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ASSESSING THE DEPOSITION OF AIRBORNE MICROPLASTICS USING VEGETATION

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Content

Abstract

The increasing global concern over the prevalence of microplastics (MPs, plastic particles <5 mm) in the environment, coupled with their recent identification in the atmosphere, underscores the potential for direct human exposure. Traditional studies on airborne MP deposition often rely on specialized infrastructure and labor-intensive sampling networks, limiting spatial observations. This PhD thesis addresses this challenge by focusing on the novel use of passive biomonitors —an accessible and cost-effective tool for monitoring atmospheric pollution— to assess airborne MP deposition. The different chapters of this thesis highlight the evolution of the research, emphasizing key issues. Chapter 1 compares the suitability of the epiphytic lichen *Evernia prunastri* to the epigeic moss *Pseudoscleropodium purum* as biomonitors in central Italy, and suggests that epigeic moss is preferable for passive biomonitoring due to its structural characteristics and habitat position. Chapter 2 investigates the deposition of airborne MPs in Tuscany, central Italy, using pleurocarpous moss at 33 remote sites, revealing the effectiveness of passive biomonitoring for high spatial resolution assessments of regional-scale MP deposition. In Chapter 3, the study expands to the European scale, assessing MPs using moss biomonitoring across 37 remote sites from 17 countries, indicating the feasibility of mosses for large-scale assessments of atmospheric MP deposition, and highlighting the influence of population density on atmospheric MP abundance (count and mass). Chapter 4 focuses on understanding the contribution of tire wear particles (TWPs) using *Robinia pseudoacacia* L. (black locust) leaflets in Siena, Italy, which was motivated by the discovery of TWPs in moss samples collected throughout the region of Tuscany, coupled with the abundant presence of this invasive tree species along roads. The results showed the suitability of *Robinia* as a biomonitor for airborne MPs, including TWPs. Overall, this thesis establishes the effectiveness of biomonitors in assessing airborne MP deposition, providing valuable insights for environmental research and air quality management.

Riassunto

La crescente preoccupazione globale per la persistenza delle microplastiche (MP, particelle di plastica <5 mm) nell'ambiente, unita alla loro recente identificazione nell'atmosfera, sottolinea la potenzialità di esposizione per l'uomo. Gli studi tradizionali sulla deposizione di MP nell'aria si basano spesso su infrastrutture specializzate e reti di campionamento ad alta intensità di lavoro, limitando le osservazioni spaziali. Questa tesi di dottorato affronta questa sfida concentrandosi sull'uso di biomonitor passivi - uno strumento accessibile ed economico per il monitoraggio dell'inquinamento atmosferico - per valutare la deposizione atmosferica di MP. I diversi capitoli della tesi evidenziano l'evoluzione della ricerca, sottolineandone gli aspetti salienti. Il capitolo 1 confronta l'efficacia del lichene epifita *Evernia prunastri* e del muschio epigeo *Pseudoscleropodium purum* come biomonitor in Italia centrale e suggerisce che il muschio epigeo è preferibile per il biomonitoraggio passivo grazie alle sue caratteristiche strutturali e al suo habitat. Il capitolo 2 analizza la deposizione atmosferica di MP in Toscana (Italia centrale) utilizzando muschi pleurocarpi in 33 siti remoti, mostrando l'efficacia del biomonitoraggio passivo tramite muschi per valutazioni ad alta risoluzione spaziale della deposizione di MP su scala regionale. Nel capitolo 3 lo studio si espande alla scala europea, valutando la deposizione di MP attraverso il biomonitoraggio con muschi in 37 siti remoti di 17 diversi Paesi. I risultati mostrano l'utizzabilità dei muschi per valutazioni su larga scala della deposizione di MP, evidenziando l'influenza della densita abitativa sulla quantità di MP atmosferiche. Il capitolo 4 si concentra sulla comprensione del contributo delle particelle di usura dei pneumatici (tire wear particles, TWP) utilizzando le foglie di *Robinia pseudoacacia* L. a Siena (Italia) motivato dalla scoperta di TWP in campioni di muschio raccolti in Toscana e dall'abbondante presenza di questa specie invasiva lungo le strade. I risultati dimostrano l'idoneità della *Robinia* come biomonitor per le MP aerodisperse, comprese le TWP. Nel complesso, questa tesi stabilisce l'efficacia dei biomonitor nella valutazione della deposizione atmosferica di MP, fornendo preziose indicazioni per la ricerca e la gestione della qualità dell'aria.

Overview of the PhD thesis

The aim of this PhD thesis was to investigate the suitability of plant biomonitors to assess the atmospheric deposition of microplastics (MPs). The initial phase involved the comparative analysis of lichens and mosses as potential biomonitoring tools for assessing airborne MP deposition. The research determined mosses to be superior to lichens for passive biomonitoring of airborne MPs. This investigation was conducted in remote areas of the Tuscan and Umbrian regions in Italy. Subsequently, leveraging the superior suitability of mosses, a comprehensive assessment of MPs at a high spatial resolution was conducted in the region of Tuscany in Italy. Building upon their effectiveness for regional-scale biomonitoring, mosses were then utilized for a broader European-scale biomonitoring effort under the framework of the MADAME project. This research highlighted the influence of population density on the abundance (count and mass) of microplastics.

Following the discovery of tire wear particles (TWPs) in moss samples collected from certain Tuscan sites, subsequent studies delved deeper into understanding and characterizing these particles. A focused investigation on TWPs was initiated, with a small-scale biomonitoring study conducted in Siena, central Italy. In this study, the leaves of *Robinia pseudoacacia* L (black locust) were utilized due to their waxy surface, which is conducive to capturing airborne particles, and their widespread presence along roadsides as a common invasive plant. Additionally, the suitability of *Robinia* as a biomonitor for MP and TWP deposition was highlighted, suggesting its potential use in environments where mosses are not readily available.

Institutions where analysis for each chapter took place

Chapter 1 – University of Siena, Department of Life Sciences, for microplastic extractions and their identification by stereomicroscope.

Chapter 2 – University of Siena, Department of Life Sciences, for microplastic extractions and their identification by stereomicroscope; Trent University (Canada) for polymer identification using μ-FTIR; Joint Nuclear Institute (Dubna, Russia) for metal analysis using ICP-OES; Istituto

Nazionale di Geofisica e Vulcanologia (Rome, Italy) for magnetic properties; Bioscience Research Center (Grosseto, Italy) for nitrogen analysis.

Chapter 3 – Trent University (Canada) for entire chapter analyses, microplastic extractions and their identification by stereomicroscope, and polymer identification using μ-FTIR.

Chapter 4 – University of Siena, Department of Life Sciences, for microplastic extractions and their identification by stereomicroscope; Istituto Nazionale di Geofisica e Vulcanologia (Rome, Italy) for magnetic properties, and Bioscience Research Center (Grosseto, Italy) for polymer identification using μ-FTIR.

Author contribution for published chapters

Chapter 1 – Jafarova, M.; Grifoni, L.; Aherne, J.; Loppi, S. Comparison of Lichens and Mosses as Biomonitors of Airborne Microplastics. *Atmosphere* 2023, 14, 1007. Author contribution: S.L. conceived the research; M.J. and L.G. performed the experiments; M.J. and S.L. analyzed the data; M.J. wrote the article; S.L. and J.A. supervised the text.

Chapter 4 – Jafarova, M., Grifoni, L., Renzi, M., Bentivoglio, T., Anselmi, S., Winkler, A., Di Lella, L.A., Spagnuolo, L., Aherne, J. and Loppi, S., 2023. *Robinia pseudoacaci*a L.(Black Locust) Leaflets as Biomonitors of Airborne Microplastics. *Biology*, *12*(12), p.1456. Author contribution: S.L. conceived the research; M.J., L.G., T.B., S.A., L.A.D.L. and L.S. performed the experiments; M.J. and L.G. analysed the data; M.J. wrote the article; S.L., A.W., M.R. and J.A. supervised the text.

Thesis Introduction

1. Microplastics in the environment – a global concern

Due to their durability and stability, plastic particles have the potential to persist in the environment for extended periods, contributing to their widespread distribution worldwide and raising significant concerns for ecosystems. Plastic particles come in various sizes, including macro-, micro-, and nanoplastics, and can originate from the abrasion of plastic products while in use (Boucher & Friot, 2017). Microplastics (MP), measuring less than 5 mm in size, have been found across all environments including aquatic (Nuelle et al., 2014; Welsh et al., 2022), atmospheric (Allen et al., 2019; Roblin et al., 2020), and terrestrial systems (de Souza Machado et al., 2018), and even in living organisms such as plants (Li et al., 2020) and animal tissues (Renzi et al., 2018), and the human body (Cobanoglu et al., 2021). Studies have demonstrated the presence of MPs in uninhabited areas, such as pristine mountain catchment (Allen et al., 2019), polar regions (Hamilton et al., 2022), and deep sea (Woodall et al. 2014). Primary microplastics are released directly into the environment, while secondary microplastics originate from degradation and fragmentation of larger plastic wastes (Boucher & Friot, 2017). Microplastics enter the environment through various pathways: for instance, wastewater treatment plants, domestic sewage, urban pollution, industry activities, and stormwater are the main sources of plastic debris to the aquatic environment (Koelmans et al., 2019); plastic waste, wind erosion, and urban dust are the main reasons for the presence of MPs in the atmosphere (Dris et al., 2017); soil amendment with compost and sewage sludge, plastic mulching, irrigation and flooding, littering, and atmospheric deposition are the sources of MPs in terrestrial environments (Bläsing & Amelung, 2018). Consequently, plastic pollution is a global issue with no boundaries in the world owing to long-distance transport in different systems.

2. Microplastics in the atmosphere

While plastic pollution in aquatic environments has been extensively documented (Nava et al., 2023), our comprehension of MP pollution in the atmosphere remains limited. Unlike MPs found in other systems, those present in the air can be directly and continuously inhaled into the human body, presenting a potential health risk (Gasperi et al., 2018; Prata, 2018). Hence, there is an urgent need for an understanding of the concentration, characteristics, sources, and risks associated with MPs in the atmosphere. Microplastics have the capability to be carried into the air and easily transported by wind due to their small size and low density, as such, atmospheric transport is likely the reason for their presence in remote, sparsely inhabited areas (Allen et al., 2019).

Microplastics originate from different sources; one of the main sources of MPs in air is synthetic textiles (Dris et al., 2016; Dris et al., 2017). More than 60 million tons of synthetic textiles were produced in 2016, with textile production increasing at a rate of approximately 6% per year (Gasperi et al., 2018). Small fibres easily tear from clothes during wearing, cleaning and drying; similarly, the grinding, chopping and cutting of synthetic textiles in industry can produce many tiny fibres (Napper & Thompson, 2016; Salvador Cesa et al., 2017). In addition to synthetic textiles, MPs in air have other possible sources: the degradation of macroplastics, waste in landfills, incineration, industrial emissions, and dust re-suspension (Dris et al., 2016; Liu et al., 2019b). Traffic serves as another significant source of airborne MPs, including particles released from traffic emissions, the resuspension of road dust (Abbasi et al., 2019; Liu et al., 2019), and abrasion from tires and roads contributing to the release of MPs (Klein & Fischer, 2019). Tire wear particles (TWPs) result from a complex physio-chemical process driven by frictional energy between the rolling shear of the tire tread and the road surface (Kreider et al., 2010). The composition of tire wear varies based on the brand and purpose of the tire, typically consisting of 40–60% rubber content (both synthetic and natural), 20–35% filler (such as carbon black and silica), and 12–15% oils (Wagner et al., 2018). The abundance and rate of TWP generation fluctuate depending on factors like vehicle load, tire inflation levels, wheel alignment, wheel position (front wheels wear preferentially), exposure to environmental elements, braking, acceleration, and high-speed driving (Kole et al., 2017; Verschoor et al., 2016).

Identification of polymers can aid in determining the sources of MPs and the studies focusing on airborne MPs, have revealed polypropylene (PP), polyethylene (PE), polystyrene (PS), and polyethylene terephthalate (PET) as the primary polymer components present in atmospheric deposition (Roblin et al., 2020; Purwiyanto et al., 2022; Capozzi et al., 2023a; Abbasi et al., 2023). Polypropylene and PET are commonly employed in the production of polyester fibres, fabrics, textiles, packaging materials, and reusable products; PE is widely utilized in materials like plastic bags; PS finds its application in packaging and manufacturing industries, valued for its thermal insulation properties (PlasticsEurope, 2019). However, very few studies have identified the polymer type of atmospheric MP deposition using biomonitors (Capozzi et al., 2023a; Jafarova et al., 2023b; Capozzi et al., 2023b).

3. Biomonitors as effective tools for airborne pollutants

Environmental biomonitoring involves utilizing bio-materials, such as plants and animals, to gather quantitative information on specific characteristics of the biosphere. It serves as a valuable complement to instrumental methods for assessing environmental quality, offering numerous advantages (Wolterbeek 2002), such as cost-effectiveness and accessibility. Traditional studies on atmospheric contamination have often faced challenges related to high costs and the complexity of conducting extensive sampling across both time and space. Therefore, the integration of biomonitors in atmospheric monitoring has proven central in addressing these limitations.

Mosses, lichens, and tree leaves have been widely employed in monitoring air pollution for many years (Loppi 1996; Loppi & Bonini 2000; Harmens et al., 2000). Mosses, the predominant plant species utilized in biomonitoring (Harmens et al., 2004; Capozzi et al., 2020), exhibit a dependency on both wet and dry atmospheric deposition for nutrients (Figure 1). This reliance, combined with the lack of a waxy cuticle and stomata, contributes to the accumulation of airborne particles on or in their tissue. Moreover, the high surface-to-volume ratio inherent in moss tissue further facilitates this accumulation. One of the key advantages of utilizing mosses in biomonitoring is their practical ease of application, wide distribution, and straightforward interpretation of results, which has led to their widespread adoption in environmental studies (Harmens et al., 2004). They have been successfully utilized in monitoring potentially toxic elements (PTEs; Loppi et al., 2021a), nitrogen (N) content (Harmens et al., 2010, 2012; Olmstead & Aherne 2019), radionuclides (Wilkins et al., 2023), and persistent organic pollutants (POPs; Holoubek et al., 2000). However, there are a limited number of studies utilizing mosses to assess airborne MP deposition (Roblin & Aherne 2020; Loppi et. al 2021b; Jafarova et al., 2023a; Capozzi et al., 2023a, 2023b); this PhD thesis aims to provide a more in-depth understanding of the efficacy of moss as a biomonitor for the assessment of atmospheric MP deposition.

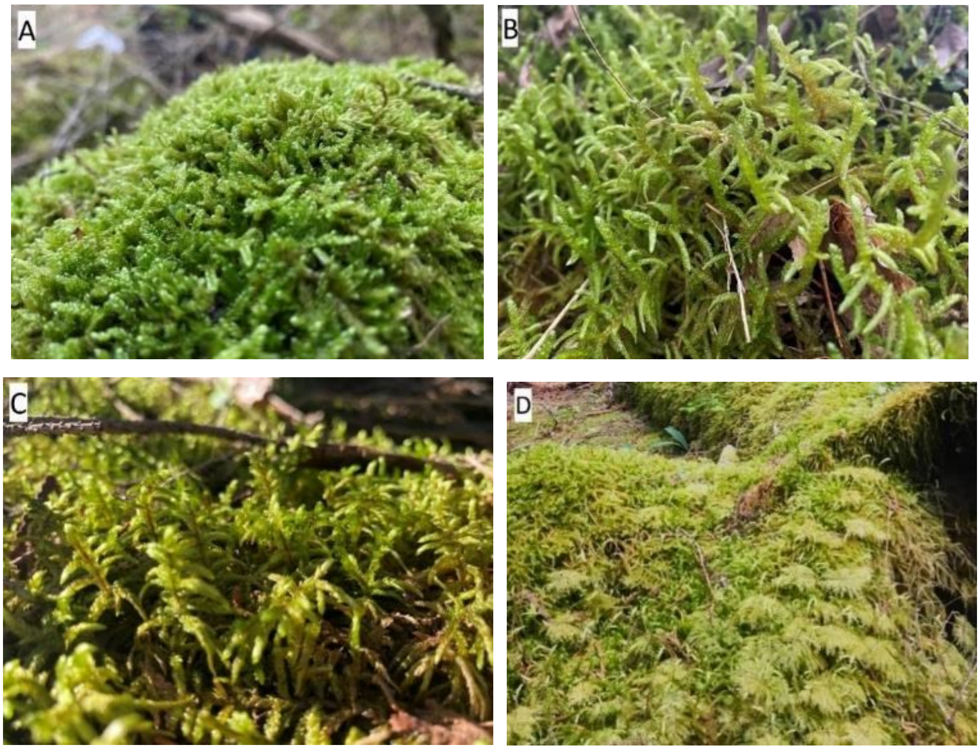

Figure 1. Pleurocarpous moss species commonly used for biomonitoring (A – *Hypnum cupressiforme*; B – *Pseudoscleropodium purum*; C – *Pleurozium schreberi*; D – *Hylocomium splendens*) in biomonitoring of air pollution.

Lichens are the product of a symbiotic relationship between a fungus and an alga, showcasing durability and longevity, by enduring harsh conditions spanning from the frigid Himalayas to arid deserts (Lalley et al. 2006; Devkota et al., 2017; Figure 2). Given their lack of roots, and therefore their reliance on atmospheric deposition for mineral nutrients, their slow growth rate and extended lifespan, lichens excel at indicating the presence and concentrations of airborne pollutants. Lichens have been widely used in monitoring of PTEs (Bačkor & Loppi, 2009; Loppi et al., 2020; Loppi et al., 2021a), N (Munzi et al., 2010), POPs (Herzig et al., 2019) and magnetic susceptibility (Winkler et al., 2020; Grifoni et al., 2024). However, very few studies have used lichens to study airborne MP deposition (Loppi et al., 2021b; Jafarova et al., 2022; Jafarova et al., 2023a; Capozzi et al., 2023b).

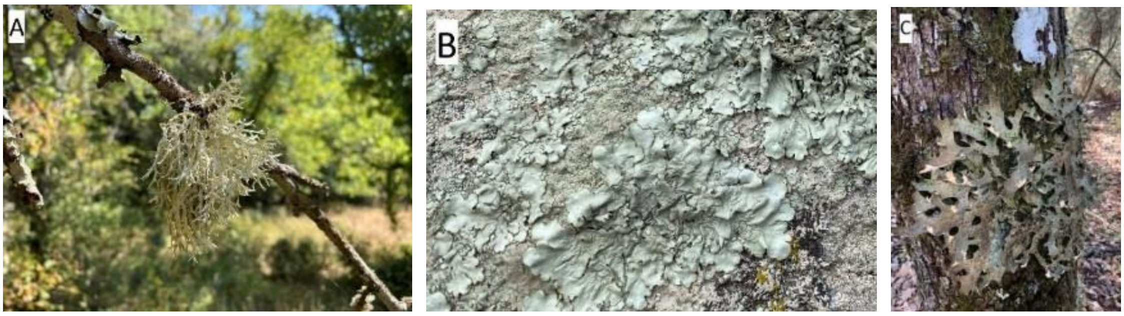

Figure 2. Lichen species commonly used for biomonitoring (A – *Evernia prunastri*; B – *Flavoparmelia caperata*; C – *Lobaria pulmonari*) in biomonitoring of air pollution.

Plant leaves possess a high capacity to intercept airborne particles and can offer insights into pollutant deposition levels. They have been utilized in various studies to monitor magnetic susceptibility (Winkler et al., 2022), and POPs (Ratola et al., 2011), as well as gaseous pollutants (Oishi, 2018; Simonich & Hites, 1995). Although some research has explored the use of leaves from higher plants for MP deposition studies (Canha et al., 2023), very few studies have employed plant leaves for assessing the deposition of MPs in the atmosphere, specifically with a focus on TWPs.

Furthermore, despite the extensive utilization of vegetation to evaluate air pollution at high spatial resolutions, there remains a notable absence of studies specifically assessing the atmospheric deposition of MPs at high spatial resolution. Additionally, no study until now has focused on the assessment of airborne MPs at both regional and European scales using plant biomonitors.

CHAPTER 1.

Comparison of Lichens and Mosses as Biomonitors of Airborne Microplastics

Summary

The atmosphere is an important pathway for microplastic (MP) transport; however, observations are limited, as traditional sampling methods are generally labor-intensive. Biological monitors (biomonitors) have been widely used as a simple alternative to determine the abundance or presence of anthropogenic pollutants. Here, we compared the effectiveness of co-located lichen and moss species as biomonitors of the atmospheric deposition of microplastics. Samples of the epiphytic lichen *Evernia prunastri* and the epigeic moss *Pseudoscleropodium purum* were collected from five remote areas of central Italy. A total of 154 MPs were found across all samples, 93.5% of which were fibres and 6.5% were fragments. The accumulation of MPs for lichens (range of 8–12 MP/g) was significantly lower than for mosses (12–17 MP/g), which might be related to their structural characteristics or habitat positions (epiphytic versus epigeic). Nonetheless, higher accumulation facilitates analytical determination and provides greater separation from the limit of detection, suggesting that mosses are preferred over lichens for studying the deposition of airborne MPs. This study further suggests that biomonitoring may be an effective tool to assess the spatial distribution of atmospheric microplastics, which is a key requirement for the development of waste mitigation policies.

1. Introduction

Microplastics (MPs, plastic pieces <5 mm) are generally produced as a consequence of photodegradation and biological, mechanical, thermo-oxidative, and hydrolytic degradation of plastic debris in the environment (Cai et al., 2017; Andrady 2011; Webb et al., 2012). Microplastics have been found globally across all environmental compartments: aquatic (Wright et al., 2013; Nerland et al., 2014; Yang et al., 2021), terrestrial (Riling 2012; de Sousca

Machado et al., 2018; He et al., 2020), and atmospheric (Dris et al., 2015; Dris et al., 2016; Allen et al., 2019), as well as in plant (Qi et al., 2018; Li et al., 2022) and animal (Li et al., 2018; Nelms et al., 2019; Carlin et al., 2020) tissue, and even in human blood (Hwang et al., 2019; Çobanoğlu et al., 2021). However, research has primarily focused on the marine environment, and studies on the presence of MPs in the atmosphere are scarce, despite the growing recognition of its importance as a transport pathway.

Microplastics in the atmosphere are mostly derived from fabric textiles [Napper et al., 2016; Henry et al 2019; De Falco et al., 2019), tire wear particles (Munyaneza et al., 2022), and the construction industry (Wang et al., 2022). MPs have been found in urban (Dris et al., 2015; Wright et al., 2020; Roblin et al., 2020; Abbasi et al., 2023) and remote areas as a consequence of long-range atmospheric transport (Allen et al., 2019; Wang et al., 2022; Roblin et al., 2020; Brahney et al., 2021; Aves et al., 2022) and also at high atmospheric elevations (González-Pleiter et al., 2021). Further, many studies have noted the dominance of plastic microfibres over fragments, especially in remote regions (Wright et al., 2020; Roblin et al., 2020; Brahney et al., 2021; Aves et al., 2022), suggesting preferential transport mechanisms.

This ubiquity of MPs in the atmosphere means that humans are potentially at risk. Humans can be exposed to MPs through inhalation, ingestion, and dermal contact, with potential consequences such as lung inflammation, immune and metabolic issues, DNA damage, and oxidative stress, causing issues in the respiratory, digestive, immune, reproductive, and nervous systems (Warheit et al., 2001; Prata, 2018; Chen et al., 2020; Yao et al., 2022; Sangkham et al., 2022). All of these health risks show the urgency of increasing our knowledge about the level of airborne MPs.

Lichens and mosses are well-established biomonitors of atmospheric pollution by potentially toxic elements (PTEs), nitrogen, persistent organic pollutants, and radionuclides, as they are cost-effective and are a simple alternative to instrument deposition monitoring (Bargagli et al., 2002; Loppi et al., 2003). Moreover, they possess a high capacity to effectively capture pollutants from the atmosphere, due to their structural (physical) characteristics. However, only a very few studies have used lichens (Loppi et al., 2021; Jafarova et al., 2022) and mosses (Roblin & Aherne 2020; Jafarova et l., 2023a; Bertrim & Aherne 2023; Capozzi et al., 2023a;

Capozzi et al., 2023b) to assess the deposition of airborne MPs. The aim of this study was to evaluate if co-located lichens and mosses have the same ability to accumulate MPs, specifically focusing on the epiphytic (tree-inhabiting) lichen *Evernia prunastri* (L.) Ach. and the epigeic (soil-inhabiting) pleurocarpous moss *Pseudoscleropodium purum* (Hedw.) M. Fleisch. Given that our aim was to compare the effectiveness of lichen and moss rather than the polymeric composition of atmospheric deposition, we focused our analysis on microplastic count only. To the best of our knowledge, this is the first study comparing the suitability of mosses and lichens for the assessment of the atmospheric deposition of anthropogenic MPs.

2. Materials and methods

2.1. Study area

The study was carried out at five remote sites in central Italy, three in Tuscany and two in Umbria (Figure 1). Site selection focused on remote areas (i.e., distance from urban centres) that received similar levels of regional background pollutant deposition. All study sites were based on prior studies that used similar background areas remote from local sources of pollution (Vannini et al., 2020). The elevation of the sites ranged from 440–1140 m asl. The climate was Mediterranean, with mild winters and warm, dry summers; mean annual temperature was 13–14°C, and annual rainfall was 600–1000 mm (Pavan et al., 2019).

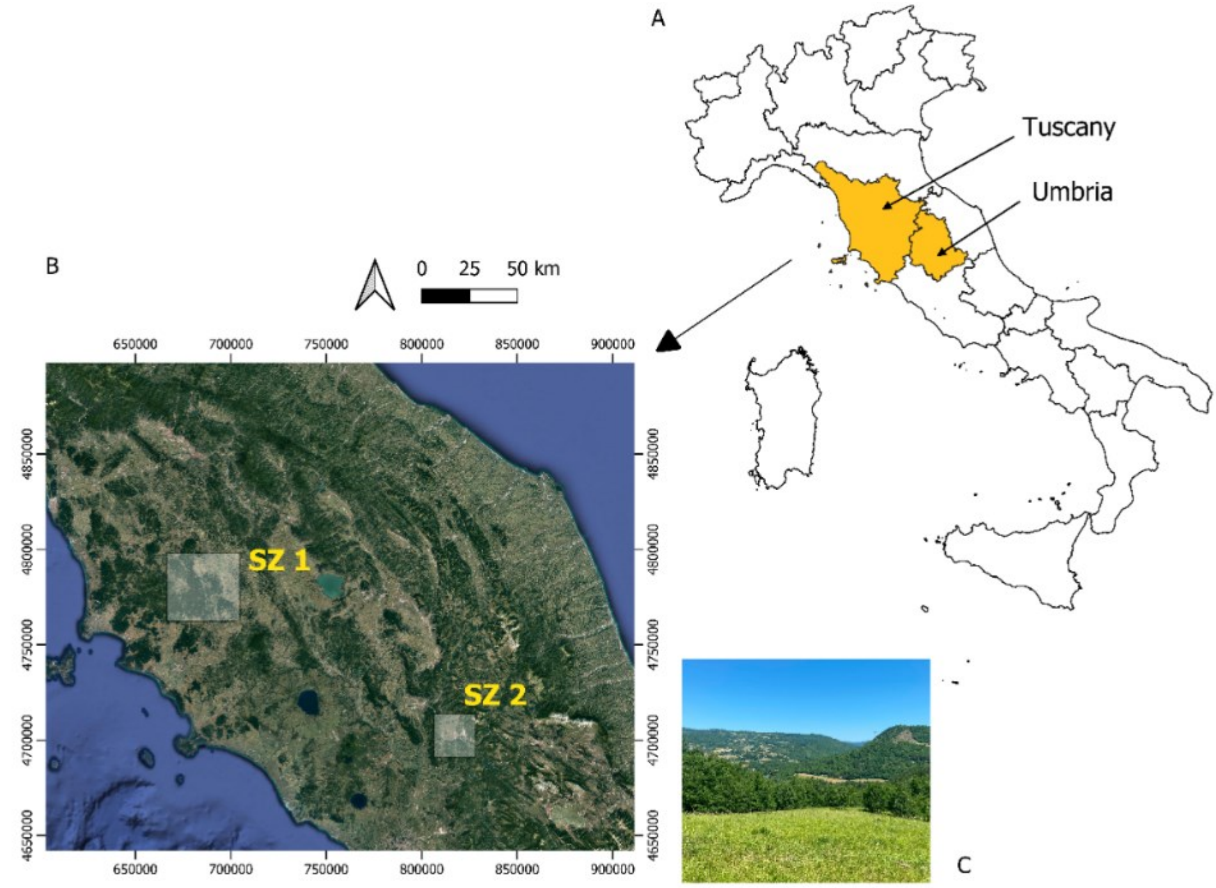

Figure 1. Study area in central Italy showing the (A) map of Italy with the Tuscan and Umbria regions highlighted; (B) sampling zones in Tuscany—SZ1, with three sampling sites (N 43.64353° E 011.97958°; N 43.34555° E 11.15181°; N 43.337333° E 11.171333°), and in Umbria—SZ2, with two sampling sites (N 42.4844759° E 12.707810°; N 42.4846855° E 12.707860°); and (C) an image of a sampling site.

2.2. Sample collection

The epiphytic lichen *Evernia prunastri* and the epigeic moss *Pseudoscleropodium purum* (Figure 2) were selected as study species, as they have been widely used in biomonitoring studies to assess the atmospheric deposition of airborne pollutants (Jafarova et al., 2022; Ares et al., 2011). They are widely distributed, easily identified, and were both present at all five study sites. From June–August 2022, lichen thalli were randomly collected from tree (*Quercus cerris*) branches as composites from a minimum of three trees, and moss samples were randomly collected in nearby open areas (50 m by 50 m), at least 3 m away from tree canopies, as composites (5–10 g) from a minimum of five points. All samples were placed into paper bags, which were tightly closed to avoid contamination, and stored in a dry place until analysis. Five 0.25 g subsamples from the individual lichen and moss composite samples at each study site were subsequently analyzed for microplastics.

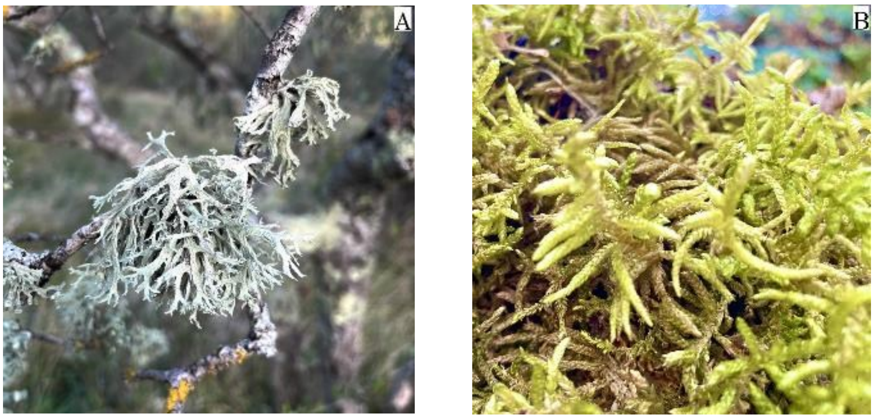

Figure 2. Epiphytic lichen species *Evernia prunastri* (L.) Ach. (A) and epigeic pleurocarpous moss species *Pseudoscleropodium purum* (Hedw.) M. Fleisch (B).

2.3. Microplastic analysis

In the laboratory, air-dried (residual water <10%) whole lichen thalli and the green parts of moss samples (roughly corresponding to the last 2–3 years of growth) were digested (0.25 g per site in quintuplicate) using the wet peroxide oxidation method (Roblin & Aherne 2020; Loppi et al., 2021). Samples were then vacuum-filtered onto cellulose filter papers (Whatman Grade 1, Maidstone, UK, 1001-090, 11 µm) and placed into glass petri dishes for storage. The filter papers were examined for MPs under a stereomicroscope (Eurotek OXTL101TUSB, Eurotek, Inc, Eatontown, NJ, USA) equipped with a digital camera (MDCE-5C, NINGBO YONGXIN OPTICS CO., LTD. Ningbo, China), following a five-criteria method (Roblin & Aherne 2020; Loppi et al., 2021; Jafarova et al., 2022). Microplastics were further verified using a hot needle test; if a particle melted or curled under the presence of a hot needle, it was counted as a microplastic. All MPs were classified (fibre or fragment) and measured using the open-source image-processing software ImageJ. In general, visual analysis was limited to particles >50 µm. Overall, 50 subsamples (25 lichens and 25 mosses; two species each at five sites, with five replicates) were analyzed.

Strict quality-control procedures were followed to ensure that contamination was minimized during sampling and analysis. Laboratory contamination was routinely controlled using analytical process blanks. All solutions were vacuum-filtered prior to use in the extraction process. All laboratory glassware used during digesting and filtering were covered with aluminum foil to prevent airborne contamination, and all glassware were rinsed in triplicate with filtered, deionized water. Surfaces were wiped down with paper towels and deionized water between the digestion of each sample. Digestion (process) blanks were vacuum-filtered using filtered, deionized water in place of sample media and analyzed for microplastic contamination. Finally, cotton clothing was worn during the collection of the moss and lichen samples and the laboratory extraction of microplastics.

2.4. Statistical analysis

A linear mixed-effect model (LMEM) was fitted to check the differences between moss and lichen samples, with the type of biomonitor (i.e., lichen or moss) as the fixed factor and the site as the random factor. The significance of the LMEM ($p < 0.05$) was checked with the analysis of deviance (type II Wald chi-square). For model validation, scatterplots of the residual and fitted values were used to check for homoscedasticity (Figure 1A), and the Shapiro–Wilk test was used to check for data normality. All calculations were run with the R software (R Core Team 2022).

3. Results

Overall, a total of 154 MPs were found across all subsamples (n = 50) from the five study sites; 62 MPs were found in lichens, and 92 MPs were found in mosses. The vast majority (93.5%) of MPs were classified as fibres, and only 6.5% were classified as fragments (Figure 2A). In mosses, the MP proportion consisted of 90% fibres and 10% fragments, while in lichens, it was 98% fibres and 2% fragments (Figure 3).

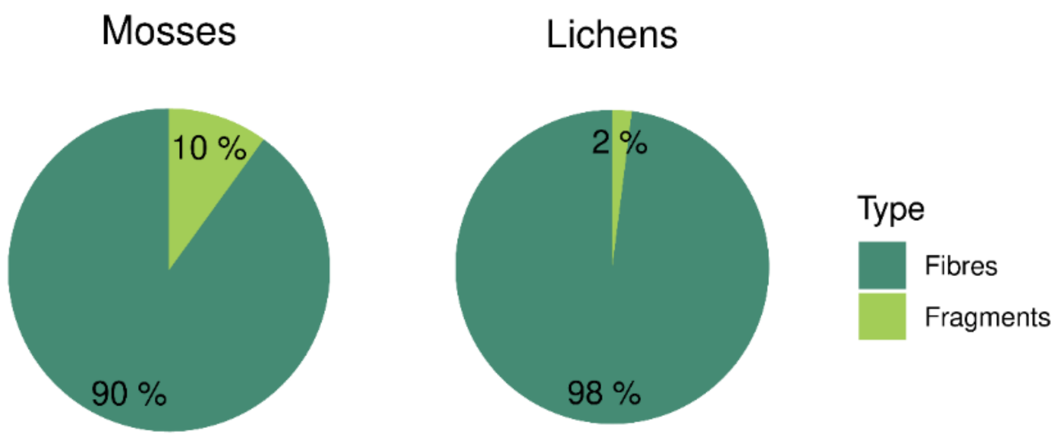

Figure 3. Proportions of fibres and fragments in moss and lichen samples from the five study sites.

Across all of the five study sites, the mean MP concentrations (per gram of dry mass) ranged from 7.9–12.5 in lichens and 12.5–21.2 in mosses; fibres ranged from 7.9–11.7 in lichens and 9.5–19.6 in mosses; fragments ranged from 0–0.8 in lichens and 0–4.0 in mosses. The mean concentration of MPs across all sites was ~50% higher for moss (14.5 MP/g), compared to lichen (9.7 MP/g). Further variability (relative standard deviation) between sites was higher for moss (26%), compared with lichen (18%), suggesting that lichens had a lower capacity to trap MPs (Table 1). Mean MP concentrations at the Tuscan sites (lichen: 8.7 MP/g, moss: 12.6 MP/g; sites 1–3) were slightly lower than Umbria (lichen: 11.4 MP/g, moss: 17.4 MP/g; sites 4 and 5); however, there was no significant difference among the sites.

Table 1. Mean (±standard error) number of microplastics (MPs), fibres, and fragments accumulated (per gram of dry matter) by the fruticose lichen *Evernia prunastri* (L) and the moss *Pseudoscleropodium purum* (M), along with fibre lengths (μm) at five remote sites of Central Italy (Tuscany 1–3; Umbria 4 and 5). MP concentrations were significantly higher in moss ($p < 0.05$).

Site	Microplastics		Fibres		Fragments		Fibre Length (μm)	
	L	M	L	M	L	M	L	M
1	8.6 ± 3.8	12.5 ± 5.6	8.6 ± 3.8	12.5 ± 5.6	0 ± 0	0 ± 0	2286 ± 660	1822 ± 470
2	7.9 ± 3.5	12.7 ± 5.6	7.9 ± 3.5	11.8 ± 5.3	0 ± 0	0.8 ± 0.4	1129 ± 357	469 ± 130
3	9.5 ± 4.2	12.6 ± 5.6	9.5 ± 4.2	11.8 ± 5.3	0 ± 0	0.8 ± 0.4	2844 ± 821	1272 ± 446
4	12.5 ± 5.6	13.5 ± 6.1	11.7 ± 5.2	9.5 ± 4.2	0.8 ± 0.4	4.0 ± 1.8	2157 ± 557	2033 ± 564
5	10.2 ± 4.6	21.2 ± 9.5	10.2 ± 4.6	19.6 ± 8.8	0 ± 0	1.6 ± 0.7	2025 ± 569	1631 ± 340

The fibre length distributions ranged from 147–4461 μm in lichens and 139–4075 μm in mosses (Table 1; Figure 4). The fragment length distributions ranged from 500–653 μm in lichens and 292–592 μm in mosses (Figure 5).

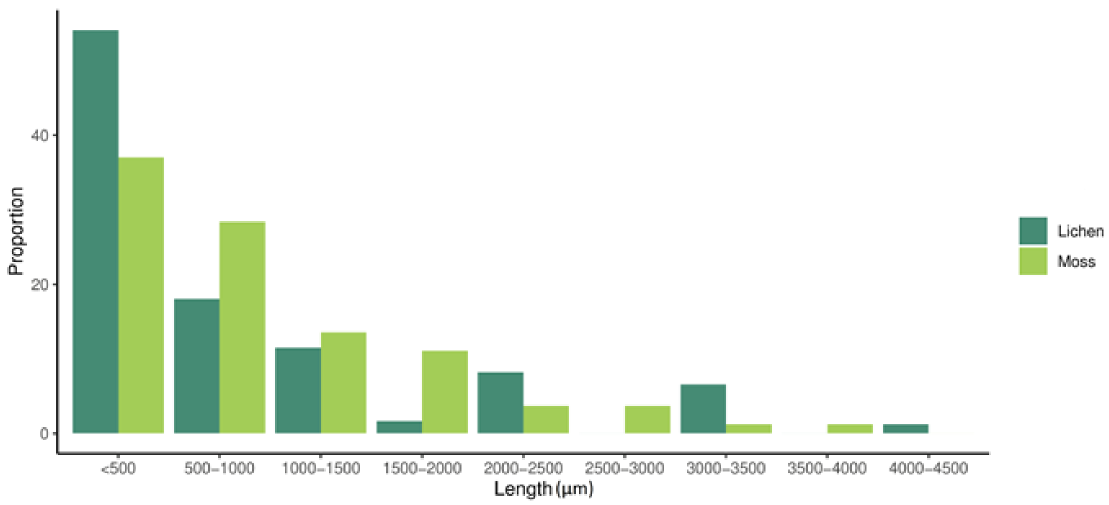

Figure 4. Frequency distributions of microfibre lengths in lichen and moss (n = 144).

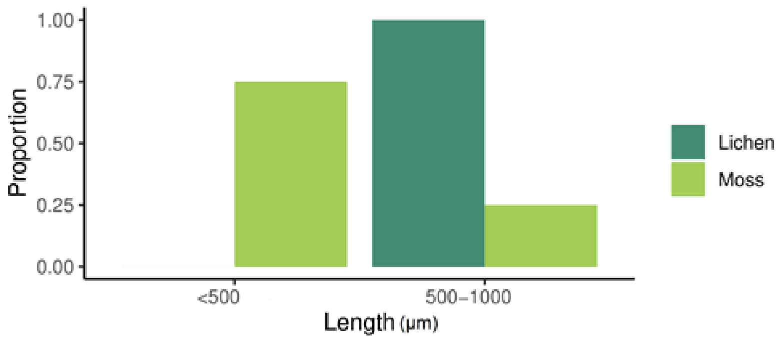

Figure 5. Frequency distributions of fragment lengths in lichen and moss (n = 10).

All of the above features (concentrations of MPs, fibres, and fragments and fragment lengths) were significantly different ($p < 0.05$) between lichen and moss (higher in moss, except for fragment length), with the only exception being fibre length.

4. Discussion

Our results suggested that the moss *P. purum* accumulated a higher number of airborne MPs than the lichen *E. prunastri*. There are several possible explanations for this outcome, including the limitations in our study design. First of all, the study lichen was epiphytic, meaning that it was, at least partially, sheltered by the tree canopy from spring to autumn (however, the tree canopy could increase the scavenging of atmospheric MPs (McCune et al., 1992), potentially leading to higher concentrations in lichens), while the moss was epigeic and was collected in open areas at least 3 m from the nearest tree canopy. Second, the structural characteristics of the two cryptogams might play an important role. The pleurocarpous branched stems with leaflets, the wider growth of mosses on the substrate, and its higher surface-to-mass ratio might make it more efficient at intercepting and retaining airborne MPs.

A study comparing the capacity of bioaccumulation of PTEs in epigeic mosses and epiphytic lichens showed higher concentrations of elements (subjected to long-range atmospheric transport) in moss (Adamo et al., 2008). Similar studies showed higher contents of PTEs and polycyclic aromatic hydrocarbons in mosses (Giordano et al., 2005; Vingiani et al., 2015).

Adamo et al. (2007) emphasized the role of the higher surface area of a moss species, compared to a lichen species, to explain the higher concentrations of trace elements in mosses. Nevertheless, despite these differences in the ability to accumulate airborne pollutants, all these studies clearly showed that both mosses and lichens can be used to monitor atmospheric deposition. Our study fell within this case: irrespective of absolute values, both organisms clearly indicated that the remote areas investigated were subjected to the deposition of atmospheric MPs.

Another important parameter to consider is the age of the biomonitor. Although the growth rate can be influenced by a wide array of factors, including species-specific differences, which we have not assessed experimentally, we can assume that the lichen was older than the analyzed moss portion. However, this should imply a higher accumulation, since longer and slower growth means longer exposure to wind, rainfall, biomass loss, MPs, etc. In order to overcome this problem, we suggest the use of lichen and moss bags, i.e., samples taken from a remote/background site and transplanted to the study area, referring the final values to the starting conditions (Loppi et al., 2019).

Only a few studies have used lichens and mosses to evaluate the deposition of atmospheric microplastics. A study at a landfill dumping site in central Italy showed an average accumulation of 79 MP/g in the foliose epiphytic lichen *Flavoparmelia caperata* (L.) Hale. However, at greater distances from the landfill (i.e., at remote sites), the concentration dropped to 7–13 MP/g (Loppi et al., 2021). In a remote area of northern Italy, lichen bags of *E. prunastri* exposed for 3 months showed 12–24 MP/g (Jafarova et al., 2022). Roblin & Aherne (2020) found 15–30 MP/g in moss collected from remote areas of Ireland, while Bertrim & Aherne (2023) reported an average of 3 MP/g in moss from a remote area of Ontario, Canada. Capozzi et al. (2023a) found an average of 53–87 MPs in moss samples collected at rural sites in southern Italy, close to the city of Naples. Our results were largely consistent with or at the lower end of the range for studies in background regions in Europe (Loppi et al., 2021; Roblin & Aherne, 2020; Jafarova et al., 2022), with lichen concentrations at 7.9–12.5 MP/g and moss at 12.5–21.2 MP/g (Table 1).

Our results were further consistent with all other similar studies (Loppi et al., 2021; Roblin &

Aherne, 2020; Jafarova et al., 2022; Bertrim & Aherne, 2023; Capozzi et al., 2023a) as fibres dominated over fragments. This was likely caused by the differences in the settling velocity of MP types, due to their weight, with fibres having a low mass-to-surface ratio and, consequently, being more easily transported over longer distances or more easily attached and retained by biomonitors, compared to fragments. This outcome was further supported by investigations using methods other than biomonitors, which yielded comparable results. For example, Ding et al. (2022) demonstrated that 88–100% of the MPs in the atmosphere over the northwestern Pacific Ocean were fibres; the study took place on a cruise ship, with an offshore distance of 400 km. Another study that investigated MPs in Antarctic snow found that 60% were fibres (Aves et al., 2022). The use of synthetic textiles was shown to be a major contributor to airborne microfibres (MFs), which were among the dominant types of MPs found at or close to inhabited regions, as shown for Ross Island (Antarctica), where the research facility and its field equipment constituted the primary origin of MFs (Aves et al., 2022).

According to our findings, MFs with a length < 500 µm were prevalent in both lichens and mosses, emphasizing their potential as indicators of long-distance MF transport. As MFs decrease in size, they become and remain airborne more easily, and thus, they are more likely to be transported over greater distances. This suggests that shorter MFs may be widespread in remote regions.

5. Conclusions

Our study shows that both lichen and moss species are effective biomonitors of the deposition of airborne microplastics. Our results further suggest that epigeic mosses may serve as a more effective indicator of atmospheric microplastic contamination, compared with epiphytic lichens, due to their more favorable structure and their open habitat position. However, we note our caution in identifying an optimum species for biomonitoring of atmospheric MPs, given the limitations of comparing an epiphytic lichen to an epigeic moss. To shed further light on the effectiveness of using lichens and mosses, we recommend that future research compare a parallel exposure of transplants of both organisms.

APPENDIX – CHAPTER 1

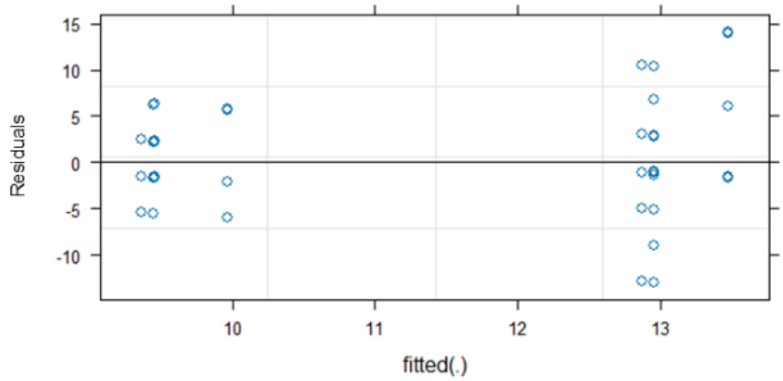

Figure 1A. Linear mixed-effect model plot performed in R (lme4 package).

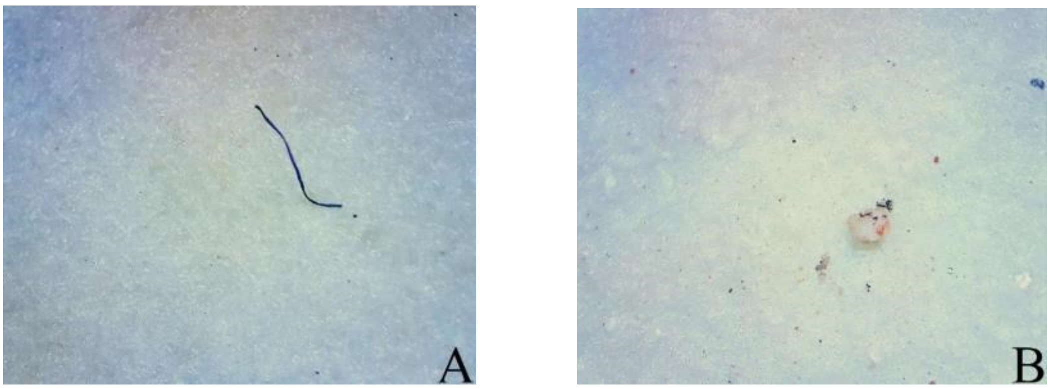

Figure 2A. Example images of the extracted microplastics from lichen and moss samples (A – blue microfibre, L = 1147 µm; B – fragment, L = 379 µm; L – length).

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Communication

Comparison of Lichens and Mosses as Biomonitors of Airborne Microplastics

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***All the authors have agreed for the published article to be used as a chapter of this PhD thesis.**

CHAPTER 2.

Atmospheric deposition of microplastics in Tuscany (C Italy) using moss biomonitoring

Summary

Biomonitors (biological or plant monitors) have been extensively utilized as straightforward alternatives for assessing anthropogenic air pollution. Here, we investigated passive biomonitoring with pleurocarpous moss species at a high spatial resolution to assess airborne microplastics (MPs) on a regional scale. Moss samples were collected from 33 randomly selected remote sites across the region of Tuscany, central Italy. A total of 322 MPs were found across all sites, 82% of which were fibres, 12.1% foams, and 3.1% tire wear particles. Given the dominance of fibres, polyethylene terephthalate (PET) was the dominant polymer type at 39%. The accumulation of MPs ranged from 2.2 to 12.9 MP/g dw (average 5.9 MP/g dw) with a coefficient of variation of 51% across all sites. The population within a 10-km buffer and nitrogen deposition were correlated with airborne MP, suggesting that MP concentration was influenced by regional-scale anthropogenic factors rather than direct local sources. This study suggests that passive moss biomonitoring can be effectively used to provide high spatial resolution assessments of MP deposition on a regional scale.

1. Introduction

Microplastics (MPs) are small plastic particles less than 5 mm in size and are primarily the result of photodegradation, biodegradation, and mechanical breakdown of plastic debris in the environment due to poor waste management. Microplastics are a worldwide concern due to their potential ecological impacts on ecosystems as they have been found in animal (Qu et al., 2018) and plant (Khalid et al., 2020) tissues, and even in human blood lymphocytes (Çobanoğlu et al., 2021). In the environment, MPs can be found in different shapes, such as fibres, fragments, foams, pellets, beads, and tire wear particles (TWPs), which is an indicator of their source.

Microplastic contamination has been observed across all environments globally, including aquatic (Rodrigues et al., 2018; Scherer et al., 2020; Nava et al., 2023), terrestrial (Möller et al., 2020) and atmospheric systems (Dris et al., 2015; Welsh et al., 2022; Loppi et al., 2021b). One of the primary contributors to the global spread of MPs is atmospheric transport, which facilitates their dispersion over regional distances, thereby enabling MPs to travel far from the source of their initial emission via the atmosphere (Allen et al., 2019; Roblin et al., 2020). Nonetheless, there are relatively few observations of atmospheric MPs, as traditional sampling networks require specialized infrastructure and are generally labor-intensive, leading to spatially limited observations.

Measurement of air pollutants via plants, i.e., biomonitoring, is an economic, convenient, and reliable method compared to traditional approaches. Biomonitors have been effectively used to investigate the atmospheric deposition of pollutants such as the concentrations of potentially toxic elements (PTEs; Loppi et al., 2021a), nitrogen (N) content (Harmens et al., 2011; Olmstead & Aherne 2019), magnetic properties (Winkler et al., 2022; Grifoni et al., 2024), radionuclides (Wilkins et al., 2023), and recently it has been shown that they can be utilized for airborne MP deposition (Jafarova et al., 2022, Jafarova et al., 2023a, 2023b). Since 2001, the International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops, known as ICP Vegetation, has coordinated the European moss biomonitoring survey, which is conducted every five years to assess the atmospheric deposition of heavy metals, N, and persistent organic pollutants (Harmens et al., 2004; Harmens et al., 2008). The Programme has established a dense biomonitoring network across Europe providing high spatial resolution measurements of airborne pollutants.

Moss biomonitoring has been used in a few studies to investigate the atmospheric deposition of MPs (Roblin & Aherne, 2020; Jafarova et al., 2023a; Capozzi et al., 2023a; Capozzi et al., 2023b; Bertrin & Aherne, 2023). However, relatively few studies have used passive moss biomonitoring for MPs (Roblin & Aherne, 2020; Jafarova et al., 2023a; Capozzi et al., 2023a) and to the best of our knowledge, no study has focused on a high spatial resolution assessment of MP deposition using moss biomonitoring.

The aim of this study was to carry out a high spatial resolution assessment of airborne MPs in Tuscany, central Italy, using passive moss biomonitoring from 33 background sites.

Microplastic source identification used PTE concentrations and N content in moss tissue, alongside magnetic properties, sample vitality, and environmental site predictors (including population density, building numbers, landcover, altitude, and distance to main roads) to understand the main drivers of MP accumulation in moss tissue. To our understanding, this is the first study to carry out a high spatial resolution assessment of airborne MPs through passive moss biomonitoring on a regional scale.

2. Materials and methods

2.1 Study area

The study took place at 33 randomly selected remote sites in Tuscany, central Italy; site selection criteria followed the well-established ICP vegetation moss survey protocol (ICP Vegetation, 2020) with all sampling sites being far from direct sources of air pollution (Figure 1). Within a geographic information system (QGIS), study sites were randomly selected that met the following criteria: at least 300 m from main roads, villages and industries (including farming) and at least 100 m away from smaller roads and houses using Corine Land Cover 2000 classes. The altitude of the sampling sites ranged from 46–1079 m above sea level (average = 454 m; Table 1A). Tuscany is mainly covered by agricultural (49%) and forested (46%) areas; in this study, all moss sampling sites were located in forest and semi-natural areas (Figure 1). The climate of the study region is Mediterranean, with mild winters and warm, dry summers; mean annual temperature is 13–14°C and annual rainfall is 600–1000 mm (Pavan et al 2019).

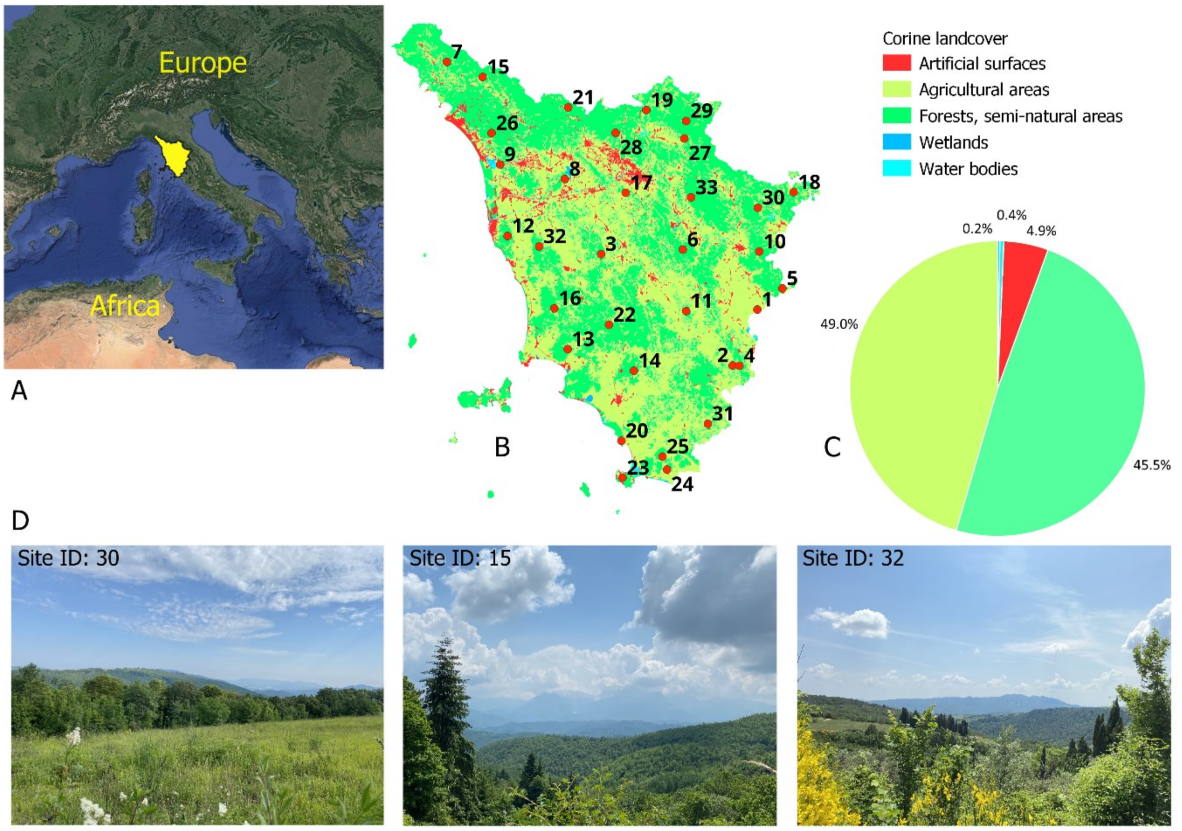


Figure 1. Map showing the study area and landcover around Tuscany, central Italy (A – Map of Italy with Tuscany highlighted; B – 33 moss sampling sites across the study area underlain by the Corine landcover classes; C – pie chart showing the landcover proportion with 49.0% agricultural areas, 45.5% forest and semi-natural areas, 4.9% artificial surfaces, 0.4% water bodies, and 0.2% wetlands; D – example images of the sampling sites 15, 30 and 32).

2.2 Experimental design

Eight moss species were collected during April–June 2021 from the 33 sampling sites in Tuscany, central Italy: *Hypnum cupressiforme*, *Pseudoscleropodium purum*, *Homalothecium sericeum*, *Eurynchium striatulum*, *Eurynchium striatum*, *Brachitecium rutabulum*, *Isothecium alopecuroides and Hylocomium splendens* (Figure 2). At the majority of the sites (64%), *Hypnum cupressiforme* was preferentially sampled; at each site, five replicates were collected from a plot of 50 m × 50 m with a distance of 5 m to the nearest projected tree canopy. The samples were put into paper lunch bags, closed tightly, and stored in a freezer until further analyses. In total, 165 moss samples (33 sites × 5 replicates) were analysed for atmospheric MPs, PTE concentrations, magnetic properties, N content, and sample vitality. All moss samples were pleurocarpous species, nonetheless, there may be interspecies differences that were not assessed in the study.

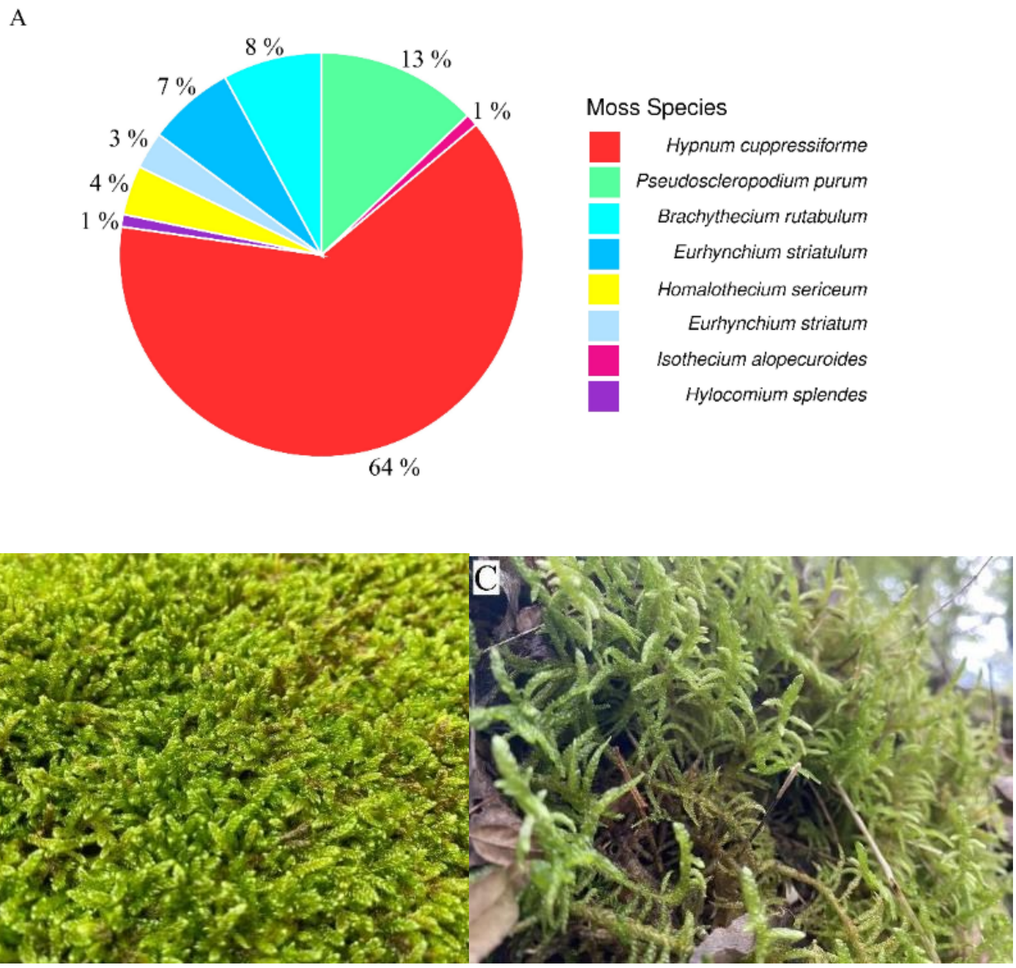


Figure 2. Pie chart showing moss species proportions (A – *Hypnum cuppressiforme* (64%), *Pseudoscleropodium purum* (13%), *Brachythecium rutabulum* (8%), *Eurhynchium striatulum* (7%), *Homalethecium sericeum* (4%), *Eurhynchium striatulum* (3%), *Isothecium aloppecuroides* (1%), and *Hylocomium splendens* (1%)) collected from 33 sites in Tuscany, central Italy, and images of the two most dominant moss species (B – *H. cuppressiforme*, and C – *P. purum*).

2.2 Microplastic extraction

In the laboratory, air-dried (residual water < 10%) green parts of moss samples (roughly corresponding to the last 2–3 years of growth) were digested (0.25 g per site in quintuplicate) using the wet peroxide oxidation method (Roblin & Aherne, 2020; Loppi et al., 2021; Jafarova et al., 2022, 2023a). Samples were then vacuum filtered onto cellulose filter papers (Whatman Grade 1, 1001-090, 11 µm) and placed into glass Petri dishes for storage. The filter papers were examined for MPs under a stereomicroscope (Eurotek OXTL101TUSB) equipped with a digital camera (MDCE-5C) using a five-criteria method. Microplastic particles were further verified using a hot needle test; if a particle melted under the presence of a hot needle, it was counted as a MP. All MPs were classified by shape (fibre, fragment, foam, pellet, and TWP) and measured using the open-source image processing software ImageJ 1.53t. In general, visual analysis was limited to particles > 50 µm. The five-criteria method included: a) unnatural colour; b) material homogeneity; c) particle resiliency; d) reflective surfaces; and e) limited fraying (Jafarova et al., 2022; Jafarova et al., 2023a; 2023b). It is difficult to identify TWPs due to their lack of reaction to a hot needle. Hence, they were classified based on distinct characteristics such as their a) black colour; b) elongated or cylindrical structure; c) rough texture; and d) flexible, rubbery feel when handled (Bertrim & Aherne, 2023; Jafarova et al., 2023b). To help identify transparent MPs that are hard to determine under normal bright light conditions, a blue light (NightSea SFA 540 nm) was used (Maes et al., 2017).

2.4 Quality control

Strict quality-control procedures were followed to ensure that contamination was minimized during the moss sampling and analyses. All solutions were vacuum filtered prior to use in the MP extraction process. Further, all laboratory glassware was covered with aluminium foil to prevent airborne contamination and was rinsed with filtered deionised water before use. Surfaces were wiped down with paper towels and filtered deionised water between the digestion of each sample. Process blanks, i.e., analytical samples without moss, followed the same extraction and identification procedure to assess potential MP contamination. In addition, a sub-set of blanks was spiked with a known number of red polyethylene beads, sized between 212–250 µm, to determine recovery. The limit of detection (LOD) was estimated as the mean MP count for the process blanks plus three times their standard deviation. However, no MPs were detected in the blanks, therefore the LOD was set to 0; further, samples were not blank corrected. Finally, cotton clothing was worn during the collection of moss and the extraction and identification of MPs.

2.5 Polymer characterisation

The polymer type of extracted MPs was identified using μ-FT-IR (LUMOS II, Brucker Scientific LLC, Germany) with attenuated total reflectance (ATR) mode. Glass slides with double-sided tape were prepared, and MPs obtained during the extraction process were adhered to the tape with their corresponding ID. The glass slides were fixed to the FTIR stage, and a spectrum (with a spectral range of 400 cm^{-1} to 4000 cm^{-1} and 32 scans) was captured for each MP particle using the LUMOS II OPUS (version 8.7.31) spectroscopy software. The ATR crystal was cleaned prior to the identification of each individual MP with an isopropyl-dipped tissue in order not to contaminate the MPs between scans. A minimum of three analyses were registered on different zones of each MP particle with the background measured prior to each run. Lastly, the spectra were analysed for polymer type using the open-source software Open Specy (openanalysis.org/openspecy; Cowger et al., 2021). The MP identification rate, verified through FTIR analysis, ranged from 95 to 100% indicating that the combination of the hot needle test, blue light examination, and the established criteria were sufficient for identifying MPs.

2.6 Metal concentration, magnetic analysis, and nitrogen content

Moss samples were cleaned of debris and carefully washed for a few seconds with 100 mL of deionized water to clean possible soil contamination. The washed samples were dried to a constant weight at 105°C, homogenised using a planetary mill Pulverisette 6 (Fritsch, Germany) and analysed for 14 PTEs, namely Fe, Al, Ni, V, Cr, Cu, Co, Sr, Cd, Pb, Zn, Ba, Mn and S using Inductively coupled plasma-Optical Emission Spectrometry (ICP-OES). Sample preparation for analysis was performed in Mars 6 microwave digestion system (CEM, USA) according to the procedure described in detail in (Zinicovscaia et al., 2021). Metal concentrations were determined using an ICP-OES PlasmaQuant 9000 Elite (Analytik Jena, Germany). To provide the quality control of the results the Oriental Basma Tobacco Leaves (INCT-OBTL-5) reference material was used. The recovery of the elements from the reference material ranged from 96% to 110%.

For the magnetic analysis, dry moss samples were placed into standard 8 cm^3 paleomagnetic plastic cubes for magnetic susceptibility. Mass magnetic susceptibility (χ, m^3 kg^{-1}) was calculated by dividing the values measured with a Agico KLY5 meter for the net weight of the samples. Further, pulverized moss tissue samples (0.5 g) were analyzed for percent N using a CNS analyzer.

2.8 Sample vitality

To evaluate the vitality of the mosses, their physiological parameters (total chlorophyll content and chlorophyll a fluorescence) and cell membrane integrity were examined. Five measurements were taken for each replicate sample, randomly selecting the moss samples to measure. The total chlorophyll content index (CCI), expressed as chlorophyll content per square metre of biological material (mg/m^2), was evaluated using a chlorophyll content metre (CCM-300, Opti-Science, Hudson, USA).

Chlorophyll a fluorescence was assessed using the widely used indicator of photosynthetic efficiency F_v/F_m, which indicates the maximum quantum efficiency of Photosystem II (PS II) photochemistry, and the performance index (PI), an overall indicator of Photosystem I (PS I) and PS II functionality. Prior to the analysis, mosses were dark-adapted for 15 min. The analysis was carried out by flashing samples with a saturating (3000 $\mu mol/m^2/s^1$) red light (650 nm) pulse for one second, using a plant efficiency analyzer (Handy PEA, Hansatech Ltd., Norfolk, UK).

The integrity of cell membranes in the moss samples was assessed through a step-by-step stress evaluation process (Marques et al., 2005; Vannini et al., 2017). Initially, moss samples weighing 100 mg were immersed in 50 mL of deionized water and agitated for 1 hour, after which the electrical conductivity (EC) of the resulting solution was measured. Then the samples were heat-stressed at 90–100°C after which EC was measured again. The results were expressed as % between the EC of the samples agitated for 1 hour and heat-stressed samples.

2.6 Statistical analysis

The normality of variables was checked using the Shapiro Wilk test ($p < 0.05$). Factor analysis was used to help identify the major sources of PTEs (Figure 3A and 4A). The Coefficient of Variation (CV%) in MP counts between all five replicates at each site was calculated as the standard deviation of the five replicates divided by their average (×100). The correlation between MP and fibre concentrations, and environmental predictors (population (pop.) in 5 km and 10 km buffers; building number, landcover and distance to main roads in a 500 m buffer; altitude (m), N (%) and PTE concentration (Cd, Cu, Pb, S, Zn, mg/kg); magnetic susceptibility (χ, 10^{-8} m^3 kg^{-1}); Factor 1 and Factor 2; F_v/F_m, CCI, (mg/m^2), and EC (%)) was assessed using the Spearman rank correlation. The influence of environmental variables on MPs and fibres accumulated in moss was further assessed using Redundancy Analysis (RDA); a requirement for RDA is that variables should be normally distributed, therefore a box-cox transformation was applied to all data. Redundancy Analysis evaluates the relationship between response (MP and fibre concentrations) and explanatory (environmental predictors, n = 15) variables.

Maps of the spatial distribution of MP and fibre concentration, population within 10 km buffer, N deposition, CV of MP replicates, and Factor 1 and 2 were prepared using QGIS 3.28.5. All statistical analysis was carried out using R 4.3.1 (R Core Team 2023) and Past 4.15 (Hammer et al., 2001).

3. Results

In total, 322 MP particles were found from the 33 remote sampling sites. Among all the MPs, fibres were the dominant shape, with a proportion of 82%, while foams, TWPs, pellets, and fragments had the proportions of 12.1%, 3.1%, 1.9% and 0.9, respectively (Figure 3). Similar to other studies (Athey et al., 2020), fibre colour was dominated by purple and blue at 57%

(see Figure 1A).

The dominant polymer type was polyethylene terephthalate (PET), making up 39% of the total. Further, polyacrylate (PAE) accounted for 13%, while polyethylene (PE), polyamide (PA), polymethyl methacrylate (PMMA), polystyrene (PS), polyvinyl formal (PVA), and TWP, each made up 6%, with the remaining 3% attributed to polymethyl pentene (PMP; Figure 3).

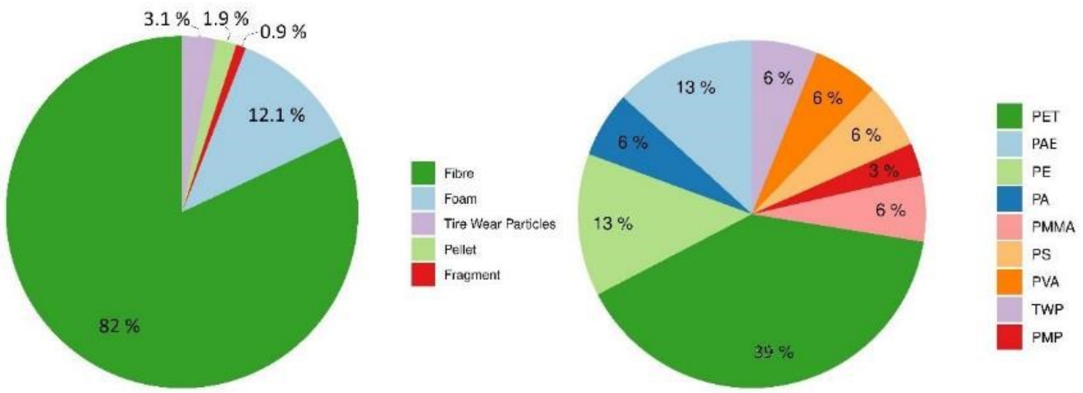


Figure 3. Proportion of microplastic shapes and polymer types (PET – polyethylene terephthalate; PAE – polyacrylate; PE – polyethylene; PA – polyamide; PMMA – polymethyl methacrylate; PS – polystyrene; PVA – polyvinyl formal; TWP – tire wear particle and PMP – polymethyl pentene).

Fibres had the longest length and ranged from 97 to 4696 µm, and only fibres were longer than 1250 µm (Figure 4). In contrast, foams measured between 116 and 1084 µm. Fragments fell within the range of 767 to 1158 µm, pellets ranged from 156 to 792 µm, and TWPs had the shortest length, ranging from 75 to 388 µm (see Figure 4 and Table 1).

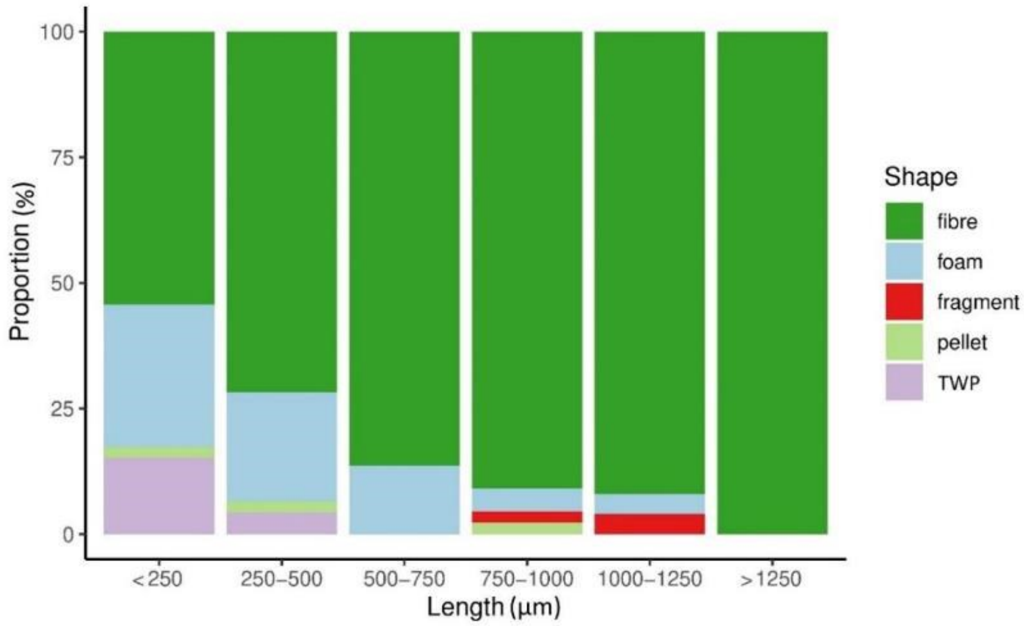


Figure 4. Classified length distribution of microplastics for each shape type (fibre, foam, fragment, pellet, and tire wear particle (TWP)).

The average for total MP concentration across the 33 sites per gram dry weight of moss was 5.92 ± 0.53 MP/g dw (CV% = 51%.). Specifically, it was 5.09 ± 0.43 MP/g for fibres, 0.05 ± 0.04 MP/g for fragments, 0.09 ± 0.04 MP/g for pellets, 0.55 ± 0.33 MP/g for foams, and 0.10 ± 0.60 MP/g for TWPs (Table 1). The highest concentrations were observed in site numbers 6, 11, 12, 19, 32, and 33, reaching a maximum of 9.6, 10.4, 8.8, 12.44, 12.89, and 8.8 MP/g, respectively (see Figure 5). The average CV% between the five replicates across all the 33 study sites was 106%. In general, there was a weak negative correlation between total numbers of MPs and CV% (r = –0.26), suggesting that sites with the highest accumulation of MPs had the highest uncertainty. Fifteen of the study sites (45%) had a higher CV (144%) than the average across all the study sites (Figure 2A).

Table 1. Count, mean (± standard error) and median (± MAD) concentration number of microplastics, minimum and maximum values, and interquartile range (IQR) of total MPs, fibres, fragments, pellets, foams, and tire wear particles (TWP) accumulated per gram dry weight of moss (MP/g dw), and the length range (µm) of microplastics at the 33 sampling sites, Tuscany, Central Italy.

Variable	Total MP	Fibre	Fragment	Pellet	Foam	TWP
Count	322	264	3	6	39	10
Mean	5.92 ± 0.53	5.09 ± 0.43	0.05 ± 0.04	0.09 ± 0.04	0.55 ± 0.33	0.10 ± 0.60
Median	6.40	5.60	0	0	0	0
Minimum	2.20	1.8	0	0	0	0
Maximum	12.90	10.4	0.9	0.8	10.7	3.1
IQR	4.09	4.0	0.5	0.0	1.75	3
Length (µm)	75–4696	97–4696	767–1158	156–792	116–1084	75–388

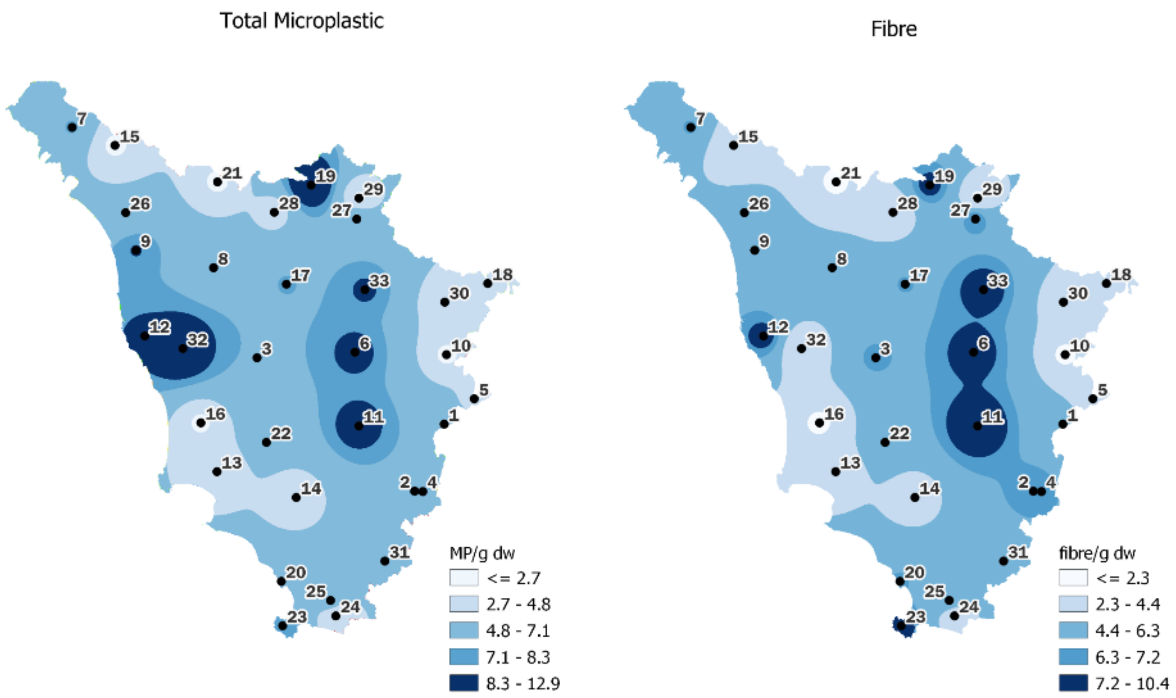


Figure 5. Spatial distribution of the total number of microplastics and fibres per gram dry weight of moss (MP/g dw) at each of the 33 sampling sites in Tuscany, central Italy.

The database of environmental variables used to predict observed MPs contained site parameters, metal concentrations and Factors, N content, magnetic susceptibility, and sample vitality (Table 2). Factor analysis returned three factors that indicated the sources of different elements; Factor 1 elements (Fe, Al, Cr, Co, V, and Ni) were from geogenic sources, Factor 2 elements (Cd, Pb, Cu, Zn, and S) were from air pollution, and Factor 3 elements (Ba, Mn, and Sr) were from an unknown source (see Figure 3A, 4A). The mean N content across all the sites was 1.15 ± 0.42% (mean ± SD), in which sites 14, 19, 20, 24 and 32 had the highest values, with 1.69%, 2.12%, 1.63%, 1.64% and 2.02%, respectively (Figure 6). Magnetic susceptibility did not show significant values across the sites with an average of $1.7 \pm 1.2 \times 10^{-8}$ m^3 kg^{-1}. Furthermore, vitality parameters had high values across all the sites, in which CCI had an average of 439 ± 42 mg/m^2, F_v/F_m of 0.663 ± 0.03, and EC of 10.5 ± 3.8 % (Table 2).

There was a significant positive correlation (Spearman) between the total MP concentration (MP/g dw) and the population number within a 10 km buffer ($r_s = 0.5$). Further, there was a weak correlation with N (%) content ($r_s = 0.32$). In general, population and N content were highest in northern and central Tuscany, consistent with MPs (Figure 6). There was no significant correlation with all the other parameters (Table 2).

Table 2. Statistical summary of site parameters (population (pop.) in 5 km and 10 km buffers; building number, landcover and distance to main roads in a 500 m buffer; altitude (m); nitrogen (N, %) and metal concentrations (Cd, Cu, Pb, S, Zn, mg/kg); magnetic susceptibility (χ, 10^{-8} m^3 kg^{-1}); Factor 1 and Factor 2; and sample vitality (photosynthetic efficiency (F_v/F_m), chlorophyll content (CCI, mg/m^2) and cell membrane integrity (EC, %)), along with Spearman's rank between environmental variables and microplastics (correlation coefficients and their *p*-value).

Variable	Mean ± SD (min–max)	Spearman's rank with MPs	
		r_s	*P* value
Site parameters			
Population in 5 km buffer	2174 ± 3617 (0–16731)	0.01	0.95
Population in 10 km buffer *	43061 ± 78411 (1222–382258)	0.50	0.007
Buildings (500 m)	2.5 ± 4 (0–15)	-0.14	0.44
Landcover (500 m)	-	0.02	0.91
Distance to main roads (500 m)	1252 ± 3601.5 (32–20814)	0.14	0.43
Altitude	454.4 ± 273.9 (46–1079)	-0.18	0.32
Nitrogen content, magnetic susceptibility, metal concentration, and Factors			
N *	1.2 ± 0.4 (0.1–2.1)	0.32	0.07
χ	1.7 ± 1.2 (0.3–5.6)	0.17	0.34
Cd	0.1 ± 0.05 (0.1–0.2)	0.14	0.43
Cu	5.8 ± 2.1 (3.02–11.5)	0.10	0.57
Pb	2.3 ± 1 (1.1–5.2)	0.22	0.21
S	1128.7 ± 196.7 (786–1640.6)	0.02	0.91
Zn	18.2 ± 5.6 (10.5–29.8)	-0.04	0.82
Factor 1	-	0.14	0.43
Factor 2	-	0.26	0.14
Factor 3	-	0.04	0.82
Sample vitality			
F_v/F_m	0.663 ± 0.03 (0.581–0.739)	-0.13	0.46
CCI	439 ± 42 (325–523)	-0.03	0.85
EC	10.5 ± 3.8 (5.4–25.3)	0.05	0.78

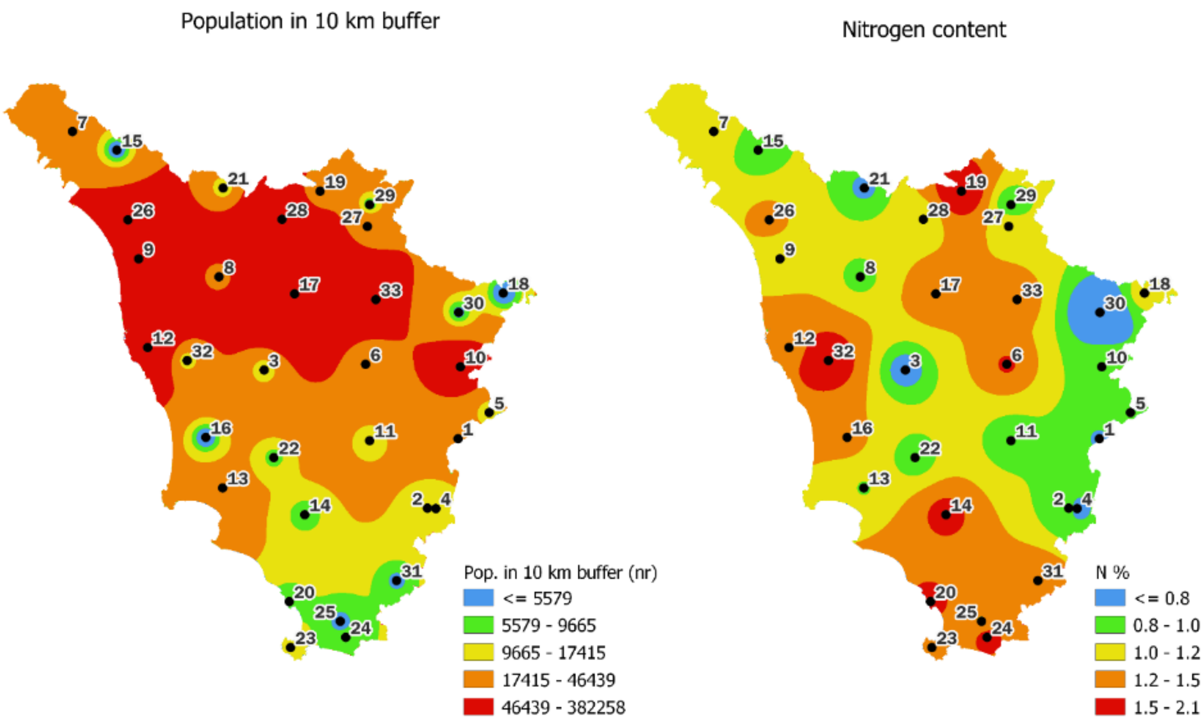


Figure 6. Spatial distribution of the population number within a 10 km buffer and nitrogen (N) content (%) in moss at each of the 33 sampling sites in Tuscany, Central Italy.

Redundancy analysis indicated that population within a buffer of 10 km and N content (%) best explained the variation in MP concentration and fibres (r = 0.72) and had the strongest associations among all the environmental predictors (Figure 7).

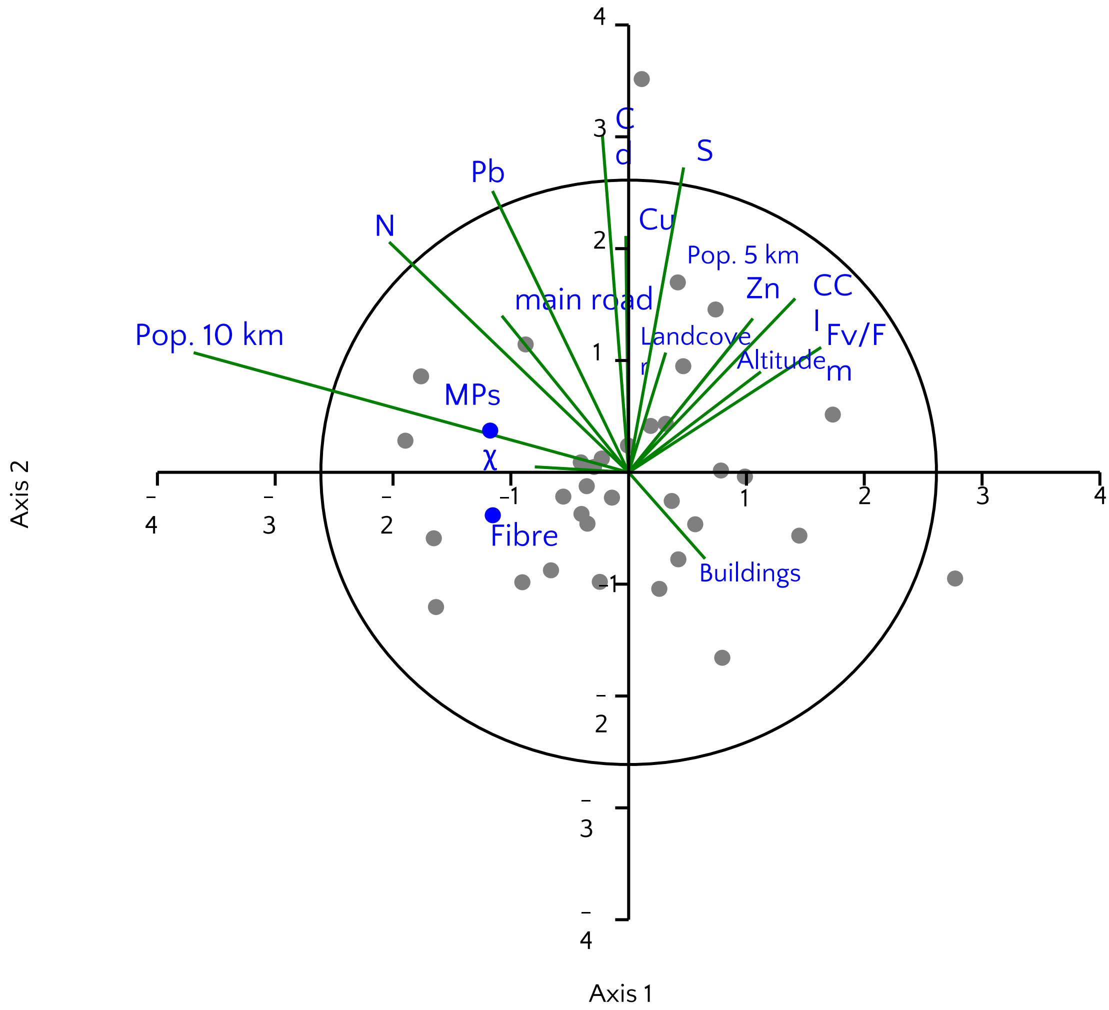


Figure 7. Biplot of Redundancy Analysis for total microplastic (MP) and fibre concentrations (MP/g dw) accumulated in moss at 33 sampling sites in Tuscany, Central Italy, with the 15 environmental predictors (population (pop.) in 5 km and 10 km buffers; building number, landcover and distance to main roads in a 500 m buffer; altitude (m); nitrogen (N, %) and metal concentrations (Cd, Cu, Pb, S, Zn, mg/kg); magnetic susceptibility (χ, 10^{-8} m^3 kg^{-1}); photosynthetic efficiency (F_v/F_m), and chlorophyll content index (CCI, mg/m^2)) as explanatory variables (R^2 = 0.42; R^2 adj = -0.1).

4. Discussions

Relatively few studies (n = 7) have assessed the efficacy of mosses or lichens as biomonitors of airborne MPs, and even fewer (n = 3) have used passive moss biomonitoring (Roblin & Aherne, 2020; Capozzi et al., 2023a; Jafarova et al., 2023a) compared with active transplants

(Loppi et al., 2021; Jafarova et al., 2023a; Bertrim & Aherne, 2023; Capozzi et al., 2023b). This is the first high spatial resolution assessment of airborne microplastics incorporating 33 sites in rural areas of Tuscany, central Italy (Table 3). In comparison, previous studies have assessed airborne microplastics using moss at three sites in Ireland (Roblin & Aherne, 2020), five sites across Tuscany and Umbria (Jafarova et al., 2023b), and seven sites in Campania, southern Italy (Capozzi et al., 2023a).

Table 3. Summary of studies that have used passive biomonitoring to assess the accumulation of airborne microplastics in moss tissue (MP/g dw). The number of study sites, mass of moss analysed per site, and the variation (CV%) in MP between sites is also shown.

Region	Number	Mass	MP/g dw	CV%	Source
Tuscany	33	1.25	5.9	51	This study
Ireland	3	4.19	3.0	42	Roblin & Aherne (2020)
Tuscany and Umbria	5	1.25	14.5	26	Jafarova et al. (2023a)
Campania	7	3.00	71.1	18	Capozzi et al. (2023a)

The average concentration of microplastics across the 33 study sites was 5.9 MP/g dw, with a variation of 51%. A wide range in airborne MPs have been reported, ranging from 3.0 MP/g dw (CV = 42%) in Ireland (Roblin & Aherne, 2020), to 14.5 MP/g dw (CV = 26%) in Tuscany and Umbria, and 71 MP/g dw (CV = 18%) in Campania (Table 3), which primarily reflects a gradient in site remoteness (or anthropogenic influence). The higher CV% between sites in the current study (and Ireland) reflects the inclusion of sites with very low MP concentrations (2.2 MP/g dw Tuscany, and 2.1 MP/g dw Ireland).

In this study, fibres were the dominant shape across all the sampling sites (82%). In general, fibres are more prone to long-range transport due to their light weight compared with other shape types (e.g. fragments), and all current sampling sites were rural, distant from pollution sources. This observation is supported by other studies that employed mosses as biomonitors of airborne MPs, all of which were conducted in remote areas characterized by a predominance of fibres. For instance, a study analyzing moss samples at remote sites in the Tuscan and Umbrian regions of central Italy identified the dominance of fibres (90%) and noted significant distances from urban areas (Jafarova et al., 2023a).

Further, the polymer type was dominated by PET (39%) compared to the other types. Given that PET is the most prevalent textile fibre globally, comprising 54% of production followed by cotton at 22%, moss sampling sites characterized by fibre accumulation primarily reflect PET polymer dominance (textileexchange.org). Similarly, several studies have reported the dominance of PET at background sites in Ireland at 71% (Roblin et al., 2020), and in the Campania region, south Italy, at ~75% (Capozzi et al., 2023a).

This study found a moderate-to-strong correlation ($r = 0.5$) between the population within a 10 km buffer of the sampling sites and the concentration of airborne MPs. However, there was no significant correlation observed with the population within a 5 km buffer ($r = 0.01$), indicating that the presence of MPs was not linked to local sources but rather was regionally distributed. This regional distribution suggests that the MPs were likely transported over longer distances, given their prevalence across the region. The RDA further suggested a strong relationship between the population number within a 10 km buffer and MP concentration. In addition, there was a moderate correlation between N deposition and MP concentration ($r = 0.32$), which was supported by the RDA ($r = 0.72$). Nitrogen content in moss tissue is related to atmospheric N deposition, and its presence is associated with anthropogenic emissions from agriculture, industrial production, and vehicles (Harmens et al., 2011; Olmstead & Aherne 2019). This suggests that the accumulation of airborne MPs in moss across the study sites is associated with regional air quality rather than direct local sources.

There was a notable weak correlation between magnetic properties and MP concentration ($r = 0.17$). It is well established that magnetic susceptibility is related to traffic emissions (Winkler et al., 2021). The weak correlation suggests the absence (or weak influence) of traffic-related MP pollution across the 33 sampling sites. However, exceptions were noted at sampling sites 9, 10, and 19, where TWP concentrations were 0.89, 0.44, and 3.11 MP/g, respectively. Among these sites, site 19 was the closest to a road, with a distance of 72 m, while sites 9 and 10 were situated at distances of 413 and 133 m from main roads, respectively. The proximity to roadways may account for the presence of TWPs at these sites. However, there are other sites located within 200 m of main roads where TWPs were not observed. This discrepancy might be attributed to factors beyond the experimental scope, such as road curvature, speed, obstruction by vegetation like trees, and the level of traffic activity, or the difficulty in visually identifying TWP. To address these nuances, Chapter 4 of this thesis specifically explored the biomonitoring of TWPs at sites close and distant from roads.

Plant physiology results, such as chlorophyll content and photosynthetic efficiency, had high values indicating that the moss samples were healthy and not affected by the concentrations of the MPs, PTEs, and N. This suggests that pollution levels in remote areas of Tuscany are not at a dangerous level to disturb the vitality of mosses. However, the high CV% between the five MP replicates at the sampling sites (Figure 5), reflected high measurement uncertainty, suggesting that the mass of each replicate (0.25 g) was not representative, and should be increased to reduce uncertainty in MP measurements. In Chapter 3, the mass of analytical samples was increased from 0.25 g to 1 g per replicate.

5. Conclusions

This is the first study to conduct a high spatial resolution evaluation of airborne MP deposition using passive moss biomonitoring on a regional scale. Microplastic deposition ranged from 2.2 to 12.9 MP/g exhibiting a coefficient of variation of 51%. Fibres constituted the predominant MP type, accounting for 82%, while PET emerged as the dominant polymer type at 39%, reflecting the prevalence of fibres likely attributable to their widespread use in textile production.

Microplastic concentration exhibited a correlation with population density within a greater distance (10 km buffer) rather than a closer one (5 km buffer). Moreover, N content showed a relationship with MPs, suggesting that at remote sites, MP deposition was influenced by regional or long-range atmospheric transport rather than site-specific local sources. In concert, the number of tire wear particles was limited even in the presence of sites near roads.

In summary, the results of this study suggest that mosses are an effective tool for evaluating atmospheric microplastic deposition at a high spatial resolution on a regional scale.

Appendix – Chapter 2.

Table 1A. The latitude, longitude, altitude, and population numbers within 5 and 10 km buffers at the 33 remote study sites in Tuscany, central Italy.

ID	Latitude	Longitude	Altitude (m)	Pop. (nr) 5 km	Pop. (nr) 10 km
S1	43°10'45.4"N	11°58'33.7"E	200	831	22269
S2	42°55'29.5"N	11°51'51.1"E	802	0	11080
S3	43°25'59.5"N	11°00'00.4"E	396	355	8160
S4	42°55'27.8"N	11°51'53.8"E	492	0	11080
S5	43°16'39.7"N	12°08'00.1"E	426	498	10579
S6	43°27'15.3"N	11°30'39.4"E	621	267	26968
S7	44°17'58.3"N	10°02'06.7"E	660	5050	24936
S8	43°46'23.5"N	10°46'23.4"E	46	499	33300
S9	43°50'19.5"N	10°22'06.9"E	163	3520	150740
S10	43°26'42.5"N	11°59'19.6"E	780	465	99469
S11	43°10'27.8"N	11°31'53.5"E	390	133	10380
S12	43°30'55.9"N	10°24'51.9"E	332	369	158916
S13	43°00'01.3"N	10°47'28.4"E	78	0	21443
S14	42°54'06.4"N	11°12'17.1"E	278	178	5591
S15	44°13'56.3"N	10°15'31.5"E	1013	1154	1920
S16	43°11'11.4"N	10°42'25.2"E	447	943	1391
S17	43°42'40.6"N	11°09'12.5"E	345	3029	382258
S18	43°42'50.2"N	12°12'14.2"E	595	1086	1222
S19	44°05'01.3"N	11°16'56.2"E	894	247	16408
S20	42°34'49.5"N	11°07'36.5"E	128	280	6100
S21	44°05'44.1"N	10°47'37.5"E	1079	1483	9582
S22	43°06'43.4"N	11°02'56.6"E	408	1807	7303
S23	42°24'33.1"N	11°07'59.9"E	176	9068	11878
S24	42°26'51.4"N	11°24'41.0"E	121	4334	5876
S25	42°30'26.0"N	11°23'00.9"E	117	0	4334
S26	43°58'49.3"N	10°18'53.0"E	467	3465	62343
S27	43°57'24.5"N	11°31'07.5"E	290	44	35272
S28	43°58'54.3"N	11°05'27.0"E	655	10314	202783
S29	44°02'05.8"N	11°31'54.8"E	831	40	5571
S30	43°38'35.7"N	11°58'47.3"E	542	231	3674
S31	42°39'37.2"N	11°39'51.0"E	350	3917	4611
S32	43°28'05.4"N	10°36'46.7"E	329	1405	9686
S33	43°41'25.7"N	11°33'45.6"E	543	16731	53884

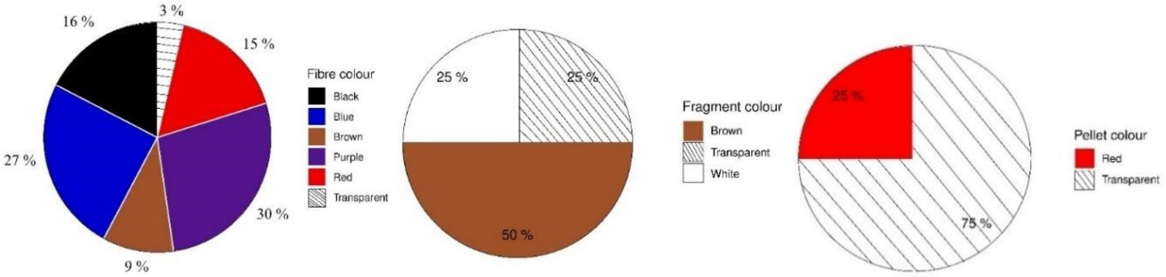


Figure 1A. Colour distribution (%) for fibres (purple – 30%, blue – 27%, black – 16%, red – 15%, brown – 9% and transparent – 3%), fragments (brown – 50%, white – 25% and brown – 25%), and pellets (transparent – 75%, red – 25%).

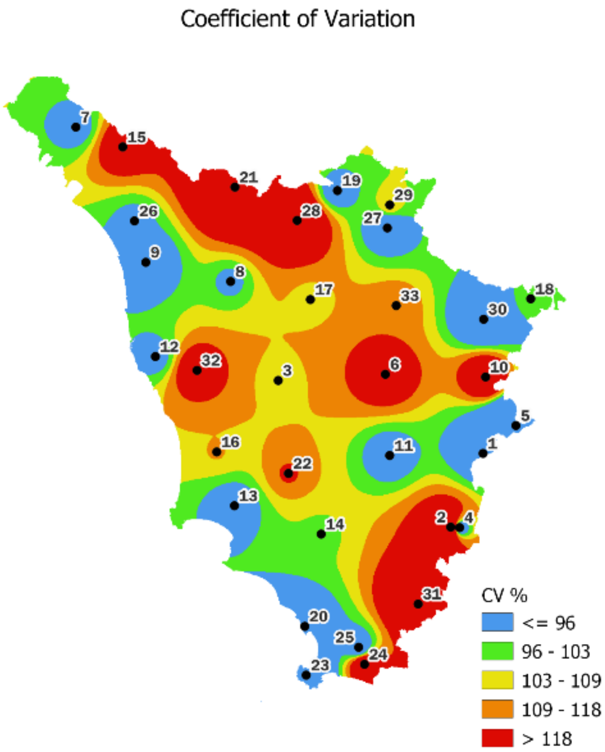


Figure 2A. The coefficient of variation (CV, %) across the five replicates at each of the 33 sampling sites in Tuscany, Central Italy.

Source identification – Factor analysis for Potentially Toxic Elements (PTE)

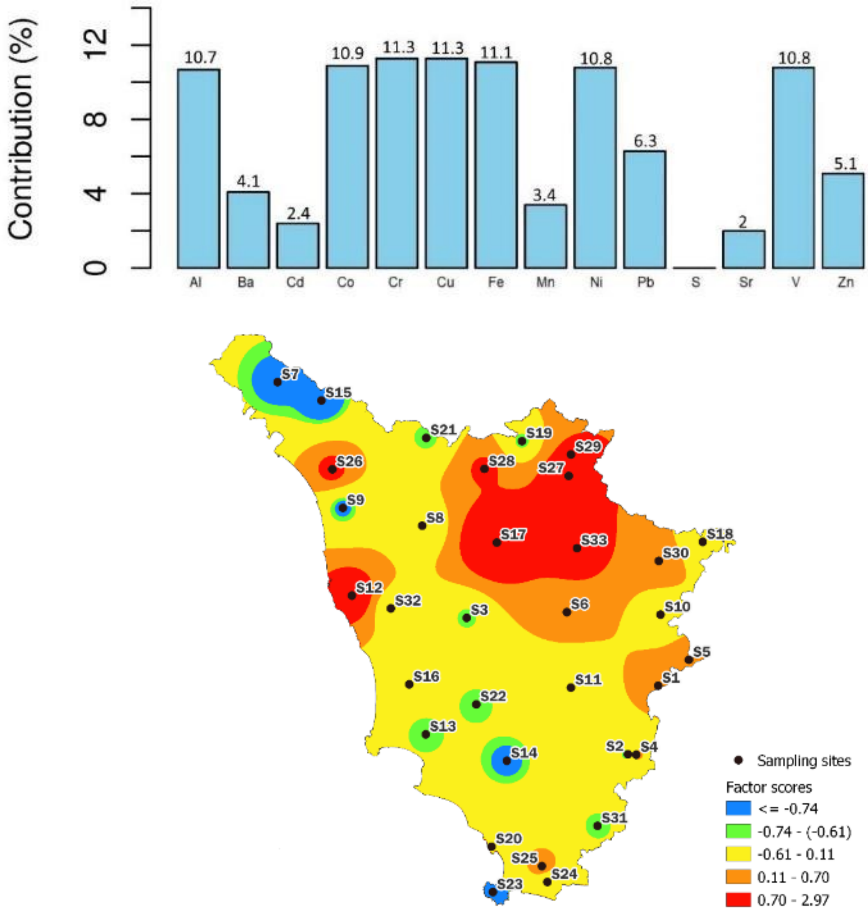


Figure 3A. Contribution (%) and spatial distribution of Factor 1 representing geogenic sources (PTE = Al, Co, Cr, Cu, Fe, Ni, and V) across 33 sites of Tuscany, Central Italy.

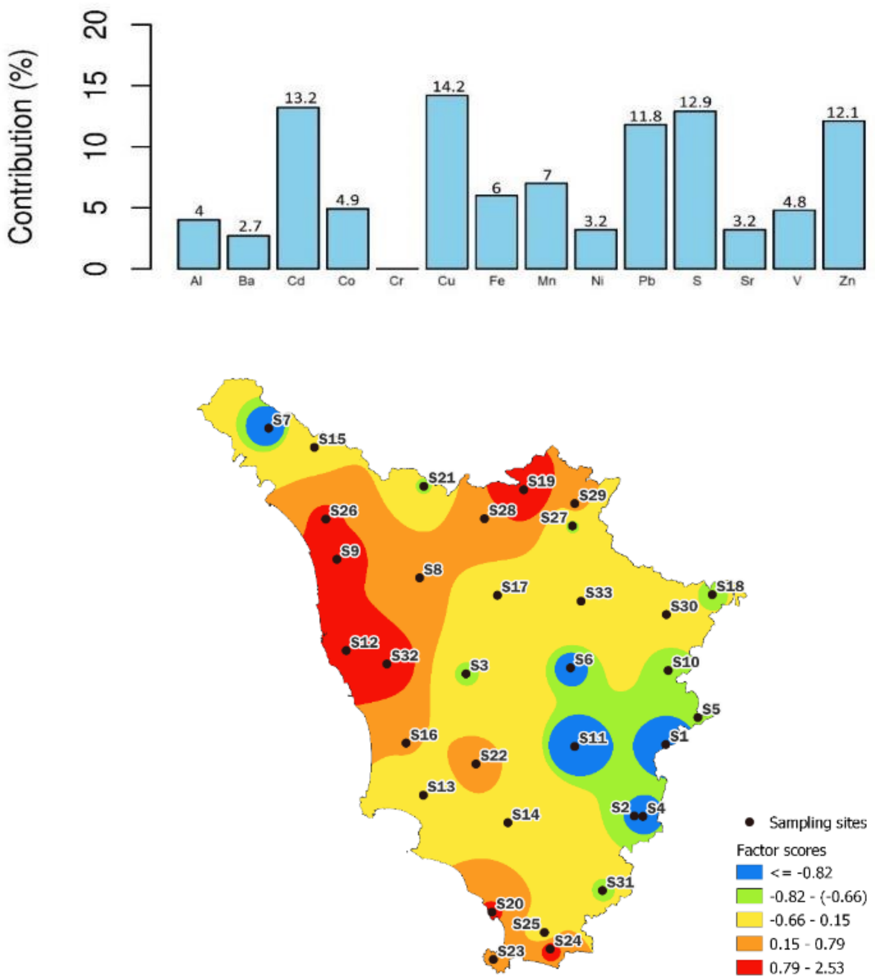


Figure 4A. Contribution (%) and spatial distribution of Factor 2 representing air pollution source (PTE = Cd, Cu, Pb, S, and Zn) across 33 remote sites of Tuscany, Central Italy.

CHAPTER 3.

Atmospheric deposition of microplastics using moss: a European pilot study (MADAME)

Summary

Here we investigate atmospheric microplastic (MP) deposition using moss passive biomonitoring on a European scale. The study took place at 37 primarily rural (remote) sites across 17 European countries following a common sampling protocol under the MADAME (Microplastic Atmospheric Deposition Assessment using Moss in Europe) project. In total, 221 MPs were found across all study sites, dominated by fibres at 91% compared with fragments at 9%. The average MP accumulation per gram dry weight of moss across all sites was 1.9 MP/g (1.6 µg/g). Polyethylene terephthalate (PET) was the dominant polymer type (72%), consistent with the dominance of fibres. Study sites were categorized based on their anthropogenic influence (e.g., population, landcover, building count, distance to roads, etc.). In general, study sites with proximity to population centres had significantly higher atmospheric MP accumulation, with an average of 3.5 MP/g (2.3 µg/g) compared with remote sites at 1.3 MP/g (1.0 µg/g). This is the first European-scale assessment of MPs using moss biomonitoring; the results suggest that mosses can be effectively used for continental-scale assessments of atmospheric MP deposition.

1. Introduction

Microplastics (MPs) are small plastic particles (< 5 mm in size) generated from the breakdown of larger plastic objects via processes such as biodegradation, exposure to UV radiation, and mechanical abrasion, or they are purposely fabricated to attain microscopic dimensions. Microplastics have been identified in various environmental compartments, spanning aquatic (Wright et al., 2013) and terrestrial ecosystems (Riling 2012). More recently, the atmosphere has been identified as a transport pathway for MPs to remote areas (Evangeliou et al., 2020), prompting global concern. However, atmospheric sampling methods are generally labor-intensive, leading to spatially limited observations.

Biomonitoring has been extensively employed for atmospheric monitoring across large spatial scales, primarily due to its cost-effectiveness and accessibility; as such, biomonitoring may offer valuable insights into the abundance and characteristics of MPs across expansive regions and continental scales. A limited number of studies have explored atmospheric MP deposition using biomonitors such as mosses, lichens, and higher plants, underscoring their efficacy in assessing airborne MP deposition (Roblin & Aherne, 2000; Loppi et al., 2019; Jafarova et al., 2022; Jafarova et al., 2023a, 2023b). Mosses have been widely used as biomonitors of atmospheric deposition since the late 1960s because they have a high capacity to trap and accumulate atmospheric particles (Loppi et al., 2000; Loppi et al., 2021a) including MPs.

Since 2001, the International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops, known as ICP Vegetation, has coordinated the European moss biomonitoring survey, which is conducted every five years to assess the atmospheric deposition of heavy metals, nitrogen, and persistent organic pollutants (Harmens et al., 2004; Harmens et al., 2008). The survey includes participants from more than 40 countries collecting pleurocarpous moss species, such as *Pleurozium schreberi*, *Hylocomium splendens*, *Hypnum cupressiforme,* and *Pseudoscleropodium purum*, from more than 5000 sites across Europe. The ICP Vegetation moss biomonitoring survey offers a potential framework for large-scale assessments of MP deposition alongside its existing objectives.

The Microplastic Atmospheric Deposition Assessment using Moss in Europe (MADAME) project, coordinated by ICP Vegetation, aims to assess the atmospheric deposition of MP across Europe through moss biomonitoring. During 2022, 33 participants collected moss from 91 sites following a common MP protocol; further, the identification of MPs was carried out at one institution to allow for unbiased comparison of results across all sampling sites. This current study presents results for 17 countries encompassing 37 moss sampling sites providing the first European scale assessment of the abundance (count and mass) and characteristics (shape, size, and polymer type) of MPs in atmospheric deposition. Further, this study assessed the potential drivers of MP deposition across remote and anthropogenically impacted sites.

2. Materials and methods

2.1 Study area

This study included 37 moss sampling sites across Europe encompassing 17 countries (Figure 1): Albania (AL-1; AL-2; AL-3), Azerbaijan (AZ-1; AZ-2), Belgium (BE-1; BE-2), Bosnia and Herzegovina (BA-1; BA-3), Croatia (HR-1), Denmark (DK-1), Estonia (EE-1; EE-2), Finland (FI-1; FI-2; FI-3), Ireland (IE-1; IE-2; IE-3), Italy (IT-1; IT-3), Kosova (XK-1; XK-2; XK-3), Latvia (LV-1; LV-2), Poland (PL-1; PL-2; PL-3), Serbia (RS-1), Switzerland (CH-1; CH-2), Slovakia (SK-1; SK-2), and the UK (UK-2; UK-3). The sites were established based on a 2° × 2° (latitude, longitude) sampling grid with generally one site per grid for a maximum of three grids per country, and where possible, the selected sampling grids were spread across each country (Figure 1). In general, sampling sites were randomly selected from sites included in the ICP Vegetation five-year moss biomonitoring survey (Harmens et al., 2010; Harmens et al., 2015) with preference given to conveniently accessible sites.

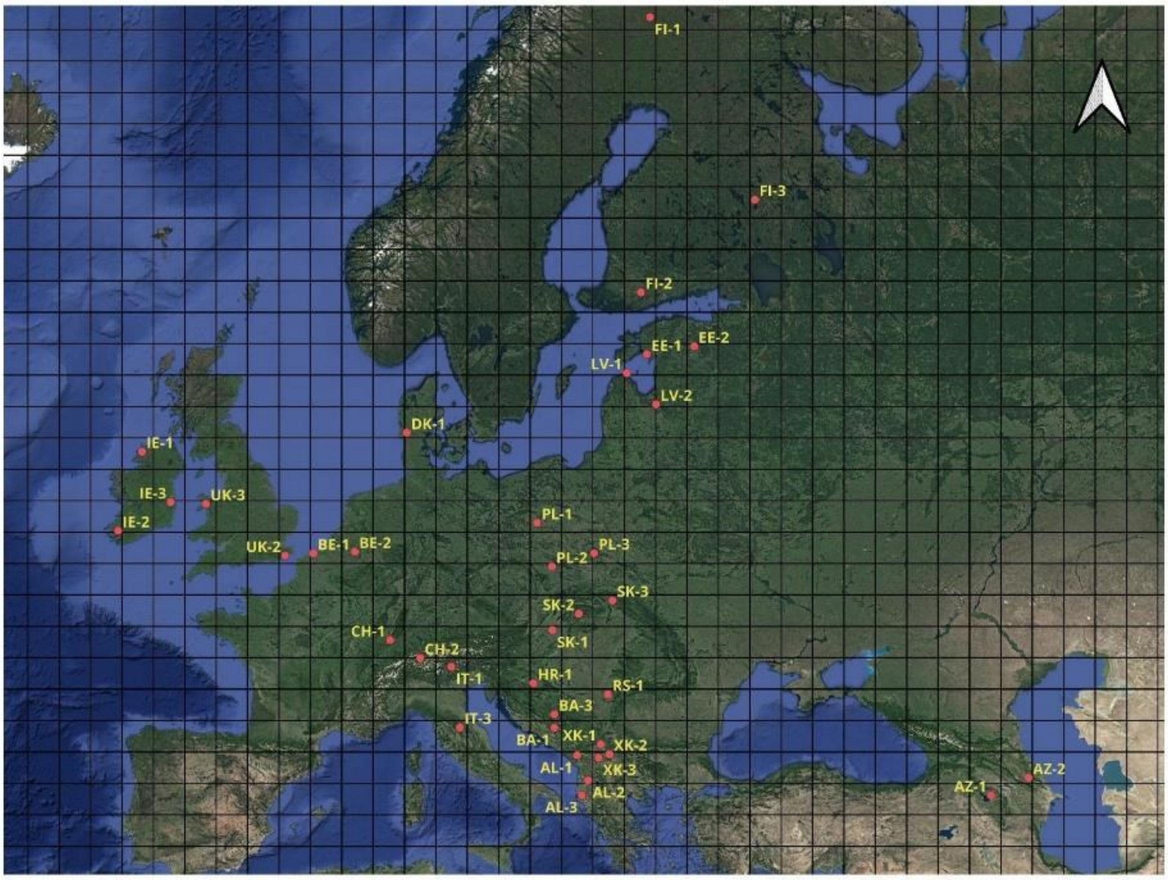


Figure 1. Map showing the pilot study area with 37 moss sampling sites located across 17 countries (Albania (AL-1; AL-2; AL-3), Azerbaijan (AZ-1; AZ-2), Belgium (BE-1; BE-2), Bosnia and Herzegovina (BA-1; BA-3), Croatia (HR-1), Denmark (DK-1), Estonia (EE-1; EE-2), Finland (FI-1; FI-2; FI-3), Ireland (IE-1; IE-2; IE-3), Italy (IT-1; IT-3), Kosova (XK-1; XK-2; XK-3), Latvia (LV-1; LV-2), Poland (PL-1; PL-2; PL-3), Serbia (RS-1), Switzerland (CH-1; CH-2), Slovakia (SK-1; SK-2), and the UK (UK-2; UK-3)).

2.2 Field sampling

Pleurocarpous moss species, i.e., *Hylocomium splendens, Hypnum cupressiforme*, *Pleurozium schreberi*, *Pseudoscleropodium purum, Homalothecium lutescens*, *Brachythecium and Homalothecium sericeum* were collected during 2022 from a 50 m × 50 m sampling plot established at each site, with only one of the species collected per site, composited from a minimum of 5–10 subsamples. In general, sampling locations were primarily remote (far from towns, large industries, and highways), at least 500 m from main roads, villages, and small industries (including industrial farms) and at least 100 m from smaller roads (including unpaved roads) and buildings. However, a number of anthropogenically influenced sites were also included. At each sampling site, moss was collected at least 15 m away from tree canopies, with composite samples (5–10 g) carefully placed in paper bags and subsequently stored in a dry environment until processing. Note: interspecies differences were not assessed in this study.

2.3 Microplastic extraction

In the laboratory, air-dried (residual water <10%) green parts of moss samples (roughly corresponding to the last 2–3 years of growth) were digested (~1 g per site in triplicate) using a wet peroxide oxidation (WPO) method (Roblin & Aherne 2020; Loppi et al., 2021; Jafarova et al., 2022, Jafarova et al., 2023a). Samples were then vacuum filtered onto filter papers (Fisherbrand Glass Fibre G6, Ø 1.6 µm) and placed into glass Petri dishes for storage. The filter papers were examined for MPs under a stereomicroscope (AmScope) equipped with a digital camera (MU1000) following a five-criteria method where the MP: (a) stands out due to its unnatural hues such as blue, red, green, purple, black, or grey, in contrast to other particles;

(b) exhibits a uniform material and texture, lacking visible cell structures or extensions; (c) retains its integrity when compressed, pulled, or prodded with delicate tweezers; (d) displays a shiny or glossy surface; and (e) shows limited fraying for fibres, being distinctly different from natural ones (Roblin & Aherne, 2020; Loppi et al., 2021; Jafarova et al., 2022; Jafarova et al., 2023a; Jafarova et al., 2023b). To help identify transparent MPs that are difficult to determine under normal lighting conditions, a blue light (NightSea SFA 540 nm) was used (Maes et al., 2017). Nonetheless, visual analysis is generally limited to particles > 50 µm. Microplastics were further verified using a hot needle test; if a particle melted under the presence of a hot needle, it was counted as a MP. All MPs were classified by shape (fibre or fragment), photographed, and measured using the open-source image processing software ImageJ 1.53t. In total, 117 moss samples (36 samples in triplicate, and one with nine replicates) were analysed for MPs.

2.4 Quality control

Strict quality-control procedures were followed to ensure that contamination was minimized during sampling and analysis. All solutions were vacuum filtered prior to use in the extraction process. All laboratory glassware used during digesting and filtering was covered with aluminium foil to prevent airborne contamination, and all glassware was rinsed five times with filtered reverse osmosis (RO) water. Surfaces were wiped down with paper towels and filtered RO water between the digestion of each sample. Process blanks used WPO digestion with the absence of sample media and were analysed for MP contamination. To evaluate the potential loss of MPs during the extraction process, blanks with and without moss were spiked with MPs (red polyethylene beads, sized between 212–250 µm), resulting in a MP recovery rate ranging from 75 to 100%. The limit of detection (LOD) was estimated as the mean MP count for the process blanks plus three times their standard deviation. However, no MPs were detected in the blanks, therefore the LOD was set to 0; further, samples were not blank corrected. Finally, cotton clothing was worn during the collection of moss and laboratory extraction of MPs.

2.5 Polymer identification

The polymer type of extracted MPs was identified using µ-FT-IR (LUMOS II, Brucker Scientific LLC, Germany) with attenuated total reflectance (ATR) mode. Glass slides with double-sided tape were prepared, and MPs obtained during the extraction process with their corresponding ID, were adhered to the tape. The glass slides were fixed to the FTIR stage, and a spectrum (with a spectral range of 400 cm^{-1} to 4000 cm^{-1} and 32 scans) was captured for each MP particle using the LUMOS II OPUS (version 8.7.31) spectroscopy software. The ATR crystal was cleaned prior to the identification of each individual MP with an isopropyl-dipped tissue in order not to contaminate the MPs between scans. A minimum of three analyses were registered on different zones of each MP particle with the background measured prior to each run. Lastly, the spectra were analysed for polymer type using the open-source software Open Specy (openanalysis.org/openspecy; Cowger et al., 2021). The MP identification rate, verified through FTIR analysis, ranged from 95 to 100% indicating that the combination of the hot needle test, blue light examination, and the established criteria were sufficient for identifying MPs.

2.6 Data analysis

The data were analyzed using the free statistical software R 4.3.1, Google Earth Pro, QGIS 3.28.5 and Past 4.15 (Hammer et al., 2001). All data were tested for normality, the association between microplastic concentrations and the sources of microplastics were evaluated using Spearman correlation (r_s), and lastly, the statistical difference between remote and anthropogenically influenced sites was assessed using a Mann–Whitney *U* test.

2.6.1 Estimation of microplastic concentration

The count concentration of MPs (MP/g dw) per site was estimated as the number of MPs divided by the mass (dry weight [dw]) of moss analysed, using the formula:

count = number (count of particles [MP]) / mass of moss (g dw)

Further, the mass concentration of MPs (µg/g dw) per site was estimated as the volume of MPs multiplied by their respective polymer density, using the formula:

$$\text{mass} = \text{volume (mm}^3\text{/g dw)} \times \text{density (g/cm}^3\text{)} \times 1000$$

The volume of each MP was estimated based on its shape; fibres, which have a cylindrical form, were assessed using the following formula:

$$V=\pi \times r^2 \times h \text{ (} r \text{ = minor axis/2, and } h \text{ = major axis)}$$

Fragments were based on an ellipsoidal shape and assessed using the formula:

$$V = 4/3\, \pi\, abc \text{ (} a \text{ = major axis, } b \text{ and c = minor axis)}$$

Where, minor axis signified the width of each microplastic particle, and major axis signified their length. Polymer densities were taken as 1.38 g/cm^3 for polyethylene terephthalate (PET) and polyvinyl chloride (PVC), 0.97 g/cm^3 for polydimethylsiloxane (PDMS), 1.16 g/cm^3 for polyamide (PA), and 1.63 g/cm^3 for polyisoprene (PI).

2.6.2 Assessment of site remoteness

Sites spanned a gradient of anthropogenic influence, ranging from extremely remote sites to those that were anthropogenically influenced. To assess the influence of anthropogenic disturbance, the sites at each end of this gradient were extracted to assess the difference between remote versus anthropogenically influenced sites. However, the majority of the sites were classified as remote given the study site selection protocol. A 500 m buffer was established around each sampling site, within which buildings were tallied, and the dominant landscape was recorded (village or town, agricultural, pasture, heathland, scrub woodland, and forest) using Google Earth Pro. Subsequently, the distance (m) from the sampling site to the nearest road and trail was measured, and distance (km) to the nearest population centre was measured (public.opendatasoft.com). Population densities within both 5 km and 10 km buffers were further estimated. Each sampling site was subsequently categorized as remote or anthropogenic; sampling sites with no buildings within a 500 m buffer and zero population within a 5 km buffer were classified as remote [n = 13; DK-1, EE-1, EE-2, FI-3, IE-1, IE-2, IE-3, LV-1, LV-2, PL-3, SK-1, SK-2, UK-3], whereas sites with more than 10,000 inhabitants within the 5 km buffer were categorized as anthropogenic [n = 7; AL-2, BA-1, BE-1, CH-1, XK-1, PL-1, SK-3] (see Table 1A).

3. Results

Across the 37 moss sampling sites, a total of 221 MP particles (Table 1) were identified in seven different Pleurocarpous moss species (Figure 1A). Fibres constituted the predominant shape at 91% compared with fragments at only 9%. Notably, fibres demonstrated longer lengths, with all microplastics > 1000 µm identified as fibres, while the longest fragment across all sites was 991 µm (Figure 2). Further, fragments dominated MPs < 250 µm in length.

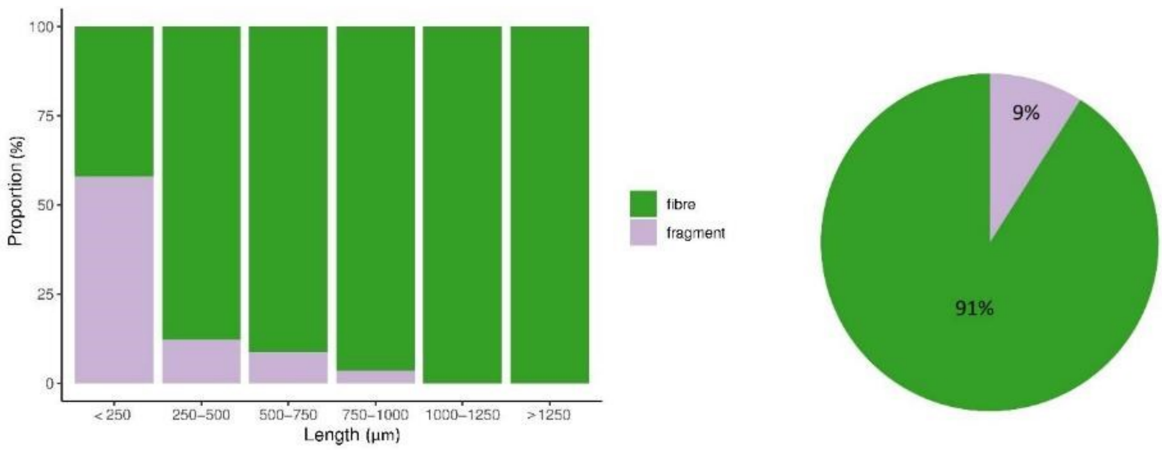


Figure 2. Length distribution (left) and shape percentage (right) of fibres and fragments across the 37 moss sampling sites.

Microplastics were mainly dominated by blue particles at 27%, while the second dominant colour was black at 22% across all the 37 sites (Figure 2A). Polymer types across the 37 sites were predominantly composed of polyethylene terephthalate (PET; 72%), with polyamide (PA) and polyvinyl chloride (PVC) each accounting for 8%, and polydimethylsiloxane (PDMS), polyethylene (PE), and polyisoprene (PI) at 4% each (Figure 3; 3A).

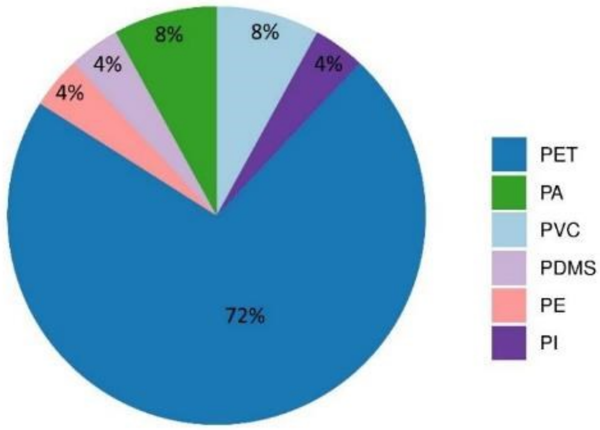


Figure 3. Distribution of microplastics polymers (polyethylene terephthalate [PET], polyamide [PA], polyvinyl chloride [PVC] polydimethylsiloxane [PDMS], polyethylene [PE], and polyisoprene [PI]) across the 37 moss sampling sites.

The abundance (count) of MP was significantly correlated with population within a 10 km buffer (r_s = 0.42), distance to nearest town (r_s = –0.38), and landcover class (r_s = 0.35), suggesting that the abundance of MP accumulated in moss increased with anthropogenic intensity (Table 1A). In total, 75 MPs were observed at the anthropogenic sites [n = 7] compared with 49 MPs at the remote sites [n = 13]; similarly, fibre count was 68 MPs at anthropogenic sites and 46 MPs at remote sites (Table 1).

The average MP concentration was 1.88 ± 0.24 (MP/g dw) and 1.58 ± 0.40 (µg/g dw) across the 37 moss sampling sites (Table 1). There were statistically significant differences ($p < 0.05$) in the MP and fibre count concentrations (MP/g dw) between anthropogenic and remote sites (Table 1, Figure 4). Similarly, MP and fibre mass concentration (µg/g dw) were significantly different between remote and anthropogenic sites (Table 1; Figure 4).

Table 1. Microplastic abundance (count), mean ± (standard error) of total microplastics, fibre, and fragment count (MP/g dw) and mass (µg/g dw) concentrations accumulated by mosses, and fibre length (µm) and proportion (%) at remote [n = 13], anthropogenic [n = 7], and all 37 moss sampling sites across Europe. An asterisk (*) indicates significant differences ($p < 0.05$) between remote and anthropogenic sites under a Mann–Whitney U test.

Variable	Remote	Anthropogenic	All sites
Number of sites	13	7	37
Microplastic count	49	75	221
Fibre count	46	68	201
Count concentration (MP/g dw)			
Total microplastics *	1.26 ± 0.20	3.45 ± 2.01	1.88 ± 0.24
Fibre *	1.18 ± 0.19	3.13 ± 0.29	1.71 ± 0.23
Fragment	0.07 ± 0.04	0.33 ± 0.05	0.17 ± 0.04
Mass concentration (µg/g dw)			
Total microplastics *	1.02 ± 0.37	2.29 ± 0.30	1.58 ± 0.40
Fibre *	0.53 ± 0.13	2.02 ± 0.81	0.97 ± 0.20
Fragment	0.50 ± 0.30	0.27 ± 0.14	0.61 ± 0.37
Fibre characteristics			
Length (µm)	1235 ± 222	2356 ± 444	1673 ± 187
Proportion (%)	93.9	90.7	91

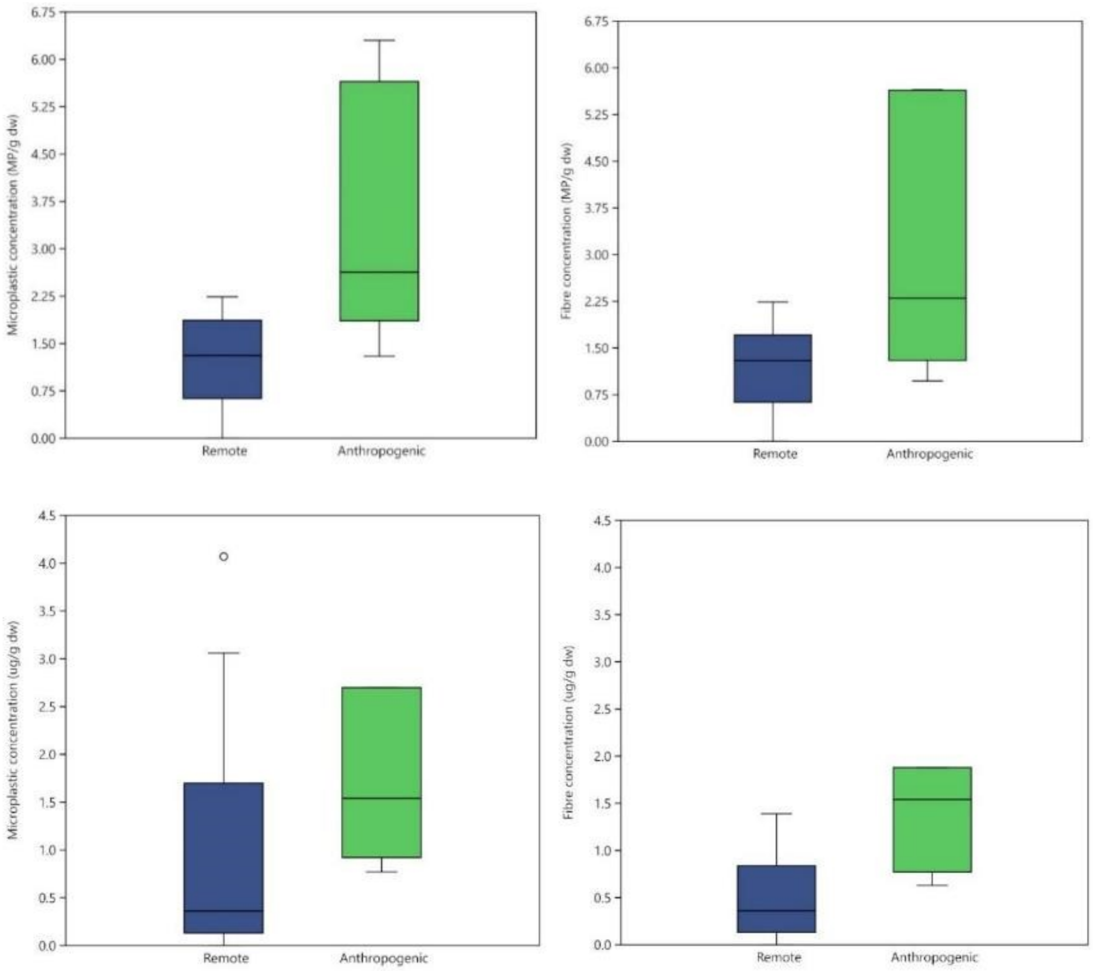


Figure 4. Boxplots showing (upper plots) the microplastic and fibre count concentration (MP/g dw) at remote [n = 13] and anthropogenic [n = 7] sites, and (lower plots) the microplastic and fibre mass concentration (µg/g dw) at remote [n = 13] and anthropogenic [n = 7] sites. Microplastic and fibre concentrations between remote and anthropogenic sites were significantly different (Mann–Whitney U, $p < 0.05$).

The average fibre length across all sites was 1673 µm (Table 1), with shorter fibres at remote sites (1235 µm) compared with anthropogenic sites (2356 µm). Nonetheless, the average length of MPs between the remote and anthropogenic sites showed no statistical difference (Figure 4A). The dominant individual MP length at remote sites was between 1500–2500 µm; in contrast, the dominant length at anthropogenic sites was between 2500–3500 µm and > 4000 µm (Figure 5A).

4. Discussions

The average MP concentration of 1.9 MP/g dw across the 37 MADAME sites (Table 1) was consistent with observations at rural or remote sites from several studies that used mosses as biomonitors of airborne MP deposition (Table 2A). For example, comparable results were observed in studies conducted in three remote lake catchments in Ireland (3.0 MP/g dw [microfibres]), rural sites in Tuscany, Italy (5.9 MP/g dw), and a rural site in Ontario, Canada (2.9 MP/g dw). In contrast, MP concentrations as high as 102 MP/g dw have been reported in urban areas (Table 2A). In the current study, the sampling site in Tuscany, Italy (IT-3), was also included in a previous regional assessment of atmospheric MPs in Tuscany (Chapter 2). The MP count for both studies was similar, also as MP/sample (3 vs. 4, and 0.8 vs. 1, respectively; Table 3A) but there was a slight difference in count per g of moss (1 MP/g versus 3.2 MP/g). This difference likely reflects differences in rainfall prior to each sampling period (Table 3A). Several studies have shown temporal variation in MP deposition (Roblin et al., 2020; Brahney et al., 2021), which is important to note when comparing between studies.

Microplastic concentration (MP/g and µg/g dw) was significantly elevated at anthropogenic sites (3.5 MP/g and 2.3 µg/g; n = 7) compared with remote sites (1.3 MP/g and 1.0 µg/g; n = 13), due to their proximity to populated areas with potential MP sources. This divergence between remote and anthropogenic sites has been widely observed; MP deposition in Ontario, Canada, was reported at 7.9 MP/g dw across nine urban sites and 2.9 MP/g dw at a rural site (Bertrim & Aherne, 2023). Similarly, MP deposition was significantly higher in urban areas (43.5 MP/g dw) compared to a rural site (20 MP/g dw) in Milan, Italy (Jafarova et al., 2022). Lastly, MPs observed on lichens at a landfill in Tuscany, central Italy, ranged from 79 MP/g dw facing the landfill to 7 MP/g dw at a distant site (1500 m) far from the landfill MP emissions source (Loppi et al., 2019).

At all moss sampling sites, fibres outnumbered fragments, regardless of whether they were influenced by anthropogenic factors or situated far from pollution sources. The prevalence of fibres has been attributed to their greater surface-area-to-volume ratio, which increases drag force and reduces settling velocity, facilitating regional transport and allowing them to reach even the most remote locations. The length of MPs in the current study further supported this observation, as MPs longer than 250 µm were dominated by fibres (Figure 2). Similar observations were reported in biomonitoring studies investigating MP pollution in both remote and non-remote areas. For example, a moss biomonitoring study at a rural site in Ontario, Canada, found fibres dominated 93% of total MPs (Bertrim & Aherne, 2023). Further in Milan, Italy, a higher proportion of fibres (~95%) were observed on lichen thalli in both urban and non-urban settings (Jafarova et al., 2022).

Shorter fibres were observed at the remote sites (Figure 4A), with the shortest measuring 510 µm, whereas at anthropogenic sites, the shortest fibre was 878 µm, suggesting that longer fibres do not disperse as extensively. A similar pattern was observed at a remote site in northern Italy (average fibre length of 616 µm), compared with urban areas in Milan (average fibre length of 945 µm; Jafarova et al., 2022). Further, in Naples, Capozzi et al. (2023b) observed that moss from background areas contained fibres measuring less than 3 mm, whereas mosses transplanted in urban environments displayed fibres up to 5 mm in length.

Polyethylene terephthalate (PET) was the predominant polymer, which is generally attributed to synthetic textiles and fabrics as the primary source of its occurrence in the environment. Similarly, in Ireland, PET emerged as the predominant polymer type in atmospheric deposition, constituting 71% of the total polymer composition (Roblin et al., 2020). In the Campania region, South Italy, PET was the dominant polymer type (~ 75%) accumulated on moss (Capozzi et al., 2023a). In the current study, given that the majority of the 37 sites are situated in remote areas, the prevalence of PET as the primary polymer type suggests that this polymer may have a greater tendency to be suspended and transported over extended distances compared with other polymer types. This observation aligns with Kyriakoudes & Turner (2023), who indicated that PET exhibited a higher susceptibility to suspension among synthetic polymers. However, considering that PET or polyester is the dominant textile fibre produced worldwide at 54% followed by cotton at 22% (textileexchange.org), it is not surprising that sites dominated by the deposition of textile fibres are dominated by PET polymers. This suggests that PET may not exhibit a higher susceptibility to suspension, rather it is pervasive in the atmosphere owing to its dominance as a textile fibre, as previously noted (Liu et al., 2019).

5. Conclusions

This study is the first European-scale assessment of MP deposition using moss biomonitoring. The mean concentration of MPs at remote sites (1.3 MP/g dw) is the lowest reported in the literature; nonetheless, the findings imply that MP abundance in atmospheric deposition (accumulated on moss tissue) is associated with land use, particularly in relation to proximity to population centres. At anthropogenic sites, MP atmospheric deposition was almost three times greater at 3.5 MP/g.

Fibres dominated MP particles across all sampling sites, encompassing both anthropogenic and remote locations. Moreover, PET dominated polymer type, consistent with the prevalence of textile fibres. In general, the findings suggest that mosses can be effectively utilized for continental-scale assessments of atmospheric MP deposition.

APPENDIX – CHAPTER 3.

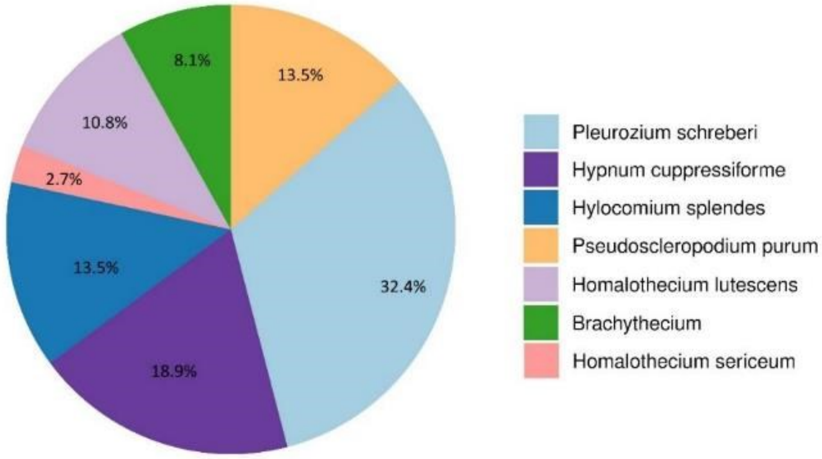


Figure 1A. Pie chart showing moss species distribution (%) across the 37 sampling sites in Europe.

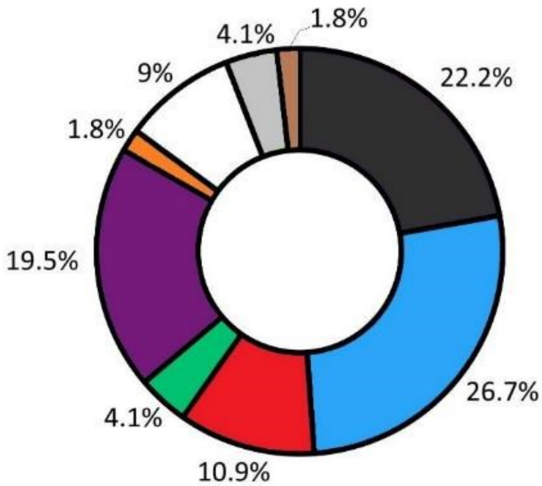
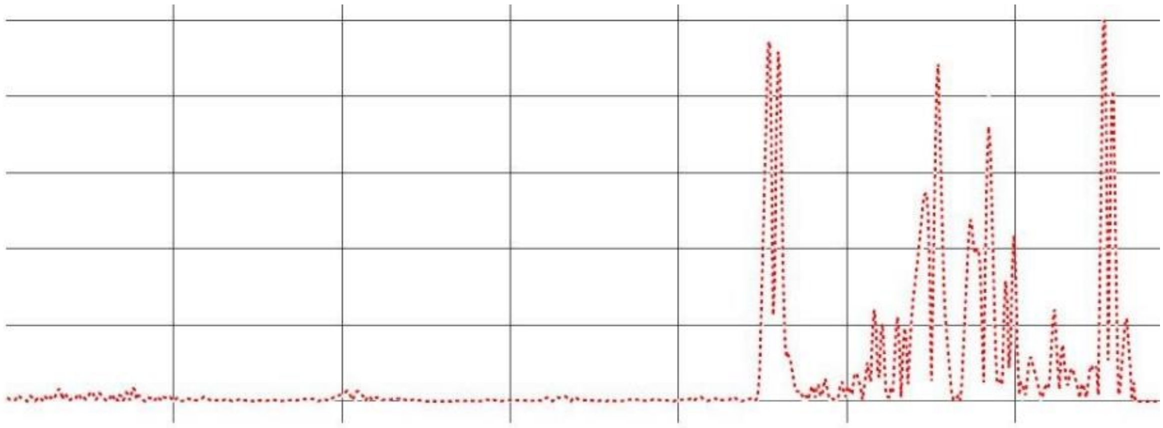


Figure 2A. Microplastic particle colour as a percentage of the total particle count (n = 221) across the 37 moss sampling sites in Europe.



Material	Plastic Pollution Category	library_id	object_id	Pearson's r	x	y	SpectrumID
polyesterterpthalate	Polyterephthalates	6baa7142cbeb279c522f44bc6db18011	intensity	0.98	11	89	27 suja

Figure 3A. Fourier-transform infrared (FTIR) spectrum from a fibre extracted from a moss sample in Switzerland (CH-1) showing a 98% match for polyester/polyethylene terephthalate (PET).

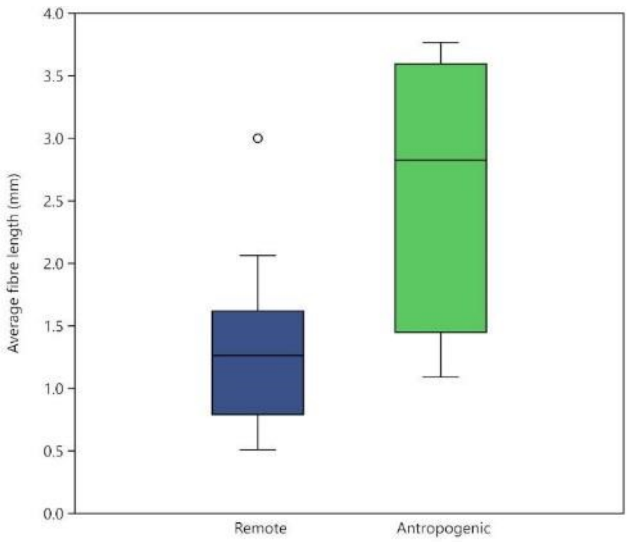


Figure 4A. Boxplot showing the distribution of average fibre length (mm) for anthropogenic (n = 7) and remote (n = 13) moss sampling sites in Europe. Average fibre length per site shows no significant difference (Mann–Whitney U, $p > 0.05$).

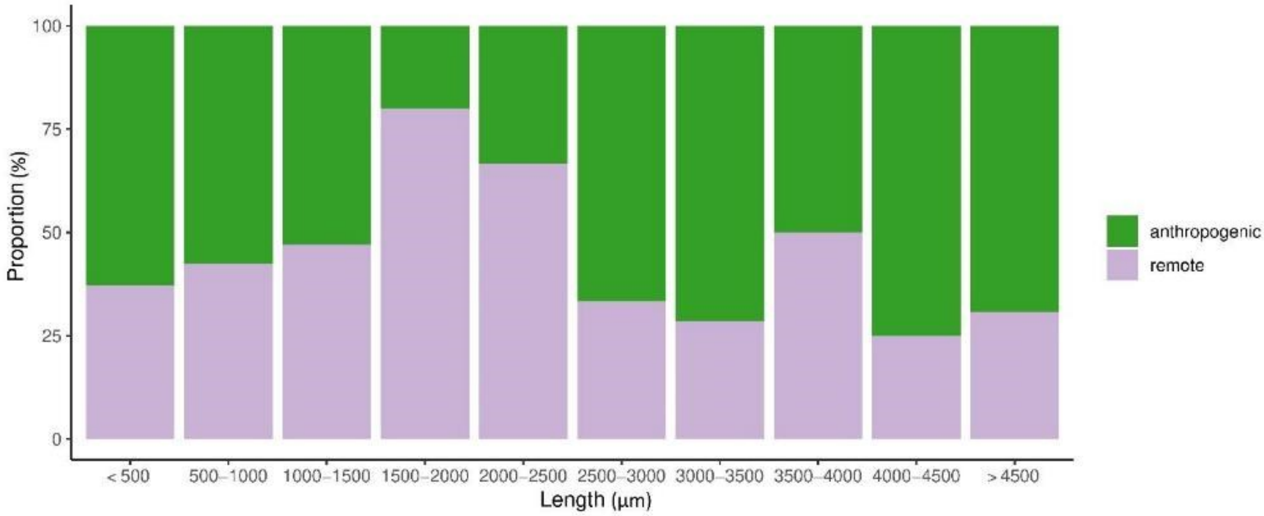


Figure 5A. Histogram showing the length (μm) across ten size categories for microplastics (n = 221) at anthropogenic (n = 7) and remote (n = 13) moss sampling sites in Europe.

Table 1A. Sampling sites (n = 37) with site ID, longitude, latitude, distance to nearest population centre (km), the population (Pop) within 10 km, along with the building count (BC) within 500 m and dominant landcover class (LCC). Note Forest = 1, Woodland Scrub = 2, Heathland = 3, Dune = 4, Pasture = 5, Agriculture = 6, and Village / Town = 7.

						10 km	500 m	
Nr	**Location**	**Site ID**	**Longitude**	**Latitude**	**Distance (km)**	**Pop. (nr)**	**BC (nr)**	**LCC**
1	Albania	AL-1	42.34615	19.42295	2.48	5944	5	2
2	Albania	AL-2	41.16697	20.11606	3.87	119355	3	2
3	Albania	AL-3	40.45775	19.72369	4.01	10029	4	1
4	Azerbaijan	AZ-1	40.44751	45.71335	13.26	0	4	2
5	Azerbaijan	AZ-2	41.28777	48.10140	21.73	0	3	5
6	Bosnia	BA-1	43.63570	17.97160	0.31	20512	287	6-7
7	Bosnia	BA-3	44.24380	17.97670	7.46	164423	16	3
8	Belgium	BE-1	51.11630	2.66480	1.22	73130	185	4-7
9	Belgium	BE-2	51.17630	5.30400	6.10	99753	8	1
10	Switzerland	CH-1	47.52483	7.53050	2.38	340707	7	1
11	Switzerland	CH-2	46.74678	9.45599	3.20	20792	6	1
12	Croatia	HR-1	45.64306	16.64722	6.10	7144	73	6-1
13	Denmark	DK-1	55.67710	8.60650	5.18	1433	0	3
14	Estonia	EE-1	58.38439	23.85188	28.43	0	0	1
15	Estonia	EE-2	58.64833	26.86511	16.54	0	0	1
16	Finland	FI-1	67.95024	24.05661	15.64	0	25	1
17	Finland	FI-2	60.38798	23.48344	9.88	1771	5	6-1
18	Finland	FI-3	63.16700	30.71700	38.85	0	0	1
19	Ireland	IE-1	54.98604	-8.21548	7.23	8764	0	3
20	Ireland	IE-2	52.00362	-9.73639	11.20	0	0	3
21	Ireland	IE-3	53.10567	-6.40233	10.11	0	0	3
22	Italy	IT-1	46.38000	11.43400	3.85	21598	1	1
23	Italy	IT-3	43.64353	11.97958	0.59	3674	52	2-7
24	Kosovo	XK-1	42.86750	20.91667	4.44	154696	117	6-7
25	Kosovo	XK-2	42.40167	21.47231	3.18	56250	3	6
26	Kosovo	XK-3	42.24639	20.78583	5.25	174610	0	2
27	Latvia	LV-1	57.75361	22.57806	31.26	0	0	1
28	Latvia	LV-2	56.67889	24.43222	7.55	5767	0	1
29	Poland	PL-1	52.31289	16.88436	1.99	63138	55	1-7
30	Poland	PL-2	50.58191	17.82452	3.37	8576	0	1
31	Poland	PL-3	51.12085	20.50306	5.15	5946	0	1
32	Serbia	RS-1	45.15019	21.38405	3.57	39446	0	2
33	Slovakia	SK-1	47.95468	17.85574	10.24	0	0	6
34	Slovakia	SK-2	48.64190	19.53097	7.14	7484	0	5
35	Slovakia	SK-3	49.19990	21.67378	1.59	10874	0	6
36	UK	UK-2	51.02750	0.87083	3.14	30594	26	6
37	UK	UK-3	53.04417	-4.15528	6.93	4723	0	3

Table 2A. Average microplastic accumulation per gram dry weight of moss (MP/g dw) estimated under passive and active biomonitoring studies (n = 8).

Region (sites)	**Moss species**	**Monitoring**	**MP/g**	**Land use**	**Source**
Europe (37)	Multiple (n = 7)	Passive	1.8	remote	Current Study
Tuscany, Italy (33)	Multiple (n = 8)	Passive	5.9	zz	PhD thesis, Chapter 2
Central Italy (5)	*Pseudoscleropodium purum*	Passive	14.5	rural	Jafarova et al., 2023
Ireland (3)	*Hylocomium splendes*	Passive	3.1	remote	Roblin & Aherne, 2020
Toronto, Canada (9)	*Pleurozium schreberi*	Active	7.9	urban	Bertrim & Aherne, 2023
Warsaw, Canada (1)	*Pleurozium schreberi*	Active	2.9	rural	Bertrim & Aherne, 2023
Campania, Italy (7)	*Hypnum cuppressiforme*	Passive	71	rural	Capozzi et al., 2023a
Campania, Italy (3)	*Hypnum cuppressiforme*	Active	102	urban	Capozzi et al., 2023b

Table 3A. Comparison of microplastic count, moss sample mass (number of replicates), microplastics per g dry weight of moss, and microplastic shape at one sampling site in Tuscany, that was sampled during May 21 and June 22 to support studies in Chapter 2 and 3.

Study	**MP Count**	**Sample mass (g)**	**MP/g**	**MP/sample**	**MP shapes**	**Sampling period**	**Rainfall (mm)**
Chapter 2	4	1.25 (0.25 g × 5)	3.2	0.8	fibre (100%)	May-21	113.4
Chapter 3	3	3 (1.0 g × 3)	1	1	fibre (66%); fragment (33%)	Jun-22	50.6

CHAPTER 4.

Robinia pseudoacacia L. (Black Locust) leaflets as biomonitors of airborne microplastics

Summary

Here we investigate the suitability of *Robinia pseudoacacia* L. (black locust) leaflets as a novel biomonitor of airborne microplastics (MPs) including tire wear particles (TWPs). Leaflets were collected from rural roadside locations (ROs, n = 5) and urban parks (UPs, n = 5) in Siena, Italy. MPs were removed by washing, identified by stereomicroscope, and analysed for polymer type by Fourier transform infrared spectroscopy. Daily MP deposition was estimated from leaf area. The mass magnetic susceptibility and the bioaccumulation of traffic-related potentially toxic elements (PTEs) were also analysed. The total number of MPs at ROs was significantly higher at 2962, dominated by TWPs, compared with 193 in UPs, where TWPs were not found. In contrast, total microfibres were significantly higher in UPs compared with ROs (185 vs. 86). Daily MP deposition was estimated to range from 4.2 to 5.1 MPs/m^2/d across UPs and 29.9–457.6 MPs/m^2/d across ROs. The polymer types at ROs were dominated by rubber (80%) from TWPs, followed by 15% polyamide (PA) and 5% polysulfone (PES), while in UPs the proportion of PES (44%) was higher than PA (22%) and polyacrylonitrile (11%). The mean mass magnetic susceptibility, a proxy of the bioaccumulation of traffic-related metallic particles, was higher at ROs (0.62 ± 0.01 10^{-8} m^3/kg) than at UPs (–0.50 ± 0.03 10^{-8} m^3/kg). The content of PTEs was similar across sites, except for significantly higher concentrations of antimony, a tracer of vehicle brake wear, at ROs (0.308 ± 0.008 µg/g) compared with UPs (0.054 ± 0.006 µg/g). Our results suggest that the waxy leaflets and easy determination of surface area make *Robinia* an effective biomonitor for airborne MPs including TWPs.

1. Introduction

Microplastics (MPs, i.e., plastic particles < 5 mm) have become a significant global concern.

They primarily arise from photo- and biodegradation, mechanical breakdown (e.g., due to wind and friction), thermo-oxidative and hydrolytic degradation of plastic materials (Cai et al., 2017; Andrady 2011; Cai et al., 2023; Maddison et al., 2023; Cao et al., 2023; Thakur et al., 2023) and can be found in different shapes such as fibres, fragments, foams, pellets, and beads (Renzi & Blaškovic).

Microplastics have been detected in all environmental compartments, including aquatic (Wright et al., 2013; Nerland et al., 2014; Yang et al., 2021; Tang et al., 2023; Mubin et al., 2023) and terrestrial ecosystems (Riling 2012; de Souza Machado et al., 2018; He & Luo 2020; Surendran et al., 2023), and the atmosphere (Dris et al., 2015; Dris et al., 2016; Allen et al., 2019; Hee et al., 2023; Klein et al., 2023; Romarate et al., 2023). Furthermore, MPs have been found in animal (Renzi et al., 2018; Nelms et al., 2019; Carlin et al., 2020; Prata & Dias-Pereira 2023; Mackenzie 2023) and plant tissues (Qi et al., 2018; Li et al., 2020), and even in human blood (Hwang et al., 2019; Çobanoğlu et al., 2021). This widespread distribution raises serious concerns about the potential impacts of MPs on ecosystem and human health.

Synthetic textiles (Akanyange et al., 2021), the fragmentation of plastic products, and industrial emissions (Chen et al., 2020) are recognized as significant contributors to airborne MPs. Furthermore, tire wear particles (TWPs), considering their chemical and physical characteristics, are classified as MPs (knight et al., 2020). The abrasion of tire treads on road surfaces, with the consequent release of TWPs is an important source of airborne MPs (Thorpe & Harrison 2008; Evangeliou et al., 2020).

It is known that plant leaves have a high capacity to intercept airborne particles from the atmosphere on their surface and can provide insights into the deposition levels of pollutants (Rai 2016). However, only a few studies have evaluated the deposition of MPs using leaves of higher plants such as shrubs (Liu et al., 2020), pine needles (Liu et al., 2022), tree species (Leonard et al., 2023), and lettuce (Canha et al., 2023). In addition, a number of studies have focused on the use of cryptogams such as mosses and lichens for monitoring the deposition of airborne MPs (Roblin & Aherne, 2020; Loppi et al., 2021; Jafarova et al., 2022; Jafarova et a l., 2023a; Bertrim & Aherne, 2023; Capozzi et al., 2023a; Wenzel et al., 2023). While some researchers have employed *Robinia pseudoacacia* L. leaves for investigating the deposition of potentially toxic elements (PTEs) from the atmosphere (Nadgórska-Socha et al., 2016; Capozzi et al., 2020), no study has yet explored its potential for the analysis of atmospheric MPs, particularly regarding TWPs.

Robinia, a fast-growing tree species, has spread extensively in temperate regions of Europe, North America, and Southern Africa, earning its classification as an invasive allochthonous species due to shading and its capacity to modify soil conditions (Crosti et al., 2016; Sitzia et al., 2016). In the Mediterranean region, it has invaded a broad range of disturbed habitats, such as abandoned fields, arid locations, roadsides, and other ecosystems where the natural vegetation has undergone severe disturbances (Crosti et al., 2016). Its wide distribution, ease of identification, and that its leaves consist of leaflets that have a hydrophobic, waxy surface (Martin 2019), potentially make it an effective biomonitor of atmospheric MP deposition.

This research explored the effectiveness of *Robinia* leaflets as a novel biomonitor to assess the deposition of airborne MPs. Leaflets were collected from urban parks and rural roadside locations reflecting opposite gradients in population and vehicle traffic densities. Samples were analysed for the accumulation and polymer composition of airborne MPs, and PTE content and magnetic analysis of traffic-related elements for source identification. To the best of our knowledge, this is the first study using *Robinia pseudoacacia* for the examination of airborne MPs, including TWPs.

2. Materials and methods

2.1. Study area

The study was conducted in Siena and its outskirts (Tuscany, central Italy) at the end of the vegetation growing season (end of September 2022). The study sites included both urban parks in Siena (UPs, n = 5), situated away from major roads, and rural locations (ROs, n = 5) distant from human settlements and any other local sources of pollution, but conveniently located close to minor asphalt roads (Figure 1). The urban parks are visited by many locals and tourists during the spring–summer period, but vehicles are strictly prohibited in the city. In contrast, the trees at the roadside locations had an approximate distance of 5–10 m from the nearest provincial (RO 2, RO 3, RO 4), tertiary (RO 1), and unclassified roads (RO 5) that connect the small towns of the province of Siena. The city of Siena has a population of about 55,000 inhabitants, while the whole province has about 270,000 inhabitants. The elevation of the sampling sites ranged from 270 to 410 m asl. The climate is Mediterranean, with warm, dry summers; mean annual temperature is 13–14 °C and annual rainfall spans a range of 600–1000 mm.

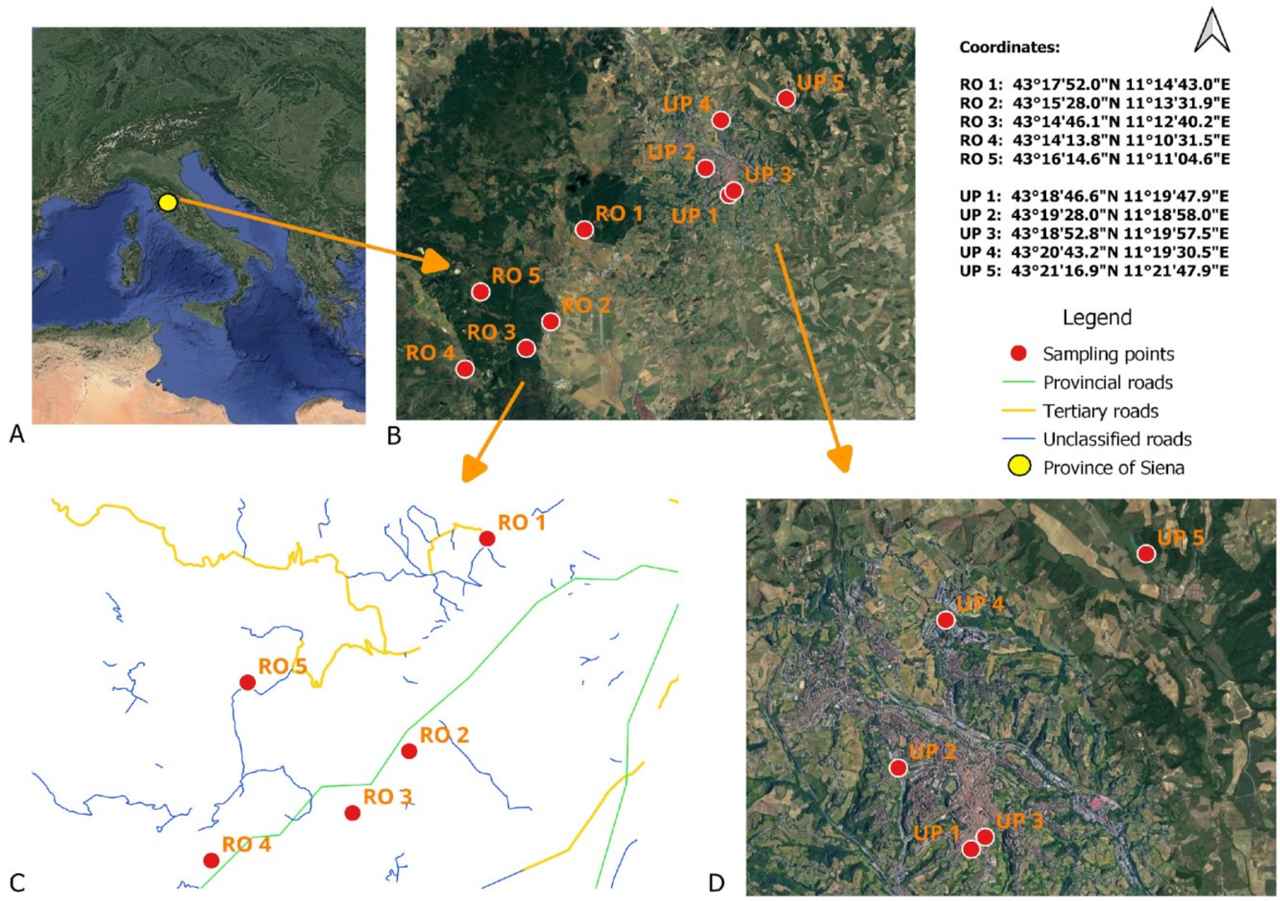


Figure 1. Study area (A); location of sampling sites: RO 1–5 = locations along rural asphalt roads, and UP 1–5 = urban parks (B); proximity of the RO sites to the nearest roads (C); and close-up of the city of Siena showing the UP sites (D).

2.2. Experimental design

In this study, the common tree species *Robinia pseudoacacia* L. (black locust) was selected as a biomonitor for MP deposition, considering its widespread presence, and the high surface area and waxy coating of its leaves. At each study site, leaves of *R. pseudacacia*, each containing multiple leaflets, were collected from five trees closest to the centre of the site. After collection, all samples were stored in paper bags and frozen for subsequent analysis. In total, 50 samples were analysed for MPs by processing five replicates of 1 g of fresh leaflets from each site.

2.3. Microplastic analysis

In the laboratory, MPs were extracted by washing each *Robinia* leaflet sample with 100 mL of deionized water inside beakers (ca. 10 fresh leaflets per 1 g sample). The leaflet samples were manually stirred in the deionised water for 3–4 min. The water samples were subsequently vacuum filtered onto cellulose filter papers (Watman Grade 1, 1001-090, 11 μm) with a diameter of 90 mm, and the filters stored in glass Petri dishes. The filter papers (n = 50, 1 filter paper per 100 mL) were examined for MPs under a stereomicroscope (Eurotek OXTL101TUSB equipped with an MDCE-5C digital camera) following a five-criteria method: unnatural colour, material homogeneity, particle resiliency, reflective surfaces, and limited fraying (Roblin & Aherne, 2020; Loppi et al., 2021; Jafarova et al., 2022; Jafarova et al., 2023a; Bertrim & Aherne, 2023). Fibres and fragments that met at least two criteria were considered anthropogenic and photographed (Windsor et al., 2019), and further verified as plastic using a hot needle (Norén 2007; Hidalgo-Ruz et al., 2012). Tire wear particles pose a challenge in terms of identification, as they do not exhibit a response to a hot needle. Therefore, they were categorized using specific criteria, including dark colour (black), elongated or cylindrical shape, rough surface texture, and rubbery flexibility when manipulated (Bertrim & Aherne, 2023; Kreider et al., 2010; Leads & Weinstein 2019; Parker et al., 2020). The length of all plastic particles was measured using the open-source image processing software ImageJ 1.53t.

2.4. Quality control

To minimize the possibility of contamination, sampling and analysis were conducted with strict adherence to standard quality control procedures. Analytical process blanks were regularly processed to ensure the systematic management of laboratory contamination. Each piece of glassware underwent a triple rinsing with filtered deionized water prior to use and aluminium foil was used to cover all laboratory glassware during the extraction of MPs to prevent airborne contamination. Surfaces were cleaned using a combination of paper towels and filtered deionized water. Furthermore, individuals wore cotton clothing throughout the procedure. A washing process blank containing only the filtered deionized water that was used to wash the leaflet samples was carried out for each site. These blanks remained open throughout the leaflet washing procedure and did not contain any substantial presence of MPs falling within the range of 0–1 MPs per blank.

2.5. Estimation of MP deposition rates

The MP deposition period spanned approximately five months from full leaf emergence at the end of April to the end of the growing season at the end of September. The leaflets were photographed, and their upper surface area was calculated using the image software Leafscan 2.1.1 (Anderson & Carlos 2020). The average daily MP deposition rate was estimated based on the accumulation of MPs on the leaflets and an exposure period of 150 days (5 months) following Jafarova et al. (2022), according to the formula:

MP deposition (MPs m^{-2} d^{-1}) = MP concentration (MPs g dw^{-1}) × sample mass (g dw^{-1} m^{-2})/150 (d).

There was no occurrence of heavy rainfall during the study period that could potentially result in the particles being washed away (WeatherSpark, weatherspark.com).

2.6. Characterization of microplastics

Leaf samples were treated in a glove box fitted with a HEPA filter to prevent contamination. Further, positive and negative controls were performed (n = 3) to ensure the quality of the entire analytical process. Extraction was performed by digesting the samples with 30% H_2O_2

solution and the filtered, digested samples were analysed using a stereomicroscope at 10–80× (SMZ-800 N; NIS-elements D software 5.11.03, Nikon, Tokyo, Japan). The polymer composition of the target particles was investigated by microscopy combined with micro-Fourier transform infrared spectroscopy (Nicolet iN10 MX, ThermoFischer Scientific, Waltham, MA, USA) using an MCT-A detector—cooled with liquid nitrogen and operated in reflection mode (spectral range between 7800 and 650 cm^{-1}). Identification was performed by determining the spectral match of target elements (%) with microplastic spectral libraries (OMNIC™ Picta™ Software 1.7.192 Libraries, ThermoFisher Scientific, Waltham, MA, USA) integrated with internal spectral libraries containing TWP spectra. A threshold for spectra acceptance was set at a match of > 80%. The detection threshold for particle size was 10 µm.

2.7. Metal content and magnetic analysis

The leaflets were further analysed for traffic-related PTE content and magnetic properties to support source identification. Plant leaflets were oven-dried at 40°C for 24 h, digested in 3 mL of 70% HNO_3 and 0.5 mL of 30% H_2O_2 using a microwave-digestion system (Milestone Ethos 900, Bergamo, Italy) at 280°C and 55 bar. The content of traffic-related PTEs (namely, Fe, Al, Cu, Zn, Ba, Cr and Sb) was then quantified by ICP-MS (Sciex Elan 6100, Perkin Elmer, Waltham, MA, USA). Analytical quality was verified using the certified reference material GBW 07603 "plant leaves"; recoveries were in the range 83–127%; the precision of the analysis was expressed by the relative standard deviation of three replicates and was within 10% for all elements. The results are expressed on a dry weight basis.

For the magnetic analysis, dry samples were placed into standard 8 cm^3 plastic cubes, and mass magnetic susceptibility (χ, m^3 kg^{-1}) was determined with an Agico KLY5 m after measuring the net weight of the sample.

2.8. Statistical analysis

The differences in the concentration of MPs, PTEs, and magnetic susceptibility between the RO and UP sites were assessed using the Mann–Whitney U test (p < 0.05). Statistical analysis was carried out using the open-source software R 4.3.1 (R Core Team 2023).

3. Results

In total, 3155 MPs were found across all sites, 193 in UPs and 2962 at ROs ($p < 0.05$; Table 1), the latter mainly due to the remarkable abundance of TWPs (Figure 2). Within the RO sites, 2872 TWPs were found, whereas no instance of TWPs was detected in UPs. The quantity of plastic fibres and fragments was notably lower at ROs compared to UP; 86 vs. 185 ($p < 0.05$) and 4 vs. 8, respectively (Figure 2). The daily deposition of MPs at the RO sites was significantly higher than at the UP sites (Table 1), consistent with the high amount of TWPs.

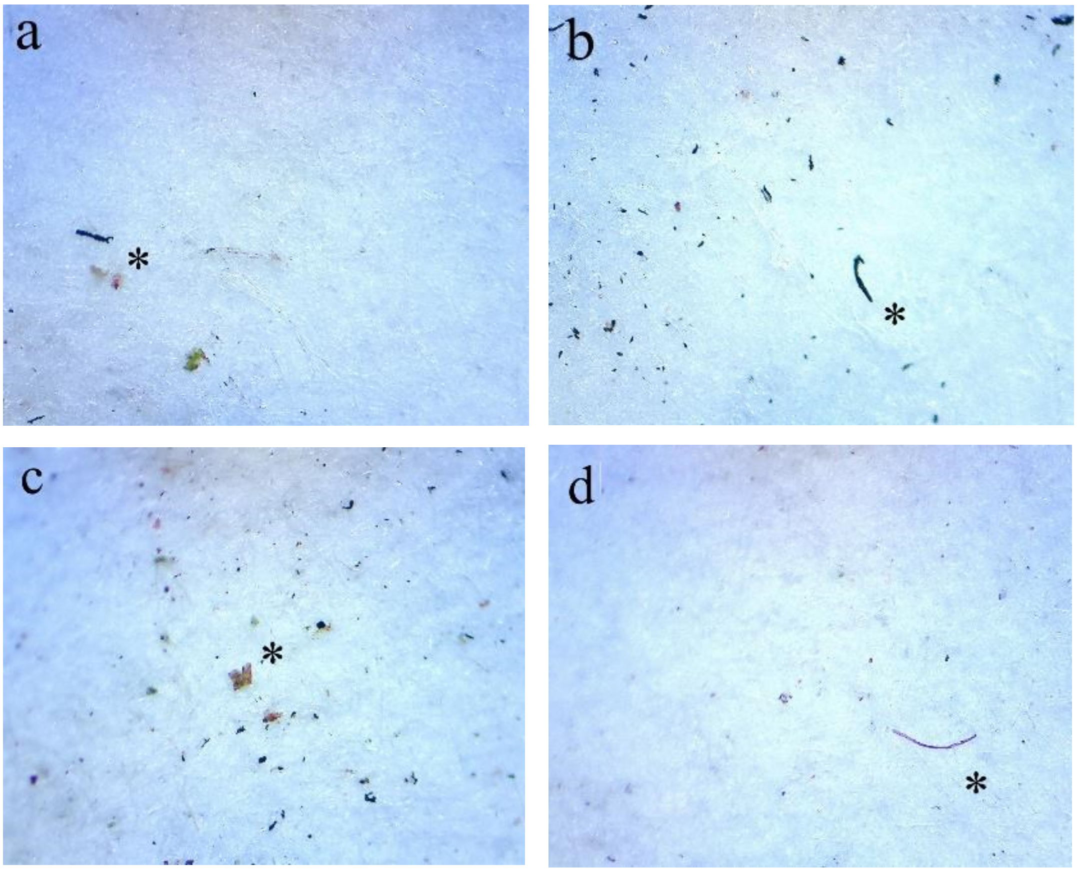


Figure 2. Images of tire wear particles (TWPs) in samples from rural roadside locations and fragments and fibres from urban parks under a stereomicroscope (tire wear particle L = 252 µm (a); tire wear particle L = 398 µm (b); black fragment L = 239 µm (c); purple fibre L = 661 µm (d); L—length, see the asterisk).

Table 1. Microplastics count (mean ± standard deviation) for total, fibre, fragment, and tire wear particles (TWP) found on *Robinia* leaflets and daily MP deposition (MPs m^{-2} d^{-1}) at ROs (rural roadside) and UPs (urban parks) locations.

Site	Total MPs (*n*)	Fibre (*n*)	Fragment (*n*)	TWP (*n*)	Mean Fibre Length (µm)	Mean TWP Length (µm)	Daily MP Deposition (MPs m^{-2} d^{-1})
RO 1	51.8 ± 1.64	3.80 ± 1.64	0.00 ± 0.00	48 ± 0.15	612 ± 370	50.03 ± 7.50	29.86 ± 0.95
RO 2	543.2 ± 2.17	4.80 ± 1.29	0.40 ± 0.55	538 ± 80.70	1073 ± 500	115.37 ± 36.14	313.14 ± 1.25
RO 3	741.4 ± 2.41	3.00 ± 2.00	0.40 ± 0.55	738 ± 110.70	1037 ± 501	176.40 ± 15.00	427.39 ± 1.39
RO 4	793.8 ± 0.45	1.80 ± 045	0.00 ± 0.00	792 ± 118.80	512 ± 337	222.75 ± 19.85	457.60 ± 0.26
RO 5	759.8 ± 2.59	3.80 ± 2.59	0.00 ± 0.00	756 ± 113.40	647 ± 414	368.05 ± 169.63	438.00 ± 1.49
UP 1	7.20 ± 3.49	7.00 ± 3.54	0.20 ± 0.45	−	881 ± 767	−	4.15 ± 2.01
UP 2	7.20 ± 3.03	6.80 ± 2.68	0.40 ± 0.89	−	1080 ± 922	−	4.15 ± 1.75
UP 3	8.00 ± 2.12	7.80 ± 1.79	0.20 ± 0.45	−	930 ± 555	−	4.61 ± 1.22
UP 4	8.80 ± 4.71	8.60 ± 4.70	0.20 ± 0.45	−	892 ± 468	−	5.07 ± 2.72
UP 5	7.40 ± 2.88	6.80 ± 3.42	0.60 ± 0.55	−	994 ± 743	−	4.27 ± 1.66

The proportion of total fibres showed a clear decrease with increasing fibre length, with shorter fibres being predominant in both the UP and RO locations; longer fibres were found only at the UP sites (Figure 3). At the ROs, the fibre length ranged from 173 to 2272 µm, while in UPs, the range was 129–4335 µm. In contrast, fragment length (Figure 4) did not show any pattern at either the RO or UP sites. The TWP size class of 150–200 µm was predominant, with a frequency distribution approaching a Gaussian curve (Figure 5), although the distribution may reflect the limits of particle identification at <150 µm.

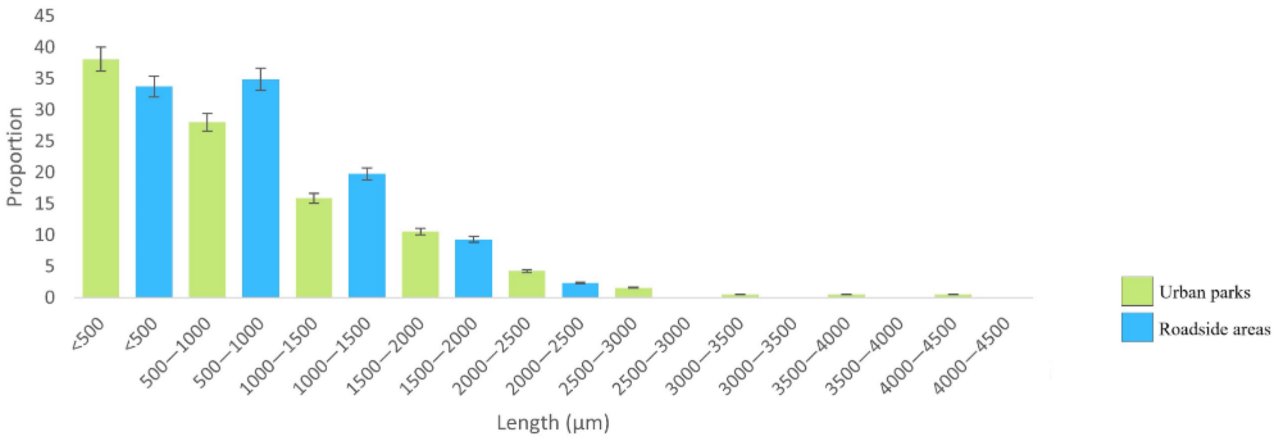


Figure 3. Distribution of microfibre lengths (µm; n = 271) in urban parks and rural roadside locations.

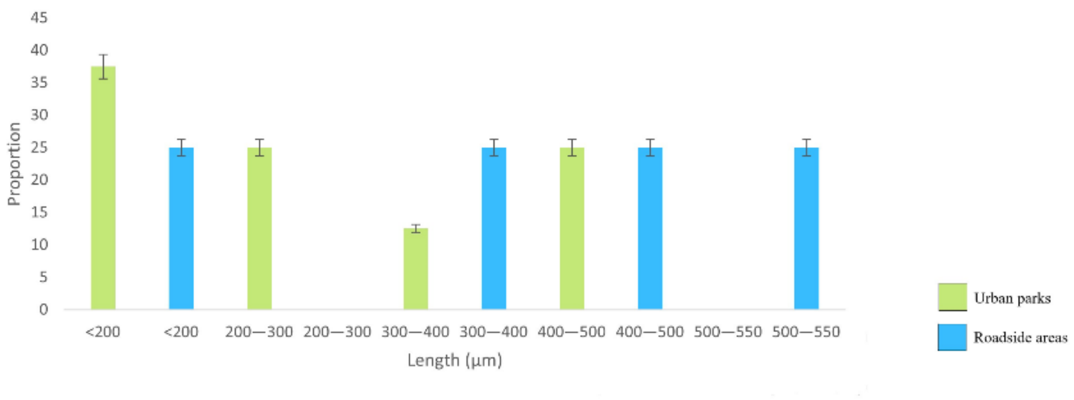


Figure 4. Distribution of fragment lengths (µm; n = 12) in urban parks and rural roadside locations.

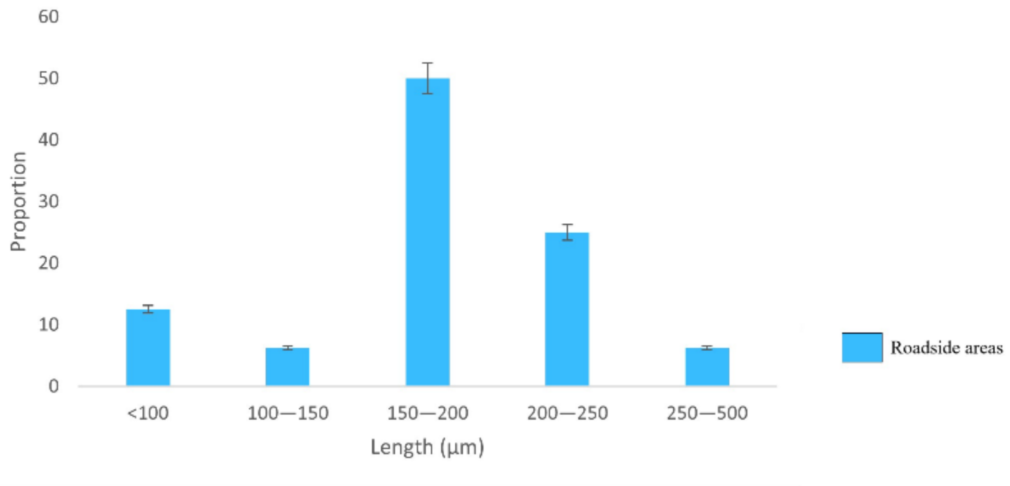


Figure 5. Distribution of tire wear particle lengths (µm; n = 2872) in rural roadside locations.

Qualitatively, at the RO locations TWP proportions (Figure 1A) were higher than polyamide (PA) and polysulfone (PES) at 80%, 15%, and 5%, respectively (Figure 6). In contrast, at the UP sites the proportion of PES was higher than PA, polyethylene terephthalate (PET) and Polyacrylonitrile (PAN), with proportions of 44%, 22%, 22%, and 11%, respectively (Figure 6).

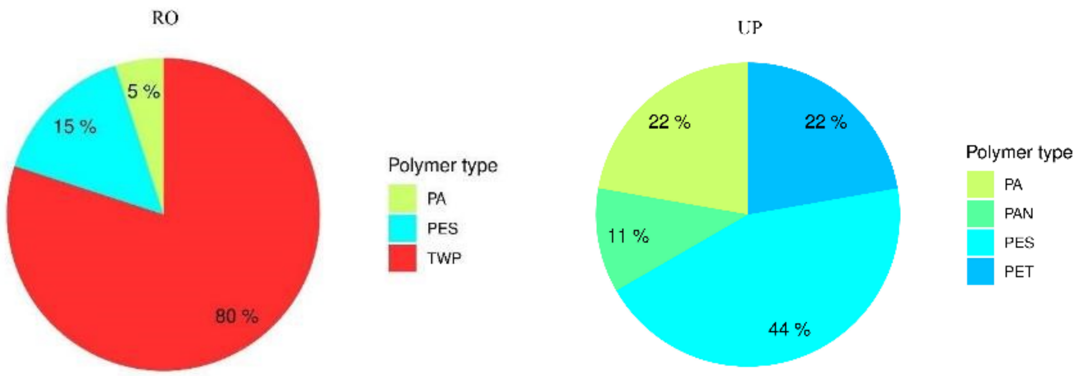


Figure 6. Proportions of polymer types (TWP—tyre wear particles; PA—polyamide; PAN—polyacrylonitrile; PES—polysulofone; PET—polyethylene terephthalate) at the RO (rural roadside) and UP (urban parks) locations.

There was no significant difference in the concentration of Fe, Al, Cu, Zn, and Cr between the RO and UP sites, while Sb concentrations were significantly different, with higher values at the ROs than in UPs; mean concentration of 0.308 ± 0.008 mg kg^{-1} and 0.054 ± 0.006 mg kg^{-1}, respectively (Table 2). Mass magnetic susceptibility was higher at the RO locations compared with the UP sites, with mean values of −0.50 ± 0.03 10^{-8} m^3 kg^{-1} in UPs and 0.62 ± 0.01 10^{-8} m kg^{-1} in ROs (Table 2).

Table 2. Concentration (mean ± standard deviation, mg kg^{-1} dw) of potentially toxic elements (Fe, Al, Cu, Zn, Ba, Cr, and Sb) and magnetic susceptibility (χ, mean ± standard deviation, 10^{-8} m^3 kg^{-1}) in leaflets of *Robinia pseudoacacia* L.

Site	Fe	Al	Cu	Zn	Cr	Sb	Ba	χ, kg
RO 1	103 ± 1	102 ± 1	8.2 ± 0.1	25.8 ± 0.6	0.6 ± 0.3	0.71 ± 0.02	17.8 ± 0.2	−0.548 ± 0.0136
RO 2	168 ± 4	117 ± 2	7.5 ± 0.3	28.2 ± 0.9	0.8 ± 0.1	0.20 ± 0.01	17.8 ± 0.1	0.670 ± 0.009
RO 3	152 ± 4	88 ± 0.4	7.6 ± 0.1	22.2 ± 1.0	0.5 ± 0.1	0.24 ± 0.002	20 ± 0.1	0.021 ± 0.0099
RO 4	181 ± 2	146 ± 3	8.1 ± 0.1	22.9 ± 0.1	0.5 ± 0.1	0.30 ± 0.01	17.7 ± 0.1	2.80 ± 0.01
RO 5	209 ± 2	162 ± 2	6.9 ± 0.1	18.8 ± 0.6	0.7 ± 0.1	0.10 ± 0.01	16.2 ± 0.1	0.155 ± 0.0096
UP 1	170 ± 3	181 ± 6	6.3 ± 0.1	14.2 ± 0.4	0.6 ± 0.1	0.10 ± 0.01	12.9 ± 0.2	−0.233 ± 0.0202
UP 2	97 ± 1	136 ± 1	9.2 ± 0.1	24.8 ± 0.5	0.3 ± 0.1	0.04 ± 0.01	7.4 ± 0.04	−0.643 ± 0.0156
UP 3	134 ± 2	92 ± 1	6.0 ± 0.2	20.6 ± 0.6	0.6 ± 0.1	0.10 ± 0.01	11.3 ± 0.1	−0.543 ± 0.0298
UP 4	87 ± 2	87 ± 1	8.0 ± 0.1	22.1 ± 0.5	0.3 ± 0.1	0.10 ± 0.01	6.4 ± 0.1	−0.555 ± 0.046
UP 5	160 ± 8	76 ± 1	7.7 ± 0.1	16.0 ± 0.5	0.4 ± 0.1	0.10 ± 0.01	33.6 ± 0.1	−0.508 ± 0.0341

4. Discussion

This study showed that the RO sites experienced a notable deposition of TWPs owing to the wearing of vehicle tires with consequent settling on nearby vegetation. At the RO sites, *Robinia* leaves were collected at 5–10 m from the roads due to the fact that this species is not native and does not penetrate deep into the local vegetation, so even at remote sites, *Robinia* trees are only available close to roads. Conversely, TWPs were not found at the UP sites, due to their central city location where traffic is very limited, thus being at a considerable distance (>500 m) from busy roads. Consequently, it can be reported that the amount of TWPs collected in atmospheric dry deposition decreased as the distance from roads increased (Järlskog et al., 2022).

In contrast, the number of plastic fibres was significantly higher at the UP sites. A likely explanation is that the UP sites are surrounded by residential areas and are frequently visited by people, especially during the summer months, while the RO sites are distant from residential areas, irrespective of their proximity to roads. Likewise, there was a greater abundance of fragments in UP sites, mirroring the pattern seen with the number of fibres, suggesting the impact of human habitation was a potential causal factor. In line with the overall MP accumulation at both sites, the daily MP deposition rate was significantly higher at the RO sites, underscoring that if MPs are present in remote locations, in cases of human habitation close to roads, there would be substantial daily exposure due to the presence of TWPs. However, when excluding traffic-related MPs, the daily deposition of MPs was greater at the UP sites, primarily owing to the higher abundance of fibres. This is consistent with a study that identified a greater daily deposition of MPs in urban parks located in Milan, Italy, compared to a remote site situated 50 km away using lichen transplants during a 3-month exposure (43–50 vs. 21–43 MPs m^{-2} d^{-1}; Jafarova et al., 2022). In another study, the daily deposition of MPs ranged from 21 to 60 MPs m^{-2} d^{-1} in three different urban locations of Southern Ontario, Canada, using moss bags as biomonitors during a 45-day exposure (Bertrim & Aherne, 2023). These studies reported a higher deposition during a shorter timeframe than our study. This suggests that plant leaves may be less efficient in capturing MPs on their surfaces, possibly serving as a temporary sink compared to lichens and mosses. Likewise, it has been suggested that higher plants act as temporary storage for microplastics, given the dynamic pattern of accumulation and loss of MPs on plant surfaces (Liu et al., 2020) (which may be enhanced in plants with a waxy cover).

Considering that Sb is a well-known indicator of non-exhaust (brake wear) traffic emissions, the higher concentrations of Sb observed at the RO sites, consistent with TWPs, can be attributed to vehicle traffic. It is noteworthy that Sb is found in the chemical content of the tire rubber (Lopez et al., 2023). However, the chemical composition of rubber particles may be influenced by factors such as tire type and vehicle specifications (Thorpe et al., 2008).

Despite their relatively low values, the mass magnetic susceptibility switched from negative values (diamagnetism) at the UP sites to positive magnetic susceptibility values for all the RO sites (up to 2.80 ± 0.013 m^3 kg^{-1}) excluding one, highlighting the modest bioaccumulation of ferromagnetic particles that are usually linked to non-exhaust metallic emissions in traffic-related contexts, as found in other studies (Winkler et al., 2020; Winkler et al., 2022).

While there was no statistically significant difference in fibre and fragment length between the UP and RO sites, it is worth noting that the longest fibres were detected at the UP sites. This suggests that fibre length was influenced by proximity to source, as the UP sites were surrounded by inhabited areas. Nonetheless, the fact that both sites primarily featured short fibres can be attributed to size fractionation during atmospheric transport of MPs, which tends to result in a reduction in fibre size with distance travelled (Jafarova et al., 2022; Jafarova et al., 2023a). Although the dimensions of TWPs are determined by many factors, including tire material, age, type of road, driving conditions, vehicle weight, etc. (Sommer et al., 2018), the almost Gaussian frequency distribution of TWP length at the RO sites is a further confirmation that the impact of vehicle traffic was only local.

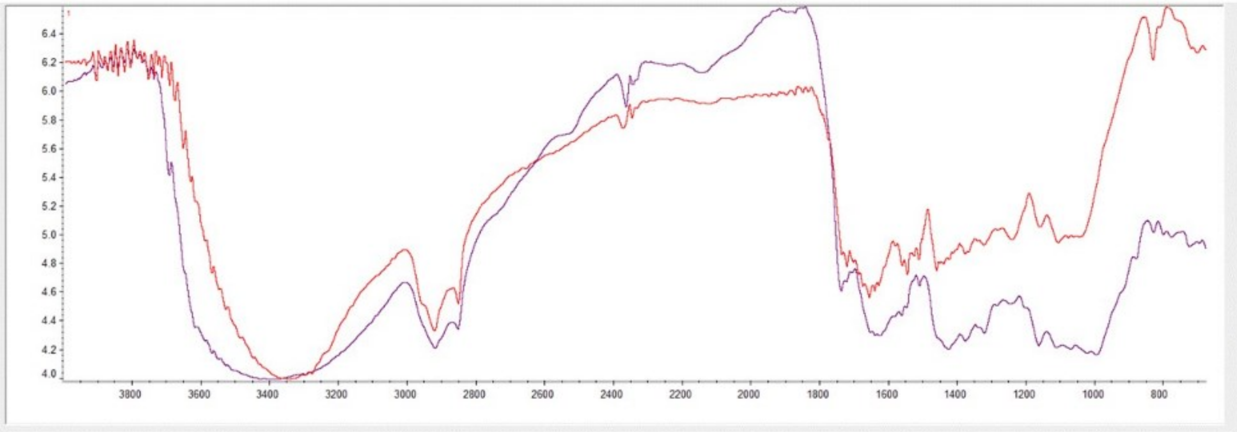
The sites at UPs had a greater diversity of polymer types, in contrast to the RO sites, which were dominated by TWPs with an average of 574.4 ± 310.4 particles per sample. The lowest number of particles was found in the analytical sample RO 1 with 48 particles, while sample RO 4 had the highest proportion of TWPs with 792 particles, which likely reflects a number of factors such as traffic density, road type, and position (e.g., road curvature) and the distance of individual trees from the road.

5. Conclusions

Despite their rural nature, the RO locations exhibited a remarkably (significantly) higher abundance of MPs compared with the UP sites located in the city centre of Siena. This disparity was due to the substantial presence of TWPs at the RO sites caused by the impact of local vehicle traffic, as suggested by chemical and magnetic analysis. In contrast, the UP sites showed higher counts of fibres, especially longer fibres, owing to their proximity to inhabited areas.

Overall, this study suggests that *Robinia*, with its widespread availability, waxy coating, and high surface-to-mass ratio, as well as ease of surface area determination for deposition rates, may serve as a valuable resource for investigating the deposition of airborne MPs, especially TWPs.

APPENDIX – CHAPTER 4



	Index	Match	Compound Name	Library Name
1	12	78.39	Tire wear	Tire wear
2	54	63.01	MONOELAIDIN	Sigma Biological Sample Library
3	19	61.10	Emcol D70-31 (melt)	Commercial Materials Polypropylene Additives
4	61	58.90	INSULIN CHAIN B. OXIDIZED FROM BO	Sigma Biological Sample Library
5	58	58.29	LIVER ACETONE POWDER BOVINE	Sigma Biological Sample Library
6	82	58.06	COLLAGENASE TYPE VII HIGH PURITY	Sigma Biological Sample Library
7	4	57.64	Polyurethane Adhesive	Cross Sections Wizard
8	66	57.05	GRAMICIDIN FROM BACILLUS BREVIS	Sigma Biological Sample Library
9	84	56.79	ARC 1004 I-1076	Commercial Materials Polypropylene Additives
10	46	56.49	Elvax 1123	Commercial Materials Polypropylene Additives

Figure 1A. FTIR spectra example of tire wear particles found during the polymer characterization using a spectral library with a match of 78.39 %.

Research article:

biology MDPI

Article

Robinia pseudoacacia L. (Black Locust) Leaflets as Biomonitors of Airborne Microplastics

Mehriban Jafarova [1], Lisa Grifoni [1,2], Monia Renzi [3], Tecla Bentivoglio [4], Serena Anselmi [4], Aldo Winkler [2], Luigi Antonello Di Lella [1], Lilla Spagnuolo [2], Julian Aherne [5,*] and Stefano Loppi [1]

***All the authors have agreed for the published article to be used as a chapter of this PhD thesis.**

Thesis Conclusion

In the light of the existing knowledge, this PhD thesis has significantly contributed to the understanding of airborne MP deposition through the use of plant biomonitors. By employing various biomonitors such as lichens, mosses, and tree leaves, this research has demonstrated the effectiveness of biomonitoring techniques in assessing airborne MP deposition across different spatial scales.

Moss (*Pesudoscleropodium purum*) emerged as a particularly suitable passive (*in situ*) biomonitor due to its structural characteristics and widespread distribution compared to lichen (*Evernia prunastri*), showing higher MP accumulation at remote sites (chapter 1). Moreover, pleurocarpous moss biomonitoring was employed at a high spatial resolution to evaluate the deposition of airborne MPs on a regional scale at 33 remote sites across the region of Tuscany, central Italy. The findings indicate that passive moss biomonitoring is a valuable tool for offering high spatial resolution assessments of atmospheric MP deposition on a regional scale, and this is the first study to provide such detailed evaluations of airborne MP deposition at this scale (chapter 2). Further, the first European-scale assessment of airborne MP deposition using moss biomonitoring at 37 remote sites across 17 countries showed that MP accumulated on moss tissue (count and mass concentration) is associated with land use, particularly in relation to proximity to population centres (chapter 3). Lastly, the investigation into TWPs using the leaves of *Robinia pseudoacacia* L. (black locust) shed light on the presence of these specific MPs in both rural and urban environments. The efficient capture of TWPs by *Robinia* leaves underscores the importance of considering vegetation type and location when selecting biomonitors for MP studies.

Overall, this PhD thesis emphasizes the pivotal role of biomonitors in assessing airborne MP deposition and underscores the need for continued research to better understand the sources, distribution, and impacts of airborne MPs on the environment and human health. The outcomes of the thesis contribute to the broader field of environmental science, guiding future efforts in addressing the global challenge of MP pollution.

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