer et al., 2001).

3. Results

All microplastic samples (PP, PET, PS, EPS) show a RR_N of 100 %, with all the number of microplastic particles found after the treatment. All microplastic samples show a high RR_W of about 97.03 ± 0.03 % on average over the whole range of applied treatments. No significant differences were observed concerning recovery rates for any tested polymer types ($H = 1.462$, $p > 0.05$). In particular, recoveries were estimated as 97.35 ± 0.038 % for PP, 99.03 ± 0.025 % for PET, 98.82 ± 0.028 % for PS and 91.10 ± 0.028 % for EPS. No colour changes were noticed.

Anthropogenic fibers and fragments were observed in all the stations considered. A total of 253 plastic particles were detected across the 90 lichen samples (98 % MPs, 25 mesoplastics), ranging from 58 (natural site) to 116 (urban site). 97 % were fibers and 3 % were fragments. Fibers were detected in 92 % of the samples, while fragments were detected in 9 % of the samples. Fragments size was in the range of 2–10 mm, with a mean length of 7 mm. The highest number of fibers were black (176), followed by green (31), blue (26), red (9), transparent (8), violet (2), and yellow (1) (Table 1). Overall, 13 mesoplastics were detected, with lengths ranging from 6 to 9 mm. The highest number of mesoplastic fibers were transparent (6), followed by black (4), green (1), violet (1), yellow (1). ANOVA test did not show a significant difference ($F = 0.9$, $p < 0.05$) between mesoplastic sample length among sites.

A gradient in the number of plastic fibers across the sites emerged, with increasing (double) accumulation of MPs from the natural site ($n = 58$) to the urban site ($n = 116$). Lichens at natural sites accumulated the lowest concentration (number per dry weight of lichen, Tab. S1) of anthropogenic microfibres (39 MPs per g dw), while values at the protected and urban sites were much higher (236 MPs per g dw and 580

Table 1
Number of lichen samples taken (n), lichen average weight (g), the total number of MPs items (it), MPs density (it/lichen), percentage of fibers found (%), average and total fiber length (mm) and colour.

	Arcinazzo	Castelporziano	Rome
Lichen samples (n)	30	30	30
Lichen weight (g)	0.012 ± 0.006	0.011 ± 0.001	0.003 ± 0.0008
Lichen size (mm)	5.0 ± 1.0	8.0 ± 1.0	3.4 ± 0.4
MPs items (it)	56	78	106
MPs (it/lichen)	1.86	2.56	3.8
Fibers (%)	99	99	94
Average fiber length (mm)	15 ± 0.2	16 ± 0.1	2 ± 0.1
Fiber colour and relative fiber cumulative length (mm)	Black 66 Blue 3.5 Green 7 Red 1	Black 100 Blue 14 Green 12 Red 2	Black 98 Blue 40 Green 36 Red 8.5 Violet 5 Transparent 9
Total fiber length (mm)	76.1	128	1965
Main plastic polymers	Cellophane, PET	Cellophane, PET	Cellophane, PAN

MPs per g dw, respectively). The median lengths of microfibers (1.5 – 2 mm) were different across sites: ANOVA test showed a significant difference ($F = 9.9$, $p < 0.05$) between samples length. Pearson Correlation Coefficient did not show a significant relationship ($r = 0.22$, $p < 0.05$) between lichen and fiber lengths.

Shapiro-Wilk test confirmed the normal distribution of the dataset (AR, $W = 0.768$, $p < 0.05$; CP, $W = 0.8159$, $p < 0.05$; RM, $W = 0.7498$, $p < 0.05$). The length of fibers shows significant differences between sites ($F = 3.559$, $p < 0.05$), with the urban site (Rome) showing the longest fibers; *t*-test highlights the differences between Arcinazzo and Rome ($t = 2.1795$, $p < 0.05$) and Castelporziano and Rome ($t = 2.2173$, $p < 0.05$). The shortest fibers were found at the natural site, with the distribution of the fibre length ranging from 0.1 to 7 mm; in the protected site the distribution of the fibre length ranged from 0.5 to 6 mm; in the urban site, the distribution of the fibre length ranged from 0.5 to 7 mm. The longest fibers (7 mm mesoplastic fibers) were found in the natural site and in the urban site (Fig. 3).

Lichen size and number of plastic items did not show any correlation ($F = 0.27$, $p = 0.84$). The bivariate linear model did not show a significant correlation between the number of plastic items and the population density of the area considered ($r = 0.90$, $p = 0.27$).

Overall, lichens from genus *Cladonia* entrapped 108 MPs with a total length of 235.7 mm, while lichens from genus *Xanthoria* entrapped 145 MPs, with a total length of 286.4 mm (Fig. 4). T-test did not show any differences between the accumulation rate and efficiency of *Cladonia* and *Xanthoria* in all three sites (AR, $t = 0.5841$, $p = 0.56$; CP, $t = 0.17196$, $p = 0.86$; RM, $t = 0.65934$, $p = 0.51$).

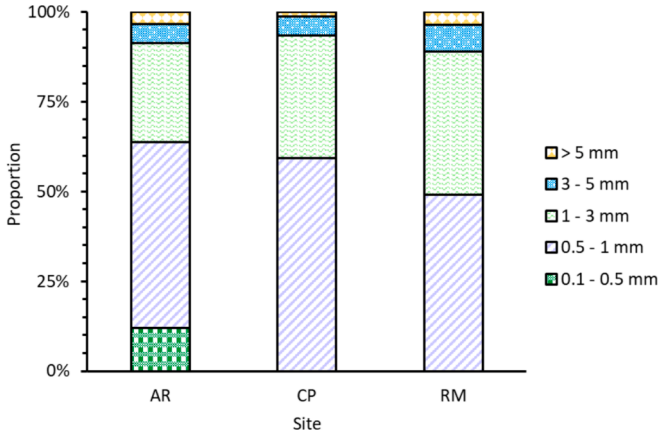

Fig. 3. Micro- and mesoplastic fibers size for each site (AR = Arcinazzo; CP = Castelporziano; RM = Rome).

FT-IR analysis identified different MPs (Fig. 5, Fig. S3). In the natural site, black, red and blue fibers were identified as cellophane, and yellow fibers were identified as polyethylene terephthalate (PET); in the protected site, blue and green fibers were identified as cellophane, red fibers as polyethylene terephthalate (PET), blue fibers as polyurethane (PU); in the urban site, blue and green fibers were identified as cellophane, transparent fibers as polyacrylonitrile. Moreover, cellulose fibers were found in all three sites both in the samples and in the control blanks (Fig. S6).

4. Discussions

This study represents the first coupled research of lichens and MPs through passive biomonitoring in Central Italy in different environments. Here we demonstrated that lichens could entrap plastic fragments and fibers in three ecosystems, characterized by different levels of pollution and urbanization. Although few studies investigated MPs in lichens, until now a recovery rate to validate the methods has not been used. However, given the importance of collecting standardised data to monitor MPs also in atmospheric environments, we proposed for the first time a validation of this method, obtaining high recovery rates for MPs in lichens ($RR_N = 100$ %, $RR_W =$ mean 97.0 %). The presented method shows a high recovery rate of MPs for lichens. We obtained a higher recovery rate for the most common polymers investigated, compared to the same polymers extracted from different media, such as sediment, water, and biota (Way et al., 2022). Furthermore, a higher recovery rate emerged comparing our data with Way et al. (2022) where, Low-Density polymers (PS, PP) show an average recovery rate of 90 % using oxidising agents (H_2O_2). Moreover, our results show similar or higher PE recovery rates compared to other studies (Weber and Kerpen, 2023). EPS recoveries are in accordance with Munno et al. (2018), demonstrating that the wet peroxide oxidation slightly impacts the recovery of EPS. In conclusion, our tests demonstrated that the oxygen peroxide digestion of lichens can be considered a valid method to recover MPs from this biological matrix.

With regards to MPs in lichens, fibers represent the larger part of MPs found, while fragments are only present in a few samples (3 %): this is in line with Zhou et al. (2017), Wright et al. (2020) and Szewc et al. (2021), confirming that fibers above 100 μm (size limit of this study) were the most frequent MPs in airborne particulate. Fragments were present with only one item in Arcinazzo and Castelporziano and six items in Rome. Focusing on these data, a comparison between sites would be statistically weak and, for this reason, this research mainly focused on MPs fibers. The low weight of fibers makes them easier to be transported by wind (Koutnik et al., 2021) and captured by lichens surface (Jafarova et al., 2023). As hypothesized, in the urban site we found the highest number of MPs, double respect to the natural site, with the protected site located in the middle. Data on airborne particulates confirm the gradient of MPs along ecosystems: in the natural site, data show an airborne particulate concentration less than 20 $\mu g/m^3$, while the protected site and the urban site share an average concentration of 24 $\mu g/m^3$. The same trend was shown by Jafarova et al. (2022) on the active bioaccumulation of MPs by lichens in different parts of the city centre of Milan (Northern Italy). At the same time, thermopluviometric data show low precipitations during the month of sampling, suggesting a reduced influence of rain in MPs entrapment by lichens. Rainfall can give a boost to airborne MPs deposition, in particular in remote areas away from sources (Wright et al., 2019). However, being the passive biomonitoring based on lichens naturally present on tree barks, it results impossible to determine the exact time of development of the lichen and the relative influence of rain and wind. Considering the important role of climatic conditions for MPs dispersion and deposition, Allen et al., (2019) demonstrated that distribution of different MP types varies with climate; it is probably that analysis conducted on other seasons could show

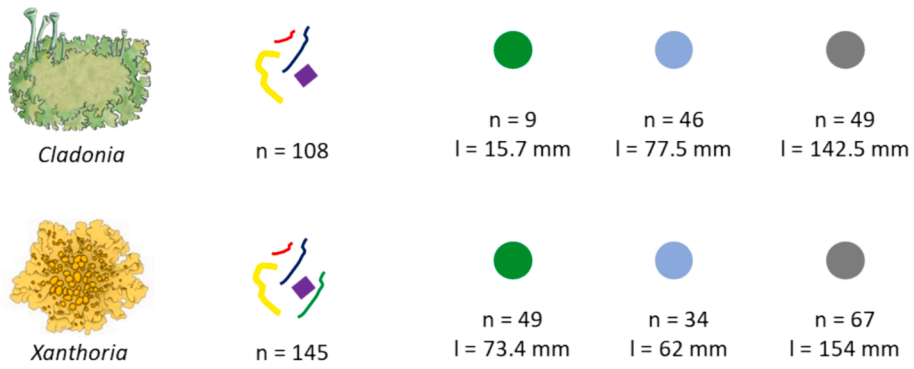

Fig. 4. Accumulation of MPs between the two genera considered. Green dot = natural site; Blue dot = protected site; Grey dot = urban site; n = number of MPs; l = MPs total length.

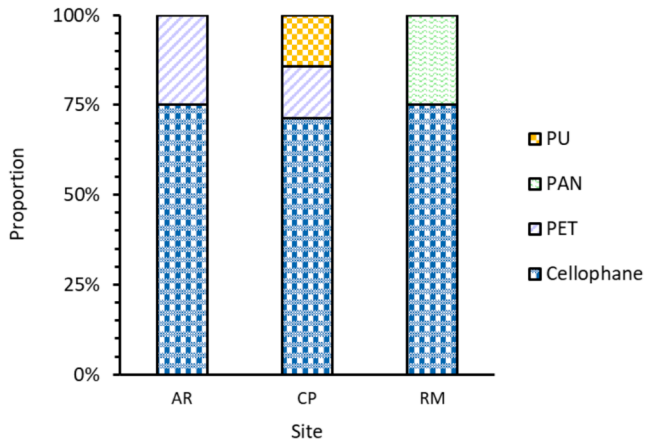

Fig. 5. MPs polymers found in the three sampling sites (AR = Arcinazzo; CP = Castelporziano; RM = Rome). PU = Polyurethane; PAN = Polyacrylonitrile; PET = polyethylene terephthalate.

different MPs deposition rates. However, our results can be explained by considering also the pollution sources near the sampling sites. The key sources of airborne MPs include synthetic textile clothing, furniture, construction materials, abrasion of rubber tyres, and urban dust (Sridharan et al., 2021). The natural site, characterized by 90 % of natural areas and with only agricultural and small human activities, didn't show any industrial activities near the sampling sites. The protected and the urban sites show, respectively, 65 % and 15 % of natural areas. Given the land use, in the natural site, MPs pollution could derive mainly from wind transport from longer distances. The wind transport could affect also protected and urban areas, but, while the former shows no anthropogenic activities inside the boundaries of the Estate, the latter shows many human activities around the sampling site. In the last two cases, we hypothesize that wind transport could be only low related to MPs pollution, with the higher pollution coming from the near anthropogenic activities.

Although the observed differences in millimetres of rain per day between the natural site (16–22 mm) and the protected and urban site (5–8 mm), the natural site had the lower number of MPs entrapped, suggesting that, in anthropized sites, lichens entrap mostly MPs, independently from rainfall (Wright et al., 2019). This result, according to Dris et al., (2016) suggests that, although rainfall is an important factor influencing MPs deposition (Jia et al., 2022), urbanization is the key factor for MPs airborne pollution and deposition (Amato-Lourenço et al., 2020).

Data about the number of MPs per dry weight of lichen are in discordance with Jafarova et al., (2022), where the highest values were found in Periphery (56), followed by the Centre (44) and urban parks (26). The control site showed an average concentration of 20 MPs/gr dw. Here we found, in the natural site, an average concentration of 36 MPs/gr dw; however, data on protected (periphery) and urban sites are considerably higher (236 MPs per g dw and 580 MPs per g dw, respectively). We did not observe an increase in the number of MPs per gram of dry lichen from the urban centre to the peripheral zones (Jafarova et al., 2022); the hypothesis of the local origin of MPs in our case is not supported by evidences. Based on our results, we could speculate that, although the local origin of MPs is an important factor for airborne plastic concentration, meteorology conditions have a major influence on the transport and deposition of MPs (Enyoh et al., 2019).

Atmospheric fallout appears to be an important source of MPs (Dris et al., 2016). Sources and deposition of MPs fibers are driven by different factors. In the Latium region, in particular in Rome Municipality, winds are primarily responsible for the dispersal of a wide spectrum of pollutants (Munzi et al., 2007). In airborne systems, two different types of man-made fibers are found: organic, derived from synthetic polymers or natural products, or inorganic, derived from compounds like carbon or oil (Gasperi et al., 2018). MPs fiber particles are lighter and more easily suspended in the air than other types of fiber particles of the same sizes (Dris et al., 2016). Fiber's length in urban ecosystems has been reported to be variable. In literature, Liu et al. (2019) found a fibers average length of 1025 μm in indoor dust from China, Dris et al. (2016) found an average fibers length of 973 μm in Paris in airborne, while fiber sizes observed by Catarino et al. (2018) in Edinburg were generally < 500 μm. Here we found an average fiber length of 1700 μm, with a size length ranging from 1500 to 2000 μm. These results are in accordance with (Roblin et al., 2020) and Dris et al. (2016), considering that 20 μm is likely the minimum size for fibers to be found in the air, and those larger than this size do not remain airborne for a long time ((Roblin et al., 2020). To the best of our knowledge, this is the first research where the entrapment of mesoplastic by lichens in remote areas is demonstrated. Mesoplastics, like MPs, can reach natural environments, away from urban settlements by wind or rain transport (Garello et al., 2021). Moreover, we hypothesize that heavier fibers can fall over lichens more easily than lighter ones, remaining trapped, while smaller fibers take longer to fall. Threats to the environment and human health can come from both airborne fiber particles (Prata, 2018) and the entrapment of MPs by lichens. Plastic is considered a persistent pollutant (Worm et al., 2017) which can biomagnify in lichen-based food chains (Kelly et al., 2001).

All the MPs detected in our research turned out to be longer than 0.1 mm. Above the threshold of 0.1 mm, our study did not confirm the inverse relationship between the length and abundance of airborne MPs proposed by Zhao et al. (2023) and Bergmann et al. (2019). Also, Bergmann et al. (2019) demonstrated that the counts of microfibers showed an increasing trend with decreasing lengths. In our case, Arcinazzo shows the lowest number of MPs found and the lowest fibers length, while Rome shows a higher number of MPs and a higher fiber

length. On average, the natural site experienced the shortest fibre length and the centre of Rome the longest. A similar higher proportion of shorter microfibers at sites more remote from urban centres has been reported also by Allen et al. (2019). These observations are consistent with the fact that smaller fibers can be dispersed over longer distances than greater ones (Yoo et al., 2017), following also other factors such as pollution concentration gradient, wind direction, temperature, and humidity (Huang et al., 2020).

Our results did not confirm the hypothesis, showing a high concentration of MPs in the protected site. The presence of MPs in the protected site can be explained considering that the atmospheric transportation spread MPs to remote regions without any local sources (Allen et al., 2019). Only 10 km divides the "protected site" and the "urban site": in this scenario, MPs can be easily carried by wind into the protected site and entrapped by lichens. In particular, the differences in fibers length between Rome (i.e., higher) and Castelporziano (i.e., lower) confirm the hypothesis of wind transport of lighter and smaller fibers rather than bigger ones. In the same way, differences in fibers length between the natural site and the urban site may be due to the weight of fibers. The importance of MPs transport over any other factor emerged also from the no-significative differences between the number of items and urbanization of sampling sites. The protected site shows an unexpectedly high airborne MPs pollution considering the low population density: only wind transport and proximity to the urban centre can explain the relatively high number of items found.

Plastic polymers identified are in accordance with the abundances found in different matrices, with a dominance of cellophane and PET, representing the main polymer materials often found in marine water (Bajt, 2021), airborne particulate (Sarathana and Winijkul, 2022) and freshwaters (Yan et al., 2019). Cellophane and PET are widely used in the packaging industry (Kim et al., 2014), which indicates that urban pollution might be an important source of these microplastics (Yan et al., 2019). PAN fibers are used on a large scale in textile industries and represent the most suitable and widely applied for making high performance carbon fibers; the presence of PAN fibers only in the urban environment can be related to their largely use in the automotive technologies (Rahaman et al., 2007). PU is involved in a variety of applications such as biomedical applications, automotive, building, construction, textiles (Das and Mahanwar, 2020), and also coatings, adhesives, constructional materials, fibers, paddings, paints, elastomers and synthetic skins. Their resistance to degradation by water, oils, and solvents makes them excellent for the replacement of plastics (Howard, 2002). PU was only found in the protected site, where no anthropogenic activities (except for agricultural ones) take place; this evidence can reinforce the hypothesis of MPs wind transport from the urban site.

Our results show that the investigated sites are impacted by plastic pollution, at least as far as it concerns airborne particulate, but also showed that there is a clear trend in MPs pollution increasing with distance from a natural environment to an urban one. The presence of fibers in all the sites highlights the air-dispersion capacity of this pollutant which can be transported to remote areas and deposited through dry or wet deposition. In this scenario, the role of lichens and vegetation in entrapping MPs and protecting pristine areas must be investigated.

5. Conclusions

Plastic pollution represents an important research topic of increasing concern. Airborne plastics, although generally low-considered and studied, are a dangerous pollutant, affecting biota in general and also humans. This study is an important starting point for investigating the use of lichens as passive bioindicators for plastic pollution. Determining the occurrence of plastic pollution in airborne particulates can be difficult and complex. The use of lichens for passive biomonitoring of plastic airborne pollution can be an important tool for investigating the impacts of anthropogenic pollution on ecosystems but also for the removal of MPs from the environment. It's also important to consider the impact that airborne MPs can have on human health: if inhaled or ingested, microplastics may accumulate and exert localized particle toxicity by inducing or enhancing an immune response. This is expected to be dose-dependent, and a robust evidence-base of exposure levels is currently lacking. Moreover, the use of lichens is low-cost and favoured by the large distribution of these organisms. Here we demonstrated the capacity of lichens to entrap MPs from the atmosphere, but we only considered two target species: further investigations about the existence of a potential species richness gradient from the natural to the urban site are required.

CRediT authorship contribution statement

Davide Taurozzi: Software, Investigation, Formal analysis, Data curation, Validation, Visualization, Writing – original draft, Writing – review & editing. **Luca Gallitelli:** Conceptualization, Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing. **Giulia Cesarini:** Investigation, Validation, Visualization, Writing – review & editing. **Susanna Romano:** Writing – review & editing, Visualization, Validation, Investigation. **Monica Orsini:** Writing – review & editing, Visualization, Validation, Investigation. **Massimiliano Scalici:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition.

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.

Data availability

All the data are available in the manuscript and Supplementary files.

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Appendix A. Supplementary material

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