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ORIGINAL PAPER

Biomonitoring of airborne microplastics and microrubbers in Shiraz, Iran, using lichens and moss

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Highlights

- Microplastics (MPs) and microrubbers (MRs) determined in lichens and mosses around Shiraz.
- In lichens, MPs mainly thin fibres up to 1 MP g^{-1}; MRs were < 0.1 MP g^{-1}.
- In mosses, abundances were similar but with a greater fraction of larger, non-fibrous particles.
- Larger MPs and MRs decreased in abundance with distance and elevation from Shiraz.
- Around Shiraz, the common moss, *Grimmia critina*, would be the most suitable biomonitor.

Abstract Lichens and mosses have been employed as biomonitors of atmospheric particulate pollutants, like metals and industrial solids, for many decades. Here, we evaluated the potential of nine species of crustose and foliose lichens and a widely distributed moss (*Grimmia critina*) to act as biomonitors of airborne microplastics (MPs) and microrubbers (MRs). About 200 lichens and 40 mosses were sampled across different altitudinal transects in the vicinity of Shiraz City, southwest Iran, and MPs and MRs were quantified and characterised after sample peroxidation. In most species of lichen, MP and MR abundance overall was < 1 g^{-1} and < 0.1 g^{-1}, respectively, and the majority of plastics were fibres of < 10 µm in diameter and < 1000 µm in length. Respective weight normalised abundances of MPs and MRs were similar in *G. critina*, but there were greater proportions of both larger (> 1000 µm) and non-fibrous particles among the MPs. In both lichens and moss, there was a greater number of larger MPs and MRs at locations closest to and at the same elevation as Shiraz than at more distant and elevated locations, suggesting an inverse relationship between particle size and distance travelled. Among the lichens, members of the genus *Acarospora*, with their areolated form, appeared to act as the most suitable biomonitors for MPs and MRs. Overall, however, the wide distribution of the moss, *G. crinita,* and its ability to intercept and accumulate a broader range of sizes and shapes of MPs and MRs make this species a better choice, at least in the type of environment studied.

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Introduction

Biomonitoring is the use of an organism to gain information about the biosphere, and either from changes in an organism's behaviour or from the concentrations of specific substances in its tissues. Mosses and lichens are widely distributed and are among the most commonly employed biomonitors of atmospheric pollution. This is because their lack of roots ensures that their nutrients are largely acquired from aerial sources and not the substratum (Garty, 2001; Wolterbeek, 2002). With high surface area to dry mass ratios that do not vary seasonally, mosses and lichens passively take up gaseous pollutants and intercept and accumulate airborne particulate matter over a period of years (Bargagli et al., 2002; Szczepaniak & Biziuk, 2003). Consequently, they have been used as general indicators of air quality and long-term integrators of trace metals, radionuclides, persistent organic pollutants, industrial particulates and magnetic particles (Augusto et al., 2013; Bargagli, 2016; Branquinho et al., 2008; Gómez et al., 2021; Loppi & Bonini, 2000; Sawidis et al., 1997).

One form of airborne contaminant that has been gaining increasing attention over the past decade is microplastics (MPs). Although MPs are conventionally defined as being < 5 mm in size, in the atmosphere they are typically much smaller and are often fibrous in nature (Dris et al., 2016; Liu et al., 2019; Roblin et al., 2020). Their aerodynamic properties also ensure that many of the finest MPs are subject to long-range (100–1000 s of km) transport (Brahney et al., 2020). Consequently, they have been detected in the atmosphere or in atmospheric deposition in regions remote from any direct sources, including the Arctic, pristine mountain ranges and plains and subtropical deserts (Abbasi et al., 2021; Allen et al., 2019; Bergmann et al., 2019; Brahney et al., 2020; Feng et al., 2020).

Recently, the concept of employing lichens and mosses as biomonitors of MPs has been addressed. Roblin and Aherne (2020) detected fibrous MPs in the mountain fern moss, *Hylocomium splendens*, sampled from three lake catchments in Ireland and used the data to estimate the annual average atmospheric deposition of plastic microfibres. Loppi et al. (2021) investigated the association of MPs with the common greenshield foliose lichen, *Flavoparmelia caperata*, in the immediate vicinity of an Italian landfill site and concluded that lichens more generally have the potential to monitor airborne MPs. A similar conclusion was reached by Jafarova et al. (2022) after transplanting the epiphytic lichen, *Everina prunastri*, from a remote area to urban and suburbanised areas of Italy for a period of three months. Capozzi et al. (2023a) transplanted the moss, *Hypnum cupressiforme*, and the foliose lichen, *Pseudevernia furfuracea*, to rural and urban environments of Campania for a period of six weeks and found that the former was more efficient at capturing microfibres because of its looser and more articulated architecture. The same species of moss was used in situ by Capozzi et al. (2023b) to measure the accumulation of MPs throughout the Campania region, with greater quantities and longer fibres found closer to urbanised areas.

Clearly, mosses and lichens are able to intercept and capture MPs but the limited number of studies to date encompass only a few species and environmental variables and have not fully considered the effects of particle characteristics. In the present study, we provide a more comprehensive investigation into the ability of a greater diversity of common crustose and foliose lichens and a widely occurring moss to act as biomonitors of both MPs and microrubbers (MRs) in a semi-arid region. We hypothesise that the capture of MPs and MRs might be affected by the distance from and elevation above a large urbanised and industrialised centre (Shiraz City, southwest Iran), and that certain species of cryptogram might act as better biomonitors than others, or capture particles of specific properties (e.g., size, shape, composition) depending on factors like their habitat and morphology.

Methods

Sampling

Samples of epilithic lichens and moss were collected from the Shiraz district of the Fars province in southwest Iran between September and October 2021. Shiraz City itself has a population of around 1.9 million distributed over an area of about 240 km^2 and is located at an altitude of about 1600 m above sea

level (ASL) on a green plain at the foot of the Zagros Mountains. The climate is moderate semi-arid, with an annual average rainfall, humidity and temperature of 335 mm, 64% and 18 °C, respectively, and the prevailing wind direction is from the northwest. The average temperature in the adjacent mountains is lower than in Shiraz but other climatic features are similar.

Sampling took place during a dry period on foot and by operators wearing cotton clothing and latex gloves. Eight altitudinal transects were sampled (T1 to T8) through a mountainous area (Mount Baba Koohi) immediately to the northeast of the city (Fig. 1). T1–T3 are near to some small centres of population and a large petrochemical unit, T4, to the northwest, faces the prevailing wind, and T5–T8 are within the urban area of Shiraz City. Transects began at about 1600 m ASL at different locations around the foot of the mountain and ended at about 1,900 to 2,100 m ASL, with lichen and moss sampled from rocky, calcareous substrates at between three and six elevations (or heights) in each transect. Samples were also collected from two control sites along a transect of Bamu Mountain, a further 10 km to the northeast that is more remote from urban and industrial influence.

At each site, and within an open and exposed area of about 1000 m^2 at a fixed altitude, the moss, *Grimmia crinita*, was collected, along with individuals or multiple samples (composites) of all species of lichen evident. The latter included one or more of the following nine species (illustrated in Fig. 2 and described in Seaward et al., 2004; Ghiyasi & Sohrabi, 2019; Sohrabi et al., 2019): *Acarospora* sp. (*A. bullata* and *A. cervina*), *Anaptychia bryorum*, *Candelariella rhodax Poelt & Vězda*, *Calogaya biatorina*, *Collema polycarpon, Dermatocarpon miniatum, Lobothallia* sp., *Placidium squamulosum* (Ach.) Breuss, *Squamarina lentigera*.

Palm-sized samples of moss gametophytes and lichen thalli were photographed and scaled with a mm-interval ruler in situ before being removed from the substrate, taking care to avoid material from the substrate itself, with a pre-cleaned (in filtered, distilled water), stainless steel spatula and stainless steel pocketknife. Using the spatula and a wooden horse-hair brush, material was carefully transferred on to a sheet of pre-cleaned aluminium foil that was

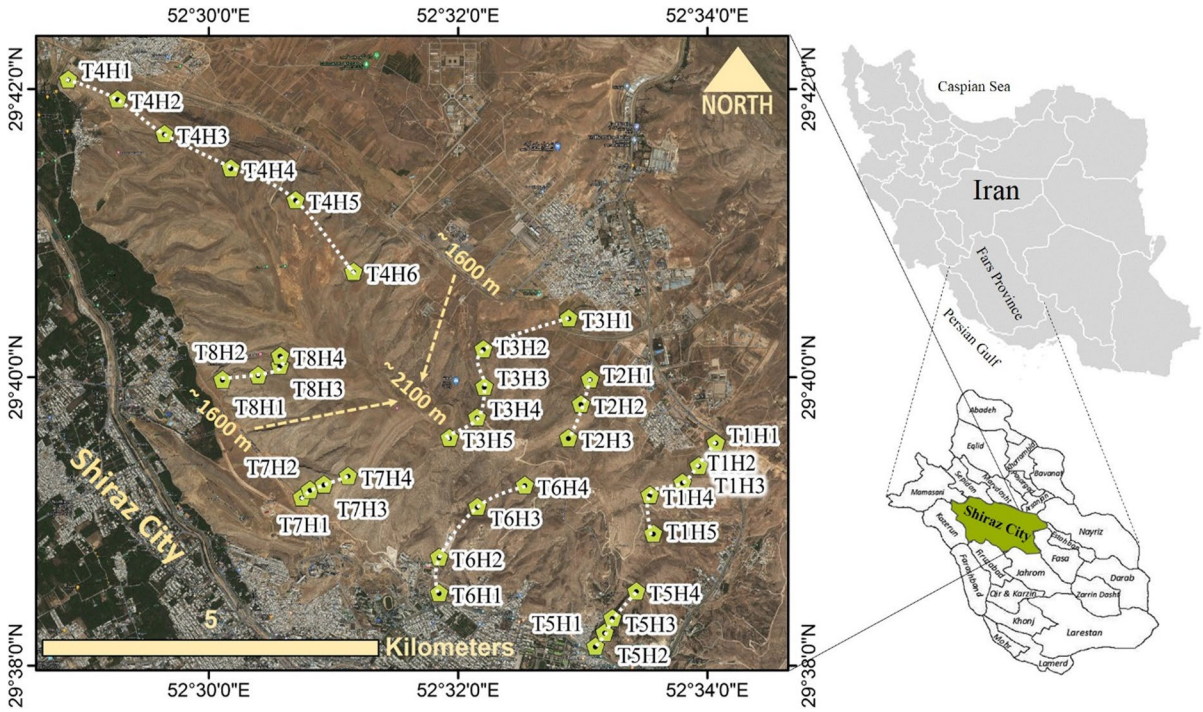

Fig. 1 Location of the eight transects (T) and different sampling sites based on height (H) on Baba Koohi Mountain

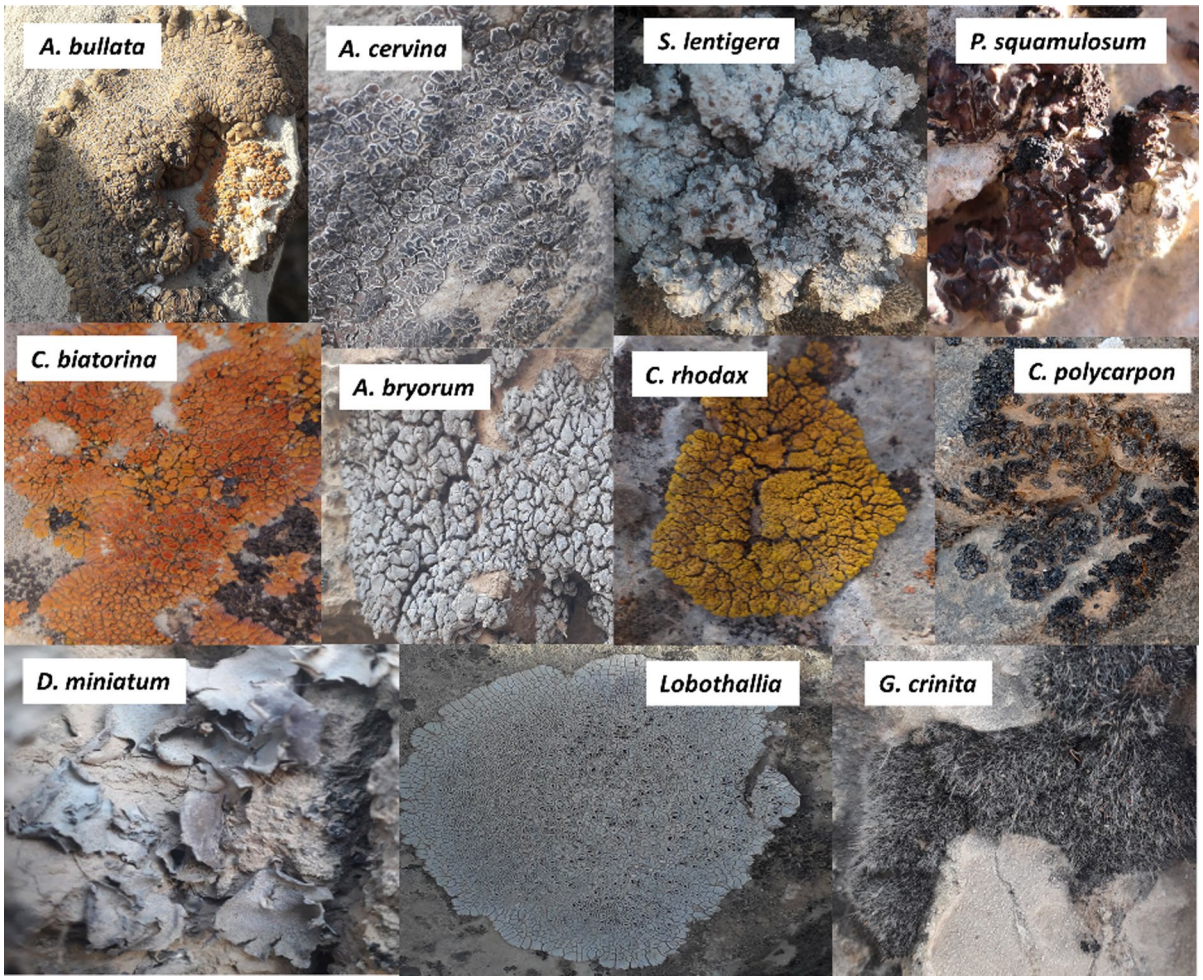

Fig. 2 Images of the different lichen species and moss sampled

subsequently wrapped around the sample, labelled and stored in a metal box. The geometric surface areas of the samples were subsequently estimated from the photographs using Digimizer image analysis software.

Sample processing

Subsequent processing of samples was undertaken in a clean laboratory where working surfaces had been wiped with ethanol, reagents had been filtered through 2 µm S&S blue band filters, glassware had been cleaned with distilled water and was wrapped in aluminium foil until use, and operators wore cotton laboratory coats (Abbasi et al., 2019). Samples were air-dried to constant weight at room temperature in a metallic cabinet for a few days before being weighed (individuals or composites up to about 35 g for lichens and 25 g for mosses) on an electronic analytical microbalance (Libror AEL-40SM; Shimadzu) and transferred to a series of pre-cleaned, elongated 100 mL glass beakers. Twenty mL of 30% hydrogen peroxide (Arman Sina, Tehran) was added to each beaker and the foil-wrapped contents were digested. Digestion proceeded at room temperature for 4 d and then at 40–50 °C for 3 d in an oven in order to evaporate any remaining liquid. Fifty mL of saturated $ZnCl_2$ solution (Arman Sina, Tehran; density 1.8 g cm^{-3}) was then added to each sample before beakers were wrapped in clean Al foil and agitated for 5 min at 350 rpm on a lateral shaker. After the contents had been allowed to settle for 24 h, remaining supernatants

were vacuum-filtered through 2 µm and rinsed with distilled water. Density separation and filtering was repeated twice more through the same filters, with filters subsequently dried at room temperature in a metal cabinet for 48 h and transferred to individual petri dishes for MP and MR identification.

Microplastic and microrubber identification and quantification

Filters arising from the lichen and moss digests were inspected using a stereo digital microscope (Sairan DSM3000) at up to 200× magnification. Commonly employed visual sorting methods and criteria were used to identify and characterise MPs and MRs according to Hidalgo-Ruz et al. (2012) and Abbasi et al. (2019). Thus, MPs were identified from their form, hardness, gloss, uniform colour and reaction to a hot needle (melting or curling) and lack of cellular or organic structures, with fibres exhibiting uniform thickness and no warping or twisting. MRs were identified by their (usually) black and non-gloss appearance, high elasticity and propensity to reversibly deform. MPs and MRs were classified according to colour as: white-transparent, yellow-orange, red-pink, blue-green or black-grey; and by shape as: fibre (with a length to diameter ratio > 10), film, fragment or spherule. With the aid of a probe and ImageJ software, MPs and MRs were classified by size in terms of length or primary diameter, L, as: $50 < L \leq 100$ µm; $100 < L \leq 250$ µm; $250 < L \leq 500$ µm; $500 < L \leq 1000$ µm; $L > 1000$ µm; and, for fibres, by diameter, d, as thin ($d \leq 10$ µm), medium ($10 < d \leq 20$ µm) and thick ($d > 20$ µm).

Three controls that were subject to digestion, separation, filtration and drying as above but in the absence of sample material returned a total of one fibre. As positive controls, triplicate samples of moss (*G. crinita*) spiked with 50 MPs of distinctive colours, shapes and polymer types were subjected to the same protocols of processing and MP identification. Recovery varied among colours and polymer types but averaged 95.4%.

In order to verify that particles identified as MPs were of plastic construction, the polymeric composition of fifteen fibres and five fragments of various colours and sizes and isolated from *G. crinita* from different locations was determined by micro-Raman spectrometry. Specifically, we used a LabRAM HR (Horiba, Japan) with a laser of 785 nm, Raman shift of 400–1800 cm^{-1} and acquisition times between 20 and 30 s, and compared Raman spectra with online, open access data hosted at Open Specy (https://openanalysis.org/openspecy/) (Cowger et al., 2021). Results revealed that all particles were thermoplastics and the dominant polymers were polyethylene terephthalate, nylon and polypropylene.

Statistics

Minitab *v*19 was employed for inferential statistics. Specifically, differences in median MP or MR abundances between transect groups were determined by signed Mann-Whitney U-tests having established non-normal data distributions (Anderson–Darling). An α-value of 0.05 was defined for statistical significance.

Results

Distribution and characteristics of lichens and moss

A total of 37 sites were sampled from the nine altitudinal transects (eight on Baba Koohi Mountain and one control). The distribution and number of epilithic lichens (or composites) sampled along each transect, and totalling 166, are shown as a function of increasing elevation in Table 1. By species, the most abundant lichen was *Acarospora* sp. ($n = 63$) and the least abundant was *D. miniatum* ($n = 2$). Transects T4 and the control exhibited the greatest diversity of lichens (all species present) while T6 was least diverse (four species present).

The mean and median dry masses of individual or composite lichens sampled were 3.72 g and 2.52 g, respectively, with a range from 0.19 to 34.33 g, and by species, median masses ranged from 0.9 g for *C. biatorina* to 10.9 g for *P. squamulosum*. Regarding geometric surface area, mean and median values for all samples were 51.4 cm^2 and 32.7 cm^2, respectively, with a range from 4.1 to 337.7 cm^2, and by species, medians ranged from 9.4 cm^2 for *C. rhodax* to 73.1 cm^2 for *S. lentigera*.

The epilithic moss, *G. crinata*, was sampled at each site ($n = 37$), with a mean and median dry mass

of 11.6 and 12.3 g, respectively, and a mass range from 2.0 to 25.2 g. Mean and median geometric surface areas were 28.9 cm^2 and 22.5 cm^2, respectively, with a range from 5.3 to 95.0 cm^2.

MPs and MRs in lichen samples

Table 1 also shows the numbers of MPs and MRs associated with the lichen samples identified. Overall, 203 MPs and 15 MRs were detected that were heterogeneously distributed among the transects, site elevations and different species. Between the transects, MPs and MRs were most abundant within T4 and T5 and least abundant (and with no MPs or MRs detected) at the control location. No significant differences in MP numbers (encompassing all heights) between transects of different land uses (urbanised versus more remote, or T5 to T8 versus T1 to T4) were established.

Table 2 summarises the number of lichens by species for the transects on Mount Baba Koohi (T1 to T8) along with the numbers of MPs and MRs for each species and numbers normalised for lichen mass and geometric surface area. Per individual or composite, MPs were most abundant and widespread in *A. bryorum*, *D. miniatum* and *S. lentigera* and were least abundant (and void of MRs) in *C. rhodax* and *C. biatorina*. On a dry mass basis, the summed number of MPs and MRs was < 0.5 g^{-1} for six species but exceeded 2 g^{-1} for *Lobothallia* sp. and *D. miniatum*. On a geometric surface area basis, the number of MPs and MRs ranged more widely among the different species and from about 0.005 cm^{-2} for *C. biatorina* to 1.15 cm^{-2} for *D. miniatum*.

The majority of MPs detected in the lichens ($n = 200$) were fibres and their distributions by colour and size are shown in Fig. 3. Thus, more than one-half of the fibres were black-grey and only two were yellow-orange, and while about 60% were ≤ 100 µm in length, all other size classifications were present. The majority of fibres detected in the largest length category (> 1000 µm) were associated with four species: *A. bryorum*, *C. polycarpon*, *S. lentigera* and *D. miniatum*; and comprised about 25% of the total number of MPs in *A. bryorum* and *D. miniatum*. About one-half of the largest MPs were also encountered along T4, the transect that faced the prevailing wind direction. About 80% of fibres detected were in the thinnest category ($d < 10$ µm) and about 3% were relatively thick ($d > 20$ µm).

The three non-fibrous MPs present were all blue-green and < 500 µm in size; specifically, a fragment in *Acarospora* sp. from T5, a film in *S. lentigera* from T1, and a film in *Acarospora* sp. from T3. Regarding the MRs, all particles were black-grey and fragmented and were encountered across all size categories but with the greatest abundance ($n = 7$) in the finest fraction.

MPs and MRs in moss samples

The numbers of MPs and MRs detected in the individual samples of moss, *G. crinita,* from each site ($n = 37$) are shown in Table 3, along with the total number for each transect normalised to both dry mass and geometric surface area. A total of 225 MPs and 22 MRs were found, and there was a greater number of both particle types at the lowest altitude ($n = 91$ and 18 for MPs and MRs, respectively) than the highest three altitudes combined ($n = 43$ and 0, respectively). Regarding individual samples, MPs were undetected in only three cases and the maximum number was 29.

Among the transects, and consistent with the lichen data, MPs and MRs were most abundant within T5 and least abundant (and with no MRs detected) at the control location. Significant differences in MP numbers (encompassing all heights) were identified statistically between transects of different land uses and aspects. Specifically, abundance was higher in T5 to T8 (urbanised) and T4 (facing the prevailing wind) than T1 to T3 (more remote regions).

On Mount Baba Koohi and Mount Bamu, the total numbers of MPs normalised to total dry mass of moss were about 0.5 g^{-1} and 0.1 g^{-1}, respectively, and normalised to total geometric surface area of moss were about 0.2 cm^{-2} and 0.03 cm^{-2}, respectively. Overall, maximum individual values normalised to weight and surface area were about 4.0 g^{-1} and 1.7 cm^{-2}, respectively, for MPs, and about 1.6 g^{-1} and 0.8 cm^{-2}, respectively, for MRs.

In contrast to the lichens, there was a significant proportion (33%) of non-fibrous particles among the MPs captured by the moss samples, with the size and colour distribution of both fibrous and non-fibrous forms shown in Fig. 4. Compared with the lichens, there was a greater abundance and more uniform distribution of fibres in size categories above 100 µm, but with similar distributions of colour (and a dominance of black-grey particles) and thickness (about

Table 1 Species of lichen sampled from each transect (T) and at different heights ASL (H), and number of MPs and, in parentheses, number of MRs detected in each species and in total

Transect/height	H1	H2	H3	H4	H5	H6	Total
T1	Ac	Ac,Ac,Ps	Ac,Ps,Sl,Sl	Ac,Cp,Ps,Ps	Ac,Ac,Ac,Ac		16
T2	Ac,Cp,Cr	Ac,Ac,Ac,Cp,Cr	Ac,Cb,Cp,S				12
T3	Ac,Ac,Ac,Cb,Cp,Cr,Sl	Ac,Cr,Sl,Sl	Ac,Cr,S1	Ac,Ac,Cp,Cr,Sl	Ab,Ac,Ac,Cp,Cr,Sl		25
T4	Ab,Ac,Ac,Ac,Cb,Cp,Cr,Dm,Lo	Ac,Ac,Ac,Cp,Cr	Ab,Ac,Cb,Cp,Cr,Lo,Sl	Ac,Ac,Ac,Cb,Cp,Cr	Ac,Ac,Cb,Cp,Cr	Ac,Ac,Cb,Cp,Cr	37
T5	Ac,Cb,Cp,Lo	Ab,Ac,Ac,Ac,Cb	Ac,Cp	Ac,Cb,Cp,Cr			15
T6	Cb	Ac,Ac,Cb,Cp	Ac,Cb,Cp,Sl	Ac,Cb,Cr			11
T7	Ac,Cb,Ps	Ab,Ac,Cb,Cp	Ab,Ac,Cp,Cr	Ab,Ac,Cp,Cr			15
T8	Ab,Ac,Ac,Cb,Ps	Ac,Cb,Cp,Sl	Ab,Cb,Cp,Sl	Ac,Ac,Cp,Cr			17
Control	Ab,Ac,Ac,Ac,Cp,Cr,Lo,Ps,Sl	Ab,Ac,Ac,Cb,Cp,Cr,Dm,Ps,Sl					18
T1	0	0,0,3	0,5,8,2	0,0,1,0	0,0,0,0		19
T2	1,0(1),0	3,2,0,0,0,	0,2,0,2				10(1)
T3	1,0,0,4,0,0,3	0,0,2,4	0,0,2	0,0,0,0,0	2(1),0,6,2,0,0		26(1)
T4	6,0,0,3,0,0,2,15(2),0	1,4,0,0,0	3,0,0,0,0,0,0	2,0,0,0,0,0	3,0,0,0,0	3,3,0,0,3	48(2)
T5	5,0,3,8(2)	4,11(3),3,2,0	1,2	1(1),1,5,1			47(6)
T6	0	0,0,0,0	2(1),0,0	0,0,5			7(1)
T7	5,0,4	2(1),3,0,0	1,0,0,1(1)	0,3,2,0			21(2)
T8	10,0,0,0,0(1)	0,0,1,4	2,0,3,5(1)	0,0,0,0			25(2)
Control	0,0,0,0,0,0,0,0,0	0,0,0,0,0,0,0,0,0					0

Note that increasing height number denotes increasing elevation with 1 about 1650–1750 m ASL (except for the control = 1850 m) and the highest number 1900–2100 m ASL. Ab = *Anaptychia bryorum*, Ac = *Acarospora* sp., Cb = *Calogaya biatorina*, Cp = *Collema polycarpon,* Cr = *Candelariella rhodax Poelt* & *Vězda*, Dm = *Dermatocarpon miniatum,* Lo = *Lobothallia* sp., Ps = *Placidium squamulosum* (Ach.) Breuss, Sl = *Squamarina lentigera*

Table 2 Total numbers of the different lichens sampled from Mount Baba Koohi (i.e., excluding the control location) and total numbers of MPs and (in parentheses) MRs by species and after normalisation to total dry sample mass and sample surface area

Species	No	No. MP(+MR)	MP(+MR)/g	MP(+MR)/cm^2
Acarospora sp.	57	65(5)	0.28(0.30)	0.017(0.019)
A. bryorum	9	33*(2)	0.97(1.03)	0.086(0.091)
C. rhodax	11	5	0.23	0.031
C. biatorina	25	7	0.31	0.0054
C. polycarpon	25	25(2)	0.39(0.42)	0.034(0.036)
D. miniatum	1	15*(2)	4.57(5.18)	1.01(1.15)
Lobothallia sp.	3	8(2)	1.86(2.31)	0.12(0.15)
P. squamulosum	5	13(1)	0.29(0.31)	0.078(0.084)
S. lentigera	12	32*(1)	0.24(0.25)	0.037(0.038)

Asterisks denote the presence of MPs in all individuals or composites of that species

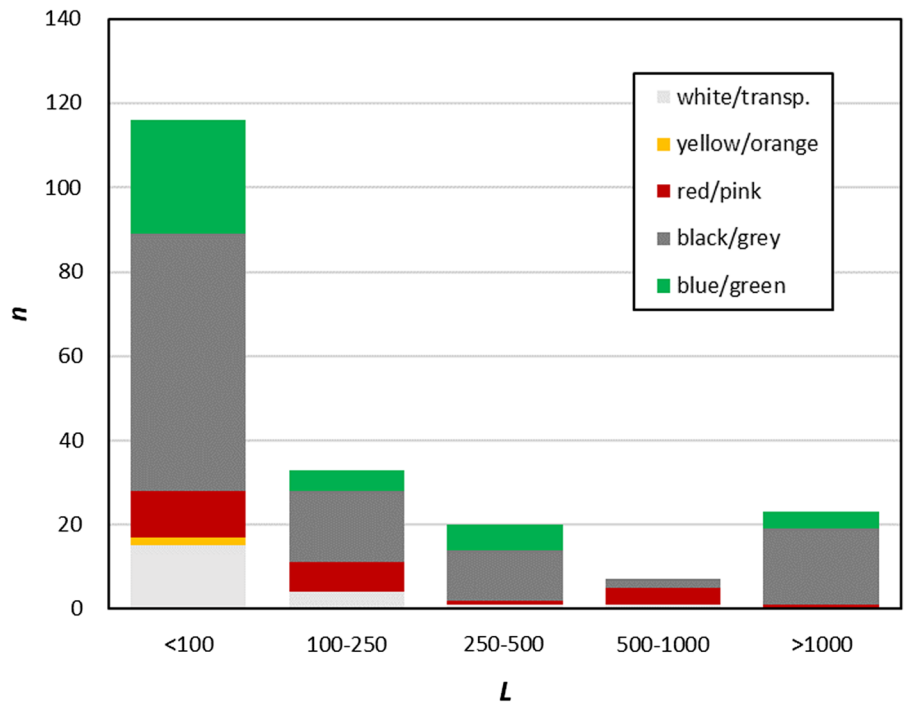

Fig. 3 Distribution of fibrous MPs in the epilithic lichens by colour and size (*L*) in µm

80% < 10 µm in diameter). Non-fibrous MPs, however, were most abundant in the largest size category (> 1000 µm) and blue-green and white-transparent were the dominant colours overall. For reference, the single MP detected at the control site was a large (> 1000 µm) white-transparent fibre.

Moss samples also captured a greater diversity of MRs. Specifically, while all particles were black, there was a roughly two to one split between numbers of fragments and fibres (although spherules were not detected), with the former represented by each size category and the latter always < 500 µm in length.

Table 3 Number of MPs and, in parentheses, number of MRs detected in the moss, *Grimmia crinita,* sampled from each transect and at different heights ASL

Transect/height	H1	H2	H3	H4	H5	H6	Total	Total/g	Total/cm^2
T1	2(1)	5	4	12	1		24(1)	0.39(0.41)	0.14(0.15)
T2	5	2	3				10	0.23(0.23)	0.15(0.15)
T3	4(2)	3(1)	2	3	1		13(3)	0.22(0.27)	0.13(0.16)
T4	16(2)	3(1)	2	2	8	0	31(3)	0.36(0.40)	0.10(0.11)
T5	29(6)	5(1)	5	3			42(7)	0.79(0.92)	0.39(0.45)
T6	6(4)	5(1)	15	8			34(5)	0.85(0.97)	0.31(0.35)
T7	7(3)	11	6	0			24(3)	0.42(0.47)	0.22(0.24)
T8	22	8	12	5			47	1.55(1.55)	0.62(0.62)
Control	1	0					1	0.10(0.10)	0.03(0.03)

Increasing height number denotes increasing elevation with 1 about 1650–1750 m ASL (except for the control = 1850 m) and the highest number about 1900–2100 m ASL. Also shown are the total number of MPs (and in parentheses, plus MRs) for each transect normalised to dry mass and geometric surface area

Distribution of largest MPs and MRs

A closer inspection of the data revealed a striking difference in the distribution of the largest (> 1000 µm) MPs and MRs between the lowest and highest elevations captured by both lichens and moss (Table 4). Thus, overall there were more large MP fibres detected in lichens at about 1600 m ASL ($n = 15$) than in those located above 1900 m ASL ($n = 2$), with the highest number encountered over T4. Regarding *G. crinita*, at the lowest elevation there was an array of particle shapes and types with a total of 65 MPs and MRs detected, but at the highest elevation only one MP fragment was found.

A similar distribution was also found for fibrous MPs based on thickness. Thus, of the four MP fibres captured by lichens whose diameters exceeded 30 µm, three were sampled from the lowest elevations of two transects, while all six fibres in this category captured by *G. crinita* were sampled from the lowest elevations of five transects.

Discussion

In our study, maximum MP (and MR) abundance was about 5 g^{-1} for *D. miniatum* but was < 1 g^{-1} for most other species. Here, transects were not close to any specific, direct sources, with many sampling sites several km from and significantly elevated relative to more diffuse inputs associated with centres of urbanisation. By contrast, Loppi et al. (2021) report MP concentrations between about 7 and 80 g^{-1} in a single, foliose species of lichen, *Flavoparmelia caperata*, in the vicinity of a landfill dumping site in Italy, with the lowest concentration and highest proportion of fibres (mainly < 1000 µm in length) and smaller fragments found at the most distant location. Jafarova et al. (2022) transplanted the epiphytic lichen, *Everina prunastri*, from a remote area of Tuscany to urban and suburban Milan, Italy, and measured MP accumulation after a period of three months. Plastics, of unknown polymeric composition, were dominated by fibres and total concentrations, ranging from about 20 to 60 g^{-1}, were used to estimate the net urban deposition of airborne MPs.

Roblin and Aherne (2020) determined the abundance of microfibres in the moss, *Hylocomium splendens*, sampled from three remote lake catchments and away from tree canopies in Ireland. An average of about 24 microfibres per g of dry moss was reported, with most < 800 µm in length, but only 13–27% of fibres were assumed to be plastic based on various identification criteria employed. Applying this range of corrections results in a MP abundance in *H. splendens* from about 3 to 6.5 g^{-1}, or an order of magnitude greater than equivalent values for most of the samples of *G. crinita* analysed in the present study (Table 3). Capozzi et al. (2023b) determined fibrous MP concentrations in the moss, *Hypnum cupressiforme*, sampled across rural and semi-natural areas of Campania, Italy, and found even higher mean concentrations (ranging from 53 to 87 g^{-1}).

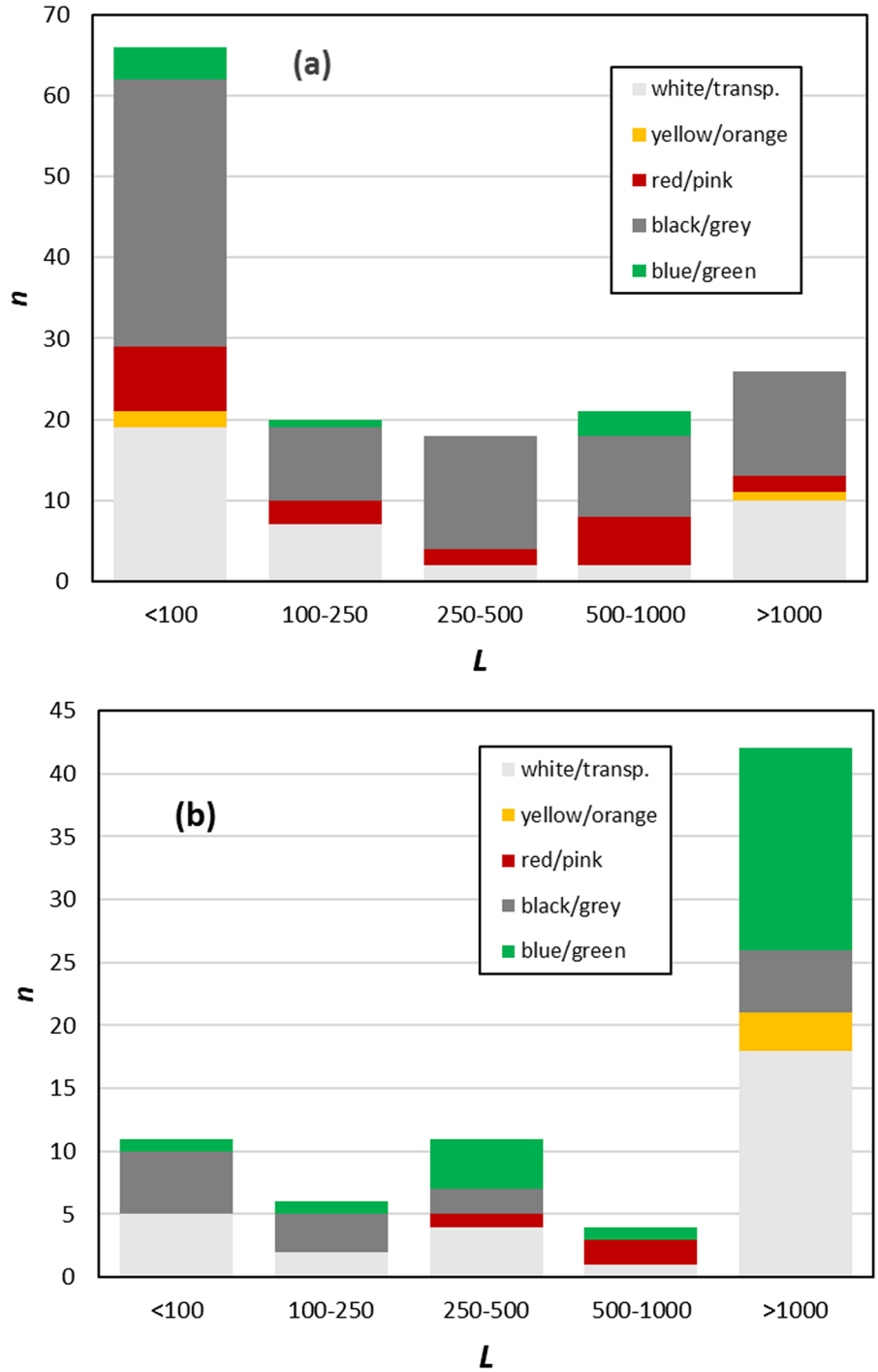

Fig. 4 Distribution of **a** fibrous MPs and **b** film and fragment MPs in the epilithic moss samples by colour and size (*L*) in µm

While the aforementioned studies demonstrate the general potential of using organisms to monitor the atmospheric deposition of MPs, significant, quantitative discrepancies with the present study are evident. These could be related to the consideration of different species of differing morphologies and

Table 4 Number of MP fibres, MP fragments and films, and MRs that were above 1000 µm in size in the epilithic lichens and moss (*G. crinita*) sampled at the lowest elevation (H1; about 1600 m ASL) and highest elevation (H_{max}; over 1900 m ASL) of each transect

	Lichens, H1	Lichens, H_{max}	Moss, H1			Moss, H_{max}		
Transect	MP fibres	MP fibres	MP fibres	MP frags/films	MRs	MP fibres	MP frags/films	MRs
T1	0	0	0	2	1	0	0	0
T2	0	1	0	3	0	0	0	0
T3	1	0	0	4	0	0	0	0
T4	8	0	2	9	0	0	0	0
T5	3	0	3	9	6	0	0	0
T6	0	1	0	0	5	0	0	0
T7	0	0	2	3	3	0	0	0
T8	3	0	7	5	0	0	1	0
Control	0	0	0	1	0	0	0	0
total	15	2	14	36	15	0	1	0

habitats, sampling from different climates, or the impacts of using different sampling, processing and identification techniques. For example, we note that *F. caperata* sampled by Loppi et al. (2021) were tree-inhabiting and particle capture by the lichen may have been enhanced by stemflow washing down the trunk (Sawidis et al., 1997).

Aside from these differences, also lacking in earlier studies is an investigation into the mechanisms by which cryptogams are able to capture and retain MPs and an evaluation of whether epilithic lichens and mosses satisfy accepted criteria as biomonitors. An understanding of the mechanisms by which lichens and mosses accumulate MPs and MRs can be gained from the extensive literature dealing with the capture of other microscopic solids, including soils, dusts, vehicular and industrial particulates, magnetic particles and pollen (Bargagli et al., 1999; Bera et al., 2012; Gómez et al., 2021; Linskens et al., 1993; Rola, 2020; Rola et al., 2016; Tretiach et al., 2011; Wu et al., 2020). Thus, lichens and mosses are likely to intercept MPs and MRs of various shapes and sizes at their surfaces both directly through atmospheric deposition and indirectly via resuspended, wind-blown dust (Abbasi et al., 2022), with the significance of each route dependent on local environmental conditions and the elevation (with respect to ground level) and inclination of the individual. Some loosely adhered material may be transient but still detected because samples were not washed in the present study, but the majority of particles will represent longer-term accumulation (Garty & Garty-Spitz, 2015). Inter-species variations are presumably the result of differences in physiology, ecological characteristics and morphology, with additional intra-species variability resulting from the heterogeneous distribution and deposition of particles and ecological variations like orientation on the substrate (Lippo et al., 1995).

Morphology is a critical characteristic that determines the ability of lichens and mosses to entrap MPs and MRs. Regarding lichens, during the formation of the cortex particles may be entrapped by the developing thallus because the upper surface is largely unsheltered (Garty & Delarea, 1987). More generally, however, the ability of lichens to entrap particles is determined by the absolute surface area (and not necessarily the measured, geometric surface area) to dry weight ratio, thallus thickness, aspect or orientation, division and extent and nature of any polygonal areolation, the development of the cortex, the incidence of surface features like cilia, pits and isidia, the ability to retain water for extended periods, and the propensity to swell and contract during wet-dry cycles (Garty, 2001; Richardson, 1995). Fine particles may also accumulate, ultimately, within the medulla where extracellular space occurs if hyphae are loosely interwoven (Dohi et al., 2021). Cushions of mosses have a very high surface area to dry mass ratio with a highly dissected surface comprising hundreds of small thin

leaflets on which particles can be trapped by various physical and electrostatic interactions (Di Palma et al., 2017; Tretiach et al., 2011). As a consequence, and more generally, mosses appear to be more effective accumulators and indicators of particulate contamination (Basile et al., 2008; Garty & Garty-Spitz, 2015), including that arising from MPs (Capozzi et al., 2023a).

Our results regarding MPs and MRs are largely consistent with the observations and characteristics of other pollutants above in terms of heterogeneity and inter-species differences. With respect to epilithic lichens, the foliose *D. miniatum*, consisting of almost circular, leathery lobes with ragged edges, is the most effective at accumulating MPs and MRs but its low regional abundance is not compatible with one of the key criteria for successful biomonitoring (Bendell et al., 2020). To this end, a more suitable biomonitor might be saxicolous members of the genus *Acarospora*, crustose lichens with an areolated and subsquamulous form. Overall, however, the abundance of the moss, *G. crinita,* and its propensity to capture a wider range of sizes and shapes of MPs and MRs make this type of bryophyte a more robust choice in the environment and climate considered. The precise ages of the organisms sampled are unknown but the literature suggests a lifespan of moss carpets and individual lichens of several years (Seaward, 2015; Wolterbeek, 2002). The integrative accumulation of MPs and MRs may, therefore, take place over a similar timescale.

Although *G. crinita* and most species of lichens exhibit the greatest accumulations of MPs and, where detected, MRs close to an urbanised district and the lowest concentrations at a control site, perhaps the most significant finding is a reduction in the number and proportion of large (> 1000 µm) MPs and MRs and the thickest fibres with increasing elevation or distance from potential regional sources. This effect has also been reported for cryptogams capturing anthropogenic dusts derived from specific industries (Branquinho et al., 2008; Garty, 2001) and MPs derived from a landfill site (Loppi et al., 2021) and is related to an inverse relationship between size and distance carried in the atmosphere. In the present study, this general relationship may be confounded by differences in particle shape and density, but it would be reasonable to assume that the largest fibrous and non-fibrous MPs and more dense MRs have local sources (for example, various industries, waste disposal, vehicle tyres) and that a large proportion of the finest (and most aerodynamic) fibres have a more distal origin. Further studies involving electron microscopy and polymer identification, for example, would be required to verify this assertion. Further research would also be required to determine if very small (< 10 µm) MPs are able to enter the intracellular environment of lichens and mosses, as has been established for fine soil and dust particles (Dohi et al., 2021), and whether intracellular accumulation could exert any adverse effects.

Conclusions

MPs and MRs have been detected among the nine species of crustose and foliose lichens and single species of moss (*G. crinita*) sampled from altitudinal transects in the vicinity of Shiraz City, Iran. Particles captured by lichens (up to about 5 g^{-1}) were mainly MP fibres and fragments of MR, with heterogeneity evident among species, transects and different altitudes. Particles captured by moss (up to about 1.5 g^{-1}) were more varied in terms of size and shape, and included non-fibrous MPs (films and fragments). Higher quantities of both MPs and MRs in the largest size category considered (> 1000 µm) were captured by lichens and moss at locations and transects closest to Shiraz and to ground level, suggesting some particle fractionation as a function of distance from the principal, urban source.

Among the lichens, those of relatively high abundance and an areolated and sub-squamulous form (*Acarospora* sp.) appear to act as the most suitable biomonitors for airborne MPs and MRs, at least in the region and climate under study. Overall, however, the wide distribution and ability to capture a greater diversity of MP and MR shapes and sizes makes *G. crinita* the most preferrable biomonitor.

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Author contributions Nafiseh Khodabakhshloo: Sampling, Lab activity, Lichenology, Data Collection, Investigation and Review. Sajjad Abbasi: Writing-original draft, Initial Idea, Methodology, Conceptualization, Investigation, Interpretation. Patryk Oleszczuk: Investigation, Review and Editing. Andrew Turner: Review and Editing/Writing-final draft, Conceptualization, Interpretation.

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Data availability Data will be available on request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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