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Atmospheric microplastic accumulation in *Ramalina celastri* (Sprengel) Krog & Swinscow Thalli: a transplant study across different levels of urbanization

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Abstract

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Lichens are widely recognized as bioindicators of atmospheric pollution, but studies on their role in monitoring microplastic (MP) deposition remain scarce. This study investigates MP accumulation in natural populations of *Ramalina celastri* across an urbanization gradient in Luján, Argentina, marking the first report for this species. MP concentrations ranged from 16.54 ± 3.59 MPs g^{-1} in baseline sites to significantly higher levels in urban zones. Fragments showed a stronger association with urbanised areas compared with fibres. Unlike larger urban centers, no significant trend was observed in MP size with urbanization, likely due to Lujan's small size, facilitating unrestricted MP movement. Comparisons with global studies revealed consistent patterns of increased MP accumulation near anthropogenic sources, while unique findings highlight the potential of *R. celastri* as a bioindicator in diverse environments. These results emphasize the influence of urbanization on MP deposition and suggest localized sources as key contributors to fragments, advancing our understanding of MP dynamics. This work underscores the need for standardized methodologies to enhance comparability in future research on terrestrial MP pollution.

Keywords

bioindicators, fragment analysis, microplastics, *Ramalina celastri*, urbanization

Introduction

Among the term microplastics (MPs) is relatively new, first mentioned in 2004 (Thomson et al., 2004). MPs have become ubiquitous in natural environments (Roblin and Aherne, 2020). These plastic residues—particles smaller than 5 mm—break down and fragment through three primary mechanisms: UV radiation, abrasion, and biological degradation (Dris et al., 2016; Peng et al., 2017). Several studies have focused on the atmospheric transport of MPs (Dris et al., 2016; Cai et al., 2017; Stanton et al., 2019; Allen et al., 2019; Wright et al., 2020). Additionally, there has been a surge in scientific research analyzing the accumulation of MPs in living organisms as bioindicators (e.g., Roblin and Aherne, 2020; Loppi et al., 2021). However, relatively few studies have investigated the accumulation or deposition of MPs in living organisms used as bioindicators (O´Brien et al., 2023). This contrasts with other anthropogenic air pollutants, for which a wide range of species has historically been employed as bioindicators (Sett and Kundu, 2016; Almeida et al., 2017; Gascon et al., 2023).

Lichens are frequently used as bioindicators of air pollution (Conti and Cecchetti, 2001; Sett and Kundu, 2016). While lichens have been employed to monitor diverse contaminants such as metals, sulfates, and nitrates, their use as bioindicators for MP accumulation or deposition remains rare (Loppi et al., 2021; Jafarova et

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al., 2022). Loppi et al. (2021) were the first to effectively document MP accumulation in lichens, particularly in the species *Flavoparmelia caperata* (L.) Hale. Their study revealed that MP accumulation correlates positively with the proximity to contamination sources, such as open-air landfills. Similarly, Javarova et al. (2022) evaluated the use of lichen transplants to measure MP deposition in urban air, using Milan (northern Italy) as a pilot area. They employed the fruticose lichen *Evernia prunastri* (L.) Ach. in four concentric zones characterized by varying land uses and observed a marked increase in MP levels in lichen thalli from the city center to the periphery. Their findings suggest that urban areas serve as sources of MPs and that urbanization intensity is closely linked to MP prevalence in the environment (Lato et al., 2021; Lloret et al., 2021).

Lichen communities are also influenced by land use (Gilbert, 1980; Stofer et al., 2006; Wolseley et al., 2006; Pinho et al., 2012). Urban, suburban, and rural areas often harbor distinct lichen communities (Szymczyk and Zalewska, 2008; Käffer et al., 2011). In the district of Luján (Buenos Aires, Argentina), García et al. (2023) conducted the first study on local lichen communities, identifying indicator species associated with three distinct land-use types: Urban, Residential, and Rural. For example, *Phaeophyscia hirsuta* (Mereschk.) Essl. was identified as an indicator species for urban areas, while *Ramalina celastri* (Sprengel) Krog & Swinscow exhibited widespread populations in rural zones (García et al., 2023). The Luján district consists of small towns embedded within an agricultural matrix, a pattern characteristic of Buenos Aires' agricultural production areas. Notably, *R. celastri* is frequently used in environmental biomonitoring projects in Argentina (Pignata et al., 2004; Estrabou et al., 2011; Mateos and González, 2016).

In Luján, there has been only one study assessing air quality using lichens as bioindicators. Gollo et al. (2024) analyzed microplastic contamination in the thalli of the lichen *Candelaria concolor* (Dicks) Arnold. Given these limitations and the growing relevance of understanding atmospheric MP contamination, this study aims to evaluate MP contamination in the Luján district across sites with varying levels of urbanization, using lichens as bioindicators.

Methods

Collection of lichen

Ramalina celastri is a fruticose lichen belonging to the order Lecanorales (family Ramalinaceae), characterized by large, flattened, and sub-pendulous thalli. According to Rodríguez et al. (2021), its colour ranges from greenish to gray, and it presents apothecia as reproductive structures, along with pseudocyphellae of various shapes. The primary substrates for this species include bark (corticolous) and rock (saxicolous). *R. celastri* is a ubiquitous species in the region (Estrabou et al., 2011) and is frequently used as a bioindicator of atmospheric pollution (Jasan et al., 2004, Pignata et al., 2024, Estrabou et al., 2011, Mateos and González, 2016).

The *R. celastri* thalli used in this study were collected from populations growing on fences within the Forest Reserve ("Arboretum") at the experimental fields of the National University of Luján, Buenos Aires, Argentina (Fig. 1). To minimize the impact on the population, only thalli within the mean ± one standard deviation of the maximum length (2.13 cm ± 1.39 cm) were collected. The mean thallus length was calculated from measurements of 100 thalli. Once collected, the thalli were placed in cellulose bags and mixed carefully to ensure homogeneity. Subsamples from this mixed sample were then used for the subsequent transplants.

Fig. 1. Study area (Luján, Buenos Aires, Argentina). The cells selected for the transplantation of R. celastri thalli can be seen. Each cell corresponds to a level of urbanization. The blue cells correspond to the urban area, the orange cells to the suburban area, the green cells to the rural area, and the lilac area corresponds to the region covered by the Arboretum (where the baseline was established). The red dot indicates the centre of the city of Luján. Each cell has a size of 500 m × 500 m.

Study area

The study was conducted in the district of Luján, located in Buenos Aires Province, Argentina (Fig. 1). This district is part of the "Pampa Ondulada" region, characterized by a temperate-humid climate with hot summers and rainfall distributed throughout the year (Bonvecchi et al., 2006).

For this study, we classified land use based on the district's *Land Use Code* (2019), defining three categories: Urban, Suburban, and Rural. These categories were spatially divided into 500 m × 500 m grid cells (Fig. 1). Three cells were randomly selected for each category to serve as transplant sites.

Sampling protocol

Within each selected cell, three trees were identified, resulting in nine trees per land use category. On each tree, a 1 g sample of *Ramalina celastri* was enclosed in cylindrical cotton mesh bags (5 cm in diameter) with a polygonal mesh size of ~2 cm per side. These bags were suspended ~2 m above ground level to prevent theft or interference from animals, particularly livestock in rural areas. The samples were left in place for 21 weeks (April 2021–September 2021).

Baseline samples

To establish baseline microplastic (MP) levels, additional *R. celastri* samples were collected from a population growing in the Forest Reserve ("Arboretum"). This 207,000 m^2 forested area, surrounded by rural zones and located ~5 km from the nearest urban area, was established 38 years ago and contains mixed tree species such as poplars (*Populus* spp.), eucalyptus (*Eucalyptus* spp.), European oaks, and chestnuts (*Castanea sativa*) (Pedreira et al., 2017). Three 1 g subsamples were collected and designated as baseline samples. Before microplastic extraction, all subsamples underwent seven consecutive washes with distilled water. The average microplastic counts from these baseline samples were subtracted from the experimental samples to distinguish contamination accumulated during exposure.

Microplastic extraction

The lichenized fungal samples were dried at 50 °C for 48 hours. Under a laminar flow hood, the samples were digested using wet peroxide oxidation (Masura et al., 2015, Herrera et al., 2018). Dry material was fragmented with a mortar to facilitate digestion. Afterward, the digested material was vacuum-filtered onto glass fiber filter papers (1.6 µm pore diameter) following Dris et al. (2016) and stained with 1 ml of ferroin (Phenanthroline Ferrous Sulphate, 0.02 N) to differentiate organic from synthetic material. Beakers used for digestion were rinsed with Milli-Q® water, which was also filtered to capture residual particles. Stained filter papers were stored in Petri dishes for further observation.

Microscopy and identification of microplastics

Microplastics on the filter papers were analyzed using a stereoscopic microscope, applying a modified five-criteria visual identification method (Roblin and Aherne, 2020, Loppi et al., 2021). Particles meeting at least two criteria were considered microplastics (Windsor et al., 2018). Additionally, a hot needle test was conducted to confirm microplastic identity (Lusher et al., 2020).

Variables analyzed included: total MPs, MPs per gram (MPs g^{-1}), fragments per gram (fragments g^{-1}), fibers per gram (fibers g^{-1}), and particle length (µm). Microplastic lengths were measured using ImageJ® software. Precautions against contamination included using non-plastic tools (e.g., metal tweezers, glass beakers) and procedural blanks, which confirmed no contamination during processing.

Recovery rate

Recovery rate (RR%) was calculated to assess microplastic extraction efficiency (Lares et al., 2019, Way et al., 2022). Ten 1 g samples of *R. celastri* were artificially enriched with 10 microplastic fragments each (e.g., Nylon, PVC, HDPE, Polyester, Polyurethane, PP). The digestion procedure was applied, and the recovered fragments were quantified.

Data analysis

Exploratory analyses were conducted for all variables, calculating means and standard deviations. Kruskal-Wallis tests with Bonferroni correction were used to evaluate differences in recovery rates. Relationships between urbanization levels and dependent variables were modelled using LOESS regression. Area categories (Urban = 1, Suburban = 2, Rural = 3) were numerically coded.

Difficulties

Unequal sample recovery occurred due to lost lichen bags in the Rural area, likely due to foraging by *Callosciurus erythraeus* (red-bellied squirrel), which was observed damaging the bags.

Results

Recovery rate

The average recovery rate (RR %) for all types of MPs was higher than 70% (Fig. 2). For five of the six polymers analyzed, RR % of more than 80% were achieved. The % RR of the PU polymer was significantly lower compared to three of the polymers analysed (Fig. 2).

Exploratory and correlation analysis

A total of 1,410 MPs were extracted, analyzed, photographed and measured. Of these MPs, 93.80% correspond-

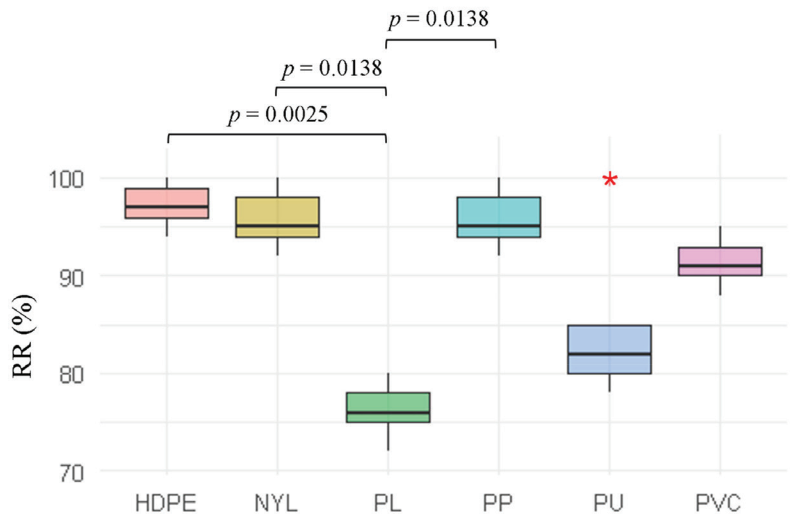

Fig. 2. Percentage recovery rates for each of the analyzed microplastic types: Nylon (NYL), Polyvinyl Chloride (PVC), High-Density Polyethylene (HDPE), Polyester (PL), Polyurethane (PU), and Polypropylene (PP). The percentage Recovery Rate (RR%) between polymers that showed significant differences are also presented through the p-value (Kruskal-Wallis test, Bonferroni correction, $p < 0.05$). The asterisk represents an outlier.

Table 1. Exploratory summary for the variables grouped by area type. The average value ± standard deviation is shown. The sample size (n) is shown for each area.

Area	Dry weight (g)	MPs g^{-1}	Fibres g^{-1}	Fragments g^{-1}	Length (µm)
Base Line n = 3	0.72 ± 0.13	16.54 ± 3.59	16.67 ± 4.97	0.45 ± 0.78	1,165.87 ± 307.32
Urban n = 9	0.84 ± 0.06	33.27 ± 14.23	29.43 ± 13.39	5.62 ± 6.09	1,247.65 ± 303.54
Suburban n = 9	0.88 ± 0.19	29.59 ± 13.74	29.77 ± 12.69	0.65 ± 0.89	1,553.75 ± 317.01
Rural n = 6	0.81 ± 0.14	53.67 ± 34.53	54.44 ± 36.12	0.44 ± 0.68	1,544.21 ± 237.81
Total	0.83 ± 0.14	34.72 ± 21.94	33.68 ± 22.40	2.24 ± 4.21	1,406.50 ± 323.59

ed to the fibre morphotype and 6.20% to the fragment morphotype. Procedural controls showed low MP counts (minimum = 0 and maximum = 6). The *R. celastri* samples that were exposed during the 21 weeks of the experiment showed a loss of dry weight of approximately 20% (Table 1). At least one microplastic particle was recorded in each of the samples (not procedural controls). The average number of fibres g^{-1} for *R. celastri* was 33.68 ± 22.40; the number of fragments g^{-1} was low (Table 1) and the average length (µm) of the microplastic particles was always greater than 1 mm (Table 1).

The results of the LOESS modelling revealed varying degrees of association between Levels of Urbanization and MPs variables. The pseudo-R^2 values ranged from 0.128 for particle length to 0.342 for fragments per gram (Fig. 3). These values indicate that, while the LOESS models provide a reasonable fit to the data, the explained variability is moderate. Crucially, the permutation test revealed that the p-values associated with the LOESS models were all significantly less than 0.05 (Fig. 3), providing strong evidence against the null hypothesis and supporting the robustness of the observed relationships, except for the variable 'length'.

Discussion

The control population of this study represents the first record of microplastic (MP) accumulation levels in natural populations of *Ramalina celastri*. Interestingly, these populations showed MP concentrations (16.54 ± 3.59 MPs g^{-1}) similar to those reported for *Evernia prunastri* in control zones by Jafarova et al. (2022), who documented 20.00 ± 4.00 MPs g^{-1} in a forested area north of Milan. At first glance, the contrasting nature of the control sites (a forest surrounded by rural zones in Luján and a mountainous area 50 km from Milan's urban center) might suggest notable differences in MP accumulation. However, the results indicate comparable levels of MP deposition. Forest ecosystems may act as natural filters, reducing atmospheric MP loads through interception by vegetation (Huang et al., 2022; Bhattacharjee et al., 2024). Supporting this, Jafarova et al. (2022) observed that urban parks in Milan had significantly fewer MPs than heavily urbanized city-center areas.

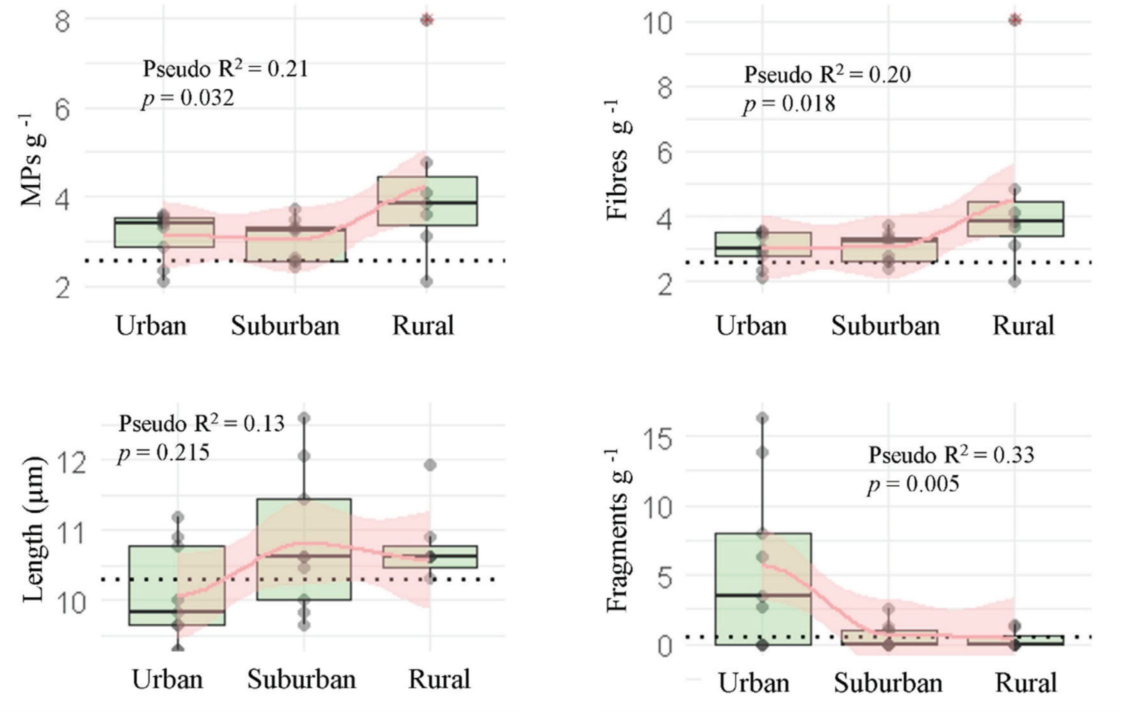

Fig. 3. Boxplot of the variables under study, ordered by urbanization level. Additionally, the corresponding LOESS model is superimposed, and the confidence intervals are displayed. Except for fragments g^{-1}, all variables were transformed (cubic root). The dotted line represents the cubic root of the mean value for each variable corresponding to the baseline samples. The values of Pseudo-R^2 and the corresponding p-value are displayed.

Our findings indicate a positive correlation between MP accumulation in *R. celastri* and urbanization levels in the Luján locality. This relationship aligns with previous studies on lichens and MP deposition. Loppi et al. (2021) documented a gradient of MP accumulation near an open-air landfill, with higher concentrations near the source. Similarly, Jafarova et al. (2022) observed an increase in MPs in lichen thalli transplanted closer to Milan's city center. In Luján, Gollo et al. (2024) reported a significant negative correlation between MP accumulation and rurality using *Candelaria concolor* as a bioindicator. By contrast, Khodabakhshloo et al. (2024) found no clear differences in MP accumulation among lichens across altitudinal transects with varying land uses in Shiraz, Iran. Meanwhile, Taurozzi et al. (2024) noted a positive relationship between MP bioaccumulation and urbanization using *Cladonia* and *Xanthoria* genera in Italy. Çobanoglu and Özen (2024) also identified higher MP loads in *Xanthoria parietina* from urbanized sites in Istanbul.

The predominant MP morphotype across these studies, including ours, is fibers, as noted in Loppi et al. (2021), Jafarova et al. (2022), and Taurozzi et al. (2024). However, our findings reveal a significant positive relationship between urbanisation and fragments g^{-1}. Conversely, fibres g^{-1} showed a distinct negative relationship with urbanisation, increasing towards rural areas. This contrasts with Jafarova et al. (2022), who reported higher fiber prevalence in Milan's urban parks. Fragments, being denser and less mobile than fibers, are often deposited closer to their sources of production, potentially explaining their association with urbanized areas (Jafarova et al., 2022).

Regarding MP size, our study does not observe a clear and significant trend in average MP length (µm) with increasing urbanization. This lack of a trend does not align with the notion that urban areas generate higher quantities of smaller plastic debris through mechanical abrasion and localized deposition (Taurozzi et al., 2024). This is likely due to the small size of Luján, which facilitates the movement, inflow, and outflow of various sizes of microplastics without substantial resistance.

Globally, urbanization-driven MP deposition in lichens has been extensively reported. For example, *Flavoparmelia caperata* (Loppi et al., 2021) and *Usnea amblyoclada* (Gollo et al., 2023) have demonstrated MP accumulation patterns tied to anthropogenic activity. Furthermore, Gomez et al. (2023) highlighted that lichen structural complexity, measured as fractal dimension, enhances MP deposition and accumulation. This supports the preference for fruticose lichens, like *R. celastri*, as bioindicators in MP studies due to their intricate structures and ease of detachment from substrates.

Potential sources of MPs in the Luján area include vehicular emissions, industrial activities, and plastic waste mismanagement. Wang et al. (2022) reported that road traffic contributes significantly to MP loads through tire wear particles, a probable source in urban and suburban sites of Luján. In rural areas, agricultural activities, such as the use of plastic mulches, may also contribute (Huang et al., 2020). Comparative studies reveal that urban centers worldwide, such as Milan, Istanbul, and Shiraz, exhibit similar MP deposition patterns, underscoring the universal impact of urbanization on atmospheric MP dispersal.

Atmospheric transport of MPs plays a critical role in their widespread deposition, with fibers often traveling longer distances than fragments. Tatssi et al. (2023) and Dris et al. (2016) demonstrated that synthetic fibers could traverse hundreds of kilometers before settling. Our findings of fibers in all land-use categories, including base line sites, align with this dispersal mechanism. Nonetheless, the dominance of fragments in urban sites suggests localized deposition from point sources, highlighting their potential as indicators of proximate contamination.

Methodological considerations

While the recovery rates (RR%) for most polymers exceeded 80%, the RR for polyethylene (PL) was notably lower at 70%. Although this remains high, it contrasts with Taurozzi et al. (2024), who reported 100% recovery for all polymers in their study but used a narrower size range (1–5 mm). Our inclusion of smaller MPs (0.1–5 mm) and six polymer types likely contributed to the observed variability. Similarly, Gollo et al. (2024) reported RR values below 100% for *C. concolor*. Future studies should standardize MP size ranges and polymer types to improve comparability.

Conclusion

In conclusion, our study highlights the utility of *R. celastri* as a bioindicator for atmospheric MP pollution. The observed patterns of MP accumulation underscore the influence of urbanization and provide a foundation for further research into the mechanisms driving MP deposition in lichens. Expanding the geographic scope and incorporating standardized methodologies will enhance our understanding of MPs in terrestrial ecosystems and their broader environmental implications.

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