



## Research Paper

## First evidence of microplastics isolated in European citizens' lower airway

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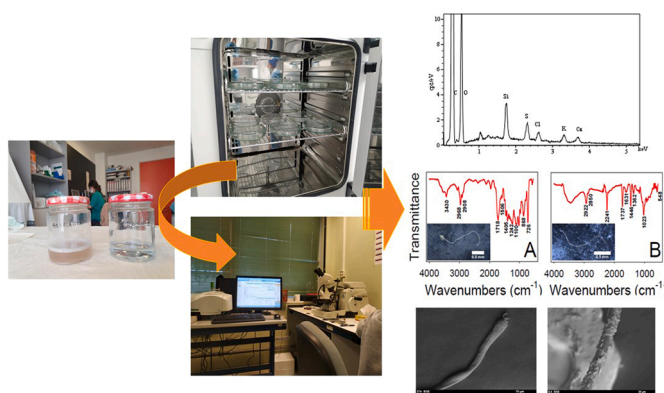
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## HIGHLIGHTS

- MPs were detected in BALF samples from the human lower airway.
- Most of them were in the shape of fiber.
- The most abundant compounds were rayon and polyester.
- There was an inverse correlation between fiber concentration and lung function.
- Inhaled MPs could play a role in respiratory pathology.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Microplastics (MPs) have been detected in all environmental locations, including the atmosphere. However, few studies have investigated the presence of airborne MPs in the human respiratory system. Our research purpose was to investigate these pollutants in the lower human airways of 44 adult European citizens, using bronchoalveolar lavage fluid (BALF) collection as a minimally invasive method, that enables the detection of these pollutants in living patients. We studied the relationship between the patients' life habits and physiological parameters, based on background information and medical and occupational history, and the concentration of MPs isolated from their respiratory systems. Our results indicate that most MPs were in the form of microfibers (MFs) (97.06%), with an average concentration of  $9.18 \pm 2.45$  items/100 mL BALF, and only 5.88% ( $0.57 \pm 0.27$  items/100 mL BALF) were particulate MPs, without a significant relationship with environmental, physiological, or clinical factors. The average size was  $1.73 \pm 0.15$  mm, with the longest dimension (9.96 mm) corresponding to a polyacrylic fiber. Taken together, the results demonstrated the occurrence of MPs in the lower human airway, although more studies are necessary to elucidate the negative effects these pollutants could induce in the human respiratory system and its associated diseases.

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## 1. Introduction

Plastic pollution is a global concern because plastic material is used worldwide. In 2020 alone, 367 million tons of plastics, not including fibers made of polyethylene terephthalate (PET), polyamide, and polyacrylate, were produced around the world (PlasticsEurope, 2021). To understand the magnitude of this issue, consider the face mask, a plastic product daily consumed during the COVID-19 pandemic. The monthly consumption of face masks during the pandemic totaled 129 billion for 7.8 billion people across the globe (Tilley and Kalina, 2020). A portion of the total plastic produced is directly manufactured as “primary MPs”, i. e., plastic particles smaller than 5 mm used in personal care products or various manufacturing industries. However, most of these plastics are “secondary MPs”, originating from plastic fragmentation processes into micro-sized particles, because of abiotic (i. e., hydrolytic, mechanical, thermal, or oxidative degradation), or biotic degradation.

MPs are ingested by various living organisms. This includes humans, because MPs are present in the foods, drinks, and condiments that are regularly consumed (Ferrante et al., 2022; Shruti et al., 2021; Da Costa Filho et al., 2021). Consequently, MPs have been found in different biological human samples, such as colectomy specimens (Ibrahim et al., 2021), feces (Pérez-Guevara et al., 2021; Yan et al., 2022), urine (Wang et al., 2021), placenta (Braun et al., 2021), saliva (Abbasi and Turner, 2021), sputum (Huang et al., 2022), and blood (Leslie et al., 2022).

Some of these MPs can also remain suspended in the air, contributing to the atmospheric fallout in several cities (Dris et al., 2016; Cai et al., 2017; Liao et al., 2021; Kashfi et al., 2022; Nematollahi et al., 2022). The concentration of these suspensions, particularly for MFs, tends to be higher indoors than outdoors (Gasperi et al., 2015), and in urban areas than rural locations (Liao et al., 2021). However, they have also been monitored in the Antarctic atmosphere (Marina-Montes et al., 2022), suggesting that airborne transport is an important pathway for these micropollutants to reach remote regions (Evangelidou et al., 2020; Petersen and Hubbard, 2021). Plastic MFs are released into the air from various sources; for instance, and in addition to the industrial and washing processes of synthetic clothes that affect MFs release into the water cycle (Napper and Thompson, 2016), a direct release from textiles to the air has been discussed as an important contributor to MFs pollution, although this area has received less attention (De Falco et al., 2020; Zhang et al., 2022). Moreover, industrial processes, 3D printing, landfills (Amato-Lourenço et al., 2020), household tumble dryers (O'Brien et al., 2020; Tao et al., 2022), and air conditioner filters (Chen et al., 2022b) may serve as significant MFs sources in indoor and outdoor environments.

Although the presence of MPs in the air has been well established, the consequences of their possible inhalation for human health have yet to be sufficiently investigated (Wright and Kelly, 2017). These particles often contain toxic additives (Verla et al., 2019) and may adsorb and carry other organic or inorganic pollutants, such as heavy metals or polychlorinated biphenyls (PCBs) (Bayo et al., 2017), antibiotics (González-Pleiter et al., 2021), pesticides (Verdú et al., 2021), and pathogenic microorganisms (Amato-Lourenço et al., 2020). The presence of some of these polymeric MFs, made of high- and low-density polyethylene, acrylate, polyamide, polyester, or PET, among others, has been reported in human lung tissues obtained from surgical resections (Pauly et al., 1998; Chen et al., 2022a; Jenner et al., 2022), and autopsies (Amato-Lourenço et al., 2021). In addition, an association between occupational exposure and respiratory symptoms has been demonstrated (Atis et al., 2005). Yee et al. (2021) reported the presence of MPs in the distal airway, with a proinflammatory effect that induces the release of reactive oxygen species, and Goodman et al. (2021) showed that exposing human lung cells to small amounts of polystyrene altered their metabolism, inhibited cell proliferation, and altered cohesion between cells.

Bronchoalveolar lavage is a minimally invasive procedure routinely performed during flexible bronchoscopy that allows cells and non-

cellular elements to be recovered in a representative sample of the lower airway (Kebbe and Abdo, 2017). Bronchoalveolar lavage fluid (BALF) is obtained through instillation and subsequent recovery of a saline solution from one or more lung segments, providing useful information about the environmental status of alveoli and terminal bronchioles (Sartorelli et al., 2020). BALF has been used for in vivo assessment of nonfibrous mineral particles (including aluminum, titanium, or calcite) in the respiratory tract (Pairon et al., 1994), determining the presence of asbestos bodies and the fiber burden of exposed workers (Dumortier et al., 2001; Alexopoulos et al., 2011; Sartorelli et al., 2020), and the identification of mineral fibers and particles in alveolar macrophages (Perna et al., 2002), among others. This study aimed to determine whether MPs are present in BALF obtained from living patients, and their relationship with environmental, physiological, and clinical factors according to each patient's background information and medical history, to identify potential risk factors associated with MPs. To the best of our knowledge, this is the first time this procedure has been used to identify these micropollutants in patients with pulmonary pathologies.

## 2. Materials and methods

### 2.1. Ethics approval and participant consent

A total of 44 adult patients, undergoing a bronchoscopy between March and September 2021 at Hospital General Universitari d'Elx (HGUE, Alicante, Spain) according to standard clinical practice were prospectively included in this study. All patients were provided with study information and signed a written consent document prior to their participation in this study. The research was approved by the HGUE Health Department's Ethics Committee (ID of the ethics approval: PI 7/2021), and the principles of the revised Declaration of Helsinki were followed. Patients with oximetry lower than 94%, hemodynamic instability, or with a suspected or known contagious disease were excluded from the study.

### 2.2. Background information and medical history

All patients underwent anamnesis, physical examination, blood tests, and chest computer tomography (CT scan). Sex, age, smoking habits, occupation, place of residence, and building type were collected on self-report, as depicted in Table 1. Cumulative tobacco consumption was calculated in pack-years, based on age at smoking initiation (plus cessation for former smokers), number of cigarettes consumed daily, and duration of tobacco consumption, i. e., a pack-year was 20 cigarettes, or the equivalent smoked daily for a year (Pedersen et al., 2020). Occupations were classified into two groups: (1) high risk of occupational exposure to MPs, and (2) low risk of occupational exposure to MPs (Table 1). CT scans were reviewed by expert chest radiologists. The diagnosis of lung cancer was based on a histological examination performed by a pathologist, and the remaining diagnoses were established according to the physician responsible for each patient. The radiological diagnosis was divided into three types: (1) patients with pulmonary parenchymal pathology, (2) patients with other anomalies detected with CT scans, and (3) patients without radiological abnormalities (Table 1).

### 2.3. Pulmonary functional tests

Pulmonary function tests were performed on 30 patients before sampling through a MasterScreen spirometer model (Jaeger Carefusion, San Diego, USA), following the ATS/ERS (American Thoracic Society/European Respiratory Society) standards (Miller et al., 2005). Forced expiratory volume in the first second (FEV<sub>1</sub>) and forced vital capacity (FVC) were measured. Airway obstruction was detected according to the definition proposed by the GOLD Initiative for Chronic Obstructive Lung Disease (GOLD): a ratio FEV<sub>1</sub>/FVC < 0.70, confirming a diagnosis of

**Table 1**  
Main characteristics of participants in the study.

Participant	Sex	Age (years)	Smoking habit	Cumulative tobacco consumption	Occupation (risk group)	Residence	Building	Radiological diagnosis (risk group)	PMG	FVE <sub>1</sub>	FVC	FVE <sub>1</sub> /FVC	BALF volume (mL)
B1BRLA_004	M	64	2	35	Salesman (2)	1	1	Pulmonary infiltrate (1)	1				20.65
B1BRLA_006	F	79	3		Shoemaker (2)	1	1	Lymphadenopathy (2)	0	125	129	0.97	31.33
B1BRLA_008	M	35	1	5	Stocker (2)	1	1	No abnormalities (3)	0	109	116	0.94	25.05
BIBRLA_010	F	47	1	11	Civil servant (2)	1	1	Pulmonary infiltrate (1)	1				32.43
BIBRLA_021	M	78	1	60	Cleaner (2)	1	1	Lung mass (1)	1	66	70	0.94	35.56
BIBRLA_022	M	76	2	100	Carpentry (1)	1	2	Lung mass (1)	1				23.13
BIBRLA_023	M	80	1	60	Farmer (1)	1	1	Lung mass (1)	0				33.88
BIBRLA_024	M	66	1	100	Salesman (2)	1	1	Multiple nodules (1)	0	71	89	0.80	14.07
BIBRLA_025	M	71	1	52	Shoemaker (2)	1	1	Lung mass (1)	0	69	89	0.78	18.86
BIBRLA_026	M	82	1	30	Shoemaker (2)	1	2	Atelectasis (1)	1	73			40.16
BIBRLA_027	M	53	3		Fuel station (1)	1	1	Pulmonary embolism (2)	0	90	89	1.01	53.69
BIBRLA_028	M	46	3		Cleaner (2)	1	1	No abnormalities (3)	1	111	111	1.00	49.27
BIBRLA_029	F	75	3		Shoemaker (2)	1	1	Lung mass (1)	0	68	72	0.94	42.92
BIBRLA_030	F	52	2	6	Teacher (2)	1	1	Lung mass (1)	1	70	59	1.19	47.50
BIBRLA_031	M	86	2	30	Shoemaker (2)	1	2	Lung mass (1)	0	74	96	0.77	37.74
BIBRLA_032	M	77	1	70	Construction (1)	1	1	Lung mass (1)	0	52	79	0.66	24.10
BIBRLA_033	M	73	2	50	Salesman (2)	1	1	Lung mass (1)	1	73	121	0.60	37.32
BIBRLA_034	F	60	1	43	Shoemaker (2)	2	2	Lung mass (1)	0	76	86	0.88	47.92
B2BRLA_039	F	64	1	35	Dressmaker (2)	2	2	Pulmonary infiltrate (1)	0				56.94
B2BRLA_040	M	49	2	10	Cleaner (2)	2	2	No abnormalities (3)	0				52.62
B2BRLA_041	M	55	2	30	Shipper (1)	1	1	Lung mass (1)	0	76	88	0.86	44.38
B2BRLA_042	M	71	1	45	Sailor (1)	1	1	No abnormalities (3)	1	89	86	1.03	38.67
B2BRLA_043	M	66	1	50	Hostelry (1)	1	2	Lung mass (1)	1	78	89	0.88	21.05
B2BRLA_044	M	56	1	40	Gardener (1)	2	2	Pulmonary infiltrate (1)	1	74	86	0.86	23.01
B2BRLA_045	M	70	1	30	Shoemaker (2)	1	1	Nodule (1)	0	51	85	0.60	15.29
B2BRLA_046	M	74	2	40	Carpentry (1)	1	2	Multiple nodules (1)1	1	87	90	0.97	31.83
B2BRLA_047	M	58	1	44	Electronic (2)	2	2	Pleural effusion (2)	0				30.02
B2BRLA_048	M	57	2	15	Sanitary staff (2)	1	1	Lung mass (1)	1	62	71	0.87	21.23
B2BRLA_049	F	55	2	20	Hostelry (1)	2	2	Lung mass (1)	0				35.76
B2BRLA_050	M	51	1	20	Shipper (1)	1	1	Lung mass (1)	0	77	79	0.97	54.88
B2BRLA_051	M	62	2	35	Shoemaker (2)	1	1	Nodule (1)	0	115	127	0.91	40.60
B3BRLA_057	M	65	1	50	Shipper (1)	2	1	Lymphadenopathy (2)	1				17.94
B3BRLA_058	F	63	1	40	Farmer (1)	1	1	Pulmonary infiltrate (1)	1	48	89	0.54	8.74
B3BRLA_059	M	57	1	30	Carpentry (1)	1	2	Lung mass (1)	1	87	89	0.98	35.71
B3BRLA_060	F	75	1	40	Shoemaker (2)	1	1	Atelectasis (1)	1	92	100	0.92	22.68
B3BRLA_061	M	77	2	130	Sailor (1)	1	1	Pulmonary infiltrate (1)	0	67	91	0.74	31.45
B3BRLA_062	M	67	1	80	Civil servant (2)	1	1	Atelectasis (1)	1				29.58
B3BRLA_063	M	76	2	5	Hairdresser (1)	1	1	Pulmonary embolism (2)	0				28.31
B3BRLA_064	F	57	3		Cleaner (2)	1	1	No abnormalities (3)	0	120	119	1.01	36.95
B3BRLA_065	M	62	2	17	Construction (1)	1	1	Pulmonary infiltrate (1)	0	96	113	0.85	45.12
B3BRLA_066	M	80	2	25	Shipper (1)	1	1	Multiple nodules (1)	0				35.72
B3BRLA_067	F	55	1	30	Salesman (2)	1	2		1	94	108	0.87	48.03

(continued on next page)

Table 1 (continued)

Participant	Sex	Age (years)	Smoking habit	Cumulative tobacco consumption	Occupation (risk group)	Residence	Building	Radiological diagnosis (risk group)	PMG	FVE <sub>1</sub>	FVC	FVE <sub>1</sub> /FVC	BALF volume (mL)
B3BRLA_068	F	62	1	40	Cleaner (2)	1	1	Pulmonary infiltrate (1)	1				39.29
B3BRLA_069	M	75	3		Banker (1)	1	1	Nodule (1) Interstitial lung disease (1)	1	99	107	0.93	20.41

Gender: (F) female, (M) male; Smoking habit: (1) active smoker, (2) former smoker, (3) non-smoker; Occupation: (1) high risk, (2) low risk; Residence: (1) urban; (2) rural; Building: (1) upper storey, (2) ground level; PMG (Pathological microbial growth); (0) no, (1) yes; radiological diagnosis: (1) pulmonary parenchymal pathology, (2) other anomalies detected in CT scan, (3) without radiological abnormalities.

pathological airflow limitation (Rabe et al., 2007).

#### 2.4. BALF collection

Fiber bronchoscopies were performed with a Pentax EB15-J10 model (Pentax Medical, Tokyo, Japan). Bronchoalveolar lavage was carried out according to the standardized technique during bronchoscopy with conscious sedation (Meyer et al., 2012). Briefly, after the fiberoptic bronchoscope was wedged in a bronchus, preferably in the middle lobe or the lingula, two successive 50-mL aliquots of sterilized 0.9% sodium chloride (NaCl) solution were instilled and each was manually aspirated using a 20-mL plastic syringe and set into a sterilized glass container with a metal lid for MPs analysis (Fig. 1), with a minimum of a 10 mL required for each sample for MPs analysis. In the case of the presence of a lung mass, BALF was carried out contralateral to the lung lesion.

The risk of pollution was reduced as much as possible; plastic lab devices was limited to the maximum, although not entirely avoided, and only clothes made of natural fabric and clean cotton lab coats were worn by the analysts. Instruments were thoroughly rinsed with bi-distilled water before each experiment, and only sterilized autoclaved glassware was used for all procedures. The study was conducted in three different batches, obtaining 18, 13, and 13 BALF samples for batches #1, #2, and #3, respectively.

#### 2.5. Measurement of MPs in BALF

Pooled unfiltered BALF samples were shipped to the Technical University of Cartagena (UPCT) for MPs analysis. An average amount ( $\pm$  standard error of the mean) of  $33.68 \pm 1.81$  mL BALF per participant was analyzed, with minimum and maximum values of 8.74 and 56.94 mL, respectively (Table 1). Once in the lab, samples were carefully transferred into muffled 120-mm glass Petri dishes, and glass containers were rinsed twice with 15 mL of bi-distilled water (Fig. 1). After drying at 60 °C overnight, because higher temperatures could lead to melting of common polymers or even to the chemical degradation of common polymers (Adomat and Grischek, 2021), Petri dishes were observed with a Olympus SZ-61TR Zoom Trinocular Microscope (Olympus Co., Tokyo, Japan), coupled to a Leica MC190 HD digital camera and the image-capturing software Leica Application Suite (LAS) 4.8.0 (Leica Microsystems Ltd., Heerbrugg, Switzerland). Images of every single microparticle were captured, and color, shape, and size in its longest dimension were recorded with the assistance of the ridge detection plugin of the Image J software before they were isolated in muffled 40-mm glass Petri dishes for further  $\mu$ -FTIR analysis. Because organic matter was deemed low in BALF samples, and the use of an oxidizing agent to treat biological samples could lead to discoloration, bleaching, and even degradation of some polymers (Nuelle et al., 2014; Karami et al., 2017), no digestion procedure was carried out.

#### 2.6. $\mu$ -FTIR analysis

The chemical composition of microparticles was analyzed by  $\mu$ -FTIR at Universidad Autónoma de Madrid, using a Perkin-Elmer Spot-light™

200 Spectrum Two instrument with a mercury cadmium telluride detector. Each microparticle was placed on KBr, which was used as a slide, and its spectrum was recorded in micro-transmission mode using the following parameters: spot 50  $\mu$ m, 32 scans, and spectral range 550–4000  $\text{cm}^{-1}$ . All spectra were compared with those from the Omnic 9.1.26 (ThermoFisher Scientific Inc., Massachusetts, USA) database and our own database. Microparticles were considered to be plastics when the match confidence was  $> 70\%$ . PET was classified as “polyester” because it is a thermoplastic polymer resin of polyester. Artificial microparticles, i.e., cellulose, wool, cotton, and linen fibers with non-natural colors or with evidence of anthropogenic processing (modified cellulose in the form of rayon, viscose, cellophane, lyocell, or Tencel™) were included as MPs in the analysis.

#### 2.7. SEM-EDS analysis

The surface characteristics and chemical composition of MFs were examined using a scanning electron microscope coupled to energy dispersive X-ray spectrometry (SEM-EDS) (Hitachi S-3500 N SEM, Hitachi High-Technologies, Tokyo, Japan). Samples were mounted on carbon stubs using carbon tape, and the morphology of the MFs surface was imaged at several magnifications, operating at a 15 kV accelerating voltage and 30 Pa chamber pressure. The elemental composition of each MFs was quantified at three different points on its surface by means of a Quantax 200 EDS analyzer coupled to SEM (Bruker AXS, Madison, WI, USA), with a detector energy resolution of 128 eV. This is a very powerful method for analyzing MPs composition, providing detailed information about elements and their spatial distribution within the sample, including the presence of inorganic additives used in their formulation (Gniadek and Dąbrowska, 2019).

#### 2.8. Quality assurance and quality control (QA/QC)

The ubiquity and low concentration of the MPs in the BALF samples made sampling and post-sampling pollution a threat to the reliability of the results, potentially inserting bias into the MPs quantification and further interpretation of the conclusions (Dehaut et al., 2019; Dioses-Salinas et al., 2020). To monitor the potential occurrence of MPs contamination, 18 negative control samples or procedural blanks were analyzed throughout the entire study, corresponding to: 6 solvent blanks of 0.9% NaCl solution ( $47.35 \pm 2.79$  mL, 2 blanks per batch) and 3 airborne blanks (1 per batch), both at HGUE, and 9 bi-distilled water blanks ( $68.63 \pm 6.53$  mL, 3 blanks per batch) at UPCT. The results were normalized to the MPs found in the blanks for every batch. Blank samples had an average concentration of  $1.45 \pm 0.67$  MFs per 100 mL, and they were used as background data to efficiently calculate the MFs content, by subtracting them from the corresponding BALF batch. No MPs were detected in airborne blanks.

#### 2.9. Statistical analysis of experimental data

Statistical treatment of the data was carried out using the SPSS (Statistic Package for Social Science) 26.0 statistic software (IBM Co.





Fig. 1. BALF samples collection and preparation for microplastic analysis.

Ltd, Chicago, IL, USA). The fitting performance of one-way analysis of variance (ANOVA) was computed with an *F*-test, and Fisher's Least Significance Difference (LSD) test was used when the *F*-test indicated rejection of the null hypothesis ( $H_0$ ) to compare paired data and identify statistically significant differences. Prior to running an ANOVA, Data were tested for normality with Kolmogorov–Smirnov (K–S) test before the ANOVA was run. All data were expressed as the mean  $\pm$  standard error of the mean (SE). Possible intercorrelations between different variables were assessed using Pearson's correlation coefficient (*r*). This coefficient is typically between  $-1$ , indicating a perfect negative correlation, and  $+1$ , expressing a perfect positive correlation, whereas  $0$  indicated the absence of a relationship. All statistical analyses were considered statistically significant at a 95% confidence interval ( $p < 0.05$ ).

### 3. Results and discussion

#### 3.1. General results

In this study, MPs were identified in human BALF using stereomicroscopy,  $\mu$ -FTIR, and SEM-EDS. BALF samples are considered to be of a very good quality and highly representative of the lower airway. Wang et al. (2019) reported that BALF samples are more objective and representative than saliva or sputum in reflecting the microbial environment of the lungs, and Callejón-Leblic et al. (2016) indicated that, since BALF is in close interaction with lung tissue, it is a more representative sample of lung status than other peripheral biofluids as blood or urine. The most distal bronchus can be reached using the fiberoptic bronchoscope, there instilling and collecting the serum so that sample does not become contaminated with the remaining airway or even the mouth. It is a fairly accessible procedure, that can be done both in a hospital or in a health center, with low cost, minimally invasive and, in general, safe for the patient. In comparison to surgical or autopsy samples, BALF samples allow to access to a considerably greater number of patients, thus facilitating the study of possible pathological or exposure associations.

In our study, the age of the participants ranged between 35 and 86

years-old, with 32 participants (72.73%) men and 12 participants (27.27%) women, and an average value of  $65.10 \pm 0.98$  years-old. The most frequent indication for performing bronchoscopy was a lung mass (32%) followed by hemoptysis (27%), and the main diagnosis obtained was pulmonary neoplasia (50%). Table 1 provides data regarding the enrolled participants for this study according to living habitats, and environmental and clinical factors. Fourteen participants (31.82%) did not have any MPs in their BALF, and 12 of them (27.27%) had only one, corresponding the rest to 18 patients (40.91%) to BALF samples with two or more MPs. The average proportion of plastic vs. non-plastic particles in this study was 41.18% and 58.82%, respectively, close to that reported by Huang et al. (2022) for human sputum (32% vs. 68%), with an average concentration of  $9.75 \pm 2.49$  items/100 mL BALF. Most of these MPs were in the shape of MFs (97.06%), with an average concentration of  $9.18 \pm 2.45$  items/100 mL BALF, and only 5.88% ( $0.57 \pm 0.27$  items/100 mL BALF) turned out to be particulate MPs, with no significant or relevant relationship with environmental, physiological, or clinical factors. Hence, all statistical analyses focused only on detecting MFs because they were the dominant shape in atmospheric studies from indoor and outdoor environments (Mathalon and Hill, 2014; Dris et al., 2017; Chen et al., 2020; Li et al., 2020). These results are similar to those reported by Abbasi and Turner (2021) for human saliva, where fibers constituted more than 97% of the MPs count, or by Ibrahim et al. (2021) for human colectomy specimens, with filament forms accounting for 96.1% of all samples. Because MFs concentration data and measurements proved to fit to a normal distribution, according to the K–S test ( $K = 0.288$ ,  $p = 0.000$  and  $K = 0.155$ ,  $p = 0.000$ , respectively), they were not log-transformed for subsequent one-way ANOVA analyses.

The average MFs size was  $1.73 \pm 0.15$  mm, with the longest dimension (9.96 mm) corresponding to a polyacrylic MFs isolated from a 75-year-old active smoker and shoemaker, with pulmonary parenchymal pathology. Although these sizes may seem too large to be present, similar MFs sizes were detected by Jenner et al. (2022) in lung samples. Due to the limitation in size identification by  $\mu$ -FTIR, MFs in BALF samples smaller 20  $\mu$ m could not be detected. The minimum size determined in this study was that of a 140- $\mu$ m MFs isolated from a

46-year-old male non-smoker with no abnormalities in his radiological diagnosis. The main colors of the isolated MFs were white (51.04%) and blue (23.96%), followed by red (7.29%), black (6.25%), and brown (6.25%). Overall, opaque MFs accounted for 63.5% of all colors, and MFs smaller than 1 mm accounted for the lowest percentage (38.61%); they were mainly between 1 and 3 mm (58.34%). As reported by Warheit et al. (2001), the severity of fibers in the respiratory system is directly proportional, among other factors, to their biopersistence, being increased with higher MFs lengths. The maximum average MFs concentration (80.10 items/100 mL BALF) corresponded to a 63-years-old female active smoker, with diagnosed pneumonia working as a farmer. In fact, females displayed statistically significant higher average MFs concentration ( $5.02 \pm 0.64$  items/100 mL BALF) than males ( $3.82 \pm 0.14$  items/100 mL BALF) ( $F$ -value = 6.118,  $p = 0.015$ ) (Fig. 2a). Abbasi and Turner (2021) reported that 97% of MPs in saliva were MFs and were more abundant amongst males than females. There were also statistically significant differences in MFs concentration by age, being higher for participants older than 60 years ( $4.87 \pm 0.30$  items/100 mL BALF) than those younger than 60 years ( $2.76 \pm 0.12$  items/100 mL BALF) ( $F$ -value = 21.091,  $p = 0.000$ ) (Fig. 2b). Similar results were reported by Chen et al. (2022a) in a study of MPs in lung ground-glass nodules, where the abundance of MFs in lung tissue gradually accumulated with increasing age. Regarding smoking, 23 participants were active smokers, 15 were former smokers, and only 6 were non-smokers, with an average value for cumulative tobacco consumption of  $40.87 \pm 4.40$  (Table 1). As depicted in Fig. 2c, MFs concentration in BALF also displayed statistically significant differences according to smoking habits ( $F$ -value = 8.131,  $p = 0.001$ ), with differences in pairwise comparisons by LSD test between active ( $5.26 \pm 0.52$  items/100 mL BALF) and former smokers ( $3.88 \pm 0.18$  items/100 mL BALF) ( $p = 0.008$ ), and active smokers vs. non-smokers ( $3.14 \pm 0.21$  items/100 mL BALF) ( $p = 0.000$ ), but not between former smokers and non-smokers ( $p = 0.192$ ). Furthermore, occupations with a high risk of exposure to MPs displayed a statistically significantly higher average concentration of MFs ( $5.80 \pm 0.73$  items/100 mL BALF) than those with a low risk ( $3.65 \pm 0.13$  items/100 mL BALF) ( $F$ -value = 19.496,  $p = 0.000$ ) (Fig. 2d).

Although participants living in an urban residence appeared to have

a higher average concentration of MFs ( $4.34 \pm 0.27$  items/100 mL BALF) than those living in a rural one ( $3.39 \pm 0.24$  items/100 mL BALF), those differences were not statistically significant ( $p = 0.166$ ). In addition, participants living at the ground level showed a statistically significant lower average MFs concentration ( $3.44 \pm 0.17$  items/100 mL BALF) than those living on the upper floor ( $4.48 \pm 0.31$  items/100 mL BALF) ( $F$ -value = 3.897,  $p = 0.051$ ) (Fig. 2e), suggesting that MPs are resuspended from the floor as a result of human activities and movement (Ageel et al., 2022). A possible relationship between indoor air quality and MPs exposure should be evidenced for people in urban areas because they stay mostly indoors rather than outdoors (Kownacki et al., 2019), and indoor environments are hotspots of MPs pollution (Mbachu et al., 2020). According to Dris et al. (2017), dilution of MPs in the larger volumes outdoors could explain this pattern.

There were also statistically significant differences according to radiological diagnosis and average MFs concentration in BALF samples, with 34 patients with parenchymal pathology, 5 patients with other anomalies detected with CT scans, and 5 patients without radiological abnormalities (Table 1). As presented in Fig. 2f, the average MFs concentration was higher for patients in group (1) ( $4.85 \pm 0.32$  items/100 mL BALF), than that in groups (2) ( $3.35 \pm 0.14$  items/100 mL BALF) and (3) ( $2.43 \pm 0.16$  items/100 mL BALF) ( $F$ -value = 9.113,  $p = 0.000$ ), although pairwise comparisons by LSD test reported differences only between groups (1) and (2) ( $p = 0.007$ ), and groups (1) and (3) ( $p = 0.000$ ), but not between groups (2) and (3) ( $p = 0.225$ ).

Regarding pathological microbial growth, participants with pathological microbes isolated from their BALF showed higher average MFs concentrations ( $4.76 \pm 0.33$  items/100 mL BALF) than those reported in aseptic participants ( $3.21 \pm 0.15$  items/100 mL BALF) ( $F$ -value = 11.034,  $p = 0.001$ ) (Fig. 2g). In 9 out of 13 patients (69.23%) who were diagnosed with a respiratory infection, pathogenic organisms were isolated in microbiological cultures, in contrast to only 30.77% of patients with other diagnoses, suggesting that microbiological findings have clinical relevance. Previous studies have reported the presence of viable microorganisms on MFs collected in the atmosphere (González-Pleiter et al., 2020), and the role of MPs as a vector for pathogens is well known (Meng et al., 2021). In this sense, airborne MFs could act as a carrier of infectious microorganisms in the human respiratory system.

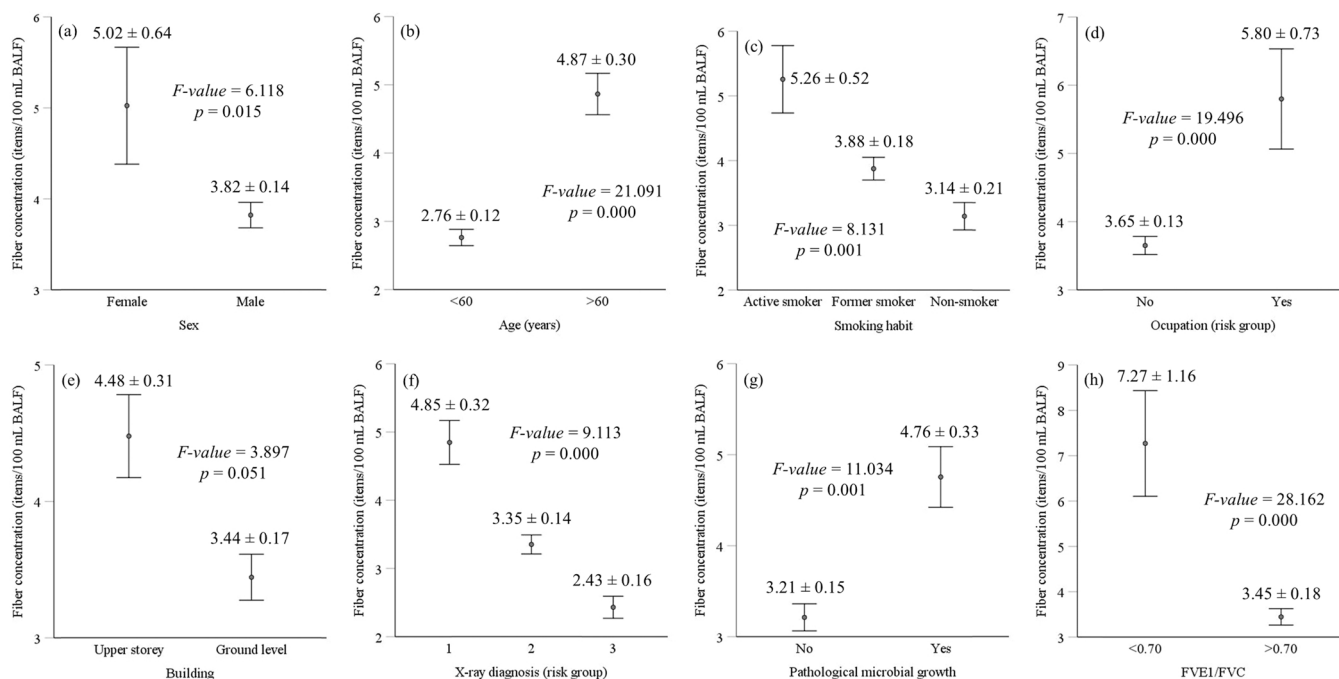


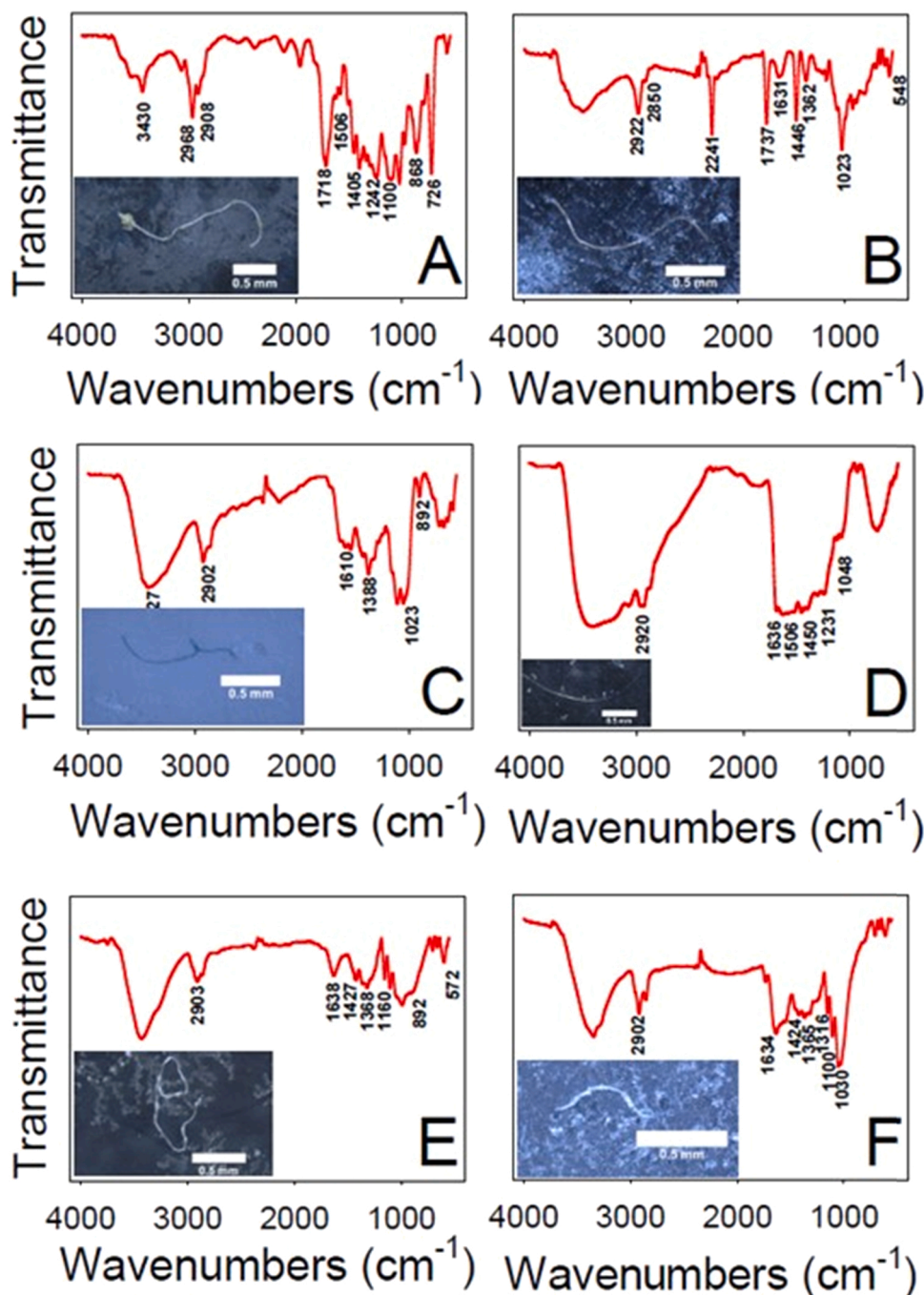
Fig. 2. One-way ANOVA plots for fiber concentration in human BALF by: (a) sex, (b) age groups, (c) smoking habits, (d) occupation, (e) building, (f) radiological diagnosis, (g) pathological microbial growth, (h) ratio FVE<sub>1</sub>/FVC (error bars represent standard error).

MPs would be included in environmental pollution as a part of particulate matter, and in some cases could represent a highly important fraction of this type of pollutant (Panko et al., 2019). Exposure to ambient particulate matter has been associated in large studies with impaired lung function (Guo et al., 2019). However, there is no previous evidence regarding the effects on respiratory function of inhaling environmental MPs. In our study, we observed a statistically significant inverse correlation between MFs concentration and the parameters FEV1 (Pearson's  $r = -0.598$ ,  $p = 0.000$ ) and FVC (Pearson's  $r = -0.355$ ,  $p = 0.005$ ). Average MFs concentration were found to be statistically higher in patients with an FEV1/FVC ratio  $< 0.70$  ( $7.27 \pm 1.16$  items/100 mL BALF) vs. patients with FEV1/FVC ratio  $\geq 0.70$  ( $3.45 \pm 0.18$  items/100 mL BALF) ( $F$ -value = 28.162,  $p = 0.000$ ), indicating

airflow limitation (Fig. 2h). This association is striking given the small size of the population analyzed. Although larger studies are required to confirm these findings, these results suggest that the inhalation of MPs could be associated with reduced lung capacity and obstructive lung diseases such as chronic obstructive pulmonary disease.

### 3.2. $\mu$ -FTIR analysis

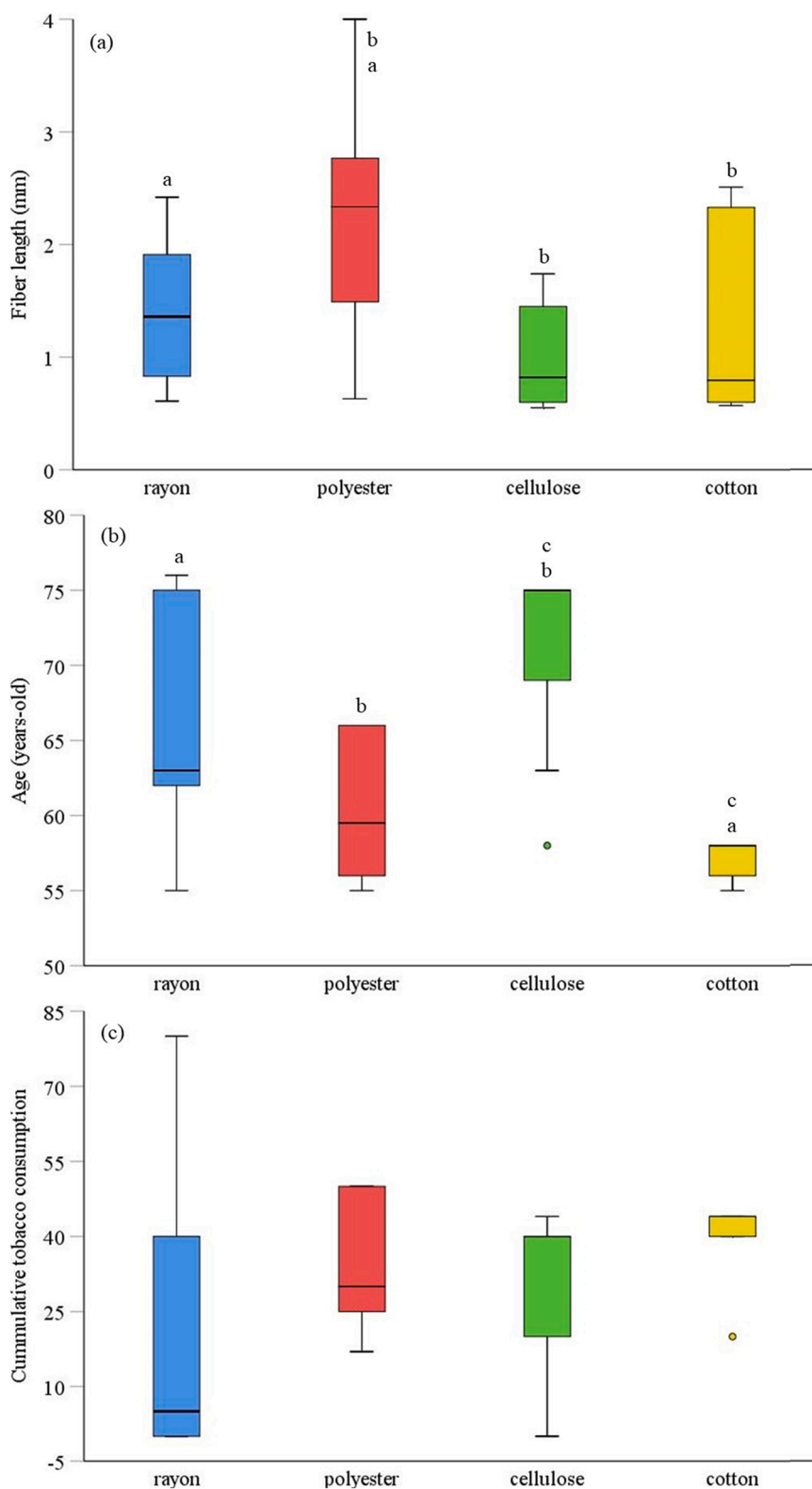
According to the  $\mu$ -FTIR analysis (Fig. 3), the vast majority of MFs were chemically identified as a semisynthetic cellulose-based polymer commonly referred to as rayon/viscose (40.48%) (Fig. 3c), followed by polyester (19.05%) (Fig. 3a), cellulose (16.67%) (Fig. 3f), and cotton (14.29%) (Fig. 3e), The remaining MFs were synthetic wool (Fig. 3d),



**Fig. 3.** Representative  $\mu$ -FTIR spectra of the polymers found in this study: (A) polyester fiber, (B) acrylic fiber, (C) rayon fiber, (D) wool fiber, (E) cotton fiber, and (F) cellulose fiber.

and polyacrylic acid (Fig. 3b) (2.38% for each one). Similar percentages of cotton (16.2%) and polyester (21.63%) were reported by [Amato-Lourenço et al. \(2021\)](#) and [Huang et al. \(2022\)](#) for human lung and sputum, respectively. Only two particulate MPs were identified as polyurethane and an unknown polymer. Furthermore, natural MFs, i.e.,

cotton and wool (Figs. 3d and 3e, respectively) displayed typical structures with no obvious disruptions of morphology, although they were often dyed and coated with chemical additives, suffering from reduced biodegradability ([Chen and Jakes, 2001](#)). Rayon MFs were mainly isolated from female participants, with a concentration of  $2.19 \pm 0.73$



**Fig. 4.** Box and whisker plots of four main polymer types according to: (a) MFs length, (b) age of patients, and (c) cumulative tobacco consumption. Circles are outliers beyond any value that lies more than  $1.5 \times \text{IQR}$ . Letters above the boxplot denote statistically significant differences.



items/100 mL BALF, in contrast to that in male patients ( $0.41 \pm 0.15$  items/100 mL BALF) ( $F$ -value = 10.769,  $p = 0.001$ ). They were also found to be higher in occupations with a high risk of exposure to MPs ( $2.71 \pm 0.92$  items/100 mL BALF) than in those with a low exposure risk ( $0.43 \pm 0.14$  items/100 mL BALF) ( $F$ -value = 14.763,  $p = 0.000$ ). In our study, the high abundance of rayon/viscose and polyester identified in BALF samples maybe related to the frequent wearing of masks during the COVID-19 pandemic because both textile materials are widely used for disposable masks (Militky et al., 2021). A recent study conducted by Li et al. (2021) reported that the source of the inhaled MFs could be the air and the mask itself, but all masks reduced the inhalation risk of global MPs even when worn continuously for 720 h continuously.

Fig. 4 displays box-and-whisker plots for the four main types of polymers identified in isolated MFs, as a graphical tool for efficiently visualizing the continuous unimodal data distribution according to MFs size (Fig. 4a), patient age (Fig. 4b), and cumulative tobacco consumption (Fig. 4c). The boxplot included in Fig. 4a shows that the median does not lie in the middle of the box, except for that of rayon, which has a more symmetrical distribution of sizes. Polyester has an asymmetric positive distribution and cellulose and cotton have a positive one. The maximum and minimum median values were those of rayon (2.42 mm) and cellulose (0.55 mm), respectively. No observations in any polyester type exceeded the upper or lower whisker, indicating that there is no outlier in the distribution, and a correct data scattering can be assumed. As presented in Fig. 4a, the median level of MFs varied substantially according to the polymer type. The highest median was that of polyester (2.34 mm), and the lowest one was that of cotton (0.80 mm); however, the lowest interquartile range (IQR) (P25–P75) was for cellulose (0.58–1.54), and cotton had the distribution with the most dispersal particle size (0.59–2.38). Kärkkäinen and Sillanpää (2021) also demonstrated that polyester had the highest mean MFs length, in the quantification of various MFs discharged from textiles in washing machines.

The type of polymer identified also varied by age (Fig. 4b). We have already determined that with the increase of age, the MFs content in BALF samples gradually increased. As depicted in Fig. 4b, cotton MFs were mostly identified in BALF samples from younger patients (58 years old) than rayon (63 years old) ( $p = 0.007$ ) or cellulose MFs (75 years old) ( $p = 0.001$ ). Rayon had the most dispersed distribution according to the IQR (59.5–75 years) and cotton had the narrowest one (55.8–58 years). According to Fig. 4c, rayon also displayed the highest IQR for cumulative tobacco consumption (0–40), although no statistically significant differences were observed among the different polymers according to this parameter ( $p = 0.213$ ). The average concentration of rayon MFs across the study was  $1.03 \pm 0.28$  items/100 mL BALF, proving to be higher for active ( $1.76 \pm 0.65$  items/100 mL BALF) than for former smokers ( $0.32 \pm 0.18$  items/100 mL BALF) ( $p = 0.027$ ). In addition, there was a statistically significant positive correlation between age and tobacco consumption (Pearson's  $r = 0.422$ ,  $p = 0.000$ ). Rayon is widely used in cigarette filters, personal hygiene products, and clothing, and it has been reported as a major source of MPs debris even in the deep sea (Woodall et al., 2014; Lusher et al., 2016) and coastal sediments (Frias et al., 2016). All those reasons could explain our results depicted in Fig. 4b and c.

### 3.3. SEM-EDS analysis of MPs

The difficulty of analyzing complex MFs with a single analytical technique is clear, and a combination of two or more analytical methods should be commonly used (Gniadek and Dąbrowska, 2019). After a first physical identification using stereomicroscopy and polymer identification by  $\mu$ -FTIR, the SEM-EDS analysis provided high-resolution imaging of MFs surface structures and morphology, as well as their elemental composition, to identify carbon-dominant plastics from other inorganic particles. It is worth obtaining precise knowledge regarding the morphology of the examined sample, together with a fast elemental

analysis with EDS, which provides semiquantitative information on its composition. Because it is a time-consuming process that requires substantial effort to prepare and examine sample, SEM-EDS analyses were limited to eight random patients (18.18%). Samples were analyzed without any covering conducting layer, and with an electron energy beam of 15.0 kV. Although images were obtained with some charging effects and less detailed morphology than with a lower voltage, a better EDS spectrum could be registered.

A total of 25 different MFs were processed using SEM-EDS. The high carbon content in all of them indicated by energy dispersive X-ray spectra was used for the validation of MPs (Tiwari et al., 2019). Fig. 5 provides SEM pictures and the EDS spectra registered for three different MFs isolated from BALF samples, i.e., MFs #12, #10, and #22, corresponding to participants 027, 008, and 022, respectively. Images revealed concave or convex smooth surfaces, with an absence of cracks or fragmentation but with some scratches. Traces of silicon and sulfur were detected on MFs #12 (Fig. 5a, b, and c), together with alkali earth calcium, alkali potassium, and non-metallic chlorine. These results could be consistent with exposure to gaseous emissions from combustion sources for participant 027, the only one working in a fuel station and with a high risk of long-term exposure to motor vehicle emissions (Morawska and Zhang, 2002; Soeroso et al., 2019).

The EDS spectrum for MFs #10 isolated in participant 008 (Fig. 5d, e, and f) showed statistically significant higher concentrations for Al, Si, and P than for the remaining analyzed samples, i.e., Al (0.58% vs. 0.09%,  $F$ -value = 28.136,  $p = 0.000$ ), Si (0.51% vs. 0.13%,  $F$ -value = 5.333,  $p = 0.025$ ), and P (0.70% vs. 0.02%,  $F$ -value = 39.008,  $p = 0.000$ ). These findings are compatible with the presence of aluminosilicate MFs or some type of asbestos MFs (Dumortier et al., 2001), although that participant did not show any abnormalities in his radiological diagnosis. As reported by Sartorelli et al. (2020), the effect of smoking on the cytological analysis of BALF from asbestos-exposed workers was significantly more evident than any other effect, and the presence of Si and Al compounds in the BALF of smokers has been widely described (Brody and Craighead, 1975), representing a confounding factor (Perna et al., 2002). The reported SEM-EDS results could also be compatible with the presence of poly(aluminum phosphate) compounds, used as flame retardants in some polymers (Naik et al., 2013). For MFs #22 (Fig. 5g, h, and i), important levels of Al in its composition may also be related to this participant's occupation as a carpenter installing assembled aluminum alloy windows. In any case, no toxic element, that could be leached into the respiratory tract of the patients and outline a potential health concern, was found to be adsorbed as a pollutant in any of the analyzed MFs.

Despite efforts required by this technique, the high-quality, high-magnification, and high-resolution images delivered by SEM, combined with energy dispersive X-ray spectroscopy for qualitative and quantitative information of the sample, provide a powerful tool for MPs identification, without the need requirement to cover samples with a metal conductive layer when working at low kV.

## 4. Conclusions

This is the first study reporting the presence of MPs in human lower airway detected with  $\mu$ -FTIR and SEM-EDS analysis of BALF samples, considered to be good quality samples and highly representative of the human lower airway. The existence of MPs was a frequent finding in our patients, particularly in smokers or in specific occupations, exposures that would be preventable. In addition, the association between the MFs concentration and pathological findings such as radiological abnormalities, pathological microbial growth, and decreased lung function, raises various possibilities regarding what the pathogenic mechanisms could be for MPs in the lung. Although larger studies are necessary to define the role of airborne MPs in respiratory pathology more effectively, these results alert us that exposure to these MFs could have important consequences on respiratory health and that it is most likely

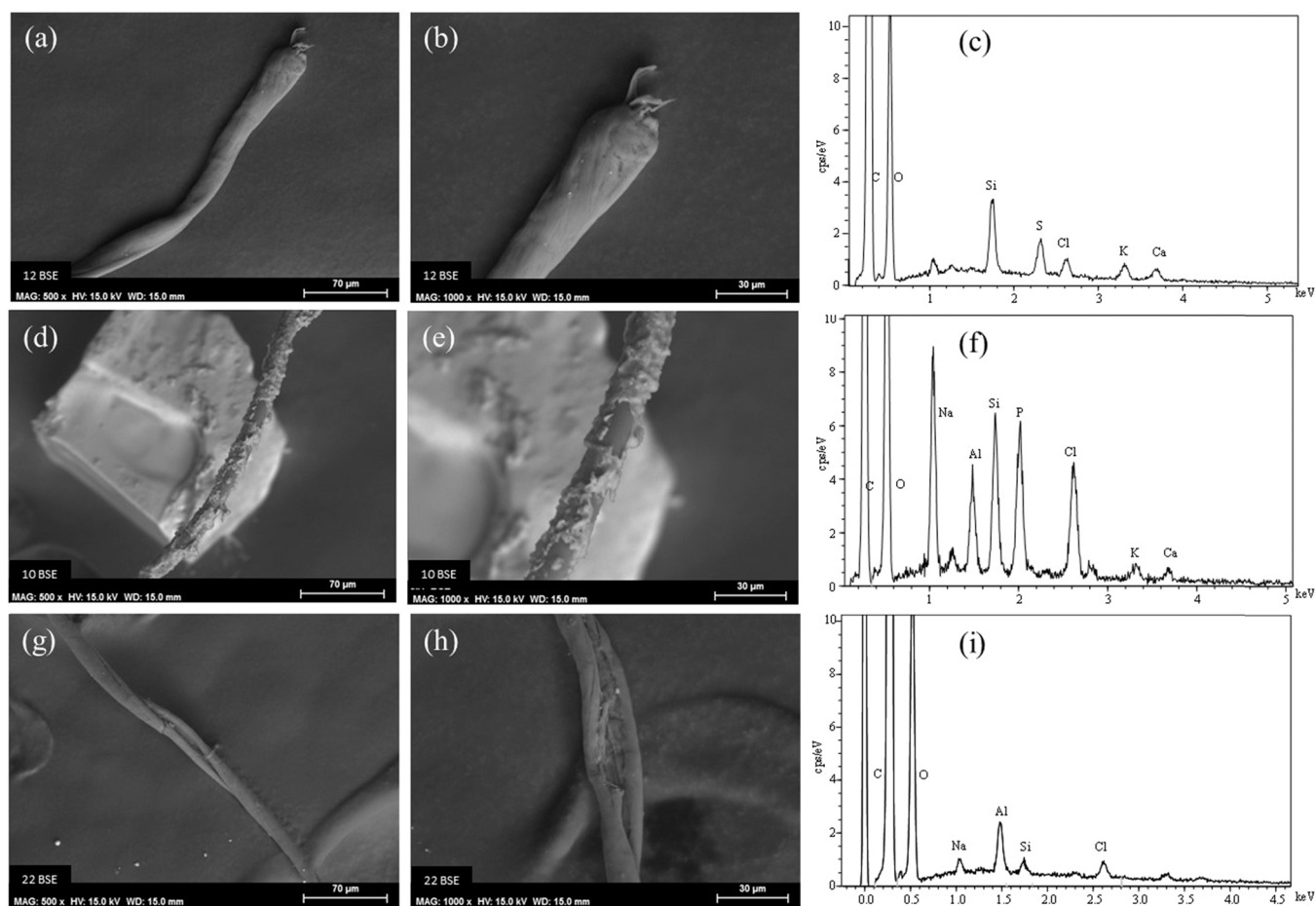


Fig. 5. SEM images and EDS spectra acquired at 15.0 KV for: (a–c) MFs #12 (participant 027); (d–f) MFs #10 (participant 008); (g–i) MFs #22 (participant 022).

necessary to implement measurements to reduce human exposure to MPs.

### Environmental implications

The term “microplastic” was first used by Carpenter and Smith (1972) to indicate the presence of these compounds in the Sargasso Sea. Since then, concern regarding these very small pollutants has grown exponentially. MPs have been detected in various environmental compartments and, even in human biological samples, i.e., feces, urine, blood, or placenta. Because they can remain suspended in atmospheric aerosols, the consequences for human health when MPs are inhaled are still not well known. In this study, we determined the presence of MPs in the human lower airway detected by means of BALF samples, most of them in the shape of fibers.

### CRedit authorship contribution statement

**Carlos Baeza-Martínez:** Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing, Funding acquisition. **Sonia Olmos:** Investigation, Resources, Supervision, Visualization. **Miguel González-Pleiter:** Conceptualization, Investigation, Supervision, Writing – original draft, Visualization. **Joaquín López-Castellanos:** Investigation, Resources, Supervision, Visualization. **Eduardo García-Pachón:** Conceptualization, Investigation, Methodology, Writing – review & editing, Visualization, Funding acquisition. **Mar Masía-Canuto:** Resources, Writing – review & editing, Supervision. **Luis Hernández-Blasco:** Resources, Writing – review & editing, Supervision. **Javier Bayo:** Methodology, Formal analysis, Writing –

original draft, Writing – review & editing, Investigation, Funding acquisition.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data Availability

The data that has been used is confidential.

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