

# IMPACT OF AIRBORNE MICROPLASTICS ON INDUCED SPUTUM OF URBAN DWELLERS: THE ROLE OF ENVIRONMENTAL AND OCCUPATIONAL FACTORS

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## ABSTRACT

As an emerging environmental problem in our society, microplastic (MP) pollution within the human body is gaining significant attention within the research community, prompting in-depth investigations. This study is based on the analysis of induced sputum samples to investigate the presence of these pollutants and to determine their level of unintentional inhalation by humans. For this purpose, samples from 25 patients with respiratory diseases were prospectively collected, who underwent anamnesis, physical examination, blood analysis and computed tomography. Besides sex, age, smoking habits, occupation, or residence, other variables were considered. After 10% KOH organic matter digestion, samples were observed under an Olympus SZ-61TR Zoom Trinocular Microscope coupled to a Leica MC190 HD digital camera, and an image capturing software Leica Application Suite (LAS) 4.8.0. The chemical composition of MP was analysed by  $\mu$ -FTIR with a Perkin-Elmer Spotlight<sup>TM</sup> 200 Spectrum Two instrument, and spectra were compared with Omnic 9.1.26 database and our own database. The abundance of microplastics ranged from 6.25 to 500 items/100 mL of induced sputum. There was no statistically significant difference for abundance between male and female, although active smokers displayed a higher average concentration than former or non-smoker patients. Furthermore, professional occupations with a high exposure risk to microplastics demonstrated significantly higher average concentration. Remarkably, participants residing in urban areas demonstrated a significantly higher average concentration of microplastics compared to those living in rural environments. Also, younger patients showed higher microplastic abundance than older ones. Microplastics smaller than 1 mm accounted for the highest percentage (52.50%), and the most represented size range for microplastics was between 1 and 2 mm (26.25%). The results revealed the identification of seven polymer types, with rayon being the most prevalent, followed by modified cellulose, acrylic, modified cotton, polyester, polyethylene terephthalate and polydimethylsiloxane.

*Keywords: microplastic, induced sputum, environment, human health, occupational factors.*

## 1 INTRODUCTION

Microplastics (MP) were first described as water pollutants in 1972, when Carpenter and Smith [1] described them floating in the Sargasso Sea, although they were first introduced in scientific references by Thompson et al. [2]. These are plastic microparticles smaller than 5 mm, except in fibres which are 15 mm in length. Furthermore, they are classified into primary and secondary MP, with the former being intentionally manufactured with that size, and the others, more important for the environment, generated from the degradation of larger mesoplastics [3]. It is a pollutant nowadays spread all over the world, so its presence poses a threat to the environment, affecting surface waters and stormwater [4], soils [5], sediments [6], food [7], drinking water [8], oceans [9], and of course the air [10]. In addition, MP have a potential toxicological effect, due to the large number of substances of organic and inorganic nature they can carry [11], [12], and because of the progressive appearance of



residual monomers that constitute the original polymer, all of which are harmful to the environment.

Human exposure, uptake, and accumulation of MP is still far from being understood, including various routes through direct food, drinks, and water ingestion [13], or inhalation [14]. Although several studies have unveiled their presence in multiple human samples, i.e., faeces [15], blood [16], placenta and meconium [17], thrombi [18], stomach and intestine [19], eye [20], testicles [21], semen [22], colectomy specimens [23], or breastmilk [24], there are still several challenges and a significant knowledge gap in the existing literature regarding MP exposure and health effects [25].

Inhalation has proved to be an important route for MP exposure and entry into the human body, because of the widespread occurrence and transport of these micropollutants in the atmosphere, even exceeding MP intake via inhalation [26], [27]. Although the presence of MP in indoor and outdoor air has been extensively established, there are still many knowledge gaps and challenges to solve, including potential human health risk, lack of a standardized methodology, or the role of environmental and social variables on MP exposure [28]–[31]. Several studies have demonstrated the presence of MP in different parts of the human respiratory system, including lung tissues [32], [33], bronchoalveolar lavage [14], [34], pleural effusion [35], sputum [36], saliva [37], or nasal lavage [38]. The presence of MP in lower airways increases the long-term MP exposure concerns for respiratory health and may adversely impact the human respiratory system [39].

Induced sputum (IS) is a relatively non-invasive technique of airway sampling, that provides the opportunity for analysing cellular components and infectious agents from respiratory tract, including bacteria and viruses, along with constituents of the liquid phase, including MP [40]. In our study, we present the detection and abundance of MP in the lower respiratory tract, as obtained from IS of 25 patients with different respiratory diseases, exploring the differences in particle size, morphology, and polymer composition, as well as their relationship with environmental, clinical, and occupational risk factors. We employed optical microscopy for visual identification and micro-Fourier transform infrared spectroscopy ( $\mu$ -FTIR) to evaluate the composition of isolated microparticles, to assess their exposure to these micropollutants.

## 2 MATERIALS AND METHODS

### 2.1 Ethics approval and participant consent

Between February and May 2023, a prospective study enrolled 25 adult patients with respiratory diseases who underwent IS analysis at Hospital General Universitari d'Elx (HGUE) in Alicante, Spain. Each participant received study details and consented to donate IS samples. The research obtained approval from the HGUE Health Department's Ethics Committee (Ethics Approval ID: PI 7/2021) and adhered to the revised Declaration of Helsinki, with sample collection conducted by trained nurses and physicians. Patients with oximetry levels below 94% or experiencing hemodynamic instability were excluded from participation.

### 2.2 Medical background and variables

As illustrated in Table 1, all patients underwent anamnesis, physical examination, blood tests, and chest computed tomography (CT scan). Self-reported data on sex, age, smoking habits, occupation, place of residence, and building type were also collected.



Table 1: Main characteristics for the study participants.

Participant	Sex	Age (years)	Smoking habit	Occupation (risk group)	Residence	Radiological diagnosis
B5INSP 078	F	60	3	2	1	3
B5INSP 079	M	61	2	1	2	1
B5INSP 080	F	54	2	2	1	1
B5INSP 081	M	51	1	1	1	1
B5INSP 082	F	58	2	2	1	2
B5INSP 083	M	67	3	2	1	1
B5INSP 084	M	57	1	1	1	1
B5INSP 085	M	64	3	2	2	1
B5INSP 086	M	49	2	1	2	1
B5INSP 087	F	65	3	2	1	1
B6INSP 094	M	56	1	2	1	1
B6INSP 095	F	48	3	2	1	1
B6INSP 097	M	77	1	2	1	1
B6INSP 098	F	60	3	1	1	1
B6INSP 099	M	63	2	1	1	1
B6INSP 100	F	47	1	2	1	1
B6INSP 101	F	74	3	1	2	3
B6INSP 102	M	49	1	1	1	1
B6INSP 103	M	69	1	2	1	1
B7INSP 109	F	73	3	2	1	1
B7INSP 111	F	61	2	1	1	1
B7INSP 112	M	49	2	1	2	2
B7INSP 113	F	72	3	2	1	3
B7INSP 114	F	66	3	2	1	1
B7INSP 115	M	66	2	2	1	1

Sex: (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; Radiological diagnosis: (1) pulmonary parenchymal pathology, (2) other abnormalities detected in CT, (3) without radiological abnormalities.

### 2.3 IS collection and quality assurance

Both the induction and processing of sputum samples are perfectly established and standardized, with manuals available for professionals to perform the procedure at the highest level [41].

Sputum induction involved inhaling nebulized sterile hypertonic saline solution, followed by coughing and expectoration to collect airway secretions. All recruited patients received a muffled sputum sampling glass container, and potential contamination from MP was monitored. This monitoring included procedural blanks, consisting of five solvent blanks of hypertonic saline solution, analyzed throughout the entire study. Additionally, five airborne blanks were collected, with one per every five IS samples. Airborne blanks were obtained using glass Petri dishes kept open during all procedures. Results were normalized, and particles matching the typology and composition of those found in control samples were excluded from statistical analyses.

The risk of contamination from devices was reduced as much as possible. Although plastic lab wares were limited to the maximum, they could not be entirely avoided. All metal, steel,





plastics. The data processing was carried out using SPSS (Statistic Package for Social Science) version 26.0 software.

### 3 RESULTS AND DISCUSSION

#### 3.1 General results

The study participants had an age range of 47 to 77 years, with an average age ( $\pm$  standard error of the mean) of  $60.64 \pm 1.76$  years. Twelve participants (48%) were female, and 13 participants (52%) male. Most of women (66.67%) were non-smokers, and the majority of men (84.61%) were active or former smokers. Only three female participants (25%) and seven out of 13 men (53.85%) had a high-risk profession for MP occupational exposure. Most of patients with respiratory diseases had pulmonary parenchymal pathology (80%), and only three women displayed no radiological abnormality. With respect to different lung diseases, most of patients had respiratory infection (56%), followed by pulmonary neoplasia (20%), and benign nodule (8%).

The techniques for MP identification through optical analysis and  $\mu$ -FTIR have proved to be of good quality and highly representatives of the lower airway. The average proportion of plastic vs. non-plastic particles in this study was 28.17% and 71.83%, respectively, close to that reported by Huang et al. [36] for human sputum in China (32% and 68% respectively). Five participants (20%) did not have any MP in IS, and the concentrations ranged from 6.25 to 500 items/100 mL IS, similar to that described by Huang et al. [36] (395 particles/100 mL) for sputum of patients suffering from different respiratory diseases. There was no statistically significant difference for abundance between male and female ( $p = 0.357$ ), however, there was statistically significant difference in MP concentration by age, being higher for participants  $\leq 50$  years old ( $176.47 \pm 12.71$  items/100 mL IS) than for those older than 50 years old ( $97.43 \pm 1.38$  items/100 mL IS) ( $F$ -value = 9.335,  $p = 0.003$ ) (Fig. 2). Chen et al. [44] and Tuna et al [45] also found no differences between sexes in bronchoalveolar or nasal lavage fluids, respectively, and Huang et al. [36] and Chen et al. [44] demonstrated an increase in MP concentrations with decreasing age. Most of MP were in the shape of fibres

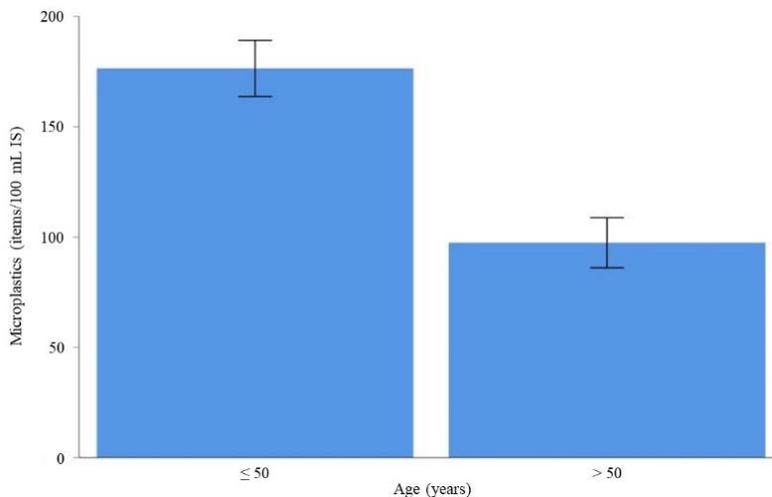


Figure 2: One-way ANOVA plot for MP concentration in human IS by age.

(87%), and only 13% turned out to be particulate MP. Similar results were reported by Abbasi and Turner [37] for human saliva, with a 97% of fibres, and Jiang et al. [38] found a 94.3% and 83.3% of fibres in sputum of couriers and office staff, respectively.

Fibers showed an average size of  $1.27 \pm 0.09$  mm, with the longest dimension (10.51 mm) corresponding to a rayon fibre isolated from a 72 year old non-smoker shoemaker woman without radiological abnormalities, and a minimum size for a 159  $\mu\text{m}$  rayon fibre, isolated in a 49 year old former smoker mechanic man, with anomalies detected in CT. The colours of the isolated MP were distributed as follows: transparent (60%), blue (16.25%), white (8.25%), brown (8.25%), red (2.50%), black (2.50%), and orange (1.25%) (Fig. 3(a)). MP smaller than 1 mm accounted for the highest percentage (52.50%), and the most represented size range was 1–2 mm (26.25%), followed by 400–600  $\mu\text{m}$  (16.25%), as presented in Fig. 3(b).

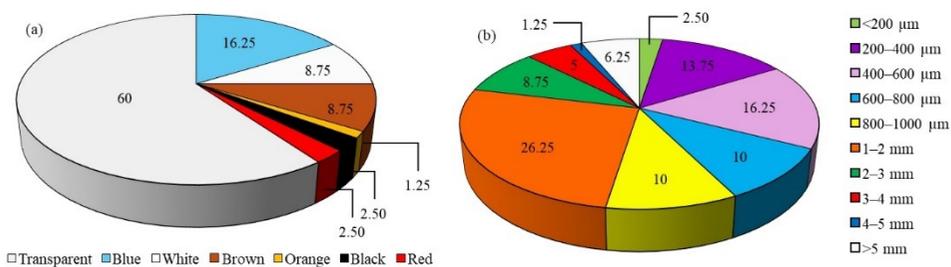


Figure 3: Accumulated percentages of MP isolated in human IS by: (a) colours; and (b) size categories, based on Spanish Environmental Ministry classification.

### 3.2 Environmental, occupational, and clinical variables

Regarding smoking habits (Table 1), out of the participants, seven were active smokers, eight were former smokers, and ten were non-smokers, with an average cumulative tobacco consumption of  $29.90 \pm 1.60$  cigarettes. MP concentration showed statistically significant differences based on smoking habits ( $F$ -value = 14.493,  $p = 0.000$ ). Pairwise comparisons by the least significant difference (LSD) test revealed significant differences between active smokers ( $220.83 \pm 18.73$  items/100 mL IS) and former smokers ( $84.24 \pm 8.69$  items/100 mL IS) ( $p = 0.000$ ), as well as between active smokers and non-smokers ( $95.88 \pm 18.48$  items/100 mL IS) ( $p = 0.000$ ), but not between former smokers and non-smokers ( $p = 0.553$ ).

Furthermore, participants residing in urban areas exhibited a statistically significant higher concentration of MP ( $134.24 \pm 12.26$  items/100 mL IS) compared to those in rural environments ( $53.64 \pm 12.77$  items/100 mL IS) ( $F$ -value = 14.902,  $p = 0.000$ ). Additionally, participants living at ground level showed a statistically significant lower average MP concentration ( $46.67 \pm 11.25$  items/100 mL IS) compared to those residing on upper floors ( $143.94 \pm 12.40$  items/100 mL IS) ( $F$ -value = 25.969,  $p = 0.000$ ).

Outdoor workers (OW), i.e., farmer, construction worker, sailor, salesperson, or gardener, displayed a lower concentration of MP ( $97.78 \pm 18.11$  items/100 mL IS) than indoor workers (IW), i.e., painter, office staff, shoemaker, cleaner, or housewife ( $117.92 \pm 12.15$  items/100 mL IS), although without statistically significant difference ( $p = 0.345$ ).

Patients with any anomaly detected with CT scan displayed a higher MP concentration ( $121.92 \pm 12.23$  items/100 mL IS) than those without radiological abnormalities ( $79.41 \pm$

16.21 items/100 mL IS), although without statistically significant difference ( $p = 0.065$ ). When the same analysis was carried out on fibres, difference appeared to be statistically significant ( $F$ -test = 4.289,  $p = 0.041$ ), being  $129.60 \pm 14.21$  items/100 mL IS for the first group, and  $79.41 \pm 16.21$  items/100 mL IS for the second one. Body mass index was inversely correlated with MP burden in IS, with a Pearson's correlation coefficient of  $-0.277$  ( $p = 0.005$ ). To the best of our knowledge, MP concentration has not been correlated with body mass index in any study carried out in humans, although both Welden and Cowie [46], and Wang et al. [47] reported a decrease in that parameter for different living beings after MP exposure. Regarding pathological microbial growth, patients with pathological microbes isolated from their IS samples displayed a higher MP abundance ( $131.31 \pm 9.94$  items/100 mL IS) than those with an aseptic IS ( $71.18 \pm 21.28$  items/100 mL IS) ( $F$ -value = 8.529,  $p = 0.004$ ).

### 3.3 MP analysis

According to the  $\mu$ -FTR analysis, a total of seven polymers were identified from IS samples. They included rayon or viscose (57%), modified cellulose (25%), acrylic (6%), modified cotton (5%), polyester (4%), PET (2%), and polydimethylsiloxane (PDMS) (1%) (Fig. 4). Similar to polyester fibres, rayon fibres produce high quality textiles widely used in personal

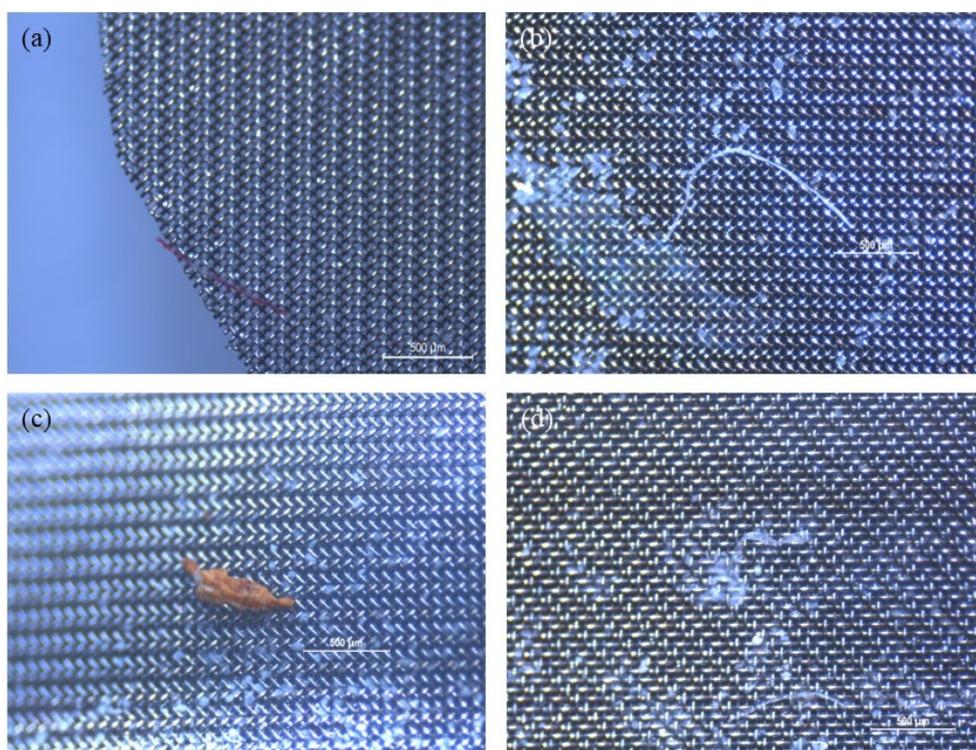


Figure 4: MP isolated from IS samples: (a) red rayon fibre from B7INSP\_111; (b) transparent modified cellulose fibre from B6INSP\_102; (c) brown acrylic particulate MP from B6INSP\_099; and (d) transparent modified cotton fibres from B6INSP\_101.

hygiene products [48], as a semisynthetic cellulose-based polymer [34]. According to Napper et al. [49], rayon proved to be the dominant polymer in air, surface water, and sediment within a major river system (54%–82%), and the most prevalent type of fibre for atmospheric MP [50]. As modified cotton, modified or dyed cellulose is considered an anthropogenic microparticle, made of synthetic, semisynthetic, or modified natural components [51].

The distribution of MP polymers did not differ between IW and OW, being rayon (40% for IW and 17% for OW) and modified cellulose (15% for IW and 10% for OW) the predominant plastic materials in the IS. However, fibres of modified cotton only appeared in IW, mainly shoemaker, cleaner, and cook, and fibres of modified cellulose were isolated in IS samples from construction worker. Again, smoking habit was important for the accumulation of cellulose fibres in IS, with statistically significant differences ( $F$ -value = 6.143,  $p = 0.008$ ). Smokers displayed the highest average cellulose abundance ( $219.05 \pm 19.05$  items/100 mL IS) than former smokers ( $44.44 \pm 16.27$  items/100 mL IS) ( $p = 0.004$ ), and non-smoker patients ( $71.88 \pm 47.74$  items/100 mL IS) ( $p = 0.009$ ), although no statistically significant difference was observed between these two groups ( $p = 0.582$ ). Cigarettes have been considered as a source of MP as their butts are usually composed of cellulose acetate [14], [34], [36].

#### 4 CONCLUSIONS

The results of this study allow us to conclude that a variety of MP were present in the lower respiratory tract of patients with respiratory diseases, as represented by IS. No differences were recorded by sex, although younger patients displayed a higher average concentration than older ones. MP in IS were predominantly transparent fibres in the ranges of 1–2 mm (26.25%) or 400–600  $\mu\text{m}$  (16.25%), and environmental and occupational factors as smoking habit, place of residence, or professional occupation proved to be important in MP burden. As much as seven different polymer types were isolated in IS samples, indicating the large amount of urban and industrial plastic sources used today, reaching the air and inhaled by humans, which should be further studied, as well as their effects on human health.

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