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RESEARCH PAPER

Detection of Atmospheric Microplastics Accumulated in *Xanthoria parietina*: A Lichen Biomonitoring Study on the Asian Side of Istanbul

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Abstract
Airborne microplastics have become invisible global threats to all living organisms today. This study was designed for the first time to monitor atmospheric microplastic pollution in the city of Istanbul (Turkiye) through lichens, known as air pollution biomonitors. Epiphytic foliose lichen *Xanthoria parietina* was sampled from forested areas in 8 different districts on the Asian side of megacity, and searched for clues of microplastics through chemical characterization and microscopic examination. Twelve compounds (aldehyde, alkene, amine, carboxylic acid, ether, hydrocarbon, hydroxide, ketone, methyl, methylene, nitrogen dioxide, and sulfur dioxide) were identified as microplastic components in urban lichen samples taken from all localities with the FT-IR technique used in polymer identification. The most accumulated compound in lichen samples was amine, which is formed as a result of the chemical degradation of plastics. Building blocks of microplastic particles (MPs) such as aldehydes, carboxylic acid and methylene, as well as air pollutants such as SO_2 and NO_2 were also detected. Analysis data were supported by microscopic observations made by applying fluorescent staining method to lichen thalli and MPs were also detected visually. The highest number of MPs seen in the lichen thalli was detected in samples taken from touristic areas in Üsküdar district. Based on the results, in addition to human impact, intense atmospheric microplastic compounds identified by lichen monitoring on the Asian side of Istanbul suggest that these pollutants may have been transported from local plastic waste or industrial areas. This study shows that biomonitoring studies of airborne organic pollutants such as microplastics can be done through lichens.

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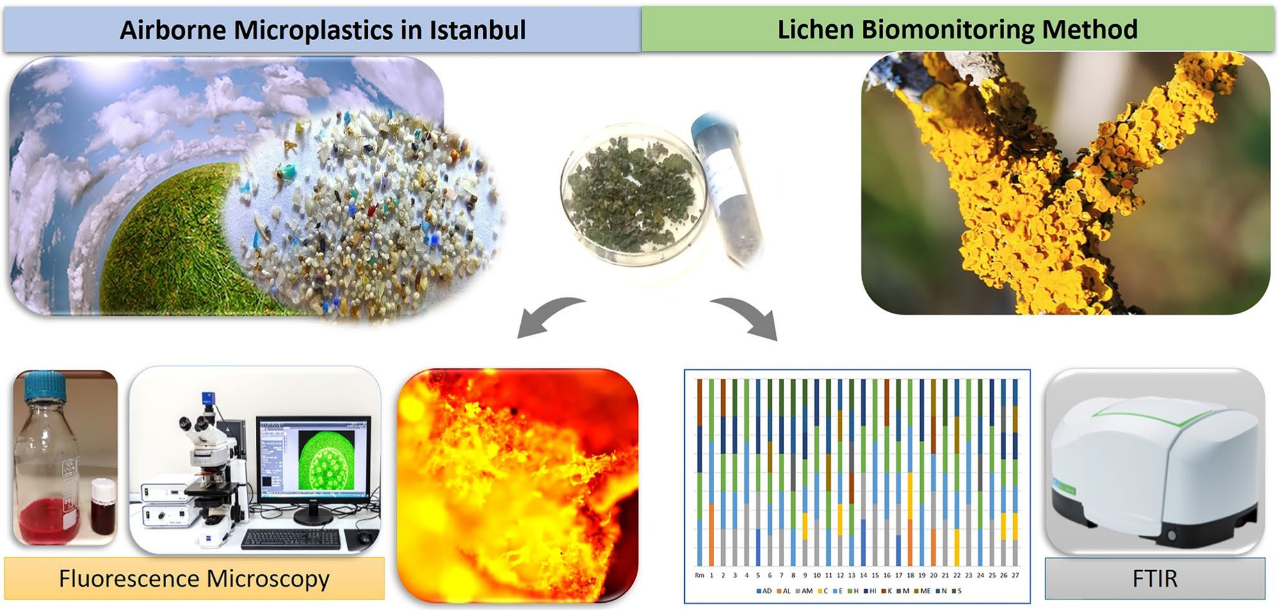
Graphical Abstract



Article Highlights

- The rapid development of industry with unsustainable technological innovations has highlighted microplastics among airborne pollutants that cause serious damage to the health of all living organisms.
- Lichen biomonitoring, a topical method for determining air quality, was applied for the first time in Istanbul metropolitan area with spectral (FT-IR) and visual (Fluorescence microscopy) analysis.
- 8 chemical compounds were found to accumulate in samples from all areas except the reference lichen.
- Microplastic particles were observed as bright yellow with the fluorescent staining technique.

Keywords Biomonitoring · Microplastic · Fluorescent staining · Lichen · Air pollution

Introduction

"Microplastic pollution", which threatens health and is harmful to all living things, has begun to attract attention on a rapidly increasing scale in recent years. Microplastics are most likely emitted into the environment at all stages of a plastic product, from production to disposal. As a result of the increase in plastic waste that last for years in nature, microplastics that break down over time in water and land lead to environmental pollution. Invisible microplastic particles (MPs), 1 µm–5 mm in size, have taken their place among other airborne pollutants today. Microbeads, a type of microplastic, are tiny pieces of fabricated polyethylene plastic added to health and beauty products. Humans are exposed to microplastics through cosmetic products containing microplastics, food, ingestion of dust indoors, and due to using plastic or painted/plastic surfaces (Smith et al. 2018).

Microplastics consist of carbon and hydrogen atoms bonded together in polymer chains (Horton et al. 2017). Microplastics, due to their lipophilic properties, contain various persistent organic contaminants (POPs) such as dichlorodiphenyltrichloroethane (DDT), polycyclic aromatic hydrocarbons (PAH), polybrominated diphenyl ether (PBDE), polychlorinated biphenyls (PCB), organochlorine pesticides, and hormone blockers on their surfaces (Silva et al. 2018). It can be mixed with the soil as a result of irrigation with treated wastewater and fertilizing applications containing microplastics. It is stated that exposure to microplastics in plants significantly affects growth, yield characteristics, and soil properties (Dhevagi et al. 2024).

Although studies on microplastic pollution are mostly concentrated in the aquatic environment, recent research has also begun to focus on the biomonitoring of airborne microplastics (Klein and Fischer 2019; Zhang et al. 2020). The degradation patterns of microplastics in the air are shown similar to those in the aquatic environment, which means that the microplastics in the air can be transported to water by atmospheric deposition (Cai et al. 2017). Airborne microplastics irritate the respiratory system and cause inflammation, as well as respiratory symptoms and even serious health problems for people such as cancer (Prata 2018). Studies conducted with animal models to examine the effects of MPs on living things reveal potential health effects such as transport of MPs to the brain, causing neurotoxic effects and increased pulmonary inflammation (Campanale et al. 2020). In addition, toxic plastic additives circulating together with microplastics can harm human health (Eberhard et al. 2024). For this reason, it is of great importance to detect MP in the air. Microplastic levels to which humans are exposed by consumption of wild mussels (*Mytilus edulis*) have been shown to be lower than those taken through house dust fibers during meals (Catarino et al. 2018). Microplastic pollution in deposited urban dust in Tehran metropolis, Iran was investigated, and street dust has been found to be a potentially important source of microplastic contamination in the urban environment (Dehghani et al. 2017).

Among the most commonly reported polymers in the aerosol are polyethylene (PE), polyether sulfone (PES), polyacrylonitrile (PAN), polyamide (PA), polyethylene terephthalate (PET), polystyrene (PS), polypropylene (PP), polyvinyl chloride (PVC), acrylic (AC), polycarbonate (PC), polyacrylic acid (PAA), and ethylene–vinyl acetate (EVA) (Habibi et al. 2022). Today, in microplastic pollution research, airborne MPs are determined by optical microscope, electron microscope, Raman spectroscopy, Fourier transform infrared spectroscopy (FT-IR), and biomonitoring methods (Silva et al. 2018; Zhang et al. 2020).

There are processes and activities that allow characterizing and monitoring the quality of the environment using biological species (Harner et al. 2006). Biomonitoring is a technique using a species of bioindicator animal (snail; Baroudi et al. 2020, *Drosophila melanogaster*; Rudenko et al. 2020, bee; Al-Alam et al. 2019), plant (pine needles; Pavlović et al. 2017), fungus (Wu et al. 2021), moss (Capozzi et al. 2020; Roblin and Aherne 2020), or lichen (Çobanoğlu 2015; Klos et al. 2018). It is well known that lichens, as natural biosensors, are among the best bioindicators for monitoring atmospheric accumulation of various air pollutants. The most important biological reason for this is that the lichen thallus, which does not have a protective layer, inevitably accumulates pollutants in the air. In addition, lichens can be easily monitored since they are spread in almost every rural and urban region on earth (Çobanoğlu Özyiğitoğlu 2020; Garty 2001; Shukla et al. 2019).

Lichens are bioindicator organisms that can also detect atmospheric PAHs (Van der Wat and Forbes 2019). It has been reported that lichens (*Pseudevernia furfuracea*) can accumulate more PAHs (*Hypnum cupressiforme*) than mosses due to the structural feature of the thallus (Capozzi et al. 2020). In addition, POPs released into the atmosphere through anthropogenic activities (especially diesel motor vehicles and industrial activities) pose a risk to the environment due to their long-range transport potential and their persistence. Indeed, Shukla et al. (2019) reported that high altitude ecosystems, particularly the Himalayas with rich biodiversity, are affected by long-distance transport of these pollutants.

There are numerous studies using lichens as a biological tool to detect levels of air pollutants such as trace elements, heavy metals, and some organic pollutants, but lichen biomonitoring studies focusing directly on airborne MPs are very limited and fairly new. The study by Loppi et al. (2021), in which the amount of MP (fibers and fragments) in the air monitored with the lichen (*Flavoparmelia caperata*) in the Italian region, is the first study on this subject. The results of this recent work clearly showed that lichens can be used effectively to monitor microplastic accumulation. In a very recent publication, Jafarova et al. (2022) used lichen transplants to monitor airborne microplastics in urban areas of Milan in northern Italy.

Air pollution in *Istanbul*, which is our study area, has been one of the most important environmental problems since the 1980s. While anthropogenic emissions were mostly from domestic heating and industrial sources at that time, today the most important anthropogenic sources are industry, as well as vehicle traffic and dust (Incecik and Im 2013). Maritime transportation is also seen as one of the sources of air pollution in Istanbul, with prominent pollutants such as SO_2 and NO_x (Doğrul et al. (2016). The sources of important pollutants such as PM_{10}, SO_2, CO, and NO_x determined by direct air measurement in the study conducted by Kara et al. (2014) in the Istanbul region are industrial, vehicle, and domestic sources. Atmospheric pollution in Istanbul has been investigated in terms of heavy metals (Içel and Çobanoğlu 2009; Kurnaz and Çobanoğlu 2017; Özkök and Çobanoğlu 2023) by lichen monitoring studies; however, there are no studies on organic pollutants and microplastics.

This study aimed to investigate airborne microplastics in the Istanbul metropolis using the lichen biomonitoring method and was carried out for the first time in Turkey. Samples of *Xanthoria parietina* (L.) Th. Fr., known as an air pollution bioindicator and growing naturally in forest areas on the Asian side, were examined microscopically and chemically to detect MPs in the air. It was also aimed to determine which organic compounds derived from MPs accumulate in lichens. Our study was designed to provide data on the air quality of the megacity on the level of microplastic components deposited in the lichen thalli. It is important to determine and periodically monitor the level of microplastics in the city atmosphere, which are increasingly considered among the air pollutants that threaten health in recent years.

Materials and Methods

Study Area

Istanbul is located at the intersection of the continents of Europe and Asia at the coordinates of 28°01′ and 29°55′ East longitudes and 41°33′ and 40°28′ North latitudes. Compared to the European side, which is dense with tourism and business centers, the Asian side is richer in terms of residential areas and green areas. The annual average temperature in Istanbul is 13.7 °C. Its climate is in the subtropical high-pressure zone and the cold-temperate low-pressure zone or on the border of continental (dry), humid, and rainy westerly winds. The prevailing wind blows from the north and mostly affects the southern parts of the region (TSMS 2021). Since microplastics in the air can be transported by wind, it is important to examine these wind systems in air pollution assessment in Istanbul. Istanbul is among the cities with the highest population density in the world. With the increase in population, the number of motor vehicles in the city is increasing day by day (TÜİK 2021).

The Asian side of Istanbul province, which is generally intertwined with industrial areas and urban settlements, is the study area. The northeastern parts of the city are forested, and the western parts are mostly industrial areas. In addition to many production facilities and 17 small industrial zones on the Asian side, there are 6 large organized industrial zones in Ümraniye (1) and Tuzla (5) districts. According to the sectoral distribution, clothing comes first with 15.59%, metal products come second with 12.51%, and machinery and equipment comes third with 10.62% (IPIS 2018).

Table 1 Location information of the sampling sites

Site#	District	Collection site—Habitat	Geographic coordinates	Elevation (m)
1	Kartal	Aydos forest	40°56′38.4″N–29°12′58.7″E	272
2	Kartal	Aydos Gülbahar Sultan countryside	40°56′40.2″N–29°13′13.1″E	272
3	Kartal	Aydos lake picnic area	40°57′24.8″N–29°13′36.5″E	232
4	Maltepe	Başıbüyük promenade area	40°57′34.6″N–29°10′04.4″E	268
5	Maltepe	Başıbüyük forest	40°57′37.4″N–29°10′04.4″E	272
6	Ataşehir	Kayışdağı forest	40°58′35.8″N–29°09′00.4″E	210
7	Adalar	Büyükada Aşıklar garden	40°51′37.1″N–29°07′01.6″E	66
8	Adalar	Büyükada Aşıklar Hill	40°51′33.8″N–29°07′07.0″E	82
9	Beykoz	Kanlıca forest	41°05′42.7″N–29°04′17.8″E	125
10	Beykoz	Kanlıca forest	41°05′42.7″N–29°04′17.8″E	125
11	Beykoz	Göztepe Nature Park	41°05′40.9″N–29°06′48.6″E	258
12	Beykoz	Göztepe Nature Park	41°05′42.7″N–29°06′49.0″E	280
13	Beykoz	Göztepe Nature Park promenade area	41°05′43.1″N–29°06′48.6″E	256
14	Beykoz	Mihrabat grove	41°05′42.7″N–29°04′17.8″E	125
15	Beykoz	Elmasburnu Nature Park, Riva	41°13′39.7″N–29°13′06.6″E	67
16	Beykoz	Elmasburnu Nature Park, forest	41°13′39.7″N–29°13′06.6″E	67
17	Şile	Hacı Kasım Pier	41°10′54.1″N–29°36′23.8″E	31
18	Şile	Atatürk forest	41°09′49.0″N–29°38′20.0″E	126
19	Şile	Serintepe picnic area	41°10′09.1″N–29°36′01.1″E	111
20	Üsküdar	Küçük Çamlıca Hill, grove	41°01′10.6″N–29°03′58.7″E	195
21	Üsküdar	Küçük Çamlıca Hill, grove	41°01′10.9″N–29°03′53.6″E	206
22	Üsküdar	K. Çamlıca Su Köşkü, grove	41°01′05.5″N–29°03′59.0″E	237
23	Üsküdar	K. Çamlıca Su Köşkü, grove	41°01′05.2″N–29°03′58.3″E	240
24	Üsküdar	Büyük Çamlıca Hill, grove	41°01′37.6″N–29°04′09.8″E	304
25	Pendik	Dumlupınar Gözdağı Hill	40°53′25.0″N–29°15′17.5″E	164
26	Pendik	Gözdağı grove	40°53′26.0″N–29°15′17.6″E	179
27	Pendik	Gözdağı Social Facility grove	40°53′29.2″N–29°15′07.0″E	162

Biomonitoring and Sampling Procedure

The epiphytic rosette-like foliose cosmopolitan lichen *X. parietina* (L.) Th.Fr. was preferred as a biomonitoring organism due to its high tolerance to atmospheric pollution and its widespread presence in both urban and forested areas in the study area. The anatomy of the upper cortex of the *X. parietina* is porous, which allows airborne elements to accumulate. This species is quite common in temperate regions of Eurasia and North America, often colonizing nitrogen-rich environments with high sunlight (Fortuna et al. 2021). Our samples were mostly taken from the trunk and branches of deciduous trees.

A total of 27 sampling sites were identified in forest areas situated in 8 different districts (Adalar, Ataşehir, Beykoz, Kartal, Maltepe, Pendik, Şile and Üsküdar) in the Asian side of Istanbul were determined. Detailed location information for each site were given in the Table 1. Lichen samples collected from urban areas in the period of 4.4.2019–7.10.2019 were taken from the trunks and branches of trees at least 1 m above the ground in order to prevent contamination from the soil, in accordance with the purpose of the biomonitoring study (Loppi et al. 2021). During the sampling, their positions were recorded with the GPS device.

Analysis Procedure

Two types of analyses, spectroscopic and microscopic, were performed to determine the chemical composition of airborne MP accumulated in the thalli of lichen samples. In the pre-analysis preparation phase, the collected lichen samples of *X. parietina* were meticulously separated from their substrate with razors and then re-examined under a stereo microscope to remove smaller contaminated materials and bark pieces.

Commercially available IAEA-336 lichen (*Evernia prunastri*) introduced as standard reference material by the International Atomic Energy Agency was obtained for use in the same analysis (Heller-Zeisler et al. 1999). In fact, no valid control reference substance for microplastics or POPs is known. For this reason, this lichen reference material, which is considered clean according to its element values, was used in this study in both chemical and in microscopic determination.

Spectral Analysis

As a chemical characterization and mapping tool for microparticle number and size, FT-IR determines which wavelengths are absorbed by particles (below 500 µm), creating a spectrum that shows their molecular components (Zhang et al. 2020; Silva et al. 2022). For quantification of organic compound types found in microplastics, Fourier transform infrared micro-spectroscopy (FT-IR) analysis was performed on lichen samples and the reference material, which were powdered and weighed in sterile falcon tubes. The analysis was made in ATR–FTIR device (Perkin Elmer FT-IR Spectrometer USA) in Sakarya University Faculty of Arts and Sciences Department of Chemistry with 0.5–1–2–4–8–16 cm^{-1} resolution and working range of 400–4000 cm^{-1} (×3). The resulting spectra were compared to KnowItAll IR (2023), available at https://sciencesolutions.wiley.com, spectral library.

To separate the organic compounds other than microplastics in the lichen thalli, 1 g lichen samples for each site were digested using a wet peroxide oxidation method (Loppi et al. 2021; Roblin and Aherne 2020). The lichen specimens were washed three times with distilled water and dried at room temperature to remove any external source of contamination such as pollen, dead insects, spider webs. Afterwards, it was placed in a 60 °C oven in glass petri dishes and incubated for 24 h. The samples, which were determined to have lost their moisture completely, were taken out of the oven and ground in a sterile ceramic mortar until they turned into powder (Augusto et al. 2010).

Visual Analysis

Fluorescence staining techniques were applied for visualization of microplastic components in the lichen samples. At total of 54 lichen samples (27 sampling sites with duplet samples) were investigated individually under fluorescence microscope. Detection of MPs was adapted following methodology in the references of Dowara et al. (2020), Maes et al. (2017), Maxwell et al. (2020), Shim et al. (2016).

Nile red is a fluorescent dye that is water soluble and helps detect hydrophobic particles and is used for microscopic imaging of MP (Zhou et al. 2022). Microplastics including polyethylene, polycarbonate, polyurethane, polyvinyl acetate, polypropylene, polystyrene, and nylon particles are successfully identified by strongly binding with this fluorescent dye (Lv et al. 2019). For the 5 µg/mL Nile Red fluorescent dye, a solution containing 1 mg/mL acetone was prepared (Maes et al. 2017). The thallus surface and anatomical sections of the freshly dried lichen samples as well as reference lichen material were stained with fluorescent dye. After waiting for 30 min, they were examined and visualized under a fluorescence microscope. Nile red-stained particles detected on thallus surface and sections of the samples were photographed. Samples were examined with a Leica DM LB2 fluorescence microscope, equipped with a 420–495 nm excitation filter and a 590 nm barrier filter for ethidium bromide.

Table 2 Chemical compounds detected in lichen samples based on FT-IR spectroscopy results

	Rm	Chemical components											
		AD	AL	AM	C	E	H	HI	K	M	ME	N	S
						*	*	*	*				
Xanthoria parietina samples	**1**		*			*	*						
	2			*		*	*	*	*				
	3			*		*	*	*					*
	4			*		*	*						
	5	*		*			*	*				*	
	6			*		*	*				*	*	*
	7			*		*	*	*					*
	8					*	*			*		*	*
	9			*	*	*	*	*				*	*
	10			*		*	*	*					
	11					*	*				*	*	*
	12			*	*	*	*				*	*	
	13					*	*		*	*		*	*
	14	*		*			*	*					
	15			*		*	*						
	16			*		*	*		*				
	17	*		*		*	*					*	*
	18		*		*	*	*						
	19					*	*	*					
	20		*	*		*			*		*		
	21			*		*	*						*
	22				*	*	*				*	*	
	23			*		*	*						
	24					*	*			*		*	*
	25			*		*	*	*					
	26			*	*	*	*	*		*		*	
	27			*	*	*	*	*			*	*	

Compounds matching Rm are shown in bold

Rm Reference material (IAEA-336), *AD* Aldehyde, R – CHO, *AL* Alkene, C = C, *AM* Amine, NH_2 – OH, *C* Carboxylic acid, C–OH, *E* Ether, R–O–R', *H* Hydrocarbon, CH, *HI* Hydroxide, OH, *K* Ketone, R – CO – R, *M* Methyl, CH_3, *ME* Methylene, CH_2, *N* Nitrogen dioxide, NO_2, *S* Sulfur dioxide, SO_2

Results and Discussion

In the current study, analytical and microscopic techniques were applied together to detect the presence of microplastics in lichen thalli, which have biomonitoring properties that accumulate airborne pollutants, and the results are shown in graphics, maps, and tables.

Spectral Analysis

Traces of microplastics in the air were searched in the thallus of lichen *X. parietina* samples collected from forest habitats on the Asian side of Istanbul. For this purpose, the detection and chemical characterization of organic compounds that may be components of airborne MP components were performed using FT-IR analysis. When the final data obtained from FT-IR spectroscopy are interpreted, a total of 12 compounds were determined in the chemical compositions of the samples determined; aldehyde, alkene, amine, carboxylic acid, ether, hydrocarbon, hydroxide, ketone, methyl, methylene, nitrogen dioxide, and sulfur dioxide (Table 2). Distribution of the compounds in control material (Fig. 1) and in the 27 lichen samples was graphically indicated (Fig. 2).

As a result of FT-IR analysis, the compounds detected in the reference material (Rm) are ether, hydrocarbon, hydroxide, and ketone. It is thought that compounds detected different from the Rm content in lichen samples taken from the Istanbul region (amine, aldehyde, alkene, carboxylic acid, methyl, methylene, NO_2, and SO_2) may be caused by airborne pollutants. Among the compounds

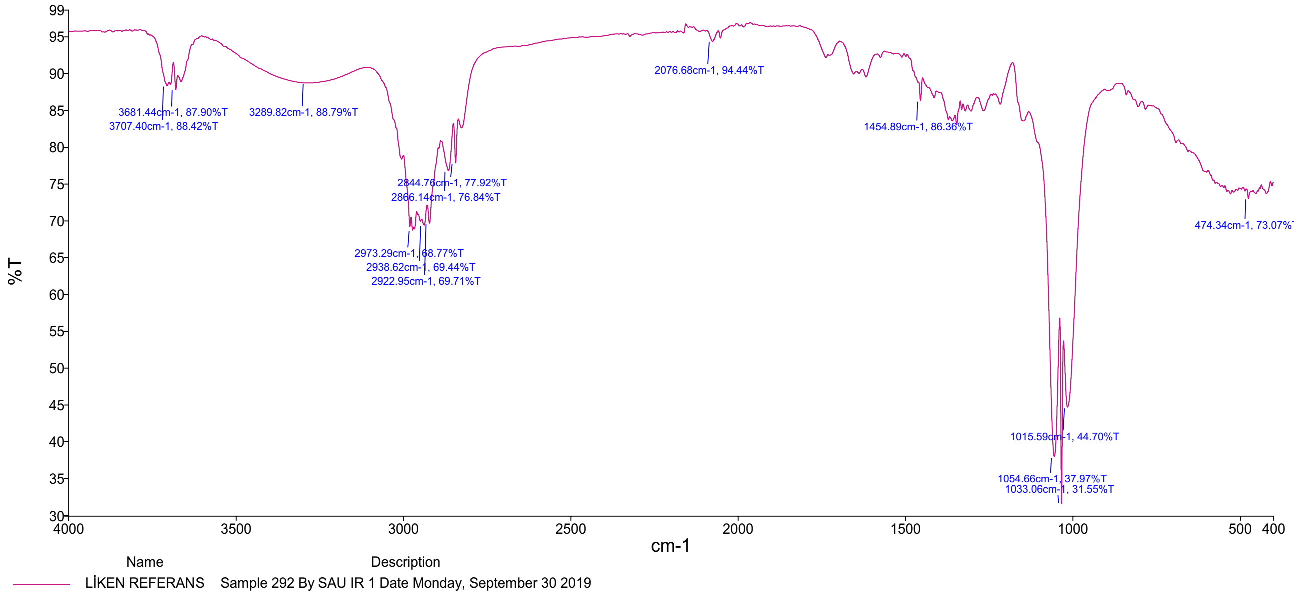


Fig. 1 FT-IR data in control reference lichen IAEA-336

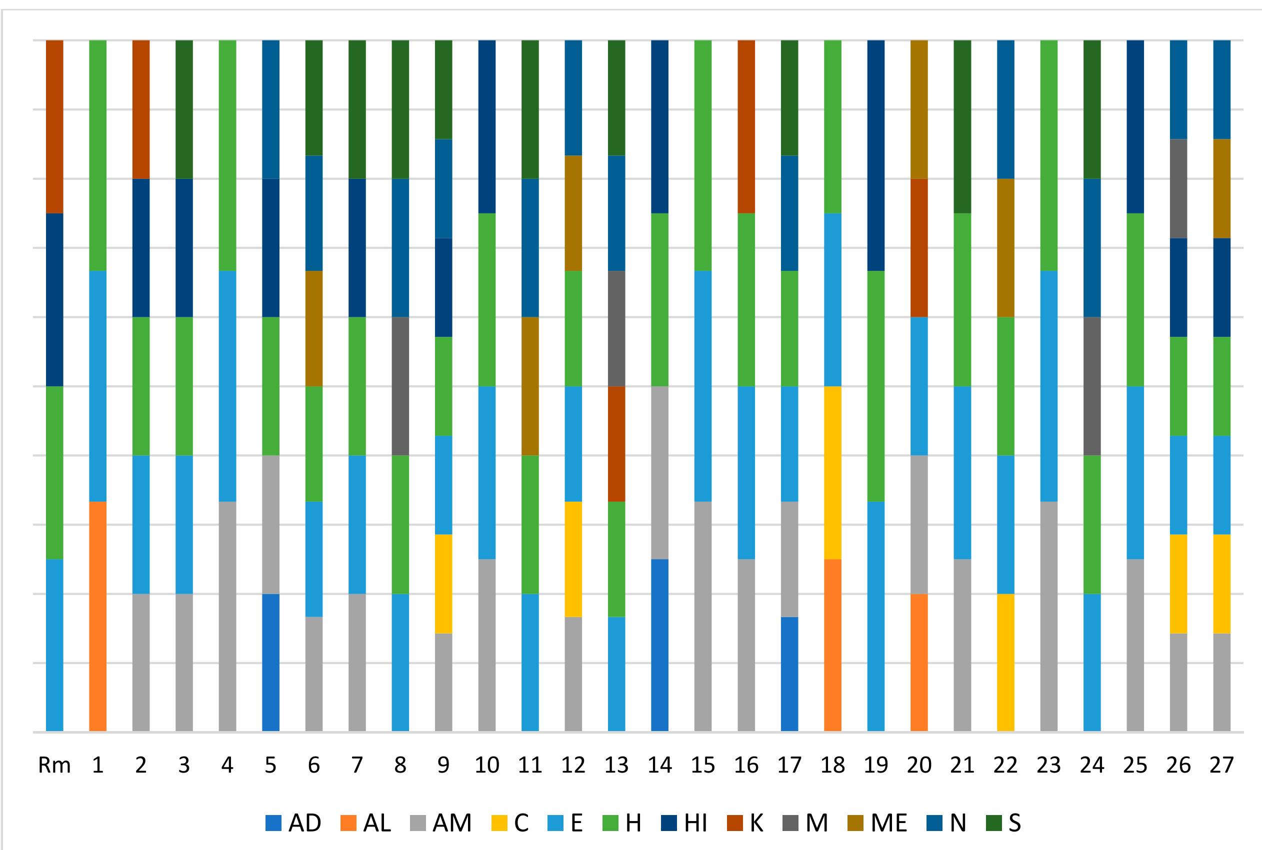


Fig. 2 Distribution of compounds found in the *X. parietina* samples, and designation of compounds E, H, HI, K in common with Rm, see Table 2 for the abbreviations

found in all samples, *amine, NO_2,* and SO_2 were in the top 3, while aldehyde and alkene were the least common compounds.

When the compounds found in the samples are evaluated in terms of pollution caused by microplastic composition in the air, aldehyde, alkene, amine, carboxylic acid, methyl, and methylene, which are not found in the reference material but detected in our samples, may be the result of the contribution of MPs. It is stated that aldehydes, alkenes and carboxylic acids are released as a result of thermal decomposition of plastics, and NO_2, SO_2, methylene and methyl are released through chemical degradation (Castelvetro et al. 2021; Gregoris et al. 2023). Amine compounds also show better compatibility on the surface of plastics than other airborne pollutants (Ortega and Cortés-Arriagada 2023). The compound found in most samples (at 19 sites) but not in the reference material is the *amine*. The atmospheric pollutants NO_2 [Maltepe (5), Ataşehir (6), Adalar (8), Beykoz (9,11,12,13), Şile (17), Üsküdar (22,24) and Pendik (26,27)] and SO_2 [Kartal (3), Ataşehir (6), Adalar (7,8), Beykoz (9,11,13), Şile (17) and Üsküdar (21,24)] were also commonly determined. Following these, carboxylic acid [Beykoz (9,12), Şile (18), Üsküdar (22) and Pendik (26,27)] and methylene [Ataşehir (6), Beykoz (11,12), Üsküdar (20,22) and Pendik (27)] were found in 6 sites in the study area.

According to the data obtained, organic pollutants in the air detected by FT-IR in lichen thalli samples generally consist of compounds or thermal degradation products found among the raw materials and solvents of plastics. Aldehydes and carboxylic acids are known to be used as raw materials of polymers such as plastics and paints. It has been determined that amines originate from exposure to thermal degradation products of polyurethane and from car repair shops. *Methyl* group compounds are formed from a mixture of petroleum hydrocarbons. Alkene, methylene, NO_2 and SO_2 compounds are also result from motor vehicle emissions (Castelvetro et al. 2021). It seems likely that these compounds may originate from heavy traffic in the study area as well as from pollutants emitted from industrial areas.

The atmosphereic pollutants exert a notable influence on air quality, contributing to phenomena like smog and turbidity, and their exposure is correlated with elevated rates of cardiopulmonary mortality and morbidity in humans (Eberhard et al. 2024). This parallel concern extends to microplastics, which originate from various sources, including commercial product development and the breakdown of larger plastics (Silva et al. 2022). Microplastics in the air negatively affect all living things, including humans, due to the presence of heavy metals and organic pollutants such as PAHs and POPS on their surfaces (Mei et al. 2020). Understanding the interactions between microplastics and organic compounds, whose adsorption mechanisms and behavior can change under the influence of environmental factors, is crucial in assessing their risks for the environment and human health.

The biomonitoring technique has been used for many years to detect pollutants that cause air pollution. Lichen species are the leading bioindicator organisms in the evaluation of air quality (Conti and Cecchetti 2001). Studies on the detection of organic pollutants by lichens began to appear in the literature later than other pollutants such as trace and heavy metals. Although MP research, which is rapidly increasing today, includes only a few studies using the lichen monitoring technique, it is thought that lichens can be a suitable system for monitoring MP pollution in the air. Some of the organic pollutants in the air, such as PAHs and POPs, which are also found in the chemical structure of MPs, were generally monitored separately in studies conducted with lichens (Shukla et al. 2019). Therefore, lichen biomonitoring studies focusing directly on airborne MPs or their components and the interactions between them will make a significant contribution.

The thallus surface structure of lichens is one of the effective factors in the detection of atmospheric MPs. Since lichen species vary depending on habitat conditions, different species are included in biomonitoring studies. Generally, foliose species with a large surface are preferred because they can absorb pollutants from the air along with moisture. In the present study, *X. parietina* species, which is a common urban lichen in the research area, was used as biomonitoring material due to its foliose thallus surface and because it can be collected from more locations. *Flavoparmelia caperata* species was used by Loppi et al. (2021) to monitor atmospheric MP pollution with lichens. The accumulation of microplastics in the air in urban areas of Milan (Italy) and in the control, zone was investigated using a three-month transplant of *Evernia prunastri* lichen (Jafarova et al. 2022), and a higher density of MP has been detected in the central and peripheral areas. PAH organic air pollutants were monitored with *Usnea longissima* in the Tibetan plateau region (Jin et al. 2020) and with *Pseudevernia furfuracea* species in southern Italy (Capozzi et al. 2020). Comparing the results of these and similar studies in the literature, there are some different applications in the habitat where lichen samples are collected, devices used to detect MP in the air, and method steps. Therefore, the findings obtained show similarities with some of them, while showing differences with some of them. The biomonitoring method used here to detect MPs followed previous similar studies (Zhang et al. 2020; Silva et al. 2022). The different points of our study are the chemical structure of microplastics resulting from FT-IR analysis, the use of reference lichen in fluorescence microscopy, and the selected bioindicator lichen species.

Many factors come into play in the evaluation of MP pollution, such as habitat characteristics in the sampled

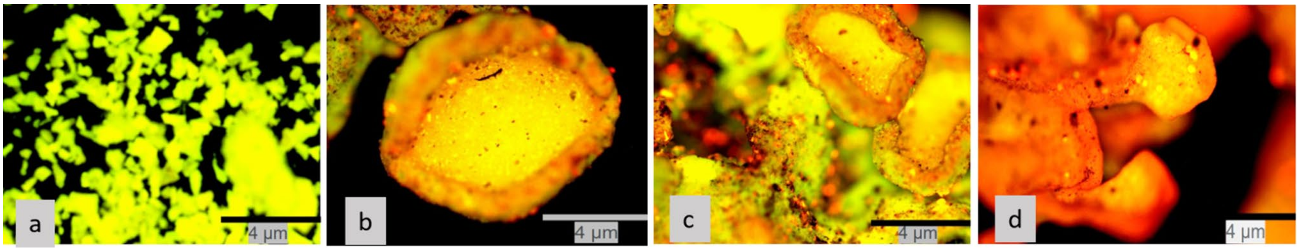


Fig. 3 Fluorescent microscopy observations stained with Nile red; **a** reference IAEA-336, **b, c** *X. parietina* apothecia surface samples from Beykoz, site#16, **d** thallus surface of the sample from Adalar district, site#7

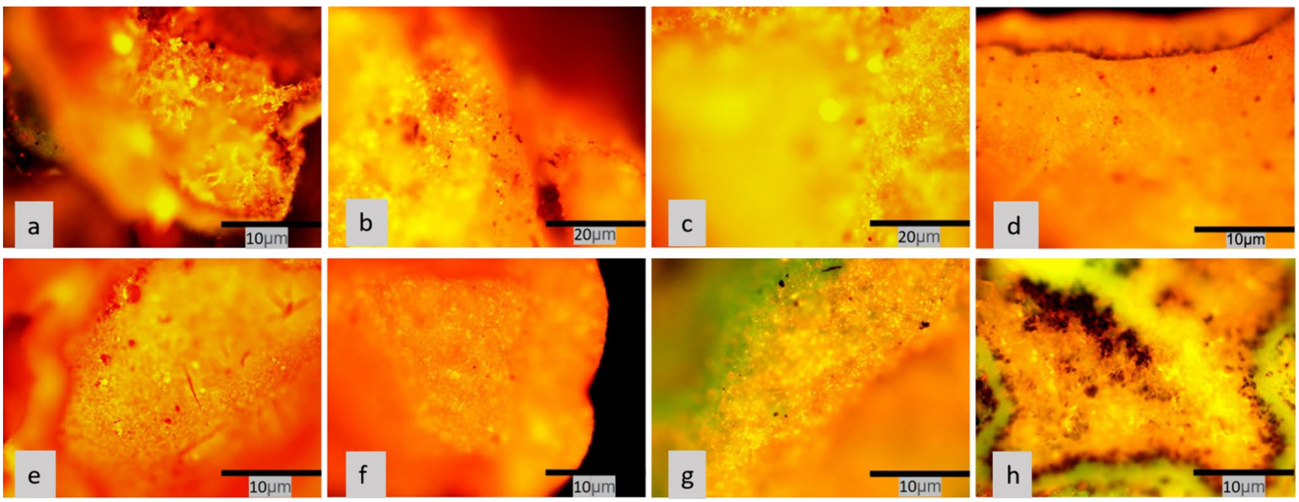


Fig. 4 Nile Red-stained fluorescent microscope images of *X. parietina* thalli from 8 districts in Istanbul with yellow shining MP; **a** Kartal, site #1, **b** Maltepe, site#4, **c** Ataşehir, site#6, **d** Adalar, site #8, **e** Beykoz, site#13, **f** Şile, site#19, **g** Üsküdar, site#24, **h** Pendik, site#25

Table 3 Number and percentages of MP counted per 2.5 µm^2 unit area in *X. parietina* samples

Sample #	#	District	Collection site—habitat	MP number (in 2.5 µm^2)		MP (%)	
1	1	Kartal	Aydos forest	73		7.46	
4	2	Maltepe	Başıbüyük promenade area	61		6.23	
6	3	Ataşehir	Kayışdağı forest	124		12.67	
7	4	Adalar	Büyükada Aşıklar garden	52		5.31	
10	5	Beykoz	Kanlıca forest	50	74.67[a]	5.11	7.63[a]
13		Beykoz	Göztepe Nature Park promenade area	94		9.60	
16		Beykoz	Elmasburnu Nature Park forest	80		8.17	
19	6	Şile	Serintepe picnic area	68		6.95	
21	7	Üsküdar	Küçük Çamlıca Hill grove	165	177.5 [b]	16.85	18.13[b]
24		Üsküdar	Büyük Çamlıca Hill grove	190		19.41	
25	8	Pendik	Dumlupınar Gözdağı Hill	22		2.25	

[a] Average of 3 sites in Beykoz

[b] Average of 2 sites in Üsküdar

locations (forest, seaside, distance to the main road), meteorological and ecological data (altitude, wind transportation) and the level of exposure to human influence. It has been stated that MPs in the atmosphere can travel long distances and can even be incorporated into water, and that it is possible to detect MP pollution even in the farthest points of urban forest areas (Zhang et al. 2020). It seems likely that the prevailing winds blowing from the north in the study area have negatively affected the middle–high MP pollution in the southern districts (Kartal and Maltepe). This observation aligns with previous studies highlighting the ability of MPs to travel over considerable distances through atmospheric transport. Wind can carry MPs from their source to remote areas, impacting ecosystems far beyond plastic manufacturing sites (Shukla et al. 2019). In addition to many small industrial zones within the city, the presence of the plastics industry in large organized industrial zones in Ümraniye in the north and Tuzla districts in the southeast (IBIS report 2018), approximately 8–60 km away from the sampling areas, increases the possibility of MPs being carried by the wind.

Microscopic Detection

The thallus surface and anatomical sections of freshly dried lichen samples stained with Nile Red fluorescent dye were examined and photographed under fluorescent microscope. Accordingly, MP were detected as bright yellow particles in lichen thalli from all sample areas, unlike the reference material, which was stained dull yellow. Images from microscopic observations of the selected samples with shining microplastics are shown in Fig. 3 and the IAEA-336 reference lichen in Fig. 4.

For quantification purposes, counting of glowing MPs in a unit area of 2.5 μm in lichen thalli from each location was performed under fluorescence microscope (Zhou et al. 2022). The average number of MPs in the lichen samples from 8 districts on the Asian side of Istanbul are shown in Table 3.

According to the percentage distribution of the average MP number in lichen samples on a district basis, it was determined that the district with the highest amount of MP in the air was Üsküdar (#24), and the district with the least amount was Pendik (#25). Adalar (#4, which is an island) and Pendik (#8), further from the city center, have the lowest MP content. The reason why Büyük Çamlıca Hill in Üsküdar stands out with its high MP rate may be that it is one of the tourist attraction centers of Istanbul. Ataşehir district (#3), which ranks second with the highest MP amount, is also a district with a high population density. Waste resulting from the plastics we constantly use in our daily lives is one of the human-related reasons for the high microplastic pollution.

The results of our study revealed that bright yellow MPs were clearly observed both on the thallus surface and inside the lichen samples. These findings were compared with the reference lichen and it was determined that lichens can accumulate the chemical components of MPs in their thallus. Similar MP biomonitoring studies in the literature show some differences in terms of the habitats of the collected lichen samples, their species, and the details of the methodologies applied. Dowara et al. (2020) focused on the analysis of MPs from beaches in India, using bioindicator organisms from two bivalve species, *Perna viridis* and *Meretrix meretrix*, while their analysis included the use of Nile Red fluorescent dye, similar to that in our study. In contrast, Loppi et al. (2021) used the Rose Bengal fluorescent dye for microplastic detection in their pioneering study investigating the use of lichens as biomonitoring agents for airborne MPs.

In our research, we contribute to the existing literature by introducing the application of fluorescence microscopy with Nile Red dye for the detection of MPs in lichen samples. This methodological innovation enhances the range of techniques available for studying MPs in environmental samples and provides a valuable addition to the evolving field of microplastic research. Moreover, these results overlap with FT-IR analysis data on lichen samples collected from the same stations and support microplastic deposition. Thus, it was determined by fluorescence microscopy analysis that these lichen species can also accumulate MPs from the air. These data showed that lichen *X. parietina* can be used for biomonitoring of organic pollutants, including microplastics.

Conclusions and Suggestions

As the first biomonitoring study for airborne microplastics in Turkey, this study showed that the foliose *X. parietina* lichen can be used effectively for this purpose. MPs were identified both visually and by their chemical composition in lichen samples growing in forest areas on the Asian side of the Istanbul megacity. Among the MP components, amine, carboxylic acid, metlylene, SO_2 and NO_2 compounds were the most prominent. It has been demonstrated that airborne MP accumulation in lichen samples can be examined in a short time and with accurate results using the fluorescent staining technique. This study was the first to use fluorescence microscopy in the visual investigation of microplastics in lichens. Although the presence, density, and location of MPs in the thallus can be detected with this technique, advanced SEM–EDS analysis is needed to understand which chemical functional groups the MPs contain.

By monitoring microplastics in lichens, it was concluded that MPs hold an important place among air pollutants on the Istanbul-Asian side. It is thought that transport of particles from urban and non-urban industry, human density and plastic waste are among the primary sources of MP pollution in the study area. Given the findings of this study, it becomes crucial to assess the potential pathways and factors influencing the dispersion of MPs. Understanding the dynamics of MP transport, including wind patterns and atmospheric deposition, is essential for a comprehensive evaluation of the environmental implications of plastic production. Further research could focus on investigating the specific types and characteristics of MPs found in the Tuzla and Ümraniye districts, exploring their potential sources within the plastic industry, and assessing their impact on surrounding ecosystems. Additionally, efforts to mitigate microplastic pollution should consider strategies at the local and regional levels, considering the role of industrial activities in contributing to this environmental challenge.

Our study recommends further research on specific types of microplastics in Istanbul organized industrial zones, their sources in the plastics industry, and their effects on surrounding ecosystems. Considering the role of industrial activities in contributing to this environmental problem, the importance of local and regional strategies to reduce microplastic pollution are gaining importance.

In our lives surrounded by plastic products, the negative effects of MPs on the living environment not only pollute the waters but also threaten our health in the air environment. Therefore, it is of great importance to limit the production and use of plastics. Whether microplastics are dissolved in lichen metabolism after they enter the lichen thallus through the pores of the upper cortex, their disintegration state, which compounds they can turn into or whether they are deposited or not are the subjects that need to be further investigated.

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Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Conflict of interest Authors do not declare competing interests.

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