Atmospheric Microplastics and the Human Lungs

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Thesis submitted for the Degree of Doctor of Philosophy (PhD)

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Hull York Medical School

March, 2025

Abstract

Microplastics (MPs) are an emerging environmental contaminant (EEC), that have recently been isolated from samples collected from the atmosphere, and are considered ubiquitous on Earth. There is a lack of knowledge regarding the properties of atmospheric MPs (AMPs), in terms of location, concentrations, plastic types, sizes and shapes. There is also limited understanding of the potential for these MPs to be inhaled, and the consequences of such exposure. Standardised approaches throughout the AMP field are called for, because incomparable datasets and varying microplastic (MP) definitions are slowing down the progression of research. It is now necessary to thoroughly investigate AMPs, and gain knowledge of the location and MP types relevant to human exposure, as well as assessing MP inhalation as an exposure route for humans. This information can direct future investigations into the potential hazards associated with AMP inhalation.

This thesis presents 3 publications, within this publication style thesis; investigating indoor AMPs, outdoor AMPs and a final investigation into the presence of MPs within human lung tissue samples, acquired from living patients.

First, passive sampling of 20 households over a 6-month discontinuous duration, reported an average concentration of 1414 MP m^{-2} day^{-1} ± 1022 (mean ± SD). This high abundance of MPs within household environments supports the importance of indoor MP sampling locations. Fibrous and fragmented Polyethylene terephthalate (PET), Polypropylene (PP) and Nylon were also stated as relevant to human exposure, at head height.

Secondly, passive sampling within a busy outdoor urban roadside location reported concentrations of MPs; 3055 ± 5072 MP m^{-2} day^{-1} (mean ± SD, 1164 median), over a yearlong investigation. Specific outdoor areas of high human activity were suggested to rival that of indoor concentrations. An additional snap-shot 2-week investigation, passively sampling 5 different areas of varying human activity, was also conducted. Roadside, commercial and industrial locations were reported with high concentration rates and relevance to human exposure. An abundance of film and fragmented particles, of Polyethylene (PE), Nylon and Resin composition, suggested these properties to be most relevant to human health studies.

Finally, digested human lung tissue analysis provided evidence to support the human inhalation of MPs. 39 MPs were identified within 13 lung tissue samples, acquired from 11 living human patients. PP was reported within samples, after strict limit of detection and limit of quantification (LOD LOQ) adjustments were applied. PP, PET and Resin synthetic plastic types, and fibre and fragment shape categories were identified and suggested to be of relevance to human inhalation. The size of most MPs identified within lung tissue samples were larger than that thought possible to inhale, whilst some were smaller and traditionally more inhalable.

These publications bridge environmental MP research and human MP health studies, providing much needed knowledge regarding the concentration and types of AMPs that humans are most likely exposed to on a daily basis, as well as supporting the potential for exposure to AMPs via inhalation. Uniquely, at the forefront of all publications, was the aim to provide novel, high quality methodologies, combatting methodological restrictions, and focusing considerably on improving the quality of research concerning AMPs and human exposure, with great emphasis on quality control. These chapters, alongside other AMP research, can form the foundations for future research improvements, leading to eventual standardised operating procedures (SOPs), policy formation, and to achieve guided, accurate and environmentally relevant MP exposure investigations.

Contents

Abbreviations

µm	Micrometres
°C	Degree Celsius
AMP(s)	Atmospheric microplastic(s)
ATP	Adenosine triphosphate
ATR	Attenuated total reflectance
BAL(F)	Bronchoalveolar lavage (fluid)
cm(s)	Centimetre(s)
CO_2	Carbon dioxide
EEC(s)	Emerging environmental contaminant(s)
EU	European Union
(µ)FTIR	µFourier-transform infrared spectroscopy
FPA	Focal plane array
GF	Glass Fibre
H_2SO_4	Sulfuric acid
H_2O	Water
H_2O_2	Hydrogen peroxide
H_2O_2 + fe	Hydrogen peroxide + Fenton's reagent
HCC	Hull City Council
HCl	Hydrochloric acid
HDPE	High density polyethylene
HEPA	High efficiency particulate air
HNO_3	Nitric acid
HYMS	Hull York Medical School
IR	Infrared

KBr	Potassium bromide
KI	Potassium iodide
km	Kilometres
KOH	Potassium hydroxide
LDIR	Laser direct infrared imaging
LDPE	Low density polyethylene
LLDPE	Linear low density polyethylene
LN_2	Liquid nitrogen
LOD	Limit of detection
LOQ	Limit of quantification
m	Metres
MCE	Mixed cellulose ester
MCT	Mercury cadmium telluride
mL	Millilitres
mm	Millimetres
MP(s)	Microplastic(s)
Mt	Million tonnes
NaCl	Sodium chloride
NaClO	Sodium hypochlorite
NaI	Sodium Iodide
NaOH	Sodium hydroxide
nm(s)	Nanometre(s)
NP(s)	Nanoplastic(s)
O_2	Oxygen
PA	Polyamide/ nylon
PAH(s)	Polyaromatic hydrocarbon(s)
PCB(s)	Polychlorinated biphenyl(s)
PE	Polyethylene
PES	Polyester
PET	Polyethylene terephthalate
PLA	Polylactic acid
PTFE	Polytetrafluoroethylene
PM	Particulate matter

$PM_{2.5}$	Particulate matter of <2.5 μm in size
PM_{10}	Particulate matter of <10 μm in size
PP	Polypropylene
PS	Polystyrene
PUR	Polyurethane
PVA	Polyvinyl acetate
PVC	Polyvinyl chloride
Pyr/GC/MS	Pyrolysis-gas chromatography-mass spectroscopy
QA/ QC	Quality assurance and quality control
SEM	Scanning electron microscopy
SEM-EDS	Scanning electron microscopy and electron dispersion spectroscopy
SD	Standard Deviation
SNR	Signal to noise ratio
SOP(s)	Standard operating procedure(s)
TEOM	Tapered element oscillating microbalance
TLR(s)	Toll like receptor(s)
UK	United Kingdom
UV	Ultraviolet
$ZnCl_2$	Zinc chloride
ZnSe	Zinc selenide

Publications

Danopoulos, E., Jenner, L., Twiddy, M., Rotchell, J. M. (2020) Microplastic contamination of salt intended for human consumption: a systematic review and meta-analysis. *SN Appl. Sci.* **2** (1950) https://doi.org/10.1007/s42452-020-03749-0.

Danopoulos, E., Jenner, L. C., Twiddy, M., Rotchell, J. M. (2020) Microplastic contamination of seafood intended for human consumption: a systematic review and meta-analysis. *Environ. Health Perspect.* **128** (126002) https://doi.org/10.1289/EHP7171.Jenner, L. C., Sadofsky, L. R., Danopoulos, E., Rotchell, J. R. (2021) Household indoor microplastics within the Humber region (United Kingdom): quantification and chemical characterisation of particles present. *Atmos. Environ.* **259** (118512) https://doi.org/10.1016/j.atmosenv.2021.118512.Jenner, L. C., Sadofsky, L. R., Danopoulos, E., Chapman, E., White, D., Jenkins, R. L., Rotchell, J. M. (2022) Outdoor atmospheric microplastics within the Humber region (United Kingdom): Quantification and chemical characterisation of deposited particles present. *Atmosphere.* **13** (265) https://doi.org/10.3390/atmos13020265.

Jenner, L. C., Kureshi, R. R., White, D., Chapman, E., Sadofsky, L. R., Rotchell, J. M. (2022) Outdoor microplastic analysis using inlet filters from an NOx regulatory air quality monitoring device. *Atmosphere.* **13** (2017); https://doi.org/10.3390/atmos13122017

Field, DT., Green J. L., Bennett, R., Jenner, L. C., Sadofsky, L. R., Chapman, E., et al. (2022) Microplastics in the surgical environment. *Environ Int.* 170:107630 https://doi.org/10.1016/j.envint.2022.107630.

Jenner, L. C., Rotchell, J. M., Bennett, R. T., Cowen, M., Tentzeris, V., Sadofsky, L. R. (2022) Detection of microplastics in human lung tissue using μFTIR spectroscopy. *Sci. Total Environ.* (154907) https://doi.org/10.1016/j.scitotenv.2022.154907

Rotchell, J. M., Jenner, L. C., Chapman, E., Bennett, R. T., Bolanle, I. O., Loubani, M., et al. (2023) Detection of microplastics in human saphenous vein tissue using muFTIR: A pilot study. Plos One. 18(2):e0280594 https://doi.org/10.1371/journal. pone.0280594.

Acknowledgements

I would like to extend my sincere thanks, to Dr Laura Sadofsky and Professor Jeanette Rotchell, for your exceptional guidance. but more importantly, for your support during my personal challenges in recent years. You have encouraged me to achieve my goals, and I couldn't have asked for better supervisors. Additionally, I wish to express my gratitude to all the people at The University of Hull, Hull York Medical School and Hull City Council, that I have had the pleasure to work alongside, throughout this degree.

On a personal note, I wish to highlight the unwavering love I have received from friends and family, from those that have been there to encourage and assist, to those that provided an appreciated distraction from life. Especially, thank you to Denise, Dave, and my best friends, Roseanna and Katie, who have been my anchors in difficult times.

To my Dad, you have shown me just how strong someone can be, and have motivated me to push and complete this work. To my Mum, you are selfless and beautiful, you will always be my rock.

To my partner, Mike, thank you for your patience and support throughout our hardest of years, I am so proud of how much we have overcome and achieved, and your love and humour was key.

Finally, to Rori and Lincoln, you are my biggest achievements and I hope you grow up to be as happy, and motivated, as you have made me.

Declaration

I confirm that this work is original and that if any passage(s) or diagram(s) have been copied from academic papers, books, the internet or any other sources these are clearly identified by the use of quotation marks and the reference(s) is fully cited. I certify that, other than where indicated, this is my own work and does not breach the regulations of HYMS, the University of Hull or the University of York regarding plagiarism or academic conduct in examinations. I have read the HYMS Code of Practice on Academic Misconduct, and state that this piece of work is my own and does not contain any unacknowledged work from any other sources. I confirm that any patient information obtained to produce this piece of work has been appropriately anonymised.

1 Chapter 1: General Introduction

1.1 Format

This publication style thesis will consist of three publications, each article aiming to provide more of an insight into AMPs and human exposure, complementing the limited literature available within these fields. Within the first two publications, indoor and outdoor sampling sites will be investigated with the aim of recording as much information about the MP particles present, such as concentration, size, shape and polymer type. A third, and final, publication aims to provide evidence to either 'support' or 'oppose' the human inhalation of MPs, by analysing human lung tissue samples acquired from living patients, for the presence of MP particles.

A general introduction will briefly describe the relevant background prior to the publications, followed by each research paper, presented within its own chapter. Finally, there will be a discussion, including post-publication knowledge and future work.

For the purpose of this thesis, solely synthetic plastics and co-polymers containing synthetic plastics, will be classified as a MP particle. Particles of a natural, or artificial polymer type, may be included within datasets but will be kept distinct from synthetic MPs. Additionally, these synthetic plastic particles will be only classified as a MP when they fall within the size ranges of 5 mm – 1 μm.

1.2 Microplastics

1.2.1 Introduction

Plastics have been produced since the Second World War, with large-scale production implemented within the last 15-20 years (1). Plastic global production is increasing annually, with 413.8 Mt of plastic generated in the year, 2023 (2). The plastic production process begins with distillation, in which extracted crude oil can be refined and 'heat separated' into hydrocarbon fractions, valuable within countless industries, one of which, being the manufacturing of plastic. The long chains of hydrocarbons can then be manipulated, via cracking, and polymerisation, to produce the resins needed to instigate plastic production (3). The introduction of plastic products and materials has heavily influenced the packaging and construction sectors (4), but also many other industries, such as, medicine, automotive, textiles and technology (Table 1). Plastic products have revolutionised and improved modern day life in countless ways (5), so much so, that it would be considered almost impossible to live a day within modern society, without encountering some form of plastic product.

An array of components can be added to the plastic resin starting product, called additives, in order to give plastics specific desirable qualities, and produce either thermosets or

thermoplastics. Engineers can essentially design plastics, with their end plastic product in mind. Thus, a plastic can be formed into a film, a moulded product or fibre, as well as obtaining other advantageous properties, such as; flexibility, rigidity, flame retardancy, chemical resistance, water resistance, biocompatibility, lightweight, heavy duty, Ultraviolet (UV) resistance, and much more, all whilst trying to achieve cost effectiveness (6-8). These adaptable qualities result in a profitable plastics industry that can accommodate countless sector demands, and provides employment to more than 1.5 million individuals within the European Union (EU) alone (4).

Such common day to day use of plastics is now under evaluation, from a governmental, environmental and a general health perspective, particularly because such a large proportion of plastic is designed for 'single use' (9) This is emphasised by the introduction of plastic carrier bag 'charges', plastic microbead 'bans' and the general public becoming more influenced by consumer products avoiding plastic content (10). The major issue of the 'booming' plastic industry is the inability to control waste, recycle plastics and achieve a fully circular plastic economy (4). The lifecycle duration of a plastic product greatly depends on the type of product it is, for instance packaging can have a life span of <1 year whilst products in the building and construction sectors can span >30 years (11). Ultimately, when the 'end of use' is reached, plastics are discarded, either directly into the environment, if waste management is not achieved, or into a waste collection and sorting process, resulting in either landfill, recycling, or energy recovery (4). The recycling rate varies throughout the globe, but approximately 9% of waste is thought to be successfully recycled (11). Consequentially, accumulation of plastic waste within the environment occurs, leading to its global transport and contamination of all ecological compartments; aquatic, terrestrial and atmospheric (12).

It is the beneficial characteristics given to plastics during their production process, that allow plastics to accumulate, disseminate and persist ubiquitously within the environment. The majority of plastics are non-degradable, and whilst their hydrocarbon backbones resist degradation, multiple environmental pressures, such as, UV radiation, biological, wave, and wind action, can cause plastics to break down into smaller particles, essentially resulting in countless MPs and nanoplastics (NPs), originating from one plastic product (5, 11) . It is these smaller plastic particles that are causing the most concern throughout literature, in terms of their impact to the environment, living organisms and most recently, to human health (13). Larger bodies of MP research exist for aquatic and terrestrial sampling, but since 2016 (14), MPs have been identified within atmospheric samples, in both deposited and suspended particle form (15). Less is known regarding the sources, abundance, type and

characteristics of MP particles captured within the atmospheric compartment, and the impact these may have, especially regarding human exposure (13).

1.2.2 Microplastics definition

MPs are commonly classified by their size. But nomenclature based on these size ranges, as well as other MP properties, remain vague throughout literature. Currently, the inclusion of specific size ranges, shapes, polymer types and other particle properties, all vary, and a common MP definition is frequently debated (5, 16, 17).

A plastic particle can be classified as primary, or secondary, in that particles are either intentionally manufactured to have microscopic dimensions, or they are the weathered bi-product of a larger (macro) plastic (12). Primary examples are pre-production pellets (nurdles), abrasives, powders, microbeads and industrial NPs. Secondary examples are; textile fibres, tyre particle wear, paint flakes, fishing equipment and fragmented plastic products (12).

It is common to encounter macro-plastic items within the environment, but at what size these can be considered a MP, is disputed. An upper size limit of 5 mm, and a lower size limit of 1 μm, is more commonly adopted within research, but not always (5, 12, 16). This ambiguity, as well as methodology variations and detection constraints, has led to studies including MPs of different size ranges, and consequently the comparison of datasets is challenging (12).

Many different polymer types are identified within MP samples, for example; synthetic, artificial, co-polymers and natural particles. Synthetic particles are man-made, derived from a petroleum source and are undisputedly considered MPs. PE, PP, Polyvinyl chloride (PVC), PET, polyurethane (PUR) and polystyrene (PS) are examples of synthetic MPs (16). Artificial particles, also referred to as ‘semi-synthetic’, are described as those that originate from a natural source, such as wool, silk and cellulose, but undergo ‘heavy’ modification during the manufacturing process. Some argue that the additives and processing of artificial particles permits them to be classified as a MP, whilst others do not include them within datasets (16). Natural, artificial, bio-based polymers, particles with high additive content, co-polymers and composites, have all been discussed as to their inclusion within the MP definition, yet no standardised definition yet exists (16).

MPs can exist, not only in an array of size ranges, but also a variety of shapes. Within literature, MPs have been reported as fibre, fragment, film, foam, flake, pellet, bead and sphere (16). However, some AMP research articles focus solely on fibrous shapes (18, 19), some fibrous alongside fragmented particles (20), and some incorporating more shape types

(21). The lack of standardisation, again, highlights a lack of harmonisation throughout literature, and begs the question; what should be included within the definition of a MP?

1.2.3 Uses

The uses for plastics are vast, and with the increase in plastic production from 370.7 Mt in 2018 to 413.8 Mt in 2023, it is clear that the demand for plastic products, remains (2). Within the year 2021, 68% of plastic production was distributed to the 'packaging' (44%) and 'building and construction' (18%) industries (4).

PE, is a thermoplastic, similar in structure to its polyolefin family member, PP. It accounts for approximately a quarter of global plastic production (2). There are many different types, or species, of PE, with low-density polyethylene (LDPE), linear low-density polyethylene (LLDPE) and high-density polyethylene (HDPE), the most common. PE originates from the polymerised ethylene starting monomer, but different processing steps and additives permits contrasting plastic properties (22). LDPE is more resistant to stress cracking than HDPE, and is the lightest species, typically clear in appearance, flexible, soft and waterproof. HDPE has a good resistance to chemicals, is more rigid, durable and has electrical insulation properties (22). The different properties allow for a magnitude of uses (Table 1).

PP is another mass-produced synthetic thermoplastic, accounting for approximately 19% of global plastic production (2). Similar to PE, it is a low-cost and versatile plastic. The tough, heat resistant, rigid but flexible nature of PP, again provides countless application opportunities (Table 1).

Whilst PE and PP constitute approximately half of global plastic production, there are a huge number of plastic types available to industries. The most common of which are included in Table 1. Global plastic production figures are an important factor in understanding the lifecycle of plastics, and the subsequent sources of environmental MPs, however it is important to note that some plastics, notably textiles, are not included in the annual 'Plastic Europe' reports (2).

The uses of plastics within textiles is immense, with clothing demand estimated to have increased 400% within the last 20 years, and approximately 70% of textile fibres being purely synthetic (23). Polyester (PES) fibre is the predominant synthetic plastic utilised within the textiles industry, followed by PP, polyamide/ nyslon (PA) and acrylic (23). Synthetic textiles can produce an array of commercial products, such as; knitted and woven fabrics, garments, carpets and textiles used within products, for instance, automobile seat belts and air bags, medical implants, etc. (24), highlighting the importance of synthetic textiles within the plastics industry.

Plastic type	%*	Common Industry Applications	Product Examples	References
PP	19.3%	Packaging, Construction, Automotive, Textiles, Medical	Water filter, air filter, piping, food containers, consumer product bottles, trays, crisp bags, straws, car parts, carpets, concrete reinforcer, surgery supplies	(7, 25, 26)
LDPE	14.4%	Packaging, Automotive, Electrical, Construction	Water bottles and containers, consumer product bottles, shopping bags, bin bags, piping and tubing, food packaging films, building membrane films	(8, 22, 26)
PVC	12.9%	Packaging, Construction, Textiles, Medical	Cleaning product containers, piping, clothes, cladding, flooring and roofing, windows, doors, vinyl records, medical containers	(6, 26, 27)
HDPE	12.5%	Packaging, Construction, Automotive, Consumer products	Food and drink storage, industrial barrels, helmets, bottle caps, fuel tanks, insulation, faux-wood and lumber, shopping bags, consumer product bottles, pipes	(22, 26, 27)
PET	6.2%	Packaging, Textiles, Automotive, Medical, Electrical	Food and drink storage, clothes, curtain and upholstery fabric, casings and insulation, medical equipment	(8, 28)
PUR	5.5%	Construction, Electrical, Automotive, Furniture, Medical	Housing insulation, flexible foams within furniture, electrical components, car seats, cell culture, pace makers	(29)
PS	5.3%	Food Packaging, Product packaging, Medical, Automotive, Construction	Food and drink containers, take-out packaging, plates and cups, electronic packing insulation, petri dishes and test tubes, housing insulation, children's car seats, building insulation	(30)

* Global Plastic Production 2021 (4)

Table 1; demonstrating common plastic types, their global production rates and common examples of application and uses

1.3 Introduction to atmospheric microplastics

1.3.1 Outdoor microplastics

The 2016 landmark study, Dris *et al.* 2016, first reported the presence of MPs within the outdoor air in, 'large amounts'(14). The study captured particles within a large glass vessel for observational and chemical analysis, in which a prevalent fibrous form was first investigated. An urban and sub-urban area of Paris were the focus of the research, and, concentration rates of 110 fibres m^{-2} day^{-1}, and 53 fibres m^{-2} day^{-1} were reported, respectively. The phenomenon of higher AMP concentration rates within an urban setting, is supported by future publications (31, 32), and also suggests population density as a promotor of AMPs. The varying methodologies within an extremely limited literature supply, make inter-study comparisons, very tough. Yet, a clear picture is beginning to emerge, in that urbanised locations are thought to be a local source and suspension of MPs, demonstrating as to why concentration levels tend to be higher.

Although urbanised areas, with dense populations, have been reported to have high levels of MPs within the air, the presence of these synthetic particles within remote regions is also stated (25, 33), signifying their global presence. Another question subsequently arises; as to what the sources of these particles are, in areas with low industrial, commercial, agricultural and human activity (25). The transportation of urbanised MPs has been a supported source to these remote regions (33, 34), with reports that these synthetic particles can travel a distance greater than 100 km, if their size, shape and density permits, highlighting their transboundary potential (25, 35). Allen *et al.* 2019 stated a concentration of 365 ± 69 MPs m^{-2} day^{-1}, and although the difficulty comparing this data has already been stated, this 'pristine' mountain range had MPs present in every sample. The outdoor atmospheric compartment introduces meteorological conditions that can potentially influence airborne MP presence, persistence and transport; rainfall, snowfall and windspeed, amongst others are proposed factors, but there is a lack of significant difference between compared variables, perhaps due to multiple factors influencing levels simultaneously (14, 25). The potential for the atmospheric compartment to influence terrestrial and aquatic compartments, and vice versa, is supported, demonstrating how difficult it is to define sources of MPs. Roadside emissions, ocean sea spray and bubble burst, as well as terrestrial dust suspension are thought to be major drivers of AMPs and show the interlink between environmental compartments (36).

Cai *et al.* 2017 applied a scanning electron microscope (SEM) analysis to capture AMPs, of many shapes, and provide for the first time, detailed images. This highlighted specific weathering patterns and adherents, and the importance of mechanical and chemical abrasion. After a plastic enters the environment, they have the ability to persist and change (31).

An array of abundant synthetic polymer types have been reported within outdoor publications, with a variety of potential sources postulated. A textile source was proposed for PET and PA synthetic fibres identified within Paris, France (14). PE, PP and PS MPs, of various shapes, were suggested to be sourced from urbanised 'dust emissions' in Dongguan, China (31). PS, PE and PP MPs, of various shapes, were proposed to originate from an urbanised source, specifically single-use plastics, packaging and textiles, within a remote Pyrenees mountain range catchment (25). Polyacrylonitrile (PAN), PET and PA were reported as multiple particle shapes, within an urbanised London location, United Kingdom (UK), and a likely source being textiles and the degradation of larger plastic products, as well as primary microbeads (37). PE, Ethyl vinyl acetate (EVA) and co-polymers that were identified within Hamburg, Germany, possibly originating from road dust (32). In offshore air samples, captured above the Pacific Ocean, the source of AMPs was suggested to be from inshore urbanised locations. Specifically, PET, epoxy resin, and PE-PP co-polymers that were most abundant, were a likely source of textiles, as well as the incomplete combustion of plastic (34). It is important to state, that although suggestions have been made, as to the sources of MPs captured, it is difficult to affirm. Thus, the importance of gaining as much information from particle characteristics and polymer composition, is stressed.

Common features regarding outdoor AMPs are starting to emerge; their ubiquitous nature, smaller MPs being the most common, fibres and sometimes fragments being most abundant, specific polymer types being commonly present, and urbanised locations, and thus, population density influencing concentration rates. There is a great need for further research into outdoor MPs, their concentration rates and characteristics, in order to gain more insight into human exposure, this knowledge will also complement the environmental MPs field, helping to understand the source-sink nature of AMPs and their role in influencing other environmental compartments (38).

1.3.2 Indoor microplastics

The body of research concerning outdoor MPs is limited, but generally greater than that of indoor MPs. Indoor MPs were first reported within Dris *et al. 2017*, where two private apartments, and one office, indoor locations were sampled in a similar fashion to their previous outdoor study in 2016, focussing on fibres (18). Importantly, within Dris *et al.* 2017, an outdoor location was sampled alongside these three indoor locations, providing the insightful ability to compare indoor MP concentrations (0.4 – 59.4 MP m^{-3}) to outdoor locations (0.3 – 1.5 fibres) (18). The higher concentration of MPs within indoor locations, compared to outdoor, is corroborated by Gaston *et al.* 2020, yet the limited publications within this field is, again, stressed (39). Although indoor concentration rates can potentially be compared to separate outdoor investigations (supporting an indoor higher concentration), again, the lack of SOPs, emphasises caution.

PA, PP and PE fibres have been reported prevalent within indoor locations, in Paris, Fance (18), with likely sources suggested to originate from textiles, such as clothing, carpets and furniture. Activities within the home, such as clothes air drying, were a proposed influence of MP prevalence, and highlights the potential for human activity to influence suspension. Zhang *et al.* 2020 later investigated the prevalence of indoor MPs within a bedroom, office and corridor and also stated high levels, particularly within the bedroom (40). The synthetic polymer types most abundant within this study were PES, PAN, PP, PS and PA, with the major potential source being textiles, and perhaps air exchange. Interestingly here, investigation into indoor sources was conducted, by collecting samples of clothes to compare to sample particles. Although the prevalence of synthetic textile fibres within an indoor environment is supported here, Vianello *et al.* 2019 reported the domination of fragmented particles, and fibres to a lesser degree (20). PES was abundant, followed by PE and PA, and these polymers were supported to be of a textiles source, such as carpet, clothing and furniture, as well as fragmentation of larger plastic products such as packaging. Gaston *et al.* 2020 stated PVC and PE dominated samples, yet, when applying different chemical analysis there was an abundance of PS, PE and PET (39). Commonly hypothesised sources of indoor MPs were again suggested to be of a textile nature; clothing, furniture, carpets and air filters.

Overall, there is an extreme lack of knowledge regarding AMPs as a whole, but even more so, regarding the indoor environment. The limited studies available, and the inability to compare, hinders the progression of research. The ubiquity of indoor MPs is clear, as well as the potential for human exposure and inhalation (20), and due to the fact that MPs in both indoor and outdoor air are most prevalent in lower size ranges, there is now a great necessity to investigate further.

1.4 Atmospheric Microplastics Methods

In order for the successful capture and analysis of AMPs, each step must be tailored to meet the needs of the study. No SOPs are yet in place to regulate AMP investigations, and there are many different factors that affect MP concentration levels. In order to improve the validity of a study, as well as allow comparability throughout literature, a detailed description of sampling, processing, characterisation and analysis methodologies is essential, and crucially, quality assurances and control measures need to be put into place to monitor MP contamination throughout an investigation.

1.4.1 Atmospheric microplastic Sampling

There are two major modes of sample collection, concerning AMPs; passive sampling and active sampling. Passive sampling requires a collection vessel, usually of known size parameters and sampling duration, in which environmental particles deposit via gravity, meteorological conditions or other activity (41, 42). In contrast, active sampling does not wait for the deposition of particles, but instead involves the ‘active’ intake of a known volume of air, through an air sampling device, in

which airborne, suspended, particles are captured and are usually impacted onto a filter for subsequent analysis (38). Although little is known regarding the behaviour of AMPs whilst suspended, realistically, the two different sample collection modes favour different types of particles, with larger and more dense particles more likely to deposit gravitationally, as opposed to smaller, less dense, particles that have the ability to remain airborne for longer periods of time, being captured actively (38, 41). Thus, the application of either method has the potential to greatly influence the dataset produced.

1.4.1.1 Passive Sample Collection

Within early AMP research, passive sampling was more commonly applied to research (14, 18, 31, 38). Collection vessels, such as a glass containers (14, 18, 31), and outdoor air samplers (25, 32), were employed. In some cases, a vacuum (18), petri dishes and filter paper (43) or dust-pan and brush allowed for the collection of settled dust (21), as well as sticky pads (44). Most of these passive sampling 'devices' provide the advantage of being simple to use, easy to adjust, easy to replicate and extremely cost-effective (45); most vessels are readily available within a laboratory environment (41). Additionally, a trained operator is not necessary, device maintenance generally is not required, and a power source during sampling is not necessary (38). This allows almost any location to be considered, for instance high altitude remote regions (25). The possibility of wet and/ or dry deposition sampling allows factors such as rainfall to be investigated (14, 31, 32), or excluded, (21), as well as continuous longitudinal sampling being a possibility (25). Plastic sampling equipment can be avoided with some passive samplers (38), and simultaneous samples could be collected at multiple sites, due to this low cost.

Passively captured MPs are expressed in the units of MP m^{-2} day^{-1}, also referred to as MP/ m^2/ day. In order for the concentration of AMPs to be presented in this format, the sampler deposition surface area is calculated, and converted to m^2 (37). The sampling duration is then converted to a daily rate, for instance a weekly sample would be divided by 7. These conversion calculations are seen within numerous publications (14, 18, 25, 32, 37, 38). The data assumes that the particles collected within a sample, accurately represent a meter squared after extrapolation, which may not be the case, however converting to a universal unit allows inter-study comparisons to be made. It is important to note, however, that converting passively sampled AMPs to a comparable unit, may cause concentrations to seem easily comparable, when in reality, the varying field and laboratory steps hinders this comparison greatly.

Unfortunately, gaining data within these units of MP m^{-2} day^{-1}, causes the inability to fully quantify human respiration rates. The number, or mass, of MPs within a known volume of air is needed, for this. Thus passive sampling does not complement the research field of AMPs and respiratory health,

as much as active methodologies. Additionally, environmental samples collected passively are often more complex, thus requiring further sample processing before accurate analysis can be achieved (41). An increase in processing steps is time consuming and also increases the likelihood of contamination. Vegetation and insects captured within samples, or possibility of vandalism could also impact results (41). Finally, a large proportion of particles are often collected, passively, leading to lengthy analysis, especially with vacuum or dust-pan collection methods (41), these methods also introduce sample loss within brushes etc (41).

1.4.1.2 Active Sample Collection

Contrastingly, active sampling typically utilises an air pump device in which known volumes of air are taken into the device by adjusting the air flow rate and sampling time. The air commonly travels from an inlet, through tubing, and passes through a filter or substrate, in which particles with specific dimensions deposit (41). It is possible to gain the mass and particle quantity data from active sampling, and the known volume of sampled air, allows for MP concentration to be presented in units of MP m^{-3}, or $\mu g\ m^{-3}$, which is generally more applicable to human exposure and respiratory health research (46), as well as air quality monitoring (47).

There are many benefits of the active sampling approach; the use of filter media to capture airborne particles allows for the option of a size exclusion approach (41), in which two or more filters with different pore sizes are used to capture larger particles and smaller particles, separately, thus different size fractions can be focussed on, or analysed differently. Actively captured samples are also typically less complex, that is, containing less undesirable particles or other components, such as rainfall and deposited organic matter (48). Consequentially, it is sometimes possible for samples to be analysed with minimal, or no, sample processing steps, compared to passive samples (48). Reducing methodology steps due to a more simple matrix, in turn, reduces chances of MP contamination and also the time taken to complete analysis. However, unlike passive sampling, active air pumps do need a power supply, have a more complex methodology, sometimes reducing the possible duration of each sample, and can add to the overall cost of a project (45).

Active sampling, has been commonly applied to air quality monitoring investigations in the past, for instance, the UK Government monitors airborne particulate matter (PM) in order to abide by Air Quality Standards Regulations, relating to PM_{10} and $PM_{2.5}$ (47). Commonly this monitoring is conducted with a Tapered Element Oscillating Microbalance (TEOM) (41). AMPs are considered a constituent of this PM (49), with PM_{10} referring to particles less than 10 μm in size and $PM_{2.5}$ referring to particles less than 2.5 μm. Although it is unclear as to what proportion of PM is synthetic MPs, the fact that MPs are a constituent of PM highlights the validity of active sampling, as a method for detecting AMPs within the air (50). The Governmental focus on regulating PM_{10} and $PM_{2.5}$ in

urbanised locations, as well as other gaseous pollutants, is linked to their association with human health (47).

In order to improve the relevance of sampling, sampling heights for both active and passive methods have been suggested to be at a height of 110-170 cm when investigating human inhalation (48). This is further demonstrated by a higher concentration of MPs present at 1.7 m height compared to higher altitudes (51). The length a sampler is left open/ on is also considered a relevant variable; this can range from 1 to 40 hours, in active studies (18, 51), and 24 hours to a month within passive (25, 40), demonstrating the different duration abilities of each sampling method. Most studies tend to be discontinuous (18, 39, 40) but continuous sampling also occurs (20, 37). Discontinuous sampling has the advantage of less complex samples, whilst continuous provides a more holistic view of atmospheric concentrations throughout the entire study. Seasonal variation, rainfall, snowfall, windspeed, wind direction, frequency and intensity of weather events are all emerging as potential factors influencing sampling, but little is known.

During sampling, conditions such as duration, continuous/discontinuous, seasonal variation, weather conditions, sampling height and the landscape including buildings and vegetation can all affect samples (52). Each sampling factor chosen within a study can greatly affect what is being investigated and consequentially affect the final MP concentration values. Therefore, in order to conduct an effective AMP investigation, each factor should be clearly evaluated and reported when published, to ensure validity and comparability.

1.4.2 Sample processing

Sample processing can be a necessary step within MP research, depending on how and where it was obtained. Within AMP research, filtration can remove rainfall from wet deposition sampling (14, 31). Removal of unwanted particles such as sediment (25), abundant particles such as skin (20), and other biogenic matter (38) that can be present is an option within research. The two most common sample preparation techniques, after filtration, are digestion and density separation (41), however there are no SOPs established, to date (41). These techniques are applied due to the buoyant properties of most plastics and the higher density of soil and sediment particles (53), as well as the ability for synthetic plastics to withstand chemical digestion, whilst other organic matter is degraded (25). Reduction of the complexity of a sample, minimises analysis time, aids the identification of MPs that may otherwise be overlooked, and improves the quality of subsequent analyses. However, the additional processing steps can introduce contamination, costs and time consuming laboratory work (25, 38).

Filtration of a sample suspended within a solution, is essentially a purification step, by removing the mixture and simplifying the sample. Filtration and complete drying of a sample can aid the

observation of particles via microscopy and allow a sample to be filtered upon a filter type that is beneficial for subsequent analytical steps (45). As well as this, it can aid chemical analysis, in which the presence of an aqueous mixture could lead to noise and unwanted spectral peaks (54).

Within AMP literature, digestion and density separation processing methods are not commonly applied to the samples (14, 20, 31, 39, 40, 55). Reducing the number of laboratory steps is a quality control technique and is a potential explanation. As well as this, captured samples are not as complex as other environmental compartments, especially within actively collected samples (39).

1.4.2.1 Digestion

Digestion can successfully remove organic material that may interfere with chemical analysis (25, 38), and hinder the identification of particles within complex samples, especially those <500 μm (38). Within wider MP research, digestion solutions such as KOH, NaOH, HNO_3, HCl, H_2O_2, H_2O_2 + H_2SO_4, H_2O_2 + Fe, and enzymatic methods, have been applied (38). But within AMP literature, H_2O_2 and NaClO are solutions infrequently applied (19, 21, 25, 32).

H_2O_2 is slowly emerging as a favoured oxidising digestion technique for MP samples (38). Exposing an environmental sample to H_2O_2 30% can remove unwanted organic matter that could otherwise hinder MP identification, such as carbonaceous particles, silica, biofilms and other organics (45). Some studies suggest the digestion process can alter or eliminate MPs from the sample, whilst others support is as being a safe digestion method concerning MPs (25). It is thought that MPs are less affected by the digestion process due to their insoluble and persistent characteristics (17). However, H_2O_2 is a known textiles bleaching agent (19), and the discolouration of plastics upon exposure to H_2O_2 can occur (25, 38). MP research typically collates colour data as a particle property of interest which is a limitation of this substance (56).

Allen *et al.* 2019 applied H_2O_2 digestion to deposited atmospheric samples (25). It was reported that biogenic and non-plastic particles were present within large amounts, thus 10 mL of H_2O_2 was used to rinse a filter holding the atmospheric sample. The sample digestion solution remained at a temperature of 55 °C, for two weeks, with the further addition of 5 mL of H_2O_2. The reduction in complexity of the sample was noted. Abbasi *et al.* 2019, also applied H_2O_2 30% digestion to street dust samples, notably not to airborne samples (21). Here, 35 mL of H_2O_2 was mixed with individual samples, for 8 days, in which bubble formation ceased, indicating a lack of digestion activity. Stanton *et al.* 2019 applied H_2O_2 at a temperature of 75 °C, showing a range of 60 – 75 °C within limited literature (19). 50 mL was added to the environmental sample and heated for 4-5 hours. This demonstrates a large range of digestion durations, ranging from 4 hours to two weeks. Comparing the

H_2O_2 digestion protocols, demonstrates the great amount of variation within studies, even when a common digestion technique is applied.

1.4.2.2 Density separation

Density separation is the other major sample processing method applied to MP samples, and involves the sample of interest being suspended within a separation solution of a known density. Essentially, plastic particles of interest float towards the top of the solution, and more dense particles descend. The bottom layer of the solution is discarded and the top layer only is analysed. The assumption here, being, that no MPs are present within the lower fraction of the density separation solution, when in fact, this may not be the case. Common examples of separation solutions and their (densities) are; NaCl (1.2 g cm^{-3}), NaI (1.60 g cm^{-3}) KI (1.67 g cm^{-3}) and ZnCl2 (3.02 g cm^{-3}) (41). Solutions of a lower density, NaCl and NaI, have the ability to cause most MPs to become buoyant, but more dense particles such as polytetrafluoroethylene (PTFE) and polyvinyl acetate (PVA) could be lost due to similar or greater densities. KI and $ZnCl_2$ solutions allow for more plastic particles to be separated within the mixture (41).

Density separation, has been applied to limited atmospheric investigations (18, 25). Here, $ZnCl_2$ solutions, with a density of 1.6 g mL^{-1}, were added to samples. Notably, vacuum dust samples were exposed to density separation methods, and not actively captured samples (18), supporting the more complex of samples undergoing sample processing. Particle loss is a possibility when applying density separation, perhaps due to adherents increasing the density of MPs (45), or the separation solution applied not having a similar or lower density to some plastic types, therefore it is important to fully assess the plastic types thought to be captured at a site, and match a density solution to this. Cost effectiveness and environmental hazards when disposing of density solutions are also factors affecting solution type. Additionally, to avoid particle loss, the gentle agitation of the solution has been conducted (25).

1.4.3 Microscopic analysis

Applying visual analysis, alone, to atmospheric samples (57) is no longer accepted as a MP verification tool, due to inaccuracy, researcher bias, differences in microscopy quality and size limitation, and complex sample matrix affecting successful categorisation (38). Stereomicroscope analysis is now required to be used in combination with a verification method, such as a ‘hot needle’ test for larger particles, but most preferably a chemical analysis approach (38). Within AMP research, stereomicroscope analysis is combined with chemical verification frequently (14, 18, 25, 37), and less frequently digital microscope (31) and fluorescence microscope (32) are applied. Generally, a visual detection limit of 50 μm is stated due to microscope resolution limits (18, 38), but using

chemical analysis tools coupled with integrated stereomicroscope analysis can push the boundaries of size limitations (20).

Using microscopy separately to verification tools introduces the difficulty of aligning particle characteristic results with polymer type data. Visually identifying a particle, marking it for analysis, and physically taking that particle for chemical analysis, is a possibility, but can cause error and particle loss, and generally is only possible with larger particles. If the sample load is heavy it is difficult to mark and handle specific particles for analysis. Separate microscopy and verification steps can lead to particle characterisation data describing the total particle load, and chemical verification data describing polymer type. The importance of gaining as much information about MPs is stressed, and therefore ideally, the size, shape and other characteristics of each particle will align with chemical analysis information.

The shape of a MP particle is one of the most commonly recorded particle properties. Particles can take many forms, a consequence of the array of manufacturing methods, but also because plastics can break down uniquely, based on exposure to certain environmental stressors, producing many irregular particle shapes (5). Typically within AMPs literature, synthetic particles are fibrous and fragmented (14, 18, 20, 31), but film, foam and microbead particles have also been identified (20, 37, 40). Cai *et al.* 2017 published the first detailed images of AMPs, using SEM, in which 'weathered' MPs demonstrated fractures, grooves and pits, as well as the presence of adhering particles upon the surface of MPs (31). Standardised shape categories for MPs have not yet been achieved, thus it is important to fully describe classification criteria and terminology within each study. Common categories do exist, and can be used as a foundation (58), however, when there is a reliance upon the human eye to categorise particles, as well as multiple researchers analysing samples within a study, it introduces subjectivity and a lack of harmonisation, especially with decreasing particle size. More stringent criteria are starting to be applied to studies, for instance the 'vague' description of particles with a small diameter and long length being categorised as a fibre (45), can be replaced by categorisation using a specific length to width ratio greater than 3 (20). This approach is utilised within asbestos studies. The importance of accurately classifying particles into correct shape categories is highlighted by the potential for shape to indicate sources of MPs, as previously stated. Particle source, history and behaviour can potentially be revealed through the analysis of particle characteristics (1).

The lipophilic fluorescent dye, Nile red has been utilised as a MP stain (59). The dye is used to stain lipids of eukaryotic cells and microorganisms. The fluorescence emission spectrum of the stain shifts, giving different coloured plastics depending on polarity which allows MPs to be broadly categorised depending on their hydrophobicity (59).

Other types of visual identification have taken place within literature, for instance, hot needle tests that essentially expose a suspected plastic particle to extreme heat in order to observe a 'melting' response, have been used but are destructive and can only be performed on larger particles, those that are already more easily identified by the human eye. Furthermore, to get the most accurate results, the methods need to be compared against known standards (60).

The colour of a particle can often reveal the history of the plastic before degradation and release into the environment (1). However, when visually analysing particles, it is easy to assume all brightly coloured particles are synthetic. But this is not the case, and stresses the importance of not solely relying on microscopy. Additionally, as previously stated, if digestion processing has been conducted upon a sample, there is a chance of MP bleaching, leading to some plastics losing their colour (25). Colour categorisation can be subjective (5), and is not considered the most important of MP properties to record. It could be prone to human error, colour blindness and difference in microscopic approach, and therefore obtaining colour data can be time consuming considering the information gained (1). Additionally, environmental stress could influence the colour of MPs (5).

1.4.4 Particle selection

After particles have undergone microscopic analysis to gain information regarding their size, abundance, shape etc. the selection of particles taken for chemical verification must take place. The number of particles within samples is generally large, and therefore only a subset of particles tend to undergo analysis. Some studies report a small number being selected for analysis; Dris *et al.* 2016 selected 24 fibres, Dris *et al.* 2017 selected 28 fibres, Cai *et al.* selected 20% of fibres and all other shapes, similar to Wright *et al.* 2019 that selected 5% of fibres and all other shapes(14, 18, 31, 37). Zhang *et al.* 2020 selected a large number of particles for analysis (2086), but remarkably Vianello *et al.* 2019 analysed all particles detected within samples (20, 40).

AMP research commonly relies upon visual criteria for the estimated number of MPs within samples (56). Here, the description of "no cellular organic structures are visible, fibers should be equally thick throughout their entire length, particles must present clear and homogeneous colors, and if they are transparent or white, they must be examined under high magnification and a fluorescence microscope." can be applied (56). Potential particles are assessed as to whether they met this visual criteria, in an attempt to reduce the misidentification of synthetic plastic particles; (56). This method allows for high numbers of particles to be rapidly identified as MPs (50). However, the differing levels of accuracy for different coloured, sized or shaped particles highlight the potential for misidentification (50). Catarino *et al.,* 2018 reported that visual identification of synthetic fibres had a success rate of approximately 50% (44). Therefore avoidance of human particle selection altogether,

and the lack of human subjectivity could rule out the bias associated with visual criteria, as seen within Vianello *et al.* 2019 (20).

1.4.5 Chemical analysis

The validity of a study relies on the accurate confirmation of plastic particles. Therefore the use of chemical 'verification' methods alongside visualisation is supported, especially for particles <500 μm, where visual analysis inaccuracy increases (41). When considering the increase in concentration of AMPs with a decrease in particle size, as previously mentioned, the importance of chemical analysis is emphasised.

Visual analysis, alone, allows for a high abundance of particles to be analysed, rapidly, but at the expense of inaccuracy and human error with decreasing particle size (50). Fourier Transform Infrared (FTIR) analysis can analyse particles, down to a 5 μm size limit (18), and Raman spectroscopy down to 1 μm (50). Both produce reliable results but are expensive and time consuming, and require operator training. Raman spectroscopy also has the disadvantage of high background fluorescence and analysis can be affected by the presence of additives (50). Scanning electron microscopy and energy dispersive x-ray spectroscopy (SEM-EDS), not observed within AMP studies, has the ability to analyse particles down to the nanometre (nm) size range, and has excellent image quality alongside particle composition data. However, SEM-EDS is time consuming, destructive and cannot be conducted on large numbers of particles, like those observed within the atmosphere (50). Thermochemical analysis, such as Py/GC/MS, cannot define particle number, shapes and other particle properties, and is destructive (38).

The two major forms of chemical analysis, apply spectroscopy for the identification plastic types. Specifically, μFTIR and μRaman spectroscopy are applied. μFTIR spectroscopy analysis is by far the most common analytical tool applied to AMP studies; μFTIR ATR (14, 18, 39), μFTIR in reflectance mode (31, 37), μFTIR in transmission mode (38) and μFTIR Focal plane array (FPA) (20). μRaman spectroscopy has been applied to a lesser degree (25, 32).

1.4.5.1 Spectroscopy

Spectroscopy, is the study of the interaction of light, with matter. In terms of MP research, light is beamed onto, or through a potential MP particle, and the change in light intensity is measured via a detector (54). Spectroscopy is considered a reliable method in the identification of MPs within environmental and biological samples (17).

There are varying types of spectroscopy depending on the type of light applied to sample. Visible, UV, Near IR, Mid IR, Far IR and microwaves are all light sources with varying properties. The

wavelength, wavenumber, frequency and energy throughout the electromagnetic spectrum varies, and these differing properties allow for different molecular interactions (54).

Light intensity is the crucial measurement of spectroscopy, where the chemical composition of an analysed particle, causes a unique interaction with the light source applied, changing the resulting intensity of that light. Electric transitions, molecular vibrations and molecular rotations are all common molecular responses to electromagnetic radiation stimulation (54).

1.4.5.2 FTIR spectroscopy

FTIR relies of molecular vibrations. FTIR is frequently applied to the field of AMPs, in order to conduct 'unknown analysis'. This is, the identification of particles of interest, in which their chemical composition, and polymer type, is not known. This type of analysis allows MPs to be analysed with a low detection limit, depending on the methods used, permitting particles <500 μm to be more accurately identified (38).

The region of electromagnetic spectrum utilised by FTIR is the IR light region, ranging from approximately 14000-4 cm^{-1} in wavenumber (54). However, AMP research, applying FTIR spectroscopy, focus solely on the mid IR region (20, 31, 39, 40, 55), due to molecular vibrations commonly have strong absorbances between 4000-400 cm^{-1} wavenumber (54). Additionally, compounds used to aid analysis, such as ZnSe windows (Vianello *et al.* 2019) and KBr (54), have light absorbance peaks around this 400cm^{-1} wavenumber threshold, and therefore known peaks can be excluded from results, without masking peaks of interest.

Microscopy when combined alongside FTIR analysis, is referred to as μFTIR spectroscopy, and benefits AMP research significantly. Information relating to the physical characteristics of the particle, for instance, the size, shape, colour or physical appearance of the particle under analysis, can be reported alongside polymer type. A combined approach therefore achieves a more holistic view of each particle in question, and has the potential to provide information relating to plastic sources and the history of the particle.

1.4.5.3 FTIR analysis

Within the spectrometer, an IR light source is beamed into the machine, upon which it hits a collimating mirror, where the light condenses and focuses. The light then redirects onto a beam splitter. In order to capitalise on the IR transparency properties of KBr, the beamsplitter is often composed of this compound. The beam splitter has the ability to separate the light in two directions; through the beam splitter itself and refracted elsewhere (54).

The light travelling through the beamsplitter, hits a fixed mirror, which remains stationary. Contrastingly, the refracted light hits a moving mirror, that as the name suggests, is not stationary (54).

The moving mirror is essentially responsible for the optical path difference. The mirror moves backwards and forwards based on the settings applied by the user. Every completion of a forward and backward motion, is referred to as a 'scan'. For instance, if a scan number of 64 is selected, the moving mirror will travel forwards and backwards a total of 64 times. (54).

The two separated light beams, after hitting the fixed or moving mirror are then recombined at the beam splitter, where the light waves can interfere. Recombining causes the light to vary between 'in' and 'out' phases, altering the energy and producing constructive and deconstructive interference. This recombined light is then directed towards the sample, where the light interacts uniquely with whatever molecules are present.

The resulting light is collected by a detector, commonly, a mercury cadmium telluride (MCT) within AMP research (54).The information from the optical path difference and light intensity is combined and plotted onto an interferogram. The light intensity decreases at specific wavenumbers, where the light has interacted with the sample molecules (54). A user setting of 64 scans, will produce 64 interferograms that are subsequently averaged, thus scan number increases the reliability of the interferogram.

In order to achieve the final IR spectrum, the data from this interferogram is transformed using 'Fourier transform' mathematics. Using information such as the frequency, light wave, the velocity of the moving mirror and the speed of light constant, an interferogram is transformed into the final product IR spectrum commonly observed within MP literature (54).

An IR spectrum is plotted with wavenumber (cm^{-1}) along the X-axis and light intensity plotted along the Y-axis. Light intensity can be presented in two ways; the percentage of light absorbed by a sample (absorbance), or the percentage of light transmitted through a sample (transmission). These two Y-axis values are related to one another, meaning that it is easy to alternate between the absorbance and transmittance with user settings, whilst the X-axis values remain the same. The IR spectrum presents itself as a continuous line, in which peaks upon the spectrum represent regions of light intensity changes at specific wavenumbers (54).

Within the IR spectrum, there contains a lot of information relating to the chemical composition of the sample under investigation. Specific peaks and specific wavenumbers often indicate molecules that are undergoing molecular vibration upon stimulation with IR light. It is possible to be fully

trained on how to interpret IR spectra and even identify changes in spectral quality based on environmental degradation of the sample. However, using the software available alongside a spectrometer, it is possible to compare the likeness of a sample IR spectrum with a database of historic IR spectra (54).

A spectral library search can identify the chemical compound that is most similar to the sample, and although each result should not be accepted without some form of interpretation, a high match index score (%) can indicate similarity (54).

An advantage of FTIR spectroscopy when applying to AMP samples is; when a particle is too thick, leading to high light absorbance and less light detected, or when a sample is small, resulting in less signal, it is possible to adjust parameters or techniques on the FTIR, to increase the match index score. There is a trade-off between scan number, resolution, signal to noise ratio and time taken to analyse each sample, and each parameter can be adjusted to best suit the sample under investigation, with spectral quality being the major goal. Additionally, liquid nitrogen (LN_2), that is IR transparent, can be used to cool the FTIR device and purge it of 'artifacts', thus improving spectral quality and library match (54).

Unfortunately, there are disadvantages of applying FTIR spectroscopy to AMP samples. Not all molecules can be identified with FTIR spectroscopy, also, whilst an array of IR spectral databases are available, and usually cost-free with the installed software, there is a lack of environmentally relevant IR spectra that have been exposed to harsh conditions resulting in altered or 'weathered' chemical structure (54). Some atmospheric components, such as oxygen (O_2), are IR transparent, but other atmospheric components are not. For example, carbon dioxide (CO_2) and water vapour are molecules are stimulated with IR light, meaning the environment in which FTIR analyses is conducted can influence the quality of an IR spectrum. It is essential for a 'background' spectrum to be taken alongside a sample in order to measure this interference, not only from the atmosphere, but from the spectroscopy device itself, and subsequently subtract it from the final spectrum. Ideally, the background interference reading will match the 'noise' measured on a sample IR spectrum, resulting in a perfect subtraction (54).

1.4.5.4 Types of FTIR Spectroscopy

Transmission mode is a type of FTIR spectroscopy in which light passes through a sample, and the resulting light intensity measured (61). Thin particles, such as films, are ideal for this analytical tool, however solid particles are possible (61). In order to avoid the majority, or all of the infrared light being absorbed by a sample, it is possible to utilise a compression cell in order to 'squash' particles into a film like form (62), the benefit of this being selection of a compression cell with an IR

transparent substrate is possible (61). The particle characteristics must be collected before compression, if this technique is applied.

In reflectance mode, IR light is passed through a sample that is placed upon a filter or substrate with an IR reflective surface, and the light then passes back through the sample and to the detector. The main problem with this technique, is similar to FTIR in transmission mode, in that there may be lack of a signal intensity. (61)

FTIR with ATR mode relies on direct contact of an ATR crystal with the sample under investigation. IR light is then beamed and scattered through the crystal where evanescent waves are subsequently collected by a detector (61). In contrast to the prior FTIR modes, ATR has the ability to analyse thick samples, but is expensive, and the crystal can become damaged with use. Additionally, ATR mode is most effective for larger particles >500 μm, due to the need for direct contact with the crystal, which could seem ineffective for most atmospheric particles. If analysis of a small particle, that does not cover the entirety of the crystal, is attempted, a weak spectrum can be produced (61).

FPA is considered the most recent type of FTIR spectroscopy to emerge, and suggested to be the most advanced (61). This detector is essentially made up of multiple IR detectors. This detector permits "simultaneous collection of spectral data from multiple points on a single plane" and when coupled with a motorised stage, allows automatic analysis of a sample (61). Vianello *et al.* 2019 has applied this technique to actively sampled atmospheric samples, reporting particle analysis down to 11 μm major dimension and 5 μm minor.

1.4.5.5 Raman Spectroscopy

Similar to μFTIR spectroscopy, μRaman also utilises molecular vibrations, but by applying visible monochromatic laser light as opposed to IR. The resulting spectra, again, provides a unique 'fingerprint' dictated by the samples chemical structure; atom type, bond strength, geometry, intermolecular interactions (63). An altering dipole moment during molecular vibration can produce peaks upon a spectrum (63). Plastics are generally considered Raman active substances, and therefore the verification of MP polymer types is frequently successful (64). Similar to FTIR spectroscopy, Raman can be coupled to a microscope, allowing for particle characteristics and polymer type to be aligned, under the right methodological conditions (64).

Firstly, a laser of single wavenumber is beamed into a spectrometer, mirrors focus the light, and the beam is then polarised. Upon the light hitting the sample it is scattered 360 degrees. This incident light can be either scattered elastically (Rayleigh) or inelastically (Raman), the latter being useful for analysis in order to obtain the 'Raman Shift'. The Raman Shift is effectively the difference in Raman scattering and incident photons. When this difference is less, the Raman Shift is negative (Stokes),

and when there is a greater difference, the Shift is positive (Anti-Stokes) (63). A sensitive detector collects light intensity data, and filters out Rayleigh scatter. A computer generates a spectrum, with wavenumber and light intensity plotted, which can be analysed and compared to a library (63).

Common advantages when applying Raman spectroscopy to MP research is that multiple particle states can be investigated, for instance samples containing water. Unlike some FTIR modes, Raman allows thick samples to be analysed because it is not necessary for the light to travel through the sample (64).Additionally, particles with a size parameter down to 1 μm can potentially analysed with this analytical tool, combatting the regularly discussed problem within MPs literature; the inability to analyse the smallest of size fractions. Raman is also considered non-destructive, when the correct laser intensity is applied (63, 64).

Because of these advantages, Raman spectroscopy is now being considered a favourable tool (38), however it is also associated with disadvantages. It is costly, can be affected by the presence of additives within plastics, fluorescence excitement can mask the Raman effect (63) and the quality of plastic spectral libraries for Raman analysis is growing, but not yet as developed as FTIR (15),

Ultimately, the selection of either analytical method is likely dependent on which is available to the researcher in question. However, it is possible to successfully verify plastic particles with both FTIR and Raman (63). If both methods are available within a laboratory, FTIR and Raman spectroscopy can complement one another, allowing for greater particle information to be gathered (54, 63).

A more recent advancement, applied to MP investigations utilising FTIR and Raman spectroscopy, is the ability to directly analyse potential MPs upon filter media. The advantage of this, being, the improvement of the size detection limit, and no requirement to handle or manipulate particles of interest. Anodisc filters and silver membrane filters have been applied, and are examples of such potential advances, and since cellulosic filters can warp, and plastic filters are not recommended for directly analysing MPs, there is a potential to improve problems surrounding research (38).

1.4.6 Quality Assurance and Control

The ubiquitous presence of indoor MPs is also applied to laboratory settings. Introduction of MPs into samples is possible. Quality assurance and control (QA/ QC) measures can reduce the likelihood of this contamination.

During environment sampling, the use of non-plastic samplers has been applied in the form of metal outdoor samplers, glass collection vessels and metal basins (14, 25, 40), as well as this, when plastic equipment is used, the plastic polymer types can be stated in order to take it into account during data interpretation (25). Due to the high abundance of AMPs, indoors and out (18), field sampling can

introduce contamination during sample collection. A lack of manipulation, covering of samples and standing downwind are supported (18, 25). Field blanks are also a method of controlling contamination within a sample, by exposing a blank sample to the same sampling conditions as the samples collected. These are often not conducted, or a poor description is given (18, 31). When field blanks are conducted, MPs identified can be compared to samples, and assessment of contamination can be discussed. Clothing is another potential source of MP contamination whilst sampling, considering textiles are often discussed as major contributors to MP pollution (12). Therefore avoiding synthetic clothing, can minimise these sources.

Within a laboratory setting, the use of a natural laboratory coat has been frequently conducted (25, 40, 55). Also, samples are generally covered at all times and briefly opened when it is essential. This is often conducted using aluminium foil coverings, as the shape is easily manipulated (65). Additionally, the use of a fume hood or laminar flow hood can reduce contamination by controlling the atmospheric environment. Since fume hoods take in air from their environment, and pass it, unfiltered, into the hood, it has been highlighted as an less effective control measure (13). Laminar flow hoods, however, process air with a high efficiency particulate air (HEPA) filter and are therefore preferred when available (13). However, the use of a fume hood has been supported to reduce contamination compared to standard laboratory bench work (43), and switching off the fume hood has also been discussed (65).

Inclusion of laboratory blanks (procedural controls) is essential in improving the validity of an investigation. Essentially collecting a blank sample that has been exposed to the same conditions and reagents as the sample under investigation, can highlight potential contamination and plastic types that may have been introduced. In the past these were not conducted (14, 18), or were conducted with little to no discussion of results (31), however these are now necessary steps, especially when the number of MPs collected is low and the potential for contamination is high (66).

The triple filtering of reagents such as water and H_2O_2 has been applied, as well as the use of MilliQ water (Allen 2019). Rinsing equipment with acid and ethanol has also been discussed (67). However, no SOPs apply to QA/ QC and therefore the quality of studies varies considerably. In order to reduce contamination and improve research, detailed QA/ QC procedures, including results should be conducted, perhaps with other control adjustments applied as well (66).

1.5 Microplastic Inhalation

1.5.1 Microplastics within Human Clinical Samples

Identification of the presence of MPs in human clinical samples is in its infancy; however more and more publications are appearing. MPs have been identified in human follicular fluid (68), human

bone, cartilage and intervertebral discs (69), faeces (70, 71), Placenta (72), colon (73), blood (74, 75). This list continues to grow and at this time, we are still unsure how these MPs are able to enter the body, pass biological barriers and what are the effects of these MPs on human health.

It is logical to assume that routes of exposure to MPs are through ingestion and the presence of MPs in faeces and food are in support of this hypothesis (70, 71, 76). Inhalation may also be an important route of exposure. As described previously, MPs are present in the air that we breathe, both inside and outside. Most MPs are likely to be removed from the airways by defence mechanisms such as the ciliary escalator and mucus trapping, causing the particles to enter the gastrointestinal tract. MPs which remain in the airways may cause inflammation and damage which has not yet been fully resolved. Indeed, MPs have been found in human lung tissue. Synthetic fibres were first identified in diseased human lung tissue back in 1998 (77) although characterisation of the particles was not done at this time. More recently, MPs have been identified in lung tissue from cadavers (78) and the use of bronchoalveolar lavage fluid (BALF) is emerging as a useful technique to collect and test for the presence of MPs (79-83).

1.5.2 Human Lung Physiology

The human respiratory system possesses defence mechanisms to help prevent unwanted matter and microorganisms from entering and damaging the lungs. The cough reflex will be triggered in response to particles and irritants which activate nociceptors on the nerve endings innervating the airways (84). The mucociliary escalator traps particles and removes them from the terminal bronchioles by the co-ordinated beating of epithelial cilia. These particles are then swallowed and processed by the gastrointestinal tract. The roles of specialised cells within the airways are also important in defending against PM. The epithelial cells, as well as having cilia, form a protective barrier lining the lumen. Immune cells, including alveolar macrophages phagocytose PM to remove it before it can cause damage.

The airways branch like a tree and consist of 24 generations starting a G0 in the trachea to G23 down in the alveoli (85, 86). According to Kelly and Fussell, deposition of PM within the respiratory tract depends on the physical properties of the matter with size being one of the biggest factors. It is roughly assumed that particles smaller than 2.5 μm will be able to enter the terminal bronchioles and even the alveoli. Particles up to 10 μm will be able to make it to the primary bronchi to be trapped and removed by mucociliary action. Larger particles up to 100 μm will be restricted to the nasopharynx (87). Shape will also be a factor. Interestingly, larger particles can make it further down the respiratory tract, especially if they are fibrous in shape as they are better able to avoid lung clearance mechanisms (88).

1.5.3 Microplastics and *in vivo* Experiments

In vivo ingestion experiments with PS MPs result in MPs accumulating in various organs in mice including the heart, liver, brain, spleen, intestines and kidney (89, 90). In the brain, chronic exposure to PS MPs leads to disruption of the blood brain barrier and may cause learning and memory dysfunction (91). Furthermore, in female mice the MPs accumulate in the uterus and ovaries resulting in reduced oocyte survival and possible reproductive toxicity (90). MPs may also cause metabolic disturbances, and in combination with a high fat diet, PS MPs caused inflammation of the intestinal mucosa and altered nutrient absorption (92). The cardiovascular effect of PS MPs have been explored in rats and various aquatic organisms (93). Effects included penetration of biological barriers including blood vessels and intestinal epithelium. The effects resulted in abnormal heart rate, myocardial fibrosis and cardiac function impairment. The mechanisms included oxidative stress and inflammation amongst others (93).

The effects of inhaled MPs are not so well understood. There is some evidence that inhalation of PS MPs also causes inflammation in rodents in the lungs and multiple other organs including liver and kidney (94). Neutrophil and macrophage infiltration was observed along with increased expression of inflammatory markers such as NF-κB and Toll Like Receptors (TLR) and this could be reversed with TLR2 and 4 inhibitors (94).

Interestingly most of the *in vivo* experiments have been performed using PS MPs as these are readily available commercially. However, PS is rarely observed in environmental samples, making these experiments not environmentally relevant. More experiments with environmentally relevant MPs and concentrations are needed to gain better insight into health effects.

1.5.4 Microplastics and *in vitro* Experiments

Like the *in vivo* experiments, PS is the most widely used plastic for *in vitro* experiments (95). Again, more environmentally relevant and realistic *in vitro* exposure investigations into the health risks of airborne MPs are needed. *In vitro* studies on the effects of MPs have shown cytotoxicity, apoptosis, autophagy, effects on barrier integrity, inflammation, endoplasmic reticulum stress, mitochondrial stress and oxidative stress in a range of human cell types (95, 96). Importantly, shape was found to significantly affect cytotoxicity as was dose and duration of exposure, unfortunately most studies seem to use unrealistic doses and more work is needed to gain a more realistic picture of the effects of MPs on human health (95, 96).

More recently, PET MP particles reduced mitochondrial respiration including adenosine triphosphate (ATP) production in human brain vascular pericytes *in vitro* without affecting oxidative stress (97).

1.5.5 Microplastics and Human Respiratory Health

Despite increasing evidence of their presence in the human body, the health effects of MPs are still not understood. Based on the *in vivo* and *in vitro* experiments summarised above MPs may affect lung health, causing oxidative damage, inflammation and exacerbations of existing lung diseases (98). Furthermore, Shi *et al*, again using PS spheres, showed the impact of MPs on lung surfactant which is produced by alveolar cells and is essential for normal lung function (99). Shi *et al* demonstrate that the PS can adsorb phospholipids from the surfactant, as well as producing H_2O_2 which can contribute to hydroxy radicals and thus oxidative stress (99).

Furthermore, as demonstrated by Shi, MPs can adsorb other molecules. They have been shown to adsorb hydrophobic pollutants such as polyaromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) (100). MPs may therefore act as vehicles to transport other pollutants into the lungs contributing to damage. Some bacteria also favour growing on plastic, again suggesting that MPs could help microorganisms to translocate in the lungs. Indeed, it is well established that clinically significant biofilms readily grow on indwelling plastic devices such as catheters and ventilator tubes (101, 102) and in aquatic environments microbial attachment and bacterial biofilm growth on plastic waste and MPs has repeatedly been shown (103, 104). Adding further to the problem, MPs often contain additives to give them the polymer properties we need to prolong their life (28). Additives, pigments, flame retardants, plasticisers, fillers, composites etc., are predominantly not bound to the polymer, leaving the potential for leachates into the environment (28), or within an organism a plastic has entered.

To date, the effects of MPs on human health including respiratory health are not known. Exposure to high levels of PM has been linked with respiratory diseases such as Chronic Obstructive Pulmonary Disease (105) and the possible role of MPs in this has not been shown. However, it is known that occupational exposure to high levels of plastic can result in respiratory disease. Indeed, the term ‘flock worker’s lung’ developed following cases of chronic interstitial lung disease seen in workers from the nylon flocking industry (106-108).

1.6 Conclusion

It is clear that there are a magnitude of ‘unknowns’ relating to the abundance of MPs within the air, that are in need of addressing. Specific locations, polymer types, sizes and shapes, identified within the environment, can highlight MPs that humans are likely exposed to, regularly. The speculation concerning MP inhalation is increasing, yet there is little evidence supporting the presence of MPs within human lung tissue. A lack of environmentally relevant toxicity investigations and evidence to either support or oppose the inhalation of MPs, emphasises the necessity of gaining more information regarding AMPs and human inhalation.

1.6.1 Problem Statement

Years of relentless plastic consumption, and the inability to achieve a circular plastic economy, have resulted in uncontrollable plastic pollution. Consequentially there is an inevitable release of MPs into the environment. The plastics economy has thrived and continues to do so, showing little suggestion of a future reduction in plastic production.

The ubiquitous nature of MPs within the environment, driven by human activity, emphasises the eventual certainty of human exposure. It is now evidenced that MPs have infiltrated, and are transported throughout, all environmental compartments. AMP research is the most recent field to be investigated, and there is a limited understanding of the types, sizes, shapes and concentrations of MPs within the air.

The consequences of AMP exposure are poorly investigated, due to both an inability to apply environmentally relevant conditions to *in vitro* and *in vivo* experiments, and a lack of knowledge regarding the types of AMPs, that humans are exposed to most. Whilst AMPs have been captured within the air, and there is also evidence to suggest human inhalation is possible, the field is considerably lacking, thus, allowing for speculation regarding the health effects of such exposure, to escalate.

Within literature, the number of AMP publications available is low, and SOPs are not achieved. Additionally, there are methodological restraints causing an inability to reliably analyse the smallest and most relevant plastic particles. Moreover, sample sizes within research are commonly low, and sampling duration short. QA/ QC measures are limited and regularly not reported fully, and adjustments that account for contamination are generally not applied.

It is accepted that MPs increase in concentration with decreasing size, AMP concentration is highest in locations of high human activity, and that there are innumerable combinations of plastic types and their additives, highlighting the complexity and importance of assessing AMPs as an EEC in relation to human respiratory health.

1.6.2 Research Objectives, Questions and Hypotheses

1.6.2.1 Chapter 2

Research objectives:

Chapter 2 will investigate the presence of indoor AMPs within 20 households, with the objective of gaining as much information relating to human exposure as possible. Households will be investigated over a year, to achieve reliable average concentration rates, and determine any fluctuations of AMPs

within a home, on an annual basis. Inter-household comparisons can also be conducted, to identify any differences in sites, as well as common AMP characteristics, such as size and shape.

Research questions:

- What concentrations of AMPs are present within the indoor household atmospheric samples, and how do these compare to the limited publications available?

- What are the characteristics of AMPs, such as size, shape and plastic type, and what conclusions can we draw regarding human exposure?

- Is it possible to identify potential sources of AMPs within the home?

- Do households reliably contain the same types of AMPs, or do they differ throughout each sampling month?

Hypotheses:

AMPs will be present within all households investigated. The concentration of AMPs within households will be similar to, or exceed that, of other indoor AMP investigations. AMP concentrations will be greater than available outdoor AMP investigations. AMP polymer types most abundant within household samples will reflect those most commonly produced by the industry, as well as the plastic types most utilised within the home. Fibrous AMPs will be most prevalent, fragmented to a lesser degree, and shapes such as foam and sphere will be least identified, if at all.

1.6.2.2 Chapter 3

Research objectives:

Chapter 3 will investigate the presence of outdoor AMPs within one high human activity site, over a yearlong sampling study, to gain accurate AMP average concentration levels, as well as observe MP fluctuations and rainfall events. Five sampling sites, all with different types of human activity levels, will also be investigated over a single 2-week sampling duration, with the aim of gaining as much information relating to site specific AMP human exposure as possible, such as size and shape.

Research questions:

- What is the average concentration of AMPs over the yearlong sampling site, and how does this compare to the snapshot investigation?

- What size, shape and plastic types are most abundant within the longitudinal study, as well as the snapshot, how do these compare?

- What are the differences in AMP characteristics and types in site specific locations?

- Is it possible to suggest sources of AMPs based on sampling site location, as well as suggesting potential drivers of AMPs, including rainfall?

Hypotheses:

AMPs will be ubiquitous within the yearlong and snapshot investigations, and concentration rates will match or exceed that of other published concentration levels. Fluctuations in concentration, throughout the yearlong sampling, as well as the greater range of AMP types and characteristics, in outdoor investigations will highlight the complexity of outdoor sampling compared to indoors. Capturing rainfall levels will complement data, but it will prove difficult to identify a correlation between rainfall and concentration rates, due to the numerous other factors driving AMPs. Within site-specific investigations, AMPs will be highest in locations of greatest human activity, and lowest in locations most 'rural', such as the residential site. The types and characteristics of AMPs will represent the locations from which they were captured, to some extent, but conclusions will be difficult due to the ability for AMPs to transport.

1.6.2.3 Chapter 4

Research objectives

Human lung tissue samples obtained from living patients will be digested and analysed with the objective of providing evidence to support, or oppose, the inhalation and deposition of AMPs within the human lungs. The site within the lungs, that tissue was obtained, will be recorded with the aim of highlighting region specific differences, if any. Data will be presented as MP/g of tissue, in order to allow for comparisons throughout literature, in the future. QA/ QC assurances will be adapted and be the greatest focus of the investigation, in order to ensure validity. Additionally, evaluation into contamination adjustments will be attempted, in order for SOPs to be achieved in the future.

Research questions

- Are MPs present within digested human lung tissue samples, and if so, what are the MP/g levels after each contamination adjustment has been applied?

- Is there evidence to support or oppose the human inhalation of AMPs?

- What are the types and characteristics of any MPs identified within samples, and are they distinct from any MPs identified within controls?

- What conclusions can be drawn, if any, in regards to lung region, gender and other factors?

- What contamination adjustment is best suited to digested human lung tissue samples, when investigating MP presence?

- How do the type and characteristics of MPs identified within lung tissue compare to indoor and outdoor AMP literature, is there a link between environmental and human samples?

Hypotheses

MPs will be identified within human lung tissue samples, in low amounts. The presence of some MPs within samples, after contamination adjustments, will support the human inhalation of AMPs. The sample size will limit conclusions drawn from lung location, as well as patient demographics. It is hypothesised that fibrous and fragmented MPs will be present within the lungs in small amounts, but their characteristics will be distinct from those identified within the rigorous controls. MP type will reflect those most commonly produced within the industry, but it will not be possible to suggest sources of those MPs due to the complexity of exposure possibilities for each patient. The smallest of MPs will be identified until the size limit of chemical analysis is reached, but MP sizes may not typically follow traditional AeD rules, and MPs larger than 10 μm will be identified, especially concerning fibrous MPs.

1.6.2.4 Thesis

It is the overall objective of this thesis, to identify and characterise MPs captured from indoor and outdoor locations, whilst improving the quality of sampling and laboratory processing, to complement this novel field. Throughout the chapters, different microscopic, filter types, laboratory processing techniques and QA/ QC will be trialled to gain a better understanding of how this field needs to progress. Additionally, by providing evidence to either support or oppose the human inhalation route of exposure for MPs, it is an additional objective to bridge the fields of environmental AMP sampling and human health. To date, these fields are often distinct, but a combined approach may be necessary to gain a better understanding of MPs and human health.

1.7 References

1. GESAMP. Guidelines for the Monitoring and Assessment of Plastic Litter in the Ocean. 2019.
2. Europe P. Plastics - the fast Facts 2024 2024 [Available from: https://plasticseurope.org/knowledge-hub/plastics-the-fast-facts-2024/.
3. Aljamali NMS, N. S. . Review on Chemical Separation of Crude Oil and Analysis of Its Components. Journal of Petroleum Engineering & Technology. 2021;11(2):35-49.
4. Europe P. Plastics - the Facts 2022 2022 [Available from: https://plasticseurope.org/knowledge-hub/plastics-the-facts-2022/.
5. Frias J, Nash R. Microplastics: Finding a consensus on the definition. Mar Pollut Bull. 2019;138:145-7.
6. Lewandowski K, Skórczewska K. A Brief Review of Poly(Vinyl Chloride) (PVC) Recycling. Polymers-Basel. 2022;14(15).
7. Hossain MT, Shahid MA, Mahmud N, Habib A, Rana MM, Khan SA, Hossain MD. Research and application of polypropylene: a review. Discov Nano. 2024;19(1):2.

8. Dhaka V, Singh S, Anil AG, Sunil Kumar Naik TS, Garg S, Samuel J, et al. Occurrence, toxicity and remediation of polyethylene terephthalate plastics. A review. Environ Chem Lett. 2022;20(3):1777-800.
9. Walker TR. (Micro)plastics and the UN Sustainable Development Goals. Curr Opin Green Sust. 2021;30.
10. Ghosh S, Sinha JK, Ghosh S, Vashisth K, Han S, Bhaskar R. Microplastics as an Emerging Threat to the Global Environment and Human Health. Sustainability-Basel. 2023;15(14).
11. Geyer R, Jambeck JR, Law KL. Production, use, and fate of all plastics ever made. Sci Adv. 2017;3(7).
12. GESAMP. Sources, fate and effects of microplastics in the marine environment: A global assessment. London UK: The Joint Group of Experts on Scientific Aspects of Marine Environmental Protection, Working Group 40. 2015.
13. Prata JC, da Costa JP, Lopes I, Duarte AC, Rocha-Santos T. Environmental exposure to microplastics: An overview on possible human health effects. Sci Total Environ. 2020;702.
14. Dris R, Gasperi J, Saad M, Mirande C, Tassin B. Synthetic fibers in atmospheric fallout: A source of microplastics in the environment? Mar Pollut Bull. 2016;104(1-2):290-3.
15. Zhang N, Zhang CC, Qin YM, Wang JF, Ge XL, Li HW, et al. A Review of Atmospheric Microplastics: Sources, Characteristics, and Detection Method. Curr Pollut Rep. 2024;10(3):412-29.
16. Hartmann NB, Hüffer T, Thompson RC, Hassellöv M, Verschoor A, Daugaard AE, et al. Are We Speaking the Same Language? Recommendations for a Definition and Categorization Framework for Plastic Debris. Environ Sci Technol. 2019;53(3):1039-47.
17. do Sul JAI. Why it is important to analyze the chemical composition of microplastics in environmental samples. Mar Pollut Bull. 2021;165.
18. Dris R, Gasperi J, Mirande C, Mandin C, Guerrouache M, Langlois V, Tassin B. A first overview of textile fibers, including microplastics, in indoor and outdoor environments. Environ Pollut. 2017;221:453-8.
19. Stanton T, Johnson M, Nathanail P, MacNaughtan W, Gomes RL. Freshwater and airborne textile fibre populations are dominated by 'natural', not microplastic, fibres. Sci Total Environ. 2019;666:377-89.
20. Vianello A, Jensen RL, Liu L, Vollertsen J. Simulating human exposure to indoor airborne microplastics using a Breathing Thermal Manikin. Sci Rep-Uk. 2019;9.
21. Abbasi S, Keshavarzi B, Moore F, Turner A, Kelly FJ, Dominguez AO, Jaafarzadeh N. Distribution and potential health impacts of microplastics and microrubbers in air and street dusts from Asaluyeh County, Iran. Environ Pollut. 2019;244:153-64.
22. Chen LL, Zewei. Polyethylene: Properties, Production and Applications. IEEE Xplore. 2022.
23. Chen XM, H. H.; Wan, Y.; Marriam, I.; Tebyetekerwa, M. Circular Economy and Sustainability of the Clothing and Textile Industry. Materials Circular Economy. 2021;3.
24. de Oliveira CRS, da Silva AJr, Mulinari J, Ferreira AJS, da Silva A. Fibrous microplastics released from textiles: Occurrence, fate, and remediation strategies. J Contam Hydrol. 2023;256.
25. Allen S, Allen D, Phoenix VR, Le Roux G, Jiménez PD, Simonneau A, et al. Atmospheric transport and deposition of microplastics in a remote mountain catchment (vol 12, pg 339, 2019). Nat Geosci. 2019;12(8):679-.
26. Evode NQ, S. A.; Bilal, M.; Barcelo, D.; Iqbal, H. M. N. Plastic waste and its management strategies for environmental sustainability. Case Studies in Chemical and Environmental Engineering. 2021;4.
27. Prasittisopin L, Ferdous W, Kamchoom V. Microplastics in construction and built environment. Dev Built Environ. 2023;15.
28. Hahladakis JN, Velis CA, Weber R, Iacovidou E, Purnell P. An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling. J Hazard Mater. 2018;344:179-99.

29. de Souza NMG, Rocha RS, Ferreira RP, Reis CBD, Bandeira RSS, Melo APF. Comparing the use of silicone and polyurethane Peripherally Inserted Central Catheters in newborns: A retrospective study. J Clin Nurs. 2021;30(23-24):3439-47.
30. Ghoshal TP, P. R.; Bhuyan, T.; Bandyopadhyay, D. Polystyrene Foams: Materials, Technology, and Applications. Polymeric Foams: Fundamentals and Types of Foams 12023. p. 121-41.
31. Cai LQ, Wang JD, Peng JP, Tan Z, Zhan ZW, Tan XL, Chen QQ. Characteristic of microplastics in the atmospheric fallout from Dongguan city, China: preliminary research and first evidence. Environ Sci Pollut R. 2017;24(32):24928-35.
32. Klein M, Fischer EK. Microplastic abundance in atmospheric deposition within the Metropolitan area of Hamburg, Germany. Sci Total Environ. 2019;685:96-103.
33. Evangeliou N, Grythe H, Klimont Z, Heyes C, Eckhardt S, Lopez-Aparicio S, Stohl A. Atmospheric transport is a major pathway of microplastics to remote regions. Nat Commun. 2020;11(1):3381.
34. Liu K, Wu TN, Wang XH, Song ZY, Zong CX, Wei NA, Li DJ. Consistent Transport of Terrestrial Microplastics to the Ocean through Atmosphere. Environ Sci Technol. 2019;53(18):10612-9.
35. Amato-Lourenco LF, Dos Santos Galvao L, Wiebeck H, Carvalho-Oliveira R, Mauad T. Atmospheric microplastic fallout in outdoor and indoor environments in Sao Paulo megacity. Sci Total Environ. 2022;821:153450.
36. Brahney J, Mahowald N, Prank M, Cornwell G, Klimont Z, Matsui H, Prather KA. Constraining the atmospheric limb of the plastic cycle. Proc Natl Acad Sci U S A. 2021;118(16).
37. Wright SL, Levermore JM, Kelly FJ. Raman Spectral Imaging for the Detection of Inhalable Microplastics in Ambient Particulate Matter Samples. Environ Sci Technol. 2019;53(15):8947-56.
38. Zhang YL, Kang SC, Allen S, Allen D, Gao TG, Sillanpää M. Atmospheric microplastics: A review on current status and perspectives. Earth-Sci Rev. 2020;203.
39. Gaston E, Woo M, Steele C, Sukumaran S, Anderson S. Microplastics Differ Between Indoor and Outdoor Air Masses: Insights from Multiple Microscopy Methodologies. Appl Spectrosc. 2020;74(9):1079-98.
40. Zhang Q, Zhao YP, Du FN, Cai HW, Wang GH, Shi HH. Microplastic Fallout in Different Indoor Environments. Environ Sci Technol. 2020;54(11):6530-9.
41. Shao LY, Li YW, Jones T, Santosh M, Liu PJ, Zhang MY, et al. Airborne microplastics: A review of current perspectives and environmental implications. J Clean Prod. 2022;347.
42. Kyriakoudes G, Turner A. Suspended and deposited microplastics in the coastal atmosphere of southwest England. Chemosphere. 2023;343:140258.
43. Wesch C, Elert AM, Worner M, Braun U, Klein R, Paulus M. Assuring quality in microplastic monitoring: About the value of clean-air devices as essentials for verified data. Sci Rep. 2017;7(1):5424.
44. Catarino AI, Macchia V, Sanderson WG, Thompson RC, Henry TB. Low levels of microplastics (MP) in wild mussels indicate that MP ingestion by humans is minimal compared to exposure via household fibres fallout during a meal. Environ Pollut. 2018;237:675-84.
45. Dos Santos Galvao L, Fernandes EMS, Ferreira RR, Dos Santos Rosa D, Wiebeck H. Critical steps for microplastics characterization from the atmosphere. J Hazard Mater. 2022;424(Pt C):127668.
46. Yuan Z, Li HX, Lin L, Pan YF, Liu S, Hou R, Xu XR. Occurrence and human exposure risks of atmospheric microplastics: A review. Gondwana Res. 2022;108:200-12.
47. Gov.UK. Accredited official statistic Particulate Matter (PM10/PM2.5) 2025 [updated 18/03/2025. Available from: https://www.gov.uk/government/statistics/air-quality-statistics/concentrations-of-particulate-matter-pm10-and-pm25.
48. Azari A, Vanoirbeek JAJ, Van Belleghem F, Vleeschouwers B, Hoet PHM, Ghosh M. Sampling strategies and analytical techniques for assessment of airborne micro and nano plastics. Environ Int. 2023;174:107885.

49. Yuk H, Jo HH, Nam J, Kim YU, Kim S. Microplastic: A particulate matter(PM) generated by deterioration of building materials. J Hazard Mater. 2022;437:129290.
50. Chen G, Feng Q, Wang J. Mini-review of microplastics in the atmosphere and their risks to humans. Sci Total Environ. 2020;703:135504.
51. Liu K, Wang XH, Fang T, Xu P, Zhu LX, Li DJ. Source and potential risk assessment of suspended atmospheric microplastics in Shanghai. Sci Total Environ. 2019;675:462-71.
52. Cheung CKH, Not C. Impacts of extreme weather events on microplastic distribution in coastal environments. Sci Total Environ. 2023;904:166723.
53. Sharma P, Sharma P, Abhishek K. Sampling, separation, and characterization methodology for quantification of microplastic from the environment. J Hazard Mater Adv. 2024;14.
54. Smith BC. Fundamentals of Fourier Transform Infrared Spectroscopy: CRC Pres; 2011.
55. Wright SL, Ulke J, Font A, Chan KLA, Kelly FJ. Atmospheric microplastic deposition in an urban environment and an evaluation of transport. Environ Int. 2020;136.
56. Hidalgo-Ruz V, Gutow L, Thompson RC, Thiel M. Microplastics in the marine environment: a review of the methods used for identification and quantification. Environ Sci Technol. 2012;46(6):3060-75.
57. Dris R, Gasperi J, Rocher V, Saad M, Renault N, Tassin B. Microplastic contamination in an urban area: a case study in Greater Paris. Environ Chem. 2015;12(5):592-9.
58. Free CM, Jensen OP, Mason SA, Eriksen M, Williamson NJ, Boldgiv B. High-levels of microplastic pollution in a large, remote, mountain lake. Mar Pollut Bull. 2014;85(1):156-63.
59. Maes T, Jessop R, Wellner N, Haupt K, Mayes AG. A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red. Sci Rep. 2017;7:44501.
60. Beckingham BA, A.; Moore, C.; Brandes, J. Hot or not: systematic review and laboratory evaluation of the hot needle test for microplastic identification. Microplastics and Nanoplastics. 2023;3.
61. Xie J, Gowen A, Xu W, Xu J. Analysing micro- and nanoplastics with cutting-edge infrared spectroscopy techniques: a critical review. Anal Methods. 2024;16(15):2177-97.
62. Finnegan A, Susserott RC, Koh LH, Teo WB, Gouramanis C. A Simple Sample Preparation Method to Significantly Improve Fourier Transform Infrared (FT-IR) Spectra of Microplastics. Appl Spectrosc. 2022;76(7):783-92.
63. Ribeiro-Claro PN, M. M.; Araujo, C. Chapter 5 - Characterization of Microplastics by Raman Spectroscopy. Comprehensive Analystical Chemistry. 2017;75:119-51.
64. Azari A, Ronsmans S, Vanoirbeek JAJ, Hoet PHM, Ghosh M. Challenges in Raman spectroscopy of (micro)Plastics: The interfering role of colourants. Environ Pollut. 2024;363(Pt 2):125250.
65. Jones NR, de Jersey AM, Lavers JL, Rodemann T, Rivers-Auty J. Identifying laboratory sources of microplastic and nanoplastic contamination from the air, water, and consumables. J Hazard Mater. 2024;465:133276.
66. Horton AA, Cross RK, Read DS, Jurgens MD, Ball HL, Svendsen C, et al. Semi-automated analysis of microplastics in complex wastewater samples. Environ Pollut. 2021;268(Pt A):115841.
67. Prata JC, Reis V, da Costa JP, Mouneyrac C, Duarte AC, Rocha-Santos T. Contamination issues as a challenge in quality control and quality assurance in microplastics analytics. J Hazard Mater. 2021;403:123660.
68. Montano L, Raimondo S, Piscopo M, Ricciardi M, Guglielmino A, Chamayou S, et al. First evidence of microplastics in human ovarian follicular fluid: An emerging threat to female fertility. Ecotoxicol Environ Saf. 2025;291:117868.
69. Yang Q, Peng Y, Wu X, Cao X, Zhang P, Liang Z, et al. Microplastics in human skeletal tissues: Presence, distribution and health implications. Environ Int. 2025;196:109316.
70. Schwabl P, Koppel S, Konigshofer P, Bucsics T, Trauner M, Reiberger T, Liebmann B. Detection of Various Microplastics in Human Stool: A Prospective Case Series. Ann Intern Med. 2019;171(7):453-7.

71. Yan Z, Liu Y, Zhang T, Zhang F, Ren H, Zhang Y. Analysis of Microplastics in Human Feces Reveals a Correlation between Fecal Microplastics and Inflammatory Bowel Disease Status. Environ Sci Technol. 2022;56(1):414-21.
72. Ragusa A, Svelato A, Santacroce C, Catalano P, Notarstefano V, Carnevali O, et al. Plasticenta: First evidence of microplastics in human placenta. Environ Int. 2021;146:106274.
73. Ibrahim YS, Tuan Anuar S, Azmi AA, Wan Mohd Khalik WMA, Lehata S, Hamzah SR, et al. Detection of microplastics in human colectomy specimens. JGH Open. 2021;5(1):116-21.
74. Leslie HA, van Velzen MJM, Brandsma SH, Vethaak AD, Garcia-Vallejo JJ, Lamoree MH. Discovery and quantification of plastic particle pollution in human blood. Environ Int. 2022;163:107199.
75. S VLL, Liddle CR, Atherall CA, Chapman E, Watkins M, S DJC, Rotchell JM. Microplastics in human blood: Polymer types, concentrations and characterisation using muFTIR. Environ Int. 2024;188:108751.
76. Kwon JH, Kim JW, Pham TD, Tarafdar A, Hong S, Chun SH, et al. Microplastics in Food: A Review on Analytical Methods and Challenges. Int J Environ Res Public Health. 2020;17(18).
77. Pauly JL, Stegmeier SJ, Allaart HA, Cheney RT, Zhang PJ, Mayer AG, Streck RJ. Inhaled cellulosic and plastic fibers found in human lung tissue. Cancer Epidemiol Biomarkers Prev. 1998;7(5):419-28.
78. Amato-Lourenco LF, Carvalho-Oliveira R, Junior GR, Dos Santos Galvao L, Ando RA, Mauad T. Presence of airborne microplastics in human lung tissue. J Hazard Mater. 2021;416:126124.
79. Uoginte I, Vailionyte A, Skapas M, Bolanos D, Bagurskiene E, Gruslys V, et al. New evidence of the presence of micro- and nanoplastic particles in bronchioalveolar lavage samples of clinical trial subjects. Heliyon. 2023;9(9):e19665.
80. Qiu L, Lu W, Tu C, Li X, Zhang H, Wang S, et al. Evidence of Microplastics in Bronchoalveolar Lavage Fluid among Never-Smokers: A Prospective Case Series. Environ Sci Technol. 2023;57(6):2435-44.
81. Lu W, Li X, Wang S, Tu C, Qiu L, Zhang H, et al. New Evidence of Microplastics in the Lower Respiratory Tract: Inhalation through Smoking. Environ Sci Technol. 2023;57(23):8496-505.
82. Baeza-Martinez C, Olmos S, Gonzalez-Pleiter M, Lopez-Castellanos J, Garcia-Pachon E, Masia-Canuto M, et al. First evidence of microplastics isolated in European citizens' lower airway. J Hazard Mater. 2022;438:129439.
83. Chen C, Liu F, Quan S, Chen L, Shen A, Jiao A, et al. Microplastics in the Bronchoalveolar Lavage Fluid of Chinese Children: Associations with Age, City Development, and Disease Features. Environ Sci Technol. 2023;57(34):12594-601.
84. Sykes DL, Morice AH. The Cough Reflex: The Janus of Respiratory Medicine. Front Physiol. 2021;12:684080.
85. Weibel ER. Geometric and Dimensional Airway Models of Conductive, Transitory and Respiratory Zones of the Human Lung. Morphometry of the Human Lung: Springer; 1963. p. 136-42.
86. Deng Q, Deng L, Miao Y, Guo X, Li Y. Particle deposition in the human lung: Health implications of particulate matter from different sources. Environ Res. 2019;169:237-45.
87. Moreno-Rios ALT-B, L. P.; Bustillo-Lecompte, C. F. Sources, characteristics, toxicity, and control of ultrafine particles: An overview. Geoscience Frontiers. 2022;13(1):101147.
88. Warheit DB, Hart GA, Hesterberg TW, Collins JJ, Dyer WM, Swaen GM, et al. Potential pulmonary effects of man-made organic fiber (MMOF) dusts. Crit Rev Toxicol. 2001;31(6):697-736.
89. Deng Y, Zhang Y, Lemos B, Ren H. Tissue accumulation of microplastics in mice and biomarker responses suggest widespread health risks of exposure. Sci Rep. 2017;7:46687.
90. Liu Z, Zhuan Q, Zhang L, Meng L, Fu X, Hou Y. Polystyrene microplastics induced female reproductive toxicity in mice. J Hazard Mater. 2022;424(Pt C):127629.

91. Jin H, Yang C, Jiang C, Li L, Pan M, Li D, et al. Evaluation of Neurotoxicity in BALB/c Mice following Chronic Exposure to Polystyrene Microplastics. Environ Health Perspect. 2022;130(10):107002.
92. Okamura T, Hamaguchi M, Hasegawa Y, Hashimoto Y, Majima S, Senmaru T, et al. Oral Exposure to Polystyrene Microplastics of Mice on a Normal or High-Fat Diet and Intestinal and Metabolic Outcomes. Environ Health Perspect. 2023;131(2):27006.
93. Zhu X, Wang C, Duan X, Liang B, Genbo Xu E, Huang Z. Micro- and nanoplastics: A new cardiovascular risk factor? Environ Int. 2023;171:107662.
94. Xia Q, Wei Y, Hu LJ, Zeng FM, Chen YW, Xu D, et al. Inhalation of Microplastics Induces Inflammatory Injuries in Multiple Murine Organs via the Toll-like Receptor Pathway. Environ Sci Technol. 2024;58(42):18603-18.
95. Bridgeman L, Cimbalo A, Lopez-Rodriguez D, Pamies D, Frangiamone M. Exploring toxicological pathways of microplastics and nanoplastics: Insights from animal and cellular models. J Hazard Mater. 2025;490:137795.
96. Danopoulos E, Twiddy M, West R, Rotchell JM. A rapid review and meta-regression analyses of the toxicological impacts of microplastic exposure in human cells. J Hazard Mater. 2022;427:127861.
97. Gettings SM, Timbury W, Dmochowska A, Sharma R, McGonigle R, MacKenzie LE, et al. Polyethylene terephthalate (PET) micro- and nanoplastic particles affect the mitochondrial efficiency of human brain vascular pericytes without inducing oxidative stress. NanoImpact. 2024;34:100508.
98. Lu K, Zhan D, Fang Y, Li L, Chen G, Chen S, Wang L. Microplastics, potential threat to patients with lung diseases. Front Toxicol. 2022;4:958414.
99. Shi W, Cao Y, Chai X, Zhao Q, Geng Y, Liu D, Tian S. Potential health risks of the interaction of microplastics and lung surfactant. J Hazard Mater. 2022;429:128109.
100. Rios LM, Jones PR, Moore C, Narayan UV. Quantitation of persistent organic pollutants adsorbed on plastic debris from the Northern Pacific Gyre's "eastern garbage patch". J Environ Monit. 2010;12(12):2226-36.
101. Dadi NCT, Radochova B, Vargova J, Bujdakova H. Impact of Healthcare-Associated Infections Connected to Medical Devices-An Update. Microorganisms. 2021;9(11).
102. Diaconu O, Siriopol I, Polosanu LI, Grigoras I. Endotracheal Tube Biofilm and its Impact on the Pathogenesis of Ventilator-Associated Pneumonia. J Crit Care Med (Targu Mures). 2018;4(2):50-5.
103. Lobelle D, Cunliffe M. Early microbial biofilm formation on marine plastic debris. Mar Pollut Bull. 2011;62(1):197-200.
104. Michels J, Stippkugel A, Lenz M, Wirtz K, Engel A. Rapid aggregation of biofilm-covered microplastics with marine biogenic particles. Proc Biol Sci. 2018;285(1885).
105. Kyung SY, Jeong SH. Particulate-Matter Related Respiratory Diseases. Tuberc Respir Dis (Seoul). 2020;83(2):116-21.
106. Lougheed MD, Roos JO, Waddell WR, Munt PW. Desquamative interstitial pneumonitis and diffuse alveolar damage in textile workers. Potential role of mycotoxins. Chest. 1995;108(5):1196-200.
107. Boag AH, Colby TV, Fraire AE, Kuhn C, 3rd, Roggli VL, Travis WD, Vallyathan V. The pathology of interstitial lung disease in nylon flock workers. Am J Surg Pathol. 1999;23(12):1539-45.
108. Kern DG, Crausman RS, Durand KT, Nayer A, Kuhn C, 3rd. Flock worker's lung: chronic interstitial lung disease in the nylon flocking industry. Ann Intern Med. 1998;129(4):261-72.

2 Chapter 2: Household Atmospheric Microplastics

University of York
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Research Degree Thesis Statement of Authorship

Note that where a paper has multiple authors, the statement of authorship can focus on the key contributing/corresponding authors.

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Title of the work (paper/chapter)	**Household indoor microplastics: quantification and chemical characterisation of particles present**	
Publication status	**Published**	✓ **15/08/2021**
	Accepted for publication	
	Submitted for publication	
	Unpublished and unsubmitted	
Citation details (if applicable)		

Description of the candidate's contribution to the work*	**The candidate (LJ as named in the paper) is listed as the first author of this paper. LJ contributed to the conceptualisation, experimental design and conduct, data analysis and writing of the manuscript.**
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By signing this Statement of Authorship, each co-author agrees that:

(i) the candidate has accurately represented their contribution to the work;

(ii) if required, permission is granted for the candidate to include the work in their thesis (note that this is separate from copyright considerations).

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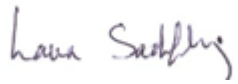
Signature of the co-author	
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Household indoor microplastics within the Humber region (United Kingdom): Quantification and chemical characterisation of particles present

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ABSTRACT

Knowledge regarding the presence of suspended microplastics (MPs) within the air is lacking, especially indoors, yet the importance of indoor air quality and human health is rising. This study is the first to report MPs within multiple homes over a 6-month period, with concentrations exceeding previous outdoor studies. Twenty households, within the City of Hull and Humber region, U.K., were passively sampled, each month, collecting atmospheric fallout at head height for subsequent particle quantification, characterisation and μFTIR validation
(n = 3061). A household average of 1414 MP m^{-2} day^{-1} ± 1022 (mean ± SD) was observed. Smaller (5–250 μm), fibrous, particles were the most abundant (90%), representing types most likely to enter the human body and cause physiological harm. Polyethylene terephthalate (PET) was present in 90% of samples and accounted for 62% of MPs. Additionally, polyamide (PA) and polypropylene (PP) were common. Results indicate that humans are exposed to significantly (1–45 times) higher concentrations, and ranges, of MPs within homes compared with the outdoor environment. In conclusion, the size range and types of MPs observed will inform laboratory experiments, using either human tissue culture or other approaches. This will allow determination of the wider implications on human health using realistic levels and representative types of indoor MPs.

2. Introduction

Plastic products are used in countless applications; from food packaging, textiles and electronics, to building, construction and medical devices (1, 2). Yet, plastic waste mismanagement is stated to be a key burden in environmental literature (3). Of the predicted 6300 million metric tons of global plastic waste generated prior to 2015, 79% has been estimated to aggregate within landfills and the environment, and only 9% recycled (1). This results in the introduction of primary plastics, secondary degradation plastics and chemical leachates into aquatic, terrestrial and atmospheric compartments (4), and ultimately leads to global MP pollution. Research has previously been directed more towards routes of MPs into the environment and prevalence (5), as well as dietary exposure via salt, seafood and drinking water (6-8). However, there is now an emerging concern surrounding MP inhalation as another human exposure route (2, 9, 10). Consequently, gaining a holistic view of MP pollution and human exposure is of increasing importance.

Since their initial identification (11), MPs have been consistently reported within atmospheric samples. MPs have been captured both passively and actively (Table S1), as well as within deposited dust samples, demonstrating their ubiquity (12, 13). MPs (defined herein as between the size ranges of 1 µm and 5 mm (14)) are not yet considered an atmospheric pollutant. They are considered an emerging contaminant of concern and have been reported as a constituent of particulate matter (PM). Questions relating to human exposure rates and health consequences have since arisen (2, 10, 12, 15). Passively sampled MPs are reported within literature as units of MPs m^{-2} day^{-1}, with publications from France (ranging from 53 to 365 m^{-2} day^{-1}) China (33-9900 m^{-2} day^{-1}), Germany (137-512 m^{-2} day^{-1}), and most recently, the UK (3-771 m^{-2} day^{-1}) (11, 16-21). In contrast, MPs captured actively are expressed in units of MP m^{-3}, with reports from France (0-59 m^{-3}), Denmark (9 m^{-3}), China (0-5 m^{-3}) and America (1-13 m^{-3}) (12, 22-25). To date, just four studies worldwide report on MPs within the home (21, 22, 26, 27). It is important to note that environmental sampling (duration, location, meteorological conditions, sample number), sample processing steps (digestion, purification), microscopic analyses (observational criteria, size and shape categories) and chemical analyses methodologies all vary, and it is difficult to conduct meaningful inter-study comparisons. Despite this, the majority of studies evidence that MPs increase in concentration with decreasing particle size (until an observational limit is reached) (18). MPs are prevalent in locations of high human activity e.g. urbanised city centres (17), and tend to be fibrous (17, 23). One passively sampled study recorded indoor atmospheric MP levels ranging from 1500 to 9900 MP m^{-2} day^{-1}, depending on the room, a rate exceptionally higher than outdoor studies (21). MP studies that have reported actively sampled indoor MP levels (ranging from 1 to 59 m^{-3}) (22, 26), again, exceed that of outdoor concentrations (which range from 0 to 6 m^{-3}) (Table S1). The significance of indoor air quality is highlighted by the fact that humans spend up to 90% of their time indoors (28) and as much as 60% within their homes (29). Whilst the health effects of exposure to indoor MPs are not yet defined, indoor PM has been linked to a number of health impacts including a decline in respiratory and cardiovascular health (30) as well as specific MP types inducing toxic impacts in recent human lung cell culture exposure studies (31, 32).

To date, there are many unknowns: from the chemical composition, size or shape of the MP, to any chemical leachate or adsorbed pollutants. The possibility of MPs entering the human body and impact of such exposure on health is of increasing concern. This study aims to provide knowledge surrounding the indoor MPs that humans are most likely exposed to, by quantifying concentration rates, and determining particle dimensions, shapes and chemical composition.

3. Materials and Methods

A total of 20 houses were selected for sampling (following a request to volunteer), all located within the city of Hull and wider East Riding of Yorkshire, U.K. region (Fig. 1). Sampling covered a six-month timescale investigation from July until December 2019. Participants were given a short questionnaire (Supplemental Information) to gain details regarding their living conditions and routines. Additionally, each month, outstanding events such as 'occupants away during sampling period' or 'building maintenance within the home' were noted. Participant ages were categorised; 18–25, 26–35, 36–50, 51–65 and > 65 (5%, 40%, 20%, 25%, 10%, respectively). Household occupancy was determined and ranged from 1 to 4 people (mean 2.2). The hours per day that participants spent within the home ranged from 10 to 21 h (mean 16 h). The hours spent within the room in which sampling was conducted ranged from 2 to 12 h (mean 7 h). Carpets and other furnishing samples were taken from the homes of willing participants to compare polymer composition to particles isolated from atmospheric samples.

2.1. Passive sampling

Identical 1 L glass beakers (0.0095 m^2) were placed at head height (1–1.8 m) within the downstairs room that residents reported was the most occupied within the home. The beakers were opened by removing the foil lid on a set date and time. The beakers were exposed to atmospheric fallout for exactly 7 days to avoid weekend or weekday bias (21), and participants were instructed to not change any of their habits or routines. A foil lid was placed tightly onto the beaker upon termination of the sampling period. Collection and transport to the laboratory was conducted by the primary researcher. Beakers were stored at room temperature in the dark until bulk processing was conducted.

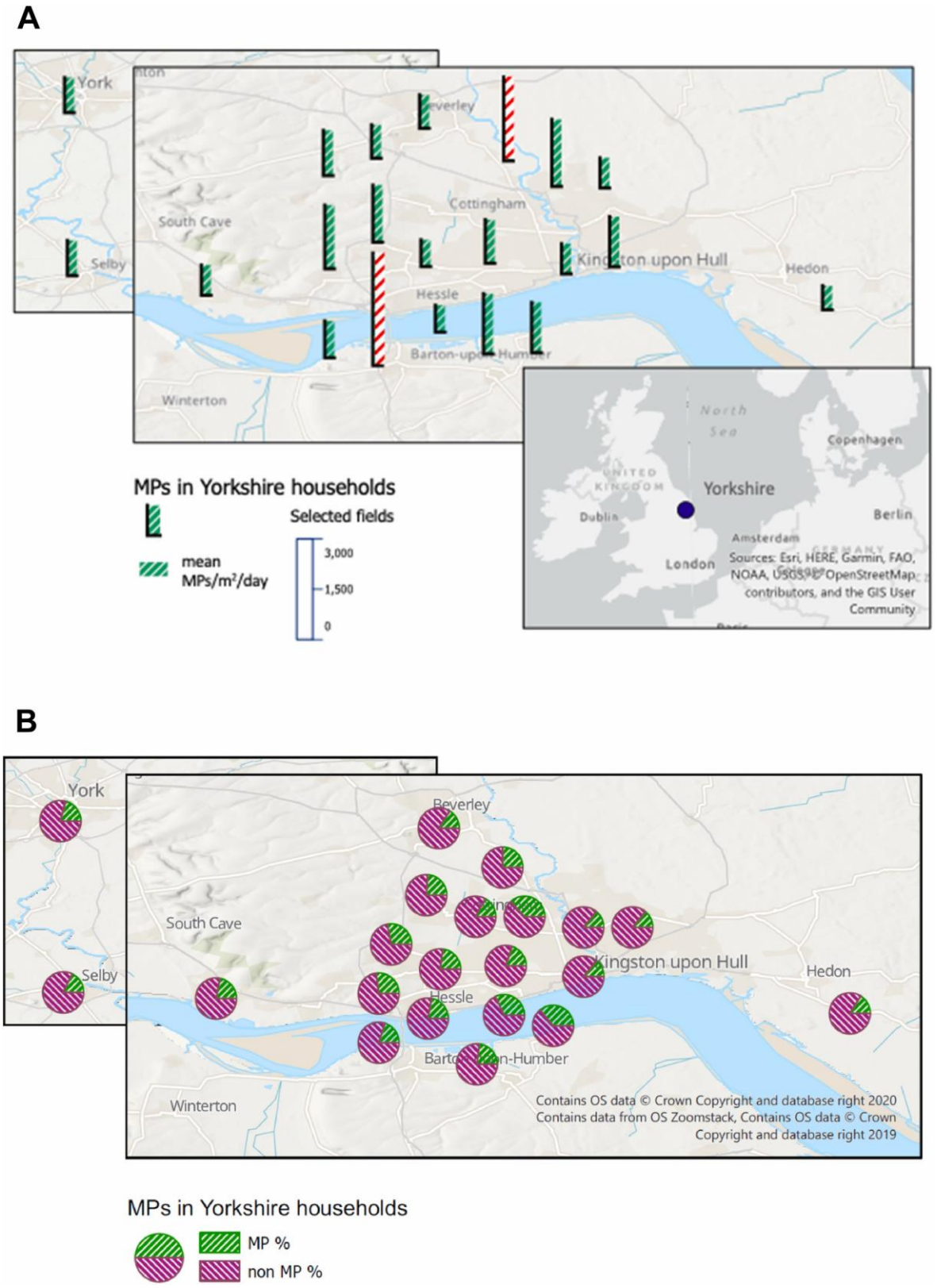


Fig. 1. Location, levels and types of particles detected in the Hull and East Riding of Yorkshire region, U.K., with plotted points indicating each household. **A.** Mean MP concentration rate for each household (MP m^{-2} day^{-1}), red/white denotes households with high MP concentration rates relative to the majority of others, and **B.** Proportion of MPs to non-MPs at each household location. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.2. Sample pre-treatment

Particle overload was a problem encountered during the pilot study. In order to significantly reduce the particle count, a digest to remove biological (non-MP) materials was conducted as follows. Each beaker was washed three times with 200 ml of 30% hydrogen peroxide solution (H_2O_2) (Fisher Scientific, Loughborough, U.K.). Beakers were rinsed in a standardised manner, ensuring the sides of the beaker were washed thoroughly (11, 23). The rinsate was collected into a 1 L glass conical flask and sealed immediately with a foil lid. Each sample was placed in a shaking incubator at 55 ˚C to ensure no destruction of polymers with a low degradation temperature (18), and rotated at 65 rpm for 3 days. This pre-treatment digestion step removed biological and other natural organic matter, such as skin and pet dander, before particle analysis (26, 33, 34), whilst not damaging any MP particles (35). Conical flask contents were then filtered using a glass vacuum filtration device and particulates collected onto mixed cellulose esters membrane filters (MCE), 47 mm diameter and 5 µm pore size (MERCK, Gillingham, U.K.), Each conical flask was washed three times with 200 ml of MilliQ water and the sides of the glassware were rinsed to reduce particle loss. Filters were stored in covered petri dishes, labelled and allowed to dry at room temperature in the dark for a minimum of 24 h.

2.3. Particle level quantification and characterisation

One quarter of each sample filter was randomly sectioned for stereomicroscope analysis (Olympus SZX10, Olympus Corporation, Japan), and extrapolation of the dataset was later calculated to represent the entire sample. Therefore, an assumption was made that even particle distribution had occurred during the filtration process, which may not always be the case. The length (largest side of particle) and width (second largest side of the particle) was recorded for each particle, as well as particle shape (36) (Table S2). Particle dimensions were measured (CellSens software, Olympus Corporation, Japan) and categorised as follows; length: 5–249 µm, 250–500 µm, 501–1000 µm,

1001–5000 µm, and width: 5–10 µm, 11–20 µm, 21–30 µm, 31–40 µm, 41–50 µm and >50 µm. Particles with a length >5 mm were removed from the filter with tweezers in order to focus the analysis of the particles to a standard MP size range (14).

2.4. Particle chemical composition analysis

For each sample, 20% of the identified particles were analysed by µFTIR analysis. Particles were placed individually onto a DC-3 diamond compression cell (Specac Limited, Orpington, U.K.) and µFTIR analysis (using a Nicolet iN10, ThermoFisher, Waltham MA, U.S.A.) was conducted in transmission mode and using the liquid nitrogen cooling system. A blank area next to the particle being analysed was chosen as a background reference, before immediate analysis using the same spatial resolution (Omnic software package, ThermoFisher, Waltham MA, U.S. A.). All background and sample spectra were obtained using a scan number of 64 and a spectral range of 4000–675 cm^{-1}. Each resulting spectrum was compared to a cluster of polymer libraries (Omnic polymer libraries). Three attempts were made to gain a spectrum with a match index ≥70%, and only these were included in the results.

2.5. Quality assurance and control

To optimise methodologies, a one-month pilot study was conducted in which the deposited dust from 10 households was continuously passively sampled and analysed, during June 2019. This facilitated subsequent standardised sampling, pre-treatment and analysis protocols to be developed.

MilliQ water and H_2O_2 were filtered three times with 47 mm glass fibre grade 6 filters (GE Healthcare Life Sciences, Marlborough MA, U.S. A.) using a glass vacuum filtration kit to reduce background particle contamination. Also, a procedural control was run alongside each batch of samples (n = 26). A blank MCE filter was opened during stereomicroscope and μFTIR analysis in order to monitor atmospheric fallout contamination during laboratory particle analysis (n = 118). All glassware were washed and rinsed three times with triple filtered MilliQ water before being immediately wrapped in foil. Foil lids were only removed when necessary and opening times kept to a minimum. Ventilation from windows, doors and airing devices was minimised and work was conducted at times of low activity to minimise particle suspension. 100% cotton laboratory coats were worn during all stages of the project and a fume cupboard used where possible. One individual researcher was responsible for all samples, ensuring standardised analyses throughout.

Limit of detection (LOD) and limit of quantification (LOQ) calculations (37) were applied to the three most common MP types identified within samples (Supplemental Materials Methods SM1 and Table S3). *2.6. Statistical analysis* MP quantity identified on each quarter was extrapolated to represent the entire filter and then the value adjusted to represent MP m^{-2} day^{-1} as follows: MP quantity per sample was multiplied by 105 which represents the factor to convert from the bottom of the beaker surface area to m^2, and then divided by 7 for a per day value (Supplementary Method 1). Statistical analyses were performed using SPSS. All data were determined not normally distributed with a Shapiro-Wilk test and either a Kruskal-Wallis test or Mann-Whitney *U* test applied. Statistical significance was accepted at a $p < 0.05$ level and extreme significance at 0.01.

3. Results

6 samples were collected from each of the 20 households, with the exception of 2 samples. Therefore, a total of 118 passive samples were collected throughout the study. μFTIR analysis was conducted on 3061 particles consisting of 2442 of ≥70% match index, 585 of 60–69% match, and 34 of <60% match.

3.1. Control and laboratory blanks

A total of 299 particles were identified from the 144 controls. From these, 25 were identified as MPs (ranging from 0 to 1 per sample, mean 0.2 ± 0.4), specifically polyurethane/polypropylene copolymer (PUR/ PP), polyvinyl chloride (PVC), PET and PP which were present in low levels (3, 2, 2, 2%, respectively). Non-MP particles consisted primarily of cellulose/cellophane (86%). MPs identified within control samples were significantly different from MPs identified within samples ($p = 1.86 \times 10^{-41}$). Due to the low contamination rates of MPs within each sample it was decided not to subtract contamination rates from final results (26) but LOD/LOQ results have been reported for the most common MP types observed (Table S3).

3.2. Total particle fallout

The number of particles collected during the sampling period varied, with a mean of 6204 ± 3122 particles m^{-2} day^{-1} (mean ± SD) and a range of 902–14,551 particles m^{-2} day^{-1}. The mean particle quantity for December was significantly lower than July ($p = 0.003$), August ($p = 0.005$) and September ($p = 0.05$), (Fig. 2), however all other months were not significantly different in terms of their mean particle quantity.

3.3. Total particle characterisation

The majority of particles were of a fibrous nature (90%). The remaining 10% were comprised of fragment, film and sphere (8%, 1%, 1%, respectively) (Fig. 3). No foam-like particles were identified within any samples.

An increase in particle concentration with decreasing particle length was observed (Fig. 4A.). Particles 5–250 µm accounted for 59% of particles, the remaining 41% were comprised of 250–500 µm, 501–1000 µm and 1001–5000 µm (18%, 13%, 10%, respectively). Particle width size categories were; 5–10 µm, 11–20 µm, 21–30 µm, 31–40 µm, 41–50 µm and >50 µm (29%, 63%, 6%, 1%, 0%, 1%, respectively) (Fig. 4B.).

3.4. Non-MP particle composition

Non-MP particles (natural and artificial particles not of a petroleum derived nature) accounted for 77% of the total particle count (July 81%, August 74%, September 78%, October 73%, November 78%, December 80%). Non-MP fractions for each household ranged from 63 to 86% (Fig. 1B.). Of the non-MP fraction, cellulose/cellophane accounted for 92% of particles. The remaining natural and artificial particles were comprised of zein, silk, cocamide and silicon dioxide (5%, 1%, 1%, 1%, respectively), and others (Figure S1).

3.5. MP concentration

The number of MP identified during the sampling period varied, with a mean of 1414 ± 1022 MP m^{-2} day^{-1} (mean ± SD), 0–5412 MP m^{-2} day^{-1} (range), 1203 MP m^{-2} day^{-1} (median). MPs were identified in all households and within 98% of samples (116/118). Lower concentration rates were noted for the month of December but there were no significant differences found between the sampling months ($p = 0.06$) (Fig. 2). Two households had significantly higher MP concentration rates compared to others throughout the sampling period (Fig. 1A).

3.6. MP particle composition

Synthetic MPs particles accounted for approximately 23% of the total particle fallout across all households, which varied by month as follows: July 19%, August 26%, September 22%, October 27%, November 22%, December 20%. MP fractions for each household ranged from 14 to 37% (Fig. 1B.). PET was present in 90% of samples and accounted for 63% of the total synthetic fraction (Fig. 5A.). Other MP polymer types identified were PA, acrylates, PP, co-polymer blends (containing at least one petroleum derived polymer), polyacrylonitrile (PAN), polyethylene (PE), polymethacrylate (PMMA) (6%, 4%, 4%, 3%, 3%, 3%, 3%, respectively) and others (Fig. 5A.). Polymer type concentrations varied, with PET being the most abundant MP within every sampling month (Fig. 5B.)

The level of PET recorded for December was significantly lower than July ($p = 0.005$), August ($p = 0.000$), September ($p = 0.004$) and October ($p = 0.001$) (Fig. 5B.). PET, PA and PP particle numbers identified within samples were above the LOD and LOQ (of 1.1 and 3.3 respectively, for PET as an example) (Table S3).

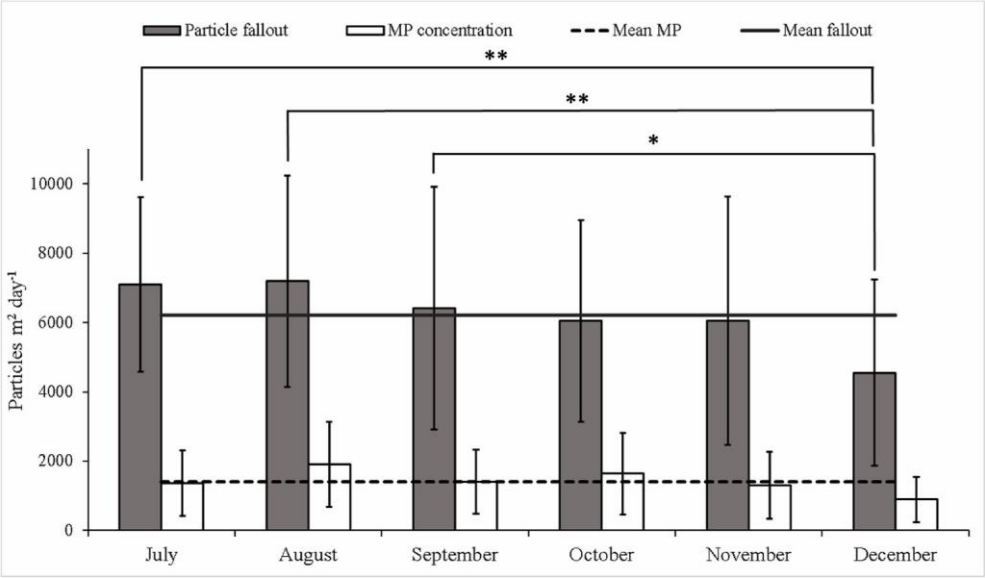


Fig. 2. Bar chart displaying monthly (mean) particle fallout and monthly (mean) MP concentrations alongside the overall means. Particle fallout significant differences Dec/Jul (p = 0.003), Dec/Aug (p = 0.005), Dec/Sept (p = 0.05). No significant differences were recorded for MP concentration and month (p = 0.06).

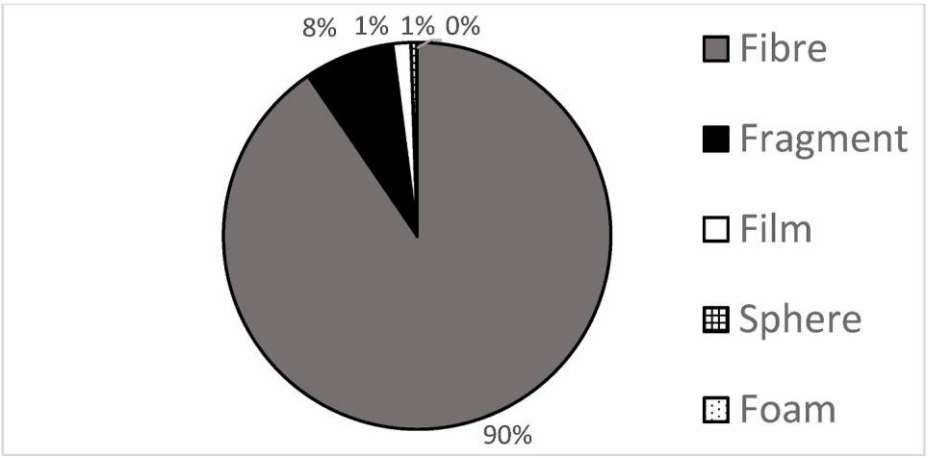


Fig. 3. Pie chart displaying the total particle shape distribution.

4. Discussion

This study addresses the limited knowledge on indoor MPs, specifically to outline what types and levels of MPs humans may be typically exposed to on a daily basis within the home. Of the three studies reporting MPs in a home environment, sampling was conducted on: one dormitory in China (2 days sampling per week, for 3 months) (21), three apartments in Denmark (3 days consecutive sampling, totalling 9 samples) (26) and two apartments in France (4–7 h sampling, for 3 days in Feb, May, Jul, Oct) (22). This study provides a more substantial indoor MP dataset for 20 UK households over a 6-month period, with sampling durations of a week. Due to the increased importance of applying chemical analysis alongside MP data, this study developed 'best practice' by; providing a large number of particles analysed and validated by µFTIR. In doing so, the approach avoids selecting particles for analysis based on observational criteria alone and provides a larger sample size and duration to represent a more longitudinal dataset (Table S1). The approach used also takes into account any background contamination (38) and includes an LOD/LOQ consideration (37). These are important since the atmospheric microplastics research field is developing, and as such, no standardised procedures are in place. This approach provides a quality assurance threshold above that of simple polymer contamination subtraction, and values below the LOQ are not included in final concentrations. Within this study, MPs within samples were high and background contamination low, therefore final concentration levels were not significantly affected (Table S3). However, this LOD/LOQ technique provides an option for future studies to apply more stringent reporting, especially for datasets in which contamination is high or MP counts within samples are low.

MPs were identified within all households and 98% of samples, thus it is evident within an indoor setting that MPs are ubiquitous. An average household atmospheric MP concentration rate of 1414 ± 1022 MP m^{-2} day^{-1} (mean ± SD) (Fig. 2) was observed. This is significantly higher than reported by all presently available passively sampled outdoor studies, and approximately 1–45 times higher than outdoor studies (Table S1). This reinforces claims of a higher contamination rate of atmospheric MPs within an indoor environment (21, 22, 26). It is also corroborated by active air sample concentration rates which range indoors from 1 to 59 MP m^{-3} and outdoors from 0 to 6 MP m^{-3} (Table S1). One study has investigated indoor atmospheric MPs in the comparable units of MP m^{-2} day^{-1} (21), in which a dormitory (9900 MP m^{-2} day^{-1}), office area (1800 MP m^{-2} day^{-1}) and corridor (1500 MP m^{-2} day^{-1}) were sampled discontinuously for 3 months, reporting concentrations similar to and higher than this current study. Importantly, cellophane and rayon, artificial polymers of natural origins were classified as MPs, unlike this study, and therefore comparable concentration rates are less than those stated. Despite this, there is a general consensus that indoor atmospheric MP levels significantly exceed that of outdoors (22, 26).

Fibres were the predominant particle form identified (90%), this is similar with the majority of other atmospheric MP studies which report a range from 67 to 90% (11, 13, 16, 20, 21), yet contradicts others that report more fragments (19, 24, 26, 27). An 8% particle fragment rate was reported here, more than film (1%), sphere (1%) and foam (0%) (Fig. 3). No foam-like particles were identified in any samples, regardless of a likely source being insulation, packing, furniture and carpet underlay (39, 40). Discrepancies between fibre and fragment prevalence may be due to differing sampling locations e.g. remote versus urban, but could also be due to ambiguity within shape classification criteria, especially with decreasing particle size (26).

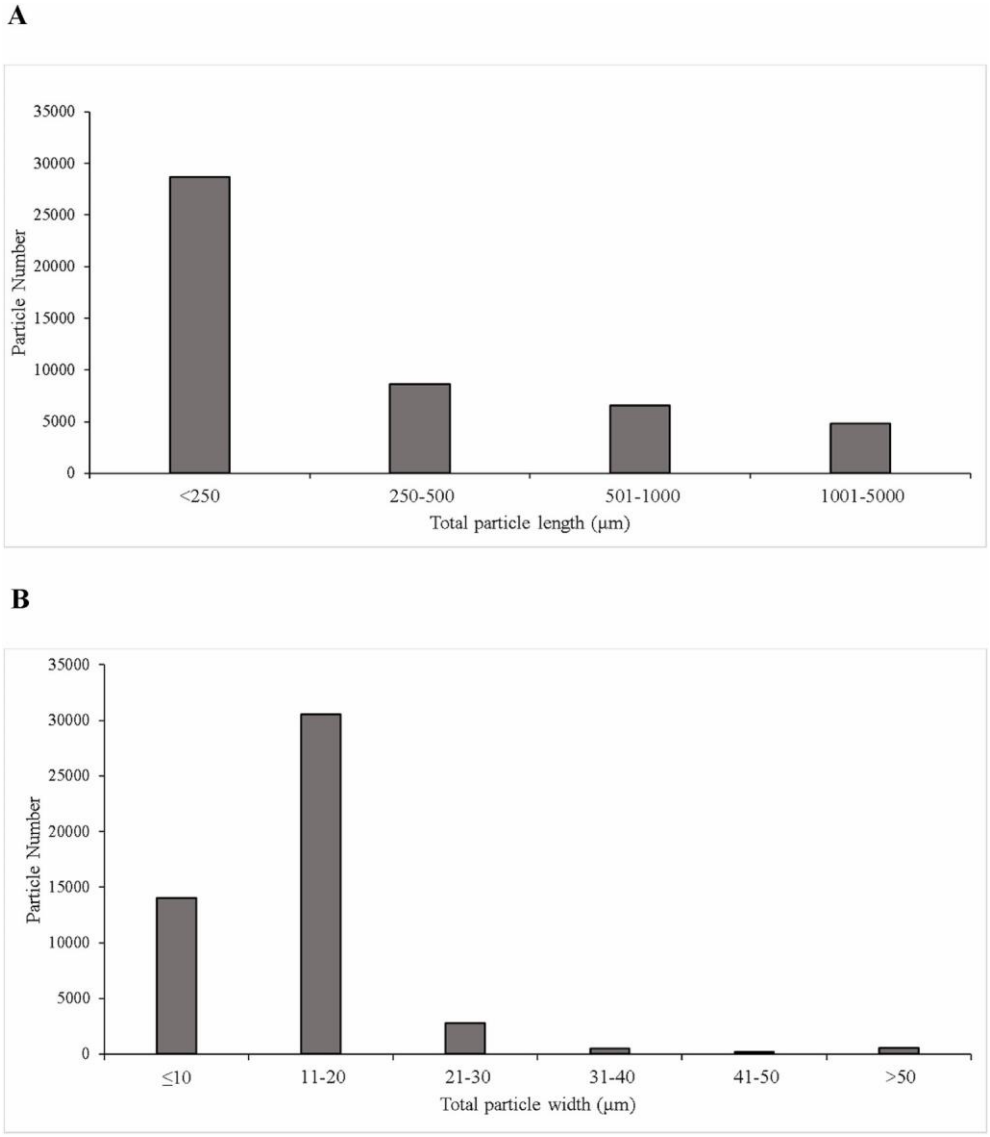
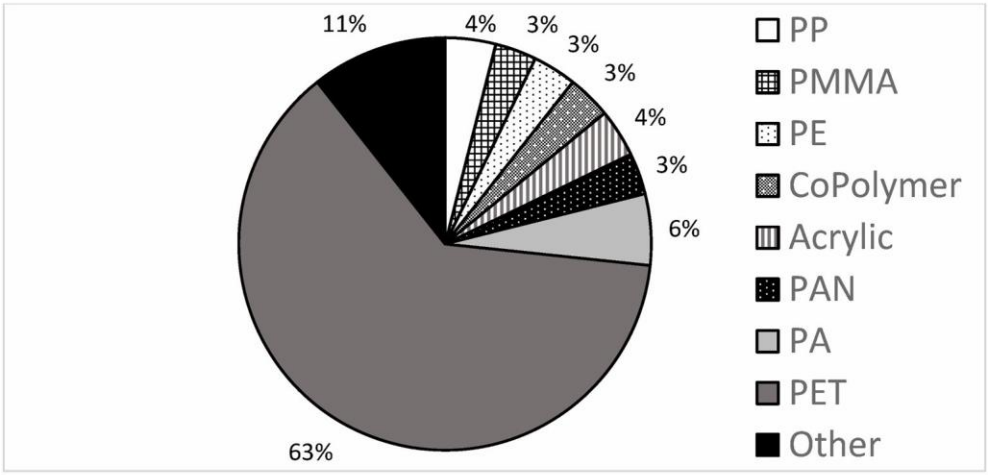


Fig. 4. Bar chart showing the particle size distributions of the entire total particle fallout. A. Particle length categories and abundance, B. Particle width categories and abundance.

The typical particle size dimensions for atmospheric MP studies observed is an increase in concentration with decreasing particle size (Fig. 4A.). Some studies report a fall in concentration before an observational size limit is reached (23, 27), as seen within width categories in this study (Fig. 4B.). This may be due to an inability to detect smaller particles, or loss of smaller particles during sample processing. Particles in the size range 5–250 µm were the most abundant throughout all particle shape categories; fibre (55%), film (88%), fragment (99%) and sphere (98%). 92% of particles had a width ≤20 µm. This is consistent in terms of length (but not width) with selected studies reporting mean particle sizes of ~50 µm (26), or 58.6 ± 55 µm for indoors and 104 ± 64.9 µm for outdoors (27) but differs from another indoor study reporting a predominant size range of 50–2000 µm (21). Particle size and shape, especially the finding of predominantly smaller fibres within households, is critical in terms of the potential health impacts discussed later.

Of the MPs identified within the households, whereby the mean value was 1414 ± 1022 MP m^{-2} day^{-1}, PET was found in 90% of samples and was 63% of the total synthetic fraction (Fig. 5A.). Other MP polymer types identified, to a lesser extent, were PA, acrylates, and PP. These findings differ significantly from previous studies of indoor MPs which report PES, PE, PA and PP as common synthetic MPs (21, 22, 26). There were more than 20 polymer types identified within this study, with PET being significantly higher in estimated concentration rate (887 ± 808 particles m^{-2} day^{-1}) compared to all other MPs (Table S3), despite PET having one of the highest densities of plastics (1.38 g/cm^3) (41). In the households studied, PET is the MP that occupants are therefore most exposed to. PET is a thermoplastic synthetic polymer used to produce drinking bottles and containers for cleaning products (42). Its transparent and flexible nature, as well as adaptability makes it a high demand material in the packaging and textiles industry (41, 43). In 2015 it was estimated that 27.8 million tons of PET were produced, globally, accounting for 8.6% of all polymer types (42). The second most detected MP in the household samples, PA, specifically synthetic nylon particles, comprised 6% of the total MP fraction, with an average 79 ± 182 particles m^{-2} day^{-1} for each household (Table S3). Compared to PET, nylon has fewer indoor applications, and is found within indoor textiles and fabrics (26). Interestingly however, Vianello et al. (2019) reported the nylon particles captured were all fragments. PP, acrylic and PAN particles were also present in household samples (4%, 4%, 3%, respectively), and are often reported in atmospheric MP studies (Table S1). These are used in packaging, textiles and reusable plastic products (18). An outdoor study in London, United Kingdom, suggested winter clothing could influence the MP polymer concentration at different times of the year, for instance PAN was higher during winter months when warmer clothes were needed (20).

A



B

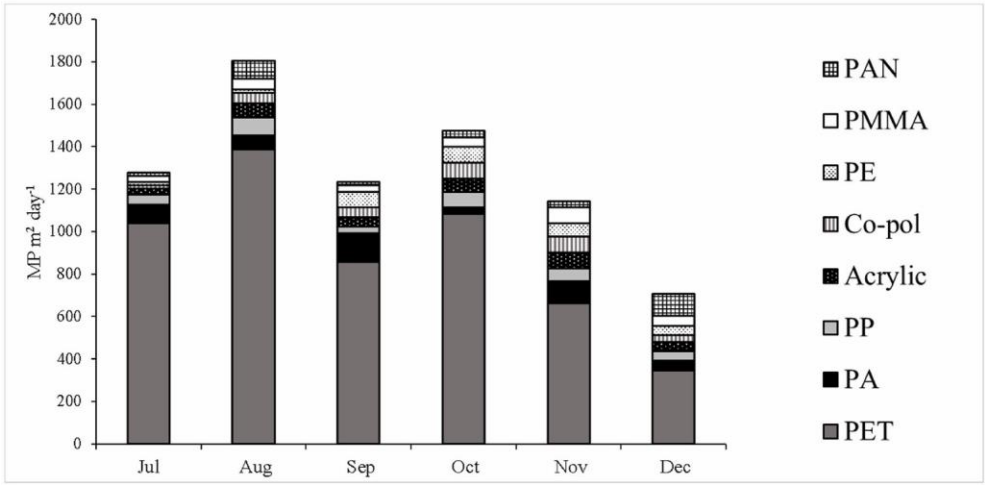


Fig. 5. A. Pie chart displaying the synthetic MP polymer types alongside their abundance, **B.** Bar chart showing the monthly concentration (mean) of each MP polymer type. **Abbreviations:** PET = polyethylene terephthalate, PA = nylon, PP = polypropylene, Co-pol = co-polymer, PE = polyethylene, PMMA = polymethacrylate, PAN = polyacrylonitrile.

In terms of seasonal differences, no variation was detected for PA, PP or the acrylic categories (p = 0.341, p = 0.749, p = 0.56, respectively). However, during December, PET was significantly lower (compared with July p = 0.005, August p = 0.000, September p = 0.004, and October p = 0.001), suggesting a reduction in PET sources and suspension during the start of the winter period. PAN was higher in mean concentration during the months of August (mean 84 ± 139 PAN particles m^{-2} day^{-1}) and December (mean 105 ± 177 PAN particles m^{-2} day^{-1}) compared with July (mean 15 ± 67 PAN particles m^{-2} day^{-1}), September (mean 15 ± 67 PAN particles m^{-2} day^{-1}), October (mean 30 ± 93 PAN particles m^{-2} day^{-1}), and November (mean 30 ± 135 PAN particles m^{-2} day^{-1}). These months are when families are traditionally on breaks from education and work. PAN being significantly higher in December supports the claim that PAN increases in winter (20) due to clothing needs. Therefore, although indoor MPs are likely not directly affected by meteorological changes, it is possible that polymers can be indirectly influenced by the outdoor compartment, changes in household occupancy, activities and clothing.

It has been suggested that through observation of MP particle characteristics, it is possible to gain knowledge of their likely origin and history (16, 36). The exact sources of MPs are not yet well understood, non-fibrous MP even less so. Fragmented particles are thought to originate from 'wear and tear' of larger plastic products, resulting in an irregular shape, such as shards from plastic bottles (36). Film shaped particles are likely from carrier bags and other thin transparent products, and spherical particles are used in personal care products (44). The most frequently mentioned probable source of atmospheric fibrous particles is their release from textiles, clothing and furnishings. Either during wear or use of a fabric item, or during the washing and drying of fibrous products, resulting in shedding (12, 16, 17, 20, 21). It is also suggested that the more a fabric item ages, the more particles it sheds, and additionally an increase in plastic fabrics within the home is likely to increase atmospheric MP concentration (21). When textiles, such as clothing and curtains, were taken from a single indoor setting, synthetic products accounted for <40% (56% cotton and 17% polyester (PES), with remaining constituents being a blend of co-polymers) (21). Further, when the study compared these textile products to those collected during sampling, natural and PES particles were prevalent polymers. If MP concentration is influenced by the number and type of plastic textiles in the home, as well as how much they are used, it is likely that high variation would be seen when comparing household MP concentrations but also when investigating a single household over a period of time, and such wide ranges were reported here; 0–5412 MP m^{-2} day^{-1}. Within an indoor environment, fabrics are common, however, other sources cannot be ruled out, such as macroplastic degradation and release of synthetic fibres from carpets (17).

After the identification of atmospheric MPs, discussion has followed on sources and factors influencing their abundance and transport. These include precipitation and meteorological events, wind speed and direction, vehicular and road emissions, human activity and population density (16-18, 20). Seasonal variance could also influence indoor polymer concentration and type, via an exchange of MPs from indoors to out, and vice versa (21, 30). Differences in building materials, furniture, cleaning routine and human activity levels may also contribute (26) making it hard to pinpoint sources. Of the recorded events within the home (derived from the participant survey), occupancy levels had no significant difference in particle fallout (p = 0.562) or MP concentration (p = 0.719) (Table S4). Building maintenance increased fallout and MP concentration in some households but not all (Table S4). Building work and maintenance has previously been reported to increase atmospheric MP concentration (21) and to be a contributor to indoor air pollution (30). Also, within the households studied, no significant difference was detected when investigating the use of regular indoor clothes hanging, tumble dryers, or presence of carpet (Table S5). The presence of pets (in 10 households reporting at least one) also had no significant impact in the total particle fallout detected (p = 0.862) or MP concentrations (p = 0.170). All households reported windows and doors

being used as the most common method for airing. Particle fallout concentrations were highest during the summer months, with a significant decline observed during winter (Fig. 2). It could therefore be suggested that ventilation increases particle suspension within the home, in the same way that wind suspends outdoor particles (18, 20). A study investigating air conditioning units and MPs also supports this phenomenon (21).

While it is unclear what the main drivers of indoor MP concentration and types are within the home, their levels are none-the-less high, especially PET, relative to outdoor environments (Table S1). Indoor air quality is a global cause for concern since humans spend the majority of their time indoors (28, 29), with the participants in this study reporting that they spend a mean of 7 h in the room sampled. The fibres detected in these households were mainly in the 5–20 μm length category, raising concerns relating to human ability to inhale particles of this size range, whether they enter lungs and what might be the health consequences. One published study highlights MP-induced human health impacts, caused by nylon fibers, in the occupational setting of nylon flock making (45). Workers displaying lung disease had been typically exposed to nylon fibres of size range 10–15 μm width and 1000 μm length at an average respirable particulate concentration of 2.2 mg m^3 (45), significantly more particles experienced in an average household environment yet consisting of the same particle size (width) range. In a controlled laboratory exposure study, human lung cell cultures have recently been exposed to nylon fibers (of approximate size shape 10 μm × 30 μm), at a level of 5000 fibers, and damage to the lung cell growth and development observed (32).

In conclusion, household environments contain significant levels of MPs, which range across homes and season from 0 to 5412 MP m^{-2} day^{-1}, with a mean of 1414 ± 1022 MP m^{-2} day^{-1} (mean ± SD). The most commonly detected MP was PET and the most abundant size dimensions detected was 5–250 μm, within the size range detected in nylon flock workers with lung disease and also found to harm lung cells in culture (32, 45). The findings herein can inform laboratory exposures using human lung cell cultures, as part of our future work to investigate environmentally-relevant levels, using the most common chemical types of MPs detected, and determine any human health impacts.

CRediT authorship contribution statement

Lauren C. Jenner: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Visualization. **Laura R. Sadofsky:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Funding acquisition. **Evangelos Danopoulos:** Formal analysis, Writing – review & editing, Visualization. **Jeanette M. Rotchell:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, 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.

Acknowledgements

The authors wish to acknowledge Maureen Twiddy for assistance with the design of the questionnaire.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atmosenv.2021.118512.

Funding sources

This research did not receive any specific grant and was is funded by a PhD scholarship in the "Human Health and Emerging Environmental Contaminants" cluster funded by the University of Hull.

References

1. Geyer R, Jambeck JR, Law KL. Production, use, and fate of all plastics ever made. Sci Adv. 2017;3(7).
2. Wright SL, Kelly FJ. Plastic and Human Health: A Micro Issue? Environ Sci Technol. 2017;51(12):6634-47.
3. Murphy M. Microplastics Expert Workshop Report EPA Office of Wetlands, Oceans and Watersheds EPA Microplastics Expert Workshop Report Executive Summary. 2017.
4. GESAMP. Science for Sustainable Oceans 2015 [Available from: www.imo.org.
5. Horton AA, Dixon SJ. Microplastics: An introduction to environmental transport processes. Wires Water. 2018;5(2).
6. Catarino AI, Macchia V, Sanderson WG, Thompson RC, Henry TB. Low levels of microplastics (MP) in wild mussels indicate that MP ingestion by humans is minimal compared to exposure via household fibres fallout during a meal. Environ Pollut. 2018;237:675-84.
7. Danopoulos E, Jenner L, Twiddy M, Rotchell JM. Microplastic contamination of salt intended for human consumption: a systematic review and meta-analysis. Sn Appl Sci. 2020;2(12).
8. Danopoulos E, Twiddy M, Rotchell JM. Microplastic contamination of drinking water: A systematic review. Plos One. 2020;15(7).
9. Gasperi J, Wright SL, Dris R, Collard F, Mandin C, Guerrouache M, et al. Microplastics in air: Are we breathing it in? Curr Opin Env Sci Hl. 2018;1:1-5.
10. Prata JC, da Costa JP, Lopes I, Duarte AC, Rocha-Santos T. Environmental exposure to microplastics: An overview on possible human health effects. Sci Total Environ. 2020;702.
11. Dris R, Gasperi J, Rocher V, Saad M, Renault N, Tassin B. Microplastic contamination in an urban area: a case study in Greater Paris. Environ Chem. 2015;12(5):592-9.
12. Abbasi S, Keshavarzi B, Moore F, Turner A, Kelly FJ, Dominguez AO, Jaafarzadeh N. Distribution and potential health impacts of microplastics and microrubbers in air and street dusts from Asaluyeh County, Iran. Environ Pollut. 2019;244:153-64.
13. Huang YM, Qing X, Wang WJ, Han G, Wang J. Mini-review on current studies of airborne microplastics: Analytical methods, occurrence, sources, fate and potential risk to human beings. Trac-Trend Anal Chem. 2020;125.
14. Hartmann NB, Hüffer T, Thompson RC, Hassellöv M, Verschoor A, Daugaard AE, et al. Are We Speaking the Same Language? Recommendations for a Definition and Categorization Framework for Plastic Debris. Environ Sci Technol. 2019;53(3):1039-47.
15. Prata JC. Airborne microplastics: Consequences to human health? Environ Pollut. 2018;234:115-26.
16. Cai LQ, Wang JD, Peng JP, Tan Z, Zhan ZW, Tan XL, Chen QQ. Characteristic of microplastics in the atmospheric fallout from Dongguan city, China: preliminary research and first evidence. Environ Sci Pollut R. 2017;24(32):24928-35.
17. Dris R, Gasperi J, Saad M, Mirande C, Tassin B. Synthetic fibers in atmospheric fallout: A source of microplastics in the environment? Mar Pollut Bull. 2016;104(1-2):290-3.
18. Allen S, Allen D, Phoenix VR, Le Roux G, Jiménez PD, Simonneau A, et al. Atmospheric transport and deposition of microplastics in a remote mountain catchment (vol 12, pg 339, 2019). Nat Geosci. 2019;12(8):679-.
19. Klein M, Fischer EK. Microplastic abundance in atmospheric deposition within the Metropolitan area of Hamburg, Germany. Sci Total Environ. 2019;685:96-103.
20. Wright SL, Levermore JM, Kelly FJ. Raman Spectral Imaging for the Detection of Inhalable Microplastics in Ambient Particulate Matter Samples. Environ Sci Technol. 2019;53(15):8947-56.

21. Zhang Q, Zhao YP, Du FN, Cai HW, Wang GH, Shi HH. Microplastic Fallout in Different Indoor Environments. Environ Sci Technol. 2020;54(11):6530-9.
22. Dris R, Gasperi J, Mirande C, Mandin C, Guerrouache M, Langlois V, Tassin B. A first overview of textile fibers, including microplastics, in indoor and outdoor environments. Environ Pollut. 2017;221:453-8.
23. Liu K, Wang XH, Fang T, Xu P, Zhu LX, Li DJ. Source and potential risk assessment of suspended atmospheric microplastics in Shanghai. Sci Total Environ. 2019;675:462-71.
24. Liu K, Wang XH, Wei N, Song ZY, Li DJ. Accurate quantification and transport estimation of suspended atmospheric microplastics in megacities: Implications for human health. Environ Int. 2019;132.
25. Li YW, Shao LY, Wang WH, Zhang MY, Feng XL, Li WJ, Zhang DZ. Airborne fiber particles: Types, size and concentration observed in Beijing. Sci Total Environ. 2020;705.
26. Vianello A, Jensen RL, Liu L, Vollertsen J. Simulating human exposure to indoor airborne microplastics using a Breathing Thermal Manikin. Sci Rep-Uk. 2019;9.
27. Gaston E, Woo M, Steele C, Sukumaran S, Anderson S. Microplastics Differ Between Indoor and Outdoor Air Masses: Insights from Multiple Microscopy Methodologies. Appl Spectrosc. 2020;74(9):1079-98.
28. Klepeis NE, Nelson WC, Ott WR, Robinson JP, Tsang AM, Switzer P, et al. The National Human Activity Pattern Survey (NHAPS): a resource for assessing exposure to environmental pollutants. J Expo Anal Env Epid. 2001;11(3):231-52.
29. Excellence) NNIfHaC. Indoor Air Quality at Home: Guideline (NG149). 2020 [Available from: https://www.nice.org.uk/guidance/ng149.
30. Health RCoPaC. Every breath we take: the lifelong impact of air pollution. Report of a Working Party. London: RCP; 2016.
31. Goodman KE, Hare JT, Khamis ZI, Hua T, Sang QXA. Exposure of Human Lung Cells to Polystyrene Microplastics Significantly Retards Cell Proliferation and Triggers Morphological Changes. Chem Res Toxicol. 2021;34(4):1069-81.
32. van Dijk F SS, van Eck GWA, Wu X, Bos IST, Boom DHA, Kooter I, Spierings DCJ, Wardnenaar R, Cole M, Salvati A, Gosens R, Melgert BN. Inhalable textile microplastic fibers impaire airway epithelial growth. BioRxiv. 2021.
33. Clark RP. Skin scales among airborne particles. J Hyg (Lond). 1974;72(1):47-51.
34. Stanton T, Johnson M, Nathanail P, MacNaughtan W, Gomes RL. Freshwater and airborne textile fibre populations are dominated by 'natural', not microplastic, fibres. Sci Total Environ. 2019;666:377-89.
35. Nuelle MT, Dekiff JH, Remy D, Fries E. A new analytical approach for monitoring microplastics in marine sediments. Environ Pollut. 2014;184:161-9.
36. Free CM, Jensen OP, Mason SA, Eriksen M, Williamson NJ, Boldgiv B. High-levels of microplastic pollution in a large, remote, mountain lake. Mar Pollut Bull. 2014;85(1):156-63.
37. Horton AA, Cross RK, Read DS, Jurgens MD, Ball HL, Svendsen C, et al. Semi-automated analysis of microplastics in complex wastewater samples. Environ Pollut. 2021;268(Pt A):115841.
38. Brander SM, Renick VC, Foley MM, Steele C, Woo M, Lusher A, et al. Sampling and Quality Assurance and Quality Control: A Guide for Scientists Investigating the Occurrence of Microplastics Across Matrices. Appl Spectrosc. 2020;74(9):1099-125.
39. Hale RC, Seeley ME, La Guardia MJ, Mai L, Zeng EY. A Global Perspective on Microplastics. J Geophys Res-Oceans. 2020;125(1).
40. Sobhani Z, Lei Y, Tang Y, Wu L, Zhang X, Naidu R, et al. Microplastics generated when opening plastic packaging. Sci Rep. 2020;10(1):4841.
41. Iniguez ME, Conesa JA, Fullana A. Microplastics in Spanish Table Salt. Sci Rep. 2017;7(1):8620.
42. Rodríguez-Hernández AG, Muñoz-Tabares JA, Aguilar-Guzmán JC, Vazquez-Duhalt R. A novel and simple method for polyethylene terephthalate (PET) nanoparticle production. Environ Sci-Nano. 2019;6(7):2031-6.

43. Hahladakis JN, Velis CA, Weber R, Iacovidou E, Purnell P. An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling. J Hazard Mater. 2018;344:179-99.
44. Leslie HA. Review of Microplastics in Cosmetics. 2014.
45. Burkhart J, Piacitelli C, Schwegler-Berry D, Jones W. Environmental study of nylon flocking process. J Toxicol Env Heal A. 1999;57(1):1-23.

3 Chapter 3: Outdoor Passively Sampled Microplastics

University of York
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Research Degree Thesis Statement of Authorship

Note that where a paper has multiple authors, the statement of authorship can focus on the key contributing/corresponding authors.

Candidate name	Lauren Jenner
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Title of the work (paper/chapter)	**Outdoor atmospheric microplastics within the Humber region (United Kingdom): Quantification and chemical characerisation of deposited particles present**	
Publication status	**Published**	✓ **02/04/2022**
	Accepted for publication	
	Submitted for publication	
	Unpublished and unsubmitted	
Citation details (if applicable)		

Description of the candidate's contribution to the work*	**The candidate (LJ as named in the paper) is listed as the first author of this paper. LJ contributed to the conceptualisation, experimental design and conduct, data analysis and writing of the manuscript.**
Approximate percentage contribution of the candidate to the work (*if possible to describe in this way*)	**85%**
Signature of the candidate	
Date (DD/MM/YY)	**21/03/2025**

Co-author contributions

By signing this Statement of Authorship, each co-author agrees that:
(i) the candidate has accurately represented their contribution to the work;
(ii) if required, permission is granted for the candidate to include the work in their thesis (note that this is separate from copyright considerations).

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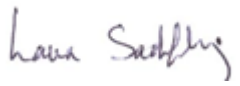
Approximate percentage contribution of the co-author to the work (*if possible to describe in this way*)	
Signature of the co-author	
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Approximate percentage contribution of the co-author to the work (*if possible to describe in this way*)	
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Approximate percentage contribution of the co-author to the work (*if possible to describe in this way*)	
Signature of the co-author	
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Copy and paste additional co-author panels as needed.

*The description of the candidate and co-authors contribution to the work may be framed in a manner appropriate to the area of research but should always include reference to key elements (e.g. for laboratory-based research this might include formulation of ideas, design of methodology, experimental work, data analysis and presentation, writing). Candidates and co-authors may find it helpful to consider the CRediT (Contributor Roles Taxonomy) approach to recognising individual author contributions.

Outdoor atmospheric microplastics within the Humber region (United Kingdom): Quantification and chemical characterisation of deposited particles present

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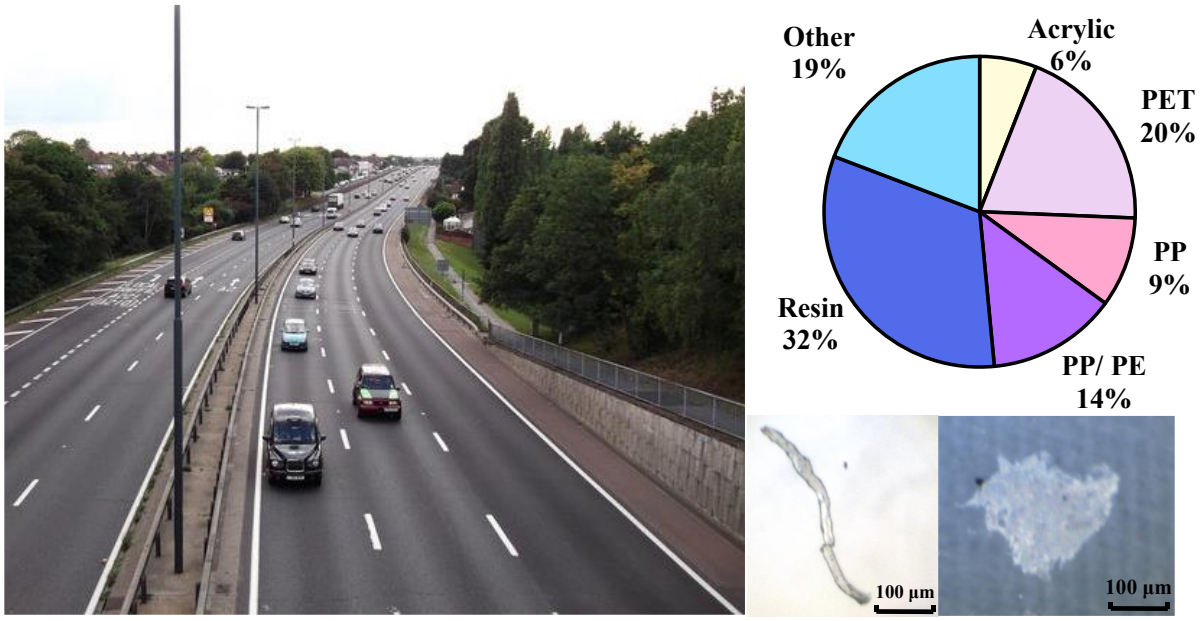
Running title: Outdoor atmospheric microplastic analysis

Graphical Abstract



Abstract

Atmospheric microplastics (MPs) have been consistently captured within air samples on a global scale. Locations with high human activity are reported to have high MP levels. An urban sampling site in the Humber region (U.K.), has been sampled over a 13-month period, providing a seasonal variation profile of MP levels, size, shape, and polymer types that humans are exposed to. Mean MP levels, measured using passive fallout into a container, were 3055±5072 MP m^{-2} day^{-1} (1164 median). An increase in levels with a decrease in MP size was observed, consisting of mainly film shaped MPs (67%) that were polyethylene (31%) and nylon (28%) polymer types. No relationship between rainfall and MP fallout levels was observed. In parallel, MPs within 5 urbanised locations relevant to human exposure were characterised over a 2-week period. An overall MP mean (and standard deviation) of 1500 ± 1279 was observed (1012 median), from which petroleum resin accounted for 32% of MP polymer type, with a higher prevalence within industrial and roadside zones. These comprised mainly fragment (52%) and film (42%) shapes and the MPs levels increased with decreasing particle size. The results provide novel information on characterising polymer levels and types, and can inform cellular toxicity studies, investigating the consequences of human MP exposure.

Keywords: **Microplastic, polymer, deposition, synthetic, outdoor, air, FTIR, urban**

1. Introduction

The intentional and unintentional release of plastic waste leads to accumulation within environmental compartments and allows for their global transport (1). Microplastics (MPs) are plastic particles, smaller than 5 mm in size (2), and can be produced through primary manufacturing or via secondary degradation of larger plastic products (1). The resulting particles have been detected within aquatic (3), terrestrial (4) and atmospheric (5) and have also been detected thousands of meters above ground level (6). Additionally, MPs have been identified within homes, offices (7), drinking water (8), salt (9) and food meant for human consumption (10). The ubiquitous nature of MPs has emphasised unavoidable human exposure, with MP inhalation being the most recent emerging cause for concern (11, 12). MPs have been observed within human lung tissue samples (13) and chemically identified in human lung cadaver samples (14), supporting inhalation as a route of exposure for MPs. It is now vital that all environments relevant to regular human exposure are investigated to gain a holistic view of the entire MP exposure likely encountered on a regular basis.

The field of atmospheric MPs is emerging, and an array of sampling and laboratory techniques have been applied to such studies. A consensus is emerging that urbanised outdoor locations display high MP levels that range from 10-712 MP m^{-2} day^{-1} (Table 1). Suburban and rural outdoor levels range from 53-132 MP m^{-2} day^{-1} (Table 1). However, there are conflicting reports regarding the types and characteristics of MPs that are most common, with fibrous MPs being the dominant shape according to some studies (15, 16), and fragmented MPs for others (17, 18). Plastic polymer types that dominate sampling locations also differ, for instance, some report polyethylene (PE) (16, 17, 19) as the predominant plastic, whilst others report polyacrylonitrile (PAN) (20), polyethylene terephthalate (PET) (15, 21, 22), polystyrene (PS) (18) and polyester (PES) (23). Such investigations highlight the need to establish the levels, plastic types, sizes, and shapes that are present within the air.

Table 1: Summary of studies reporting outdoor atmospheric microplastic deposited samples. Abbreviations: EVA, ethylene vinyl acetate; PA, polyamide (nylon); PAN, polyacrylonitrile; PE, polyethylene; PES, polyester; PET, polyethylene terephthalate; PP, polypropylene; PP/PE, polypropylene-polyethylene co-polymer; PS, polystyrene; PVC, polyvinylchloride; resin, including alkyd, hydrocarbon and phenoxy resin.

Reference	**Sample location (no. sampling sites)**	**Total sampling duration**	**Dominant MP types (total present)**	**Average MP m^{-2} day^{-1} (range)**
[20]	Urban (1)	1 month	PAN, PET, PA (15)	712 ± 162 mean ± SD (575-1008)
[17]	Urban (3)	3 months	PE, EVA (4)	261 median 247 median 137 median
	Rural (3)			331 median 512 median 343 median 275 overall median (137-512)
[16]	Urban (3)	3 months	PE, PP, PS (3)	36 ± 7 mean ± SD (175-313)
[15]	Urban (1)	13 months	PET, co-polymers, PA (3)	110 ± 96 mean ± SD (2-355)
	Suburban (1)	5 months		53 ± 38 mean ± SD
[18]	Rural, remote (1)	5 months	PS, PE, PP (>5)	365 ± 69 mean ± SD
[23]	Urban (1)	9 months	PES, PP, PE, PVC (6)	10 ± 8 mean ± SD (0-30)
[22]	Urban (1)	12 months	PET, PAN, PP, PA, resin (>8)	114 ± 40 mean ± SD
Present study	Urban (1)	13 months	PE, nylon, PET (25)	3055 ± 5072 mean ± SD (1164 median)
Present study	Urban (5)	2 weeks	resin, PET, PP, PP/PE	1500 ± 1279 mean ± SD (1014 median)

This study determines the temporal variation in levels and types of MPs evident at one urban sampling site over a 13-month sampling period (including the Covid-19 lockdown), within the city of Kingston upon Hull, U.K. In parallel, the MP profile over a 2-week time span for five sites; residential, city centre, industrial, commercial roadside, and a location in which all are relevant, has also been conducted. Having representative outdoor airborne MP levels and characteristic data from within a city environment can inform realistic human cellular toxicity studies, to investigate the consequences of MP inhalation with respect to MP levels, polymer type, shape, and size ranges.

2. Material and Methods

2.1. Passive Sampling

Using guidance from the Hull City Council (HCC) air quality monitoring department, five sampling locations were selected, representing different zones that humans are commonly exposed to (Fig. 1). Site 1 ('A63') is located along a stretch of road (the A63), with heavy traffic flow in which blocks of residential flats, shops, offices, the city centre, and industrial units are all in close vicinity. Site 2 ('roadside commercial') is situated on a city centre road that has a heavy flow of traffic, as well as numerous commercial outlets and a Police Station. Site 3 ('industrial') is in an industrial zone in which nearby units produce furniture, paper, and paint products. Site 4 ('city centre') is in the centre of Kingston Upon Hull, important for transport, commercial use, and entertainment. Site 5 ('residential') represents a residential zone, including housing and student accommodation as well as nearby playing fields.

Continuous passive sampling was conducted from October 2019 until October 2020, which included a period of National lockdown, affecting the sampling months of April and May (2020). Five rain samplers (Palmex Ltd, Zagreb, Croatia) were secured at head height, considering the placement of the inlet at the top of the sampling device. In practice this was approximately 1.8 m (1.5-2.0 m) from the ground with the aid of Council street fixtures such as the tops of fences. Each location was individually assessed to avoid wind, shadow from buildings or vegetation, and minimise air movement disturbance. Ultimately the samplers were placed in locations that best represented the zone type, but also in locations that avoided theft and damage to the samplers. The surface area (0.014 m^2) of the sampler was calculated using the diameter of the opening funnel (0.135 m). The stainless-steel housing exterior of the sampler supported an opening funnel in which deposited environmental particles, and precipitation, could enter, travel through an intake tube and deposit within a 3 L polyvinylchloride (PVC) bottle, ensuring no evaporation. A metal mesh grid (pore size 3 mm) placed inside the funnel opening prevented large particles and objects from blocking the sampler. After each 14-day sampling period (each month), the PVC bottle was unscrewed from the sampler and sealed

tightly with a PVC lid. The inside and sides of the rain sampler were then thoroughly rinsed with MilliQ water before another identical PVC bottle was screwed into the sampler for the subsequent sampling period.

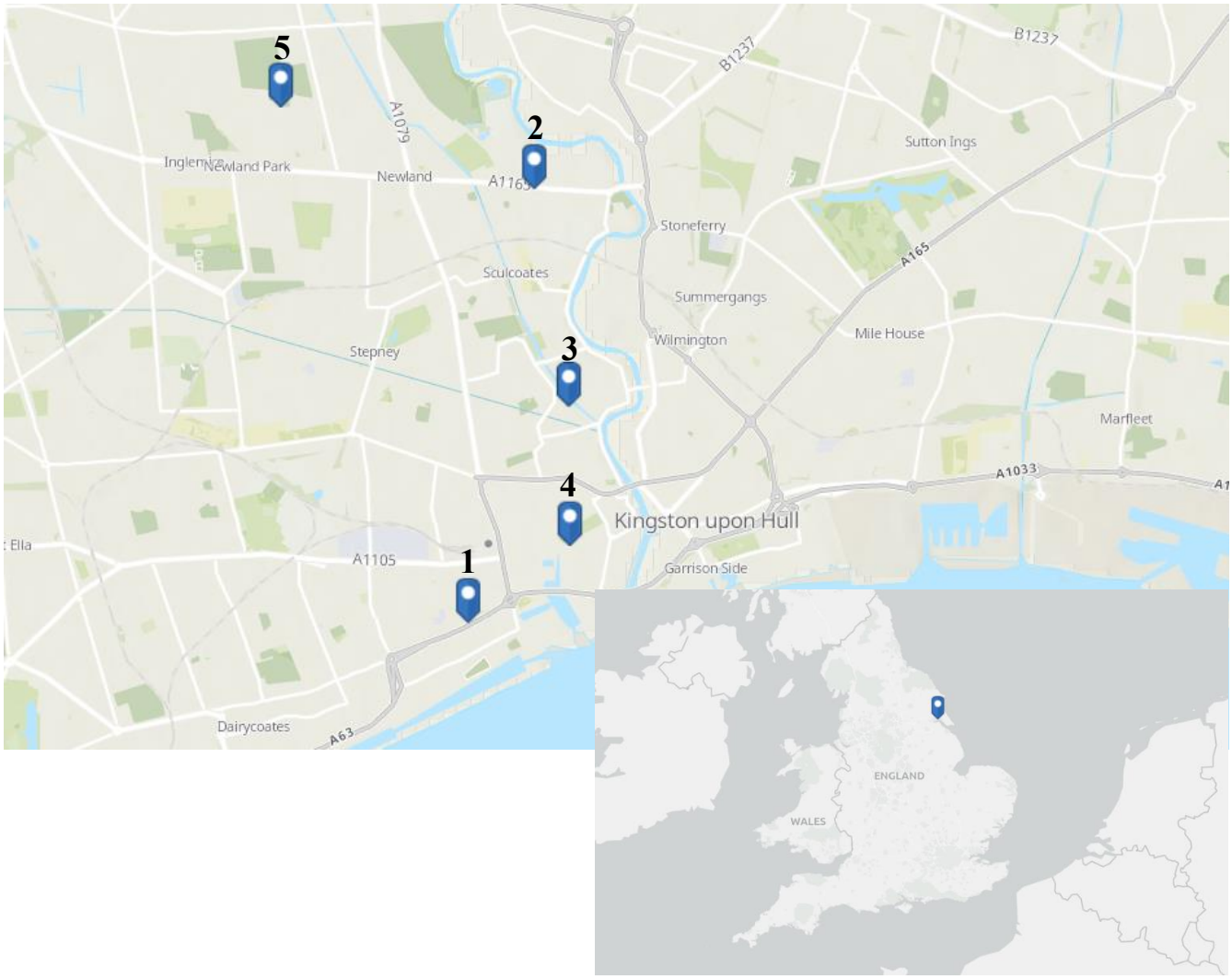


Figure 1. Map of the 5 sampling sites chosen for atmospheric deposition sampling of MPs within Kingston Upon Hull. A63 (Site 1), roadside commercial (Site 2), industrial (Site 3), city centre (Site 4), and university district (Site 5).

Rainfall data was obtained by measuring the amount of rainfall (to the nearest 10 mL) within the PVC containers at the end of the 2-week sampling period. The sampler design ensured that no evaporation occurred, allowing rainfall data to be collected on a site-specific level throughout the city, rather than acquiring regional online data. The sampling period included a national lockdown that occurred between the months of April and May 2020, during which only key workers were permitted to travel and a significant proportion of the local population worked from home. Phasing out of the national lockdown occurred from May, in which people were allowed to return to work,

schools started to reopen (June 2020) and non-essential shops and venues re-opened (June-July). Hull entered a further Tier 2 local lockdown during the month of October (2020); however, sampling had finished.

2.2. Sample Preparation

Site 1 samples were analysed for the duration of the study (13 months, n=13, 14/10/2019 – 26/10/2020). Additionally, one sample from each of the 5 sampling locations was analysed, representing the same 14-day sampling period (17/02/2020 – 02/03/2020, prior to the National Lockdown period). Many of the remaining samples were used during pilot studies in which method optimisation was achieved. The volume of rainfall (to the nearest 10 mL) within each PVC bottle was recorded. Each sample was decanted, and vacuum filtered onto mixed cellulose ester membrane filters (MCE), 47 mm diameter and 5µm pore size (MERCK, Gillingham, U.K.). Filters were dried and stored in sealed petri dishes, in the dark at room temperature.

Samples were digested in three bulks (bulk 1 and 2 comprised Site 1 longitudinal samples, bulk 3 comprised the Site 2-5 samples). For each sample, one quarter of the sample filter was randomly selected for analysis. The quarter was cut and placed into a pre-cleaned 1 L conical flask, and pre-filtered 200 mL of 30% hydrogen peroxide (H_2O_2) added. For procedural blanks (total=4, n=2 for bulk 1 and 2, n=2 for bulk 3), that underwent every stage of laboratory sample preparation, a clean MCE filter was cut, and the quarter added to a 1 L conical flask, along with 200 mL of H_2O_2. Conical flasks were sealed with aluminium tin foil, labelled, and placed in a shaking incubator for 10 days (55°C, 65rpm (24)). Samples were vacuum filtered onto aluminium oxide (Anodisc) filters, 47 mm diameter and 0.2 µm pore size (MERCK, Gillingham, U.K.). Sample Anodisc filters were placed in sealed petri dishes, allowed to dry, and stored in the dark at room temperature.

2.3. µFTIR Analysis

Each sample filter was placed directly onto the µFTIR platform for particle characterisation. One half of each sample was randomly sectioned and analysed, except for two samples in which heavy particle load dictated that one quarter of the filter undergo analysis. Extrapolation of the data was later conducted. The length (largest side) and width (second largest side) was recorded for every particle above 5 µm. Particles with a length larger than 300 µm were recorded as “>300µm” due to the selection tool size limit and field of view. Particles were sorted into shape categories: fibre, film,

fragment, foam, and sphere (24, 25). Particles with a length to width ratio >3 were categorised as fibres (26). For every particle characterised, chemical composition analysis was also conducted to identify the polymer type. This led to a total of 9983 particles undergoing analysis including the temporal and spatial variation studies, of which, 3275 (representing 33% of the total) particles were identifiable (>70% match). μFTIR analysis was conducted in transmission mode (Nicolet iN10, ThermoFisher, Waltham MA, U.S.A.), equipped with a liquid nitrogen cooling system. The cooled MCT detector allows for analysis of particles accurately to 3 μm in size, although the filter pore size cut off used was 5 μm. A background reference spectrum was taken before immediate analysis of each sample particle. A scan number of 64 and a spectral range of 4000-1250 cm^{-1} was applied. No observational criteria (3) was applied to select suspected MPs for analysis. Instead, all particles >5 μm were included in analysis. A library match index (Omnic Picta, Omnic Polymer Libraries) of ≥70% was chosen as a quality threshold and particles below were not included in the results presented.

2.4. Quality Control and Assurance

Strict quality control measures were employed to monitor background MP contamination levels. Field blanks (n=5) monitored contamination due to the opening of the PVC bottle during bottle replacement at each of the sampling sites. Procedural blanks (n=4) quantified any contamination during the sample preparation stages of laboratory processing, in which procedural blanks mimicked the digestion and filtration steps. Laboratory blanks (n=17) quantified any contamination during μFTIR analysis, in which an Anodisc filter was opened for the same period as each sample undergoing analysis. Therefore, contamination was monitored from every possible environment that each sample was exposed to (blank results are detailed in Supplementary Table S1). Field, procedural, and lab blank results for each sample were combined (as the 'blank correction') and used in later adjustments to account for background contamination (Supplemental Information Methods 1).

Strict quality assurance measures were also used to restrict background MP contamination. All equipment and glassware used were first washed by hand before a dishwasher cycle using distilled water and finally a triple rinse using MilliQ water. The MilliQ water and H_2O_2 used during digestion were triple filtered using glass fibre (GF6) filters (GE Healthcare Life Sciences, Marlborough MA, U.S.A.). All reagents and equipment were always covered with aluminium tin foil lids and a small opening made when pouring. During pouring and filtering of samples, triple rinsing of containers with MilliQ water was conducted to avoid sample particle loss. A fume cupboard was used during most stages of the laboratory processing, with the power off to minimised air flow. Other than the PVC sampler (considered during field blanks), safety goggles and nitrile gloves, plastic equipment

was avoided by using an all-glass vacuum filtration kit, glass petri dishes, a cotton laboratory coat and glass or metal laboratory equipment. Laboratory work was conducted at times of low activity and μFTIR analysis conducted in a single-person room. Windows and doors were closed with no other ventilation. Three random new Anodisc filters were chosen for μFTIR analysis, in which no particles were identified to rule these out as a source of contamination. All fieldwork sampling and sample-preparations were conducted by the primary researcher. The μFTIR analyses was conducted 'blind', by labelling samples anonymously and random allocation to one of 4 researchers.

2.5. Statistical Analysis

It was assumed that an even particle distribution occurred during the filtration process, and that the analysed portion of the filter represented the whole filter, after extrapolation (multiplied by 8 for most samples, or by 16 for the two samples with a heavy particle load). To convert the number of MPs per filter into meter squared, rather that surface area of the sampling device opening area, a correction factor (1/0.014 m^2) was used. A final division of 14 was applied to represent one day:

$$\text{MP m}^{-2}\ \text{day}^{-1} = (\text{MP on whole filter} * 71.43)/14$$

Results were adjusted using a limit of detection (LOD) and limit of quantification (LOQ) approach (24, 27) (Supplementary Information Method 1). The background contamination detected during the field, procedural and laboratory blanks were combined to give an overall value of likely contamination for each polymer type. Only MPs detected above the LOD/LOQ were included within the final levels to provide the highest quality threshold available within atmospheric MP literature.

Data were determined not normally distributed, using SPSS with a Shapiro-Wilk test. To test for a relationship between rainfall and particle fallout, as well as the rainfall and MP levels, a Spearman's Correlation test was applied. A significance of $p=0.05$ was applied and extreme significance of $p=0.005$.

3. Results

3.1. Temporal variation in the atmospheric deposition of MP levels and types at sampling site 1 ('A63')

MPs were identified within all deposited samples from site 1 (A63) (n=13) (Fig. 2). The average deposition rate of all particles was 11278 ± 15025 particles m^{-2} day^{-1} (ranging from 1429-57471) (5551 median). After LOD/ LOQ adjustments, the average deposition rate of MPs was 3055 ± 5072 MP m^{-2} day^{-1} (79-18996) (1164 median) (Table 2) (Supplementary Fig. S1). A correlation relationship

between the rainfall (Fig. 2) collected within the sampler and the atmospheric fallout captured was investigated using a Spearman's Correlation test, with no significance. Additionally, a Spearman's Correlation relationship between rainfall and MP deposition was investigated, again with no significance difference detected.

Table 2: MP levels detected at Site 1, and from all 5 sampling sites, before and after LOD and LOQ adjustments

Site 1 sampling month	**MP m^{-2} day^{-1} (no adjustments)**	**MP m^{-2} day^{-1} (LOD LOQ adjusted)**
October 2019	1225	1164
November	408	316
December	367	308
January	1469	1446
February	694	603
March	857	799
April	3184	3086
May	5551	5494
June	4857	4715
July	19266	18996
August	1061	917
September	82	79
October 2020	2612	2597
Mean ± SD (median)	3203 ± 4926 (1225 median)	3055 ± 5072 (1164 median)
February sampling site	**MP m^{-2} day^{-1} (no adjustments)**	**MP m^{-2} day^{-1} (LOD LOQ adjusted)**
Site 1 (A63)	694	603
Site 2 (Roadside)	3633	3617
Site 3 (Industrial)	1755	1746
Site 4 (City Centre)	1020	1012
Site 5 (Residential)	531	522
Mean ± SD (median)	1527 ± 1268 (1012 median)	1500 ± 1279 (1012 median)

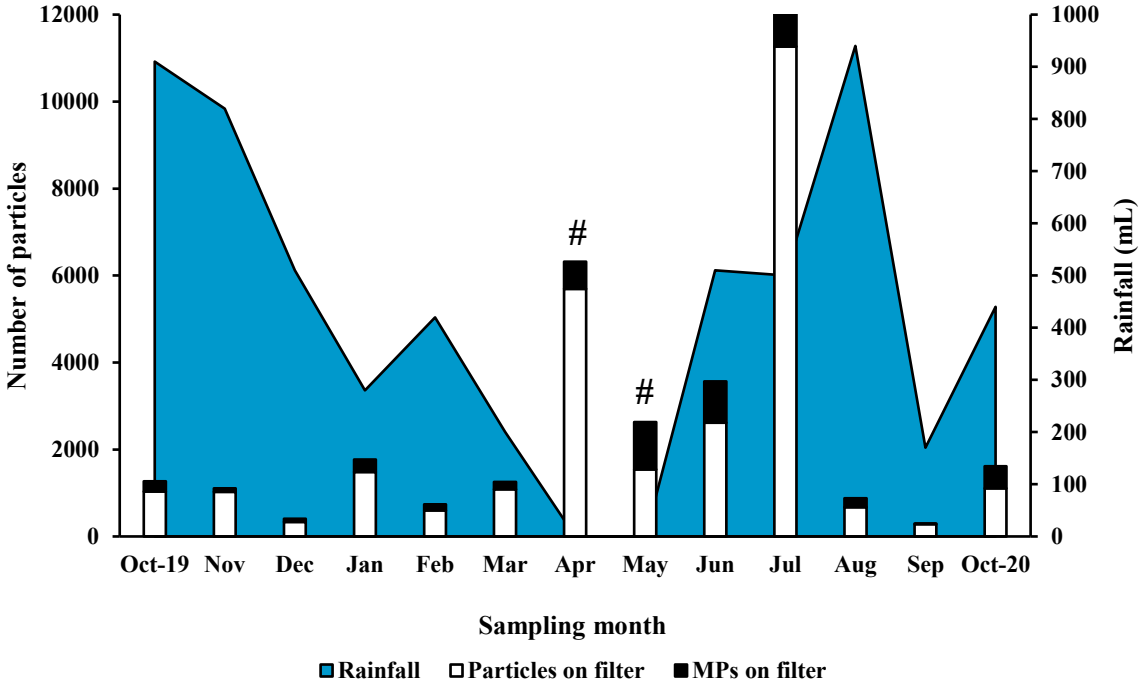


Figure 2. Total number of particles and MPs identified, within the 2-week sampling period, for sampling Site 1 (A63). The total rainfall during each 2-week sampling period is presented alongside. # Symbol indicates periods in which an official Covid-19 lockdown was issued.

With respect to MP shape, film shaped were the most abundant (67%), followed by fragment (24%) and fibrous (9%) (Fig. 3A). Film shaped MPs had a mean level of 2151 ± 3880 MP m^{-2} day^{-1} (82-14368) and were identified within every sample from Site 1. Fragmented MPs had a mean MP of 763 ± 997 MP m^{-2} day^{-1} and were identified in all but one sample. Fibrous MPs had a mean MP level of 273 ± 487 MP m^{-2} day^{-1} and were identified in all but one sample.

With respect to MP size ranges, an increase in MP levels was associated with decreasing MP length (Fig 3B). The most prevalent length category was 21-30 µm (20%), and most MPs (52 %) were <50 µm in length (Fig 3B). While MPs with a length >300 µm were present in all shape categories, 67% of these largest particles were fibrous (long but thin). An increase in MP levels with decreasing width was observed at site 1 (A63) (Fig 3C). The most prevalent width categories were 11-20 µm (24%) and 21-30 µm (26%). 67% of MPs had a width of <40 µm. The smallest MP length recorded in this study was an 8 µm long PE fragment. The smallest MP width recorded was a PE fibre with a 5 µm width.

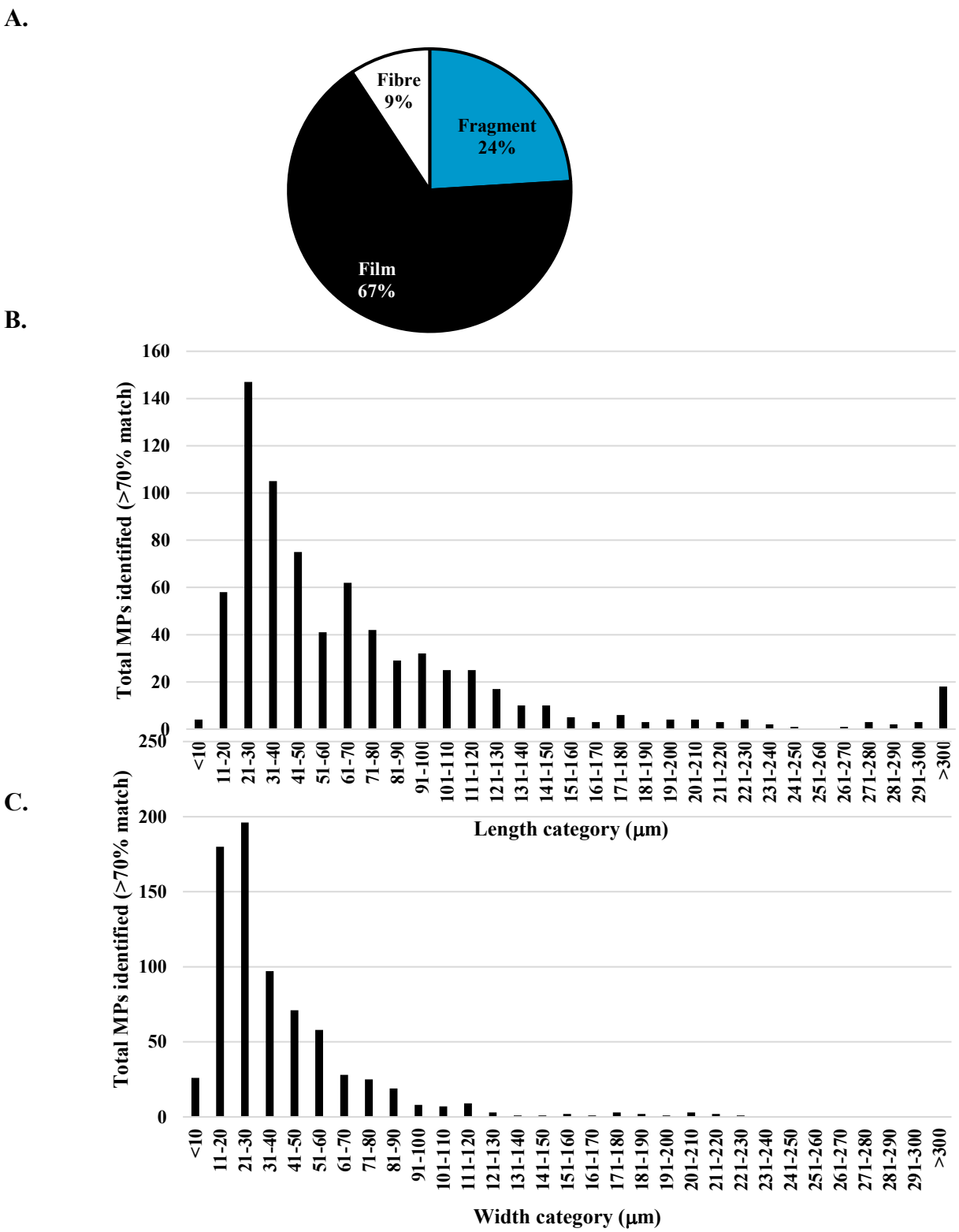


Figure 3. MP characteristics observed in the atmospheric samples obtained from sampling site 1 (A63): A) shape, B) length, and C), width.

The overall MP polymer composition of samples detected across the 13-month study at Site 1 (A63) samples was predominantly PE (31%) and nylon (28%), with 25 different polymer types identified (Fig. 4). Overall, MPs accounted for 26% of the particles that were identifiable. Film shaped MPs were predominantly PE (42%) and nylon (35%). Fragmented MPs displayed a more varied MP polymer type; with PET (15%), PP/ PE (11%), PE (10%), nylon (9%), PTFE (9%), and resin (9%) the most abundant. Fibrous MPs were predominantly nylon (32%), PE (16%), PET (9%) and acrylic (9%).

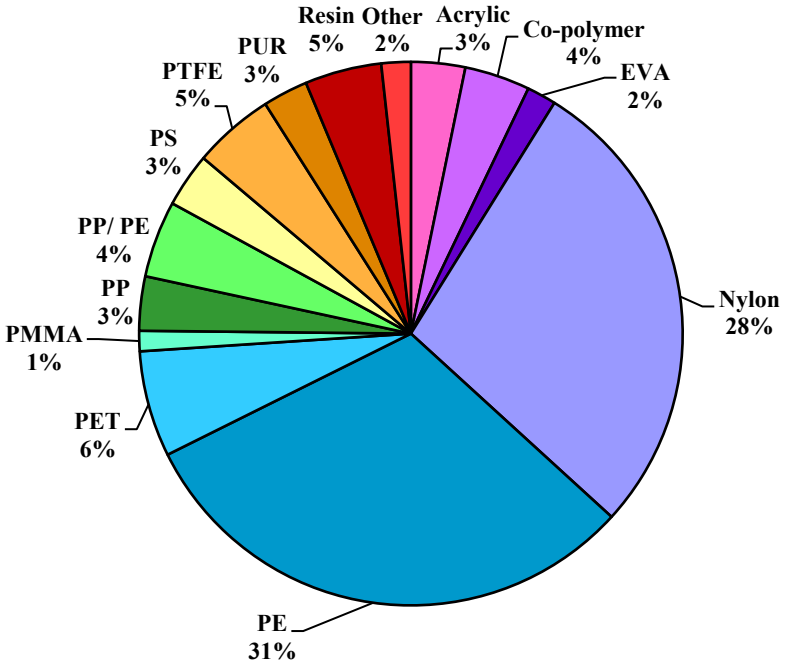


Figure 4. MP polymer types detected at sampling site 1 (A63) throughout the 13-month sampling period. Abbreviations: EVA, ethylene vinyl acetate; PE, polyethylene; PET, polyethylene terephthalate; PMMA, polymethylmethacrylate; PP, polypropylene; PP/PE, polypropylene-polyethylene co-polymer; PS, polystyrene; PTFE, polytetrafluoroethylene; PUR, polyurethane; resin, including alkyd, hydrocarbon and phenoxy resin.

3.2. Spatial variation in atmospheric deposition of MP levels and types across all sampling sites (1-5)

MPs were identified in samples from all 5 sampling sites. After LOD/ LOQ adjustments, the total MP levels for each site were: Site 1 (A63) 603 MP m^{-2} day^{-1}; Site 2 (roadside commercial) 3617 MP m^{-2} day^{-1}; Site 3 (industrial) 1746 MP m^{-2} day^{-1}; Site 4 (city centre) 1012 MP m^{-2} day^{-1}; and Site 5 (residential) 522 MP m^{-2} day^{-1}. The average MP levels detected during the February 2-week period, across the 5 sampling sites, was 1500 ± 1279 (1012 median) (Table 2) (Fig. 5).

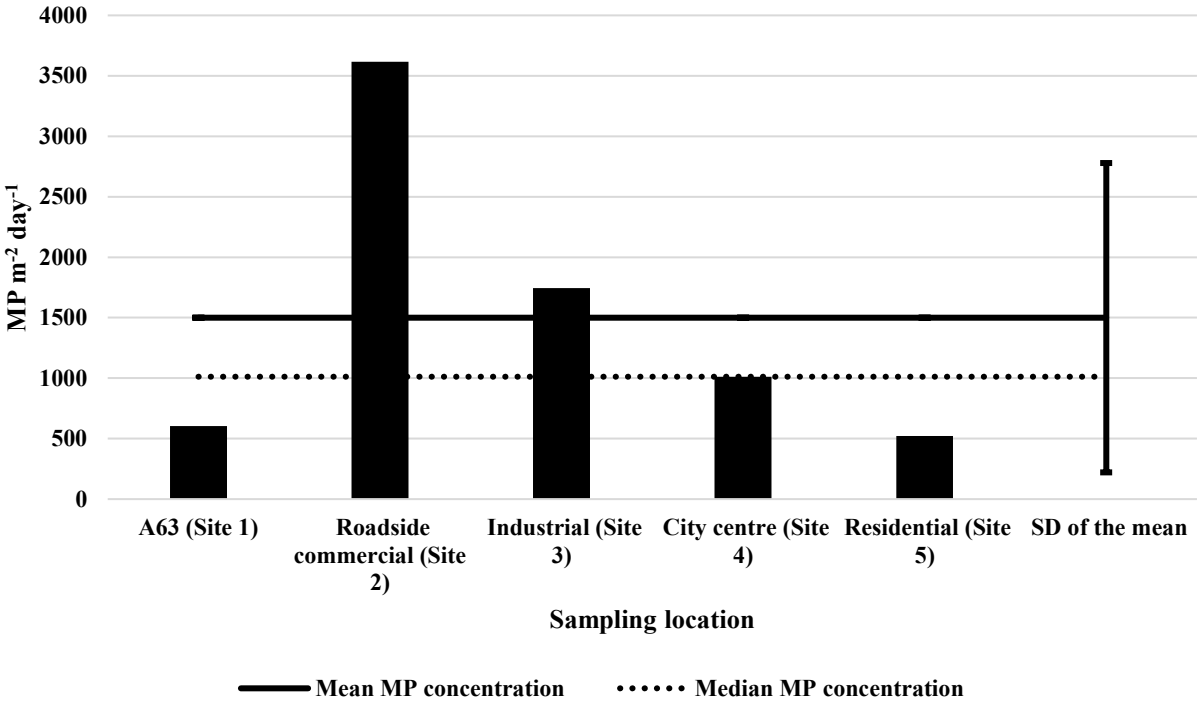


Figure 5. MP levels from the sampling month of February 2020, across all 5 sampling sites (after LOD LOQ adjustments). The solid line represents the mean MP level (MP m^{-2} day^{-1}) from the 5 sampling sites, and standard deviation of the mean included. The dotted line represents the median MP level (MP m^{-2} day^{-1}) from the 5 sampling sites.

From the 5 sampling sites, the most predominant MP shape was fragment (52%), followed by film (42%) and fibre (6%) (Supplementary Fig. S2A). With respect to MP size ranges, an increase in MP levels was associated with a decrease in particle length as well as width (Supplementary Fig. S2B and S2C). The most predominant length categories were 31-40 (14%), 41-50 (16%) and 51-60 (11%). The most prevalent MP width categories were 11-20 (17%), 21-30 (29%) and 31-40 (17%). Multiple and varied MP polymer types were identified from across each of the 5 sampling sites (Fig. 6). Overall, resin comprised 32% of MPs, specifically, hydrocarbon resin, phenoxy resin and alkyd resin

were most prevalent. PET and PP were also prevalent (20% and 9% respectively). PE was not a prevalent polymer type within the February sampling period at any of the 5 sites (Fig. 6). Fibres were detected in a higher abundance in Site 1 (A63) and Site 5 (residential) (39%, 38%, respectively), as opposed to 0% at Site 2 (roadside commercial), 20% at Site 3 (industrial) and 17% at Site 4 (city centre).

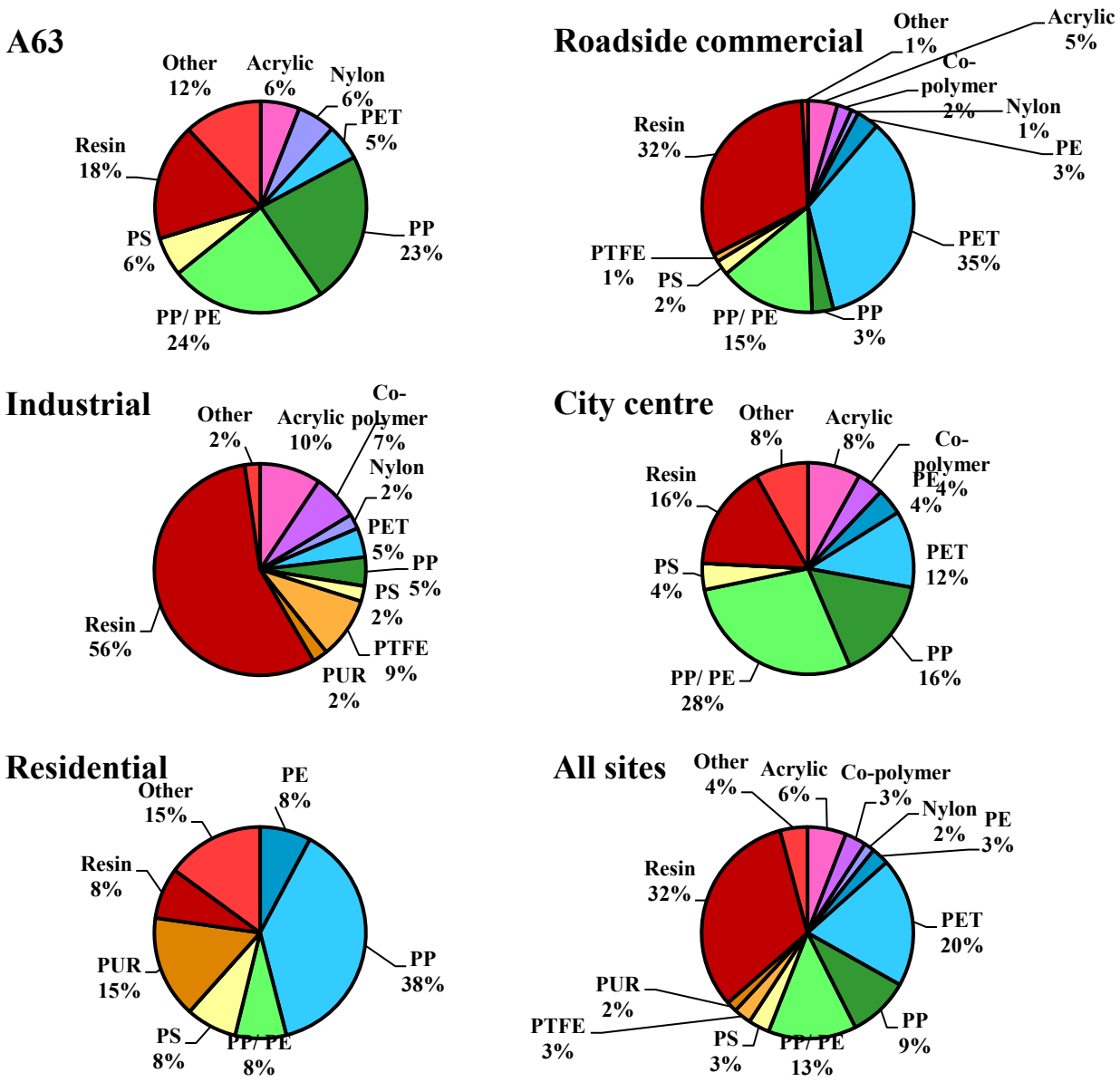


Figure 6. The proportion of MP polymer types within each sample at every site, during a single 2-week sampling period. A63 (Site 1), roadside commercial (Site 2), industrial (Site 3), city centre (Site 4), and residential (Site 5), and the overall proportion across “all sites” combined. Abbreviations: EVA, ethylene vinyl acetate; PE, polyethylene; PET, polyethylene terephthalate; PMMA, polymethylmethacrylate; PP, polypropylene; PP/PE, polypropylene-polyethylene co-polymer; PS, polystyrene; PTFE, polytetrafluoroethylene; PUR, polyurethane; resin, including alkyd, hydrocarbon, and phenoxy resin.

4. Discussion

This 13-month study conducted at sampling site 1, alongside a major trunk road, the A63, evidenced a mean levels of 3055 ± 5072 MP m^{-2} day^{-1} (79-18996 range, 1164 median) and, as such, represents the highest mean level of 'bulk' outdoor atmospheric deposited MPs reported, to date, in the literature (Table 1). Other outdoor atmospheric MP deposition investigations report average levels ranging from 10-712 MP m^{-2} day^{-1} (Table 1). A suite of quality control and quality assurance measures were employed herein, quantifying background contamination, and using combined μFTIR validation to add weight to these findings. In contrast to the current literature, a high abundance of film shaped MPs, specifically PE and nylon MP types, has been observed.

MPs were identified within all sampling site 1 (A63) samples collected during the 13-month longitudinal study, highlighting that this lower atmospheric environment within Kingston Upon Hull, U.K., is contaminated with high levels of MPs. The site represents a zone in which many forms of human activity take place, from residential, commercial, and office-based work to industrial and heavy vehicular traffic flow. The abundant thin, transparent, and flimsy 'film' shaped particles (25) made up 67% of the MPs identified. No other studies report an abundance of this shape category, with others reporting fibrous (15, 16) and fragmented (17, 18) as the predominant shapes. The film shaped MPs identified within this study alone had a greater mean level, 2151 ± 3880 MP m^{-2} day^{-1} (82-14368 range), than the total MP levels reported within similar studies (Table 1).

PE was the most abundant MP polymer type detected, similarly to other atmospheric studies (16-18, 23). However, another U.K. study comprising an urban London sampling site reported a high abundance of PAN and low PE (20). Wright *et al.* (2020) sampled over a one-month period and reported high levels of PAN fibres (712 ± 162 MP m^{-2} day^{-1}). In contrast, herein, only one PAN particle was identified throughout the 13-month sampling period. Bordering the U.K., an Irish study conducted outdoors reported predominantly PET fibres of 100 MP fibres m^{-2} day^{-1} (21). Work by the current authors conducted across 20 indoor home settings detected PET fibres as most common, followed by PP then nylon, with a total MP level of 1414 ± 1022 MP m^{-2} day^{-1} (24). This suggests that local sources and different human activities within different areas affect MP types and levels. Additionally, the outdoor levels measured within this study highlight the possibility of outdoor 'hotspots' having similar, or exceeding, MP levels compared to an indoor environment, where levels are reported as typically being the highest (7, 24, 28). PE comprised 42% of film shaped particles within this study, suggesting likely sources are degraded containers, packaging, and carrier bags with a thin, 'film like', structure (25, 29). Nylon, a MP polymer which has already been highlighted as hazardous for inhalation at industrial levels of exposure (12), was also observed in high abundance in this study, and represented 35% of film shaped particles or 28% of the total MP count. Similarly,

to other atmospheric MP studies (Table 1), no foam or spherical MPs were identified. Also of note, the quantity of MP polymer types (n=25) detected herein was exceptionally high when compared with similar investigations (Table 1). However, the 13-month sampling period, as well as the high number of particles taken to chemical composition analysis (n=8481 for Site 1, n=9983 total), could be responsible for the higher levels reported. In many atmospheric MP studies, it is common practice to apply observational criteria (3), chemical analysis on a relatively small subset of particles (6, 15, 23), or chemical analysis on a small subset of specific particle shapes (16, 20) before extrapolation, which may skew conclusions reached regarding the main polymer types.

Still focussing on the dataset from the 13-month single site (A63) analysis, the particle size trend, which showed an increase in levels of the smaller particles is commonly reported within atmospheric MP literature (17, 18), as well as a decrease just before the method detection limit is reached (15, 16) (Fig. 3B, Fig. 3C). Weathered particles may cause an inability to gain a >70% polymeric match rate, there could also be difficulty observing the smallest of particles due to heavy particle load and equipment constraints that lead to a situation where the smallest of size ranges cannot be analysed (15, 16). With MP lengths as small as 8 μm and widths as small as the method detection limit (5 μm), inhalable sized particles were captured within samples. This highlights the importance of developing analytical methodologies and techniques that include respirable and nano-plastic size ranges, which will aid the understanding of what MPs are likely to be inhaled daily.

In terms of spatial variation in MP levels, the roadside commercial sampling Site 2 had the highest MP levels compared to the other sites (Fig. 5) for the 2-week snapshot of sampling across all the sites. While Site 2 (commercial roadside) was in a zone with high human activity in terms of traffic and footfall, it was hypothesised that Site 1 (A63) representing a trunk road, with relatively heavier traffic flow, would have the highest MP levels, yet it did not. Site 5 (residential) had the lowest MP levels within the single sampling period. This residential zone is considered the lowest in human activity, with playing fields and a distance away from commercial and industrial zones, allowing for a greater air dilution. It is, however, difficult to draw conclusions based on this single set of results, illustrating the importance of conducting long-term sampling analysis.

The sizes of MPs within the snapshot 2-week sampling period followed the same trend as the 13-month investigation and other similar studies within literature (17, 18); there was an increase in MP levels with decrease in both particle length and width. Compared to Site 1, there were a higher proportion of fragment particles from the 5 combined sites. However, both fragment (52%) and film (42%) particles dominated the 5-site investigation, like Site 1 results. Whilst it is common to identify an abundance of fragmented MPs within outdoor atmospheric samples (17, 18), it is not common to identify film shapes MPs in such high abundance.

The predominant MP polymer type detected during the spatial, 2-week sampling period, across all sites, differed compared with the 13-month single site analysis. In contrast to the PE polymer detected at Site 1 overall, resin was predominant during the 2-week snapshot sampling. Resin is used in rubber tyres and road markings, paints, and industrial use (20). Almost half of the resin particles detected within the 5-site investigation were reported as fragment in shape, explaining why the investigation had a higher prevalence of fragmented MPs compared to the Site 1 investigation. It is not surprising to see lower levels at a residential site (Site 5), and higher levels in zones where traffic and manufacturing are more common (Fig. 6). The lack of PE MPs from all 5 sampling sites highlights the importance of sampling numerous months, as fluctuations in polymer types is clear. The presence of fibres within samples can be considered an indicator of urbanisation, high human activity (6) or proximity to residential areas in which fibres can dominate indoor samples (24). Results show that in Site 1, of highest human activity (A63), and Site 5, of a residential zone, fibrous MPs were highest. Other sites in which non-fibrous particles were more prevalent suggest a non-textile source of MPs and/or degradation of MPs into smaller fragmented shapes. Interestingly, PET MPs, known to be extremely abundant within indoor samples (24), were not abundant within the residential Site 5 samples.

Since the aim of this study was not to focus on transport and sources of MPs, only rainfall was investigated as a potential significant meteorological event. In this study, no relationship, using a Spearman's Correlation test, between MP levels and rainfall was observed (Fig. 2). A more in-depth investigation into other meteorological events is needed before the effects of such weather conditions can be determined. Many such factors will influence MP transport and it is therefore unsurprising that no relationship with rainfall was found. On the other hand, many studies do support a relationship between rainfall and particle and/ or MP fallout, yet the majority do not report a significance between the variables (16-18, 30). A final, and somewhat unique variable to consider herein, is the impact, if any, that the pandemic-induced national lockdown may have had in influencing the sampling site 1 (A63) 13-month dataset. The lockdown months involved high MP levels relative to selected other months, with April and May accounting for 21% of total MPs identified within Site 1. It is possible that vehicular and weather events resuspended particles already present within the environment, even while the human activity remained low due to lockdown. July, the month in which schools, non-essential shops and venues reopened, accounted for 46% of the total MPs identified in the Site 1 sample (Fig. S1). Other months, pre and post lockdown, largely remained below the MP mean and median (Fig. S1). As further data becomes published, the influence of the pandemic lockdown periods on levels of atmospheric MPs may become clearer.

While it is unclear what the main sources and drivers of outdoor MP levels and types are in specific locations, their levels are none-the-less high with a mean of 3055 ± 5072 MP m^{-2} day^{-1} (79-18996 range, 1164 median), consisting mainly of films comprised from PE and nylon (Table 1, Fig. 4). The size range of MPs detected were mainly in the 5-40 mm length category (Fig. 3C), raising concerns relating to human ability to inhale particles of this size range, whether they enter lungs and what might be the health consequences. There has been just one published study of MP-induced human health impacts, caused by nylon fibres, in the occupational setting of nylon flock making (31) . Workers displaying lung disease had been typically exposed to nylon fibres of size range 10-15 mm width and 1000 mm length at an average respirable particulate concentration of 2.2 mg m^3 (31), significantly more particles experienced than in an outdoor environment yet consisting of the same particle size (width) range. In a controlled laboratory exposure study, human lung cell cultures have recently been exposed to nylon fibres (of approximate size shape 10 mm x 30 mm), at a level of 5000 fibres, and damage to the lung cell growth and development observed (32). In parallel work, a total of 39 MPs were identified within 11 of the 13 human lung biopsy tissue samples, with 3.00 ± 2.55 MPs for individual tissue samples, adjusted to 1.42 ± 1.50 for MP/g tissue sample (33). Of these, 12 polymer types were identified with PP (23%), PET (18%), resin (15%), PES (10%) the most abundant (33). Given the levels of MPs reported herein and previously, as well as potential human health impacts from inhalation, the inclusion of such particles as an emerging contaminant should be considered for inclusion within air quality modelling and monitoring practices.

Conclusion

In conclusion, outdoor urban environments contain significant levels of MPs which range across zones and season from 79-18996 MP m^{-2} day^{-1}, with a mean of 3055 MP m^{-2} day^{-1} ± 5072 (mean ± SD) (1164 median) for Site 1 over a 13-month sampling period. A range of 522-3617 MP m^{-2} day^{-1}, with a mean of 1500 ± 1279 MP m^{-2} day^{-1} (mean ± SD) (1012 median) was determined across the 5 sites over the 2-week sampling period. The most detected MPs were PE, nylon and resin, and the most abundant size dimensions detected was 5-50 μm, within the size range detected in nylon flock workers with lung disease and found to harm lung cells in culture (31, 32). The findings herein can inform laboratory exposures using human lung cell cultures, as part of our future work to investigate environmentally relevant levels, using the most common chemical types of MPs detected (PE and nylon, plus others), and determine any human health impacts.

Acknowledgements

This research did not receive any specific grant and was funded by a PhD scholarship in the "Human Health and Emerging Environmental Contaminants" cluster funded by the University of Hull.

Author contributions.

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Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Horton AA, Dixon SJ. Microplastics: An introduction to environmental transport processes. Wires Water. 2018;5(2).
2. Hartmann NB, Hüffer T, Thompson RC, Hassellöv M, Verschoor A, Daugaard AE, et al. Are We Speaking the Same Language? Recommendations for a Definition and Categorization Framework for Plastic Debris. Environ Sci Technol. 2019;53(3):1039-47.
3. Hidalgo-Ruz V, Gutow L, Thompson RC, Thiel M. Microplastics in the marine environment: a review of the methods used for identification and quantification. Environ Sci Technol. 2012;46(6):3060-75.
4. Hale RC, Seeley ME, La Guardia MJ, Mai L, Zeng EY. A Global Perspective on Microplastics. J Geophys Res-Oceans. 2020;125(1).
5. Chen G, Feng Q, Wang J. Mini-review of microplastics in the atmosphere and their risks to humans. Sci Total Environ. 2020;703:135504.
6. Gonzalez-Pleiter M, Edo C, Aguilera A, Viudez-Moreiras D, Pulido-Reyes G, Gonzalez-Toril E, et al. Occurrence and transport of microplastics sampled within and above the planetary boundary layer. Sci Total Environ. 2021;761:143213.
7. Dris R, Gasperi J, Mirande C, Mandin C, Guerrouache M, Langlois V, Tassin B. A first overview of textile fibers, including microplastics, in indoor and outdoor environments. Environ Pollut. 2017;221:453-8.
8. Danopoulos E, Twiddy M, Rotchell JM. Microplastic contamination of drinking water: A systematic review. Plos One. 2020;15(7).
9. Danopoulos E, Jenner L, Twiddy M, Rotchell JM. Microplastic contamination of salt intended for human consumption: a systematic review and meta-analysis. Sn Appl Sci. 2020;2(12).
10. Danopoulos E, Jenner LC, Twiddy M, Rotchell JM. Microplastic Contamination of Seafood Intended for Human Consumption: A Systematic Review and Meta-Analysis. Environ Health Persp. 2020;128(12).
11. Gasperi J, Wright SL, Dris R, Collard F, Mandin C, Guerrouache M, et al. Microplastics in air: Are we breathing it in? Curr Opin Env Sci Hl. 2018;1:1-5.
12. Prata JC. Airborne microplastics: Consequences to human health? Environ Pollut. 2018;234:115-26.
13. Pauly JL, Stegmeier SJ, Allaart HA, Cheney RT, Zhang PJ, Mayer AG, Streck RJ. Inhaled cellulosic and plastic fibers found in human lung tissue. Cancer Epidemiol Biomarkers Prev. 1998;7(5):419-28.
14. Amato-Lourenco LF, Carvalho-Oliveira R, Junior GR, Dos Santos Galvao L, Ando RA, Mauad T. Presence of airborne microplastics in human lung tissue. J Hazard Mater. 2021;416:126124.
15. Dris R, Gasperi J, Saad M, Mirande C, Tassin B. Synthetic fibers in atmospheric fallout: A source of microplastics in the environment? Mar Pollut Bull. 2016;104(1-2):290-3.

16. Cai LQ, Wang JD, Peng JP, Tan Z, Zhan ZW, Tan XL, Chen QQ. Characteristic of microplastics in the atmospheric fallout from Dongguan city, China: preliminary research and first evidence. Environ Sci Pollut R. 2017;24(32):24928-35.
17. Klein M, Fischer EK. Microplastic abundance in atmospheric deposition within the Metropolitan area of Hamburg, Germany. Sci Total Environ. 2019;685:96-103.
18. Allen S, Allen D, Phoenix VR, Le Roux G, Jiménez PD, Simonneau A, et al. Atmospheric transport and deposition of microplastics in a remote mountain catchment (vol 12, pg 339, 2019). Nat Geosci. 2019;12(8):679-.
19. Brahney J, Hallerud M, Heim E, Hahnenberger M, Sukumaran S. Plastic rain in protected areas of the United States. Science. 2020;368(6496):1257-60.
20. Wright SL, Ulke J, Font A, Chan KLA, Kelly FJ. Atmospheric microplastic deposition in an urban environment and an evaluation of transport. Environ Int. 2020;136.
21. Roblin B, Ryan M, Vreugdenhil A, Aherne J. Ambient Atmospheric Deposition of Anthropogenic Microfibers and Microplastics on the Western Periphery of Europe (Ireland). Environ Sci Technol. 2020;54(18):11100-8.
22. Huang Y, He T, Yan M, Yang L, Gong H, Wang W, et al. Atmospheric transport and deposition of microplastics in a subtropical urban environment. J Hazard Mater. 2021;416:126168.
23. Szewc K, Graca B, Dolega A. Atmospheric deposition of microplastics in the coastal zone: Characteristics and relationship with meteorological factors. Sci Total Environ. 2021;761:143272.
24. Jenner LC, Sadofsky LR, Danopoulos E, Rotchell JM. Household indoor microplastics within the Humber region (United Kingdom): Quantification and chemical characterisation of particles present. Atmos Environ. 2021;259.
25. Free CM, Jensen OP, Mason SA, Eriksen M, Williamson NJ, Boldgiv B. High-levels of microplastic pollution in a large, remote, mountain lake. Mar Pollut Bull. 2014;85(1):156-63.
26. Vianello A, Jensen RL, Liu L, Vollertsen J. Simulating human exposure to indoor airborne microplastics using a Breathing Thermal Manikin. Sci Rep-Uk. 2019;9.
27. Horton AA, Cross RK, Read DS, Jurgens MD, Ball HL, Svendsen C, et al. Semi-automated analysis of microplastics in complex wastewater samples. Environ Pollut. 2021;268(Pt A):115841.
28. Zhang Q, Zhao YP, Du FN, Cai HW, Wang GH, Shi HH. Microplastic Fallout in Different Indoor Environments. Environ Sci Technol. 2020;54(11):6530-9.
29. Europe P. Plastics – the Facts 2020. An analysis of European plastics production, demand and waste data 2020 [Available from: https://www.plasticseurope.org/en/resources/publications/4312-plastics-facts-2020.
30. Dris R, Gasperi J, Rocher V, Saad M, Renault N, Tassin B. Microplastic contamination in an urban area: a case study in Greater Paris. Environ Chem. 2015;12(5):592-9.
31. Burkhart J, Jones W, Porter DW, Washko RM, Eschenbacher WL, Castellan RM. Hazardous occupational exposure and lung disease among nylon flock workers. Am J Ind Med. 1999;Suppl 1:145-6.
32. van Dijk F SS, van Eck GWA, Wu X, Bos IST, Boom DHA, Kooter I, Spierings DCJ, Wardnenaar R, Cole M, Salvati A, Gosens R, Melgert BN. Inhalable textile microplastic fibers impaire airway epithelial growth. BioRxiv. 2021.
33. Jenner LC, Rotchell JM, Bennett RT, Cowen M, Tentzeris V, Sadofsky LR. Detection of microplastics in human lung tissue using muFTIR spectroscopy. Sci Total Environ. 2022;831:154907.

4 Chapter 4: Microplastics Identified within Human Lung Tissue

University of York
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Research Degree Thesis Statement of Authorship

Note that where a paper has multiple authors, the statement of authorship can focus on the key contributing/corresponding authors.

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Thesis title	**Atmospheric Microplastics and the Human Lungs**

Title of the work (paper/chapter)	**Detection of microplastics in human lung tissue using µFTIR spectroscopy**	
Publication status	**Published**	✓ **20/07/2022**
	Accepted for publication	
	Submitted for publication	
	Unpublished and unsubmitted	
Citation details (if applicable)		

Description of the candidate's contribution to the work*	**The candidate (LJ as named in the paper) is listed as the first author of this paper. LJ contributed to the conceptualisation, experimental design and conduct, data analysis and writing of the manuscript.**
Approximate percentage contribution of the candidate to the work (*if possible to describe in this way*)	**85%**
Signature of the candidate	
Date (DD/MM/YY)	**21/03/2025**

Co-author contributions

By signing this Statement of Authorship, each co-author agrees that:

(i) the candidate has accurately represented their contribution to the work;

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*The description of the candidate and co-authors contribution to the work may be framed in a manner appropriate to the area of research but should always include reference to key elements (e.g. for laboratory-based research this might include formulation of ideas, design of methodology, experimental work, data analysis and presentation, writing). Candidates and co-authors may find it helpful to consider the CRediT (Contributor Roles Taxonomy) approach to recognising individual author contributions.

Detection of microplastics in human lung tissue using μFTIR spectroscopy

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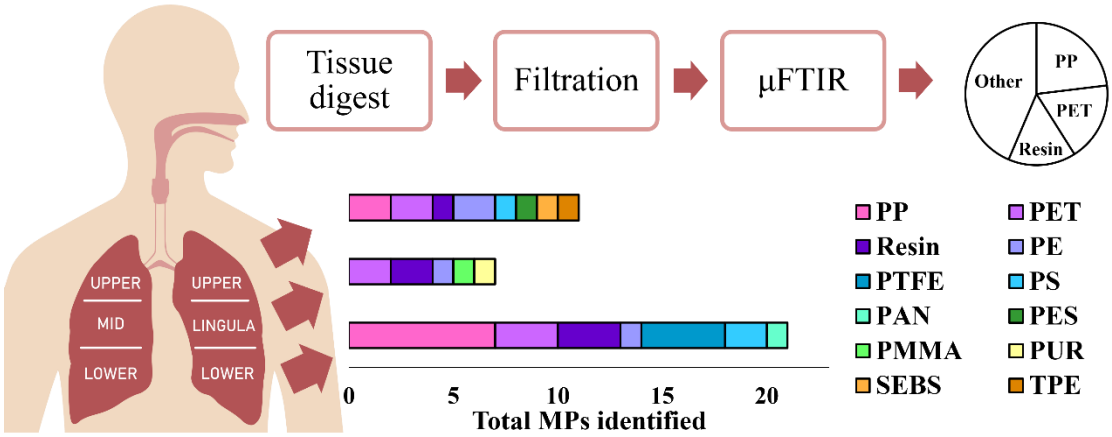
Graphical Abstract



Abstract

Airborne microplastics (MPs) have been sampled globally, and their concentration is known to increase in areas of high human population and activity, especially indoors. Respiratory symptoms and disease following exposure to occupational levels of MPs within industry settings have also been reported. It remains to be seen whether MPs from the environment can be inhaled, deposited and accumulated within the human lungs. This study analysed digested human lung tissue samples (n=13) using μFTIR spectroscopy (size limitation of 3 μm) to detect and characterise any MPs present. In total, 39 MPs were identified within 11 of the 13 lung tissue samples with an average of 1.42±1.50 MP/g of tissue (expressed as 0.69±0.84 MP/g after background subtraction adjustments). The MP levels within tissue samples were significantly higher than those identified within combined procedural/laboratory blanks (n=9 MPs, with a mean±SD of 0.53±1.07, p=0.001). Of the MPs detected, 12 polymer types were identified with polypropylene, PP (23%), polyethylene terephthalate, PET (18%) and resin (15%) the most abundant. MPs (unadjusted) were identified within all regions of the lung categorised as upper (0.80±0.96 MP/g), middle/lingular (0.41±0.37 MP/g), and with significantly higher levels detected in the lower (3.12±1.30 MP/g) region compared with the upper (p=0.026) and mid (p=0.038) lung regions. After subtracting blanks, these levels became 0.23±0.28, 0.33±0.37 and 1.65±0.88 MP/g respectively. The study demonstrates the highest level of contamination control and reports unadjusted values alongside different contamination adjustment techniques. These results support inhalation as a route of exposure for environmental MPs, and this characterisation of types and levels can now inform realistic conditions for laboratory exposure experiments, with the aim of determining health impacts.

Keywords: microplastic, lung, inhalation, human, atmospheric, airborne, air, μFTIR

Abbreviations

LOD – limit of detection

LOQ – limit of quantitation

μFTIR – micro Fourier Transform Infrared

MCT – mercury cadmium telluride

MP – microplastics between 1 μm and 5 mm

NP – nanoplastics

PAN - polyacrylonitrile

PE - polyethylene

PES - polyester

PET - polyethylene terephthalate

PMMA - polymethylmethacrylate

PP – polypropylene

PS - polystyrene,

PTFE - polytetrafluoroethylene

PUR – polyurethane

PVA – polyvinyl alcohol

ROS – reactive oxygen species

SEBS - styrene-ethylene-butylene co-polymer

TPE - thermoplastic elastomer

1. Introduction

Microplastics (MPs), defined herein as plastic particles between 1 μm and 5 mm (1), are present in all environmental compartments; from marine and freshwater bodies (2), to soil (3), food, drinking water (4, 5), and air (6-9). For the latter, suspended MP particles have been isolated from many atmospheric locations, including urbanised city centres (8, 10, 11), indoor households (7, 9, 12, 13), and remote outdoor regions (6). Previous work highlights that citizens are exposed to higher concentrations of MP within their homes (9), compared to outdoors, and this results in ubiquitous and unavoidable human exposure (14). Consequentially, there is an increasing concern regarding the hazards associated with MP ingestion, dermal contact, and inhalation (14).

Synthetic fibres have previously been observed within human lung tissue samples (15), yet limited studies confirm the presence of MPs within the lungs alongside chemical analysis tools, such as μRaman and μFTIR spectroscopy (16). Reliance upon observational criteria alone to distinguish between MP and non-MPs, can lead to over and under-estimated MP counts, and a lack of information relating to polymer or additive type (17, 18). The plausibility of MP inhalation has been highlighted (19, 20) and MPs with a width as small as 5 μm have been reported within air

samples (11, 21). Upon environmental release, plastics are exposed to oxidation, mechanical stress and biological action, resulting in embrittlement and fragmentation, forming MPs, and eventually nanoplastics (NPs)(<1μm), as well as release into the environment in their primary form (18).

Historical studies report respiratory symptoms and disease at an occupational level of exposure in synthetic textile, flock, and vinyl chloride workers (19), and as such, support inhalation as an exposure route for MPs. However, it remains unclear whether MPs can enter and remain in the lungs of the general population due to environmental exposure, rather than the chronic levels seen within industry settings. MPs are designed to be robust materials, unlikely to break down within the lungs (22), potentially leading to accumulation over time depending on aerodynamic diameter and respiratory defences (19).

The mounting concern surrounding airborne MPs stems from the unknown polymer types, levels of exposure, and consequences of their inhalation. MP characteristics such as size, shape, vectored absorbed pollutants and pathogens, as well as plastic monomer or additive leaching, have been highlighted as potential promoters of cytotoxicity (20). MPs are consistently identified within air samples, their concentration is highest indoors (7, 13, 14) and within highly populated areas (8), they are readily suspended at times of high human activity (13) and are often small and fibrous (11). Together, these concerns highlight the necessity for accurate tissue analysis to understand the potential for these synthetic polymers to penetrate the human respiratory system and cause harm.

This study aims to identify any MP particles present in digested human lung tissue samples, while also accounting for procedural and laboratory blank contamination. Any particles isolated from lung tissue have been chemically characterised using μFTIR spectroscopy (with a 3 μm lower size limit of detection).

2. Material and Methods

2.1 Human tissue acquisition

Excess human lung tissue was collected from thoracic surgical procedures at Castle Hill Hospital, Hull University Teaching Hospitals NHS Trust, following NHS Research Ethics Committee and Health Research Authority approval (REC reference 12/SC/0474). Samples of peripheral human lung tissue were collected from upper, middle (left lingula) or lower lobe specimens following surgical resection for cancer or lung volume reduction surgery. Descriptions of the tissue origin were provided by the surgical team. Care was taken to avoid the tumour margins. Details of the donors smoking status, occupation and area of residence were unavailable for the

researchers under the terms of the ethical approval obtained. Tissue samples were placed into empty glass containers with foil lids and immediately frozen (-80°C) until bulk analysis (two batches) was conducted. Lung tissue was obtained from 11 patients (numbered 1.1 to 11.1), with patients 1 and 2 providing two samples (numbered 1.2 and 2.2) from different lung positions (n=13, total tissue mass=55.41 g), resulting in a mean mass of 4.26±3.87 g (range 0.79-13.33 g). Patients mean age was 63±13 years (range 32-77), 5 females and 6 males (Table 1).

2.2 Lung tissue digestion and filtration

Thawed samples were exposed to a hydrogen peroxide (100 mL of 30% H_2O_2) bath and rinsed alongside 'procedural blanks' (n=4) (Supplementary Fig. S1). Each tissue sample was transferred to a clean glass conical flask with a foil covering, and 100 mL of 30% H_2O_2 added. The total mass of each individual tissue sample digested is detailed in Table 1. Flasks were placed in a shaking incubator at 55°C for approximately 11 days, 65 rpm, or until there was no visible tissue. After 5 days within the incubator, an additional 100 mL of 30% H_2O_2 was added. The digest, adapted from previous studies investigating MPs within different environmental and tissue samples (23), ensures removal of organic particles whilst maintaining MP integrity (6, 23). Samples were then filtered onto aluminium oxide filters (0.02 µm Anodisc, Watford, U.K.) using a glass vacuum filtration system. These were stored in clean glass petri dishes, in the dark, before chemical composition analysis alongside laboratory blanks (n=13) (Supplementary Fig. S1).

2.3 Chemical characterisation of particles using µFTIR analysis

Each tissue sample Anodisc filter was placed directly onto the µFTIR spectroscopy platform, and the length (largest side) and width (second largest side) recorded using the aperture height, width and angle size selection tool, available within the ThermoScientific Omnic Picta Nicolet iN10 microscopy software. Particles were then assigned to a shape category (fibre, film, fragment, foam, or sphere (24)), whereby fibrous particles were characterised as having a length to width ratio >3 (12).

µFTIR spectroscopy analysis was conducted in liquid nitrogen cooled transmission mode (Nicolet iN10, ThermoFisher, Waltham MA, U.S.A), without the aid of further accessories or crystals. The cooled mercury cadmium telluride (MCT) detector allowed for the analysis of particles accurately down to 3 µm in size. The Nicolet iN10 microscope used is equipped with 15x 0.7 N.A. high efficiency objective and condenser. It has a colour CCD digital video camera with an

independent reflection and transmission illuminations mounted, for capturing images of particles. This model has a standardised 123x magnification with the aperture settings used. No observational criteria (18) was applied to select specific particles for μFTIR analysis, to prevent bias. Using the aperture size selection tool, all particles upon the sample filter >3 μm were included in the analysis process. For this study, the whole filter, containing the total digested tissue sample, was analysed.

A background reference spectrum was first recorded, using identical parameters to the particles undergoing analysis. A blank area of the Anodisc filter was chosen as the site for background collection before immediate analysis of the sample particles. μFTIR parameters were; spectral range of 4000-1250 cm^{-1}, high spectral resolution 4 cm^{-1}, scan number of 64. No smoothing, baseline correction or data transformation was attempted. Resulting sample spectra were compared to a combination of polymer libraries (Omnic Picta, Omnic Polymer Libraries), available with the Omnic Picta software, and full spectral ranges were used with a match threshold of ≥70%. If particles were below the ≥70% match index threshold, three attempts were made to collect a successful match before moving on to the next particle undergoing analysis. Particles below ≥70% match, and particles not classified as a plastic were recorded but not included in the results presented (25).

During μFTIR analysis, one 'laboratory blank' Anodisc filter was opened alongside every sample filter (Supplementary Fig. S1). A total of 13 lung tissue samples were analysed, plus 4 'procedural blanks', and 13 'laboratory blanks'. The total number of particles (MPs and others) identified was 296, whereby 225 (76%) of these were above the 70% hit quality index threshold. Only the MPs data is shown in the results. Identified PET and PES MP particles were reported separately within this study, using a high match (>70 %) on a polymer database search to confirm their identities.

2.4 Quality assurance and control measures to reduce and quantify background MP contamination

Strict control measures were adhered to, in order to quantify and characterise the nature of any unavoidable background contamination. Due to the ubiquitous nature of MPs in the air, contamination upon the surface of lung tissue samples could be possible during the surgical procedure, where lung tissue was removed from live human subjects. While it was not possible to fully control the surgical environment, each tissue sample was dropped into a 100 mL 30% H_2O_2 bath, re-sealed with foil and agitated for 2 minutes. In parallel, 'procedural blanks' (n=4) were initiated. The tissue sample was removed, and the outer surface rinsed thoroughly with 100 mL 30% H_2O_2 to remove any surface contamination, employing a method similar to extracting

microplastics from whole biota (26). Analysis of solely the interior portion of the tissue was considered (15) but was not applied with the aim of maintaining a larger tissue mass. Tissue samples were digested in two batches, with two procedural blanks, which mimicked the entire tissue processing steps but lacked the lung tissue sample, alongside each batch (Supplementary Fig. S1). Reagents were filtered and prepared in bulk for each batch. When conducting μFTIR analyses, a 'laboratory blank' filter (n=13), placed in a glass sealed petri dish, was opened for the same duration as that for the tissue sample.

MPs found within 'procedural blanks' represent contamination from the laboratory reagents, equipment or fallout from the air during the transfer of samples. For each batch, the average procedural contamination was calculated and assumed to be present within each of the tissue samples. MPs within 'laboratory blanks' represent contamination from atmospheric fallout within the μFTIR laboratory room during particle characterisation. Procedural blank and laboratory blank results were combined to account for contamination at every step. No standardised protocols are currently adopted within the MPs research field to account for background contamination, so multiple contamination adjustments were applied in this study for comparison. These comprised two approaches: subtraction, routinely used in the MP research field, and a limit of detection (LOD) and limit of quantification (LOQ) technique (27) (Supplementary methods 1). Presenting raw data, subtraction, and LOD/LOQ adjusted results allows a comparison for each technique.

All H_2O_2 and MilliQ water used were triple filtered using an all-glass vacuum filtration kit and 47 mm glass fibre grade 6 filters (GE Healthcare Life Sciences, Marlborough MA, U.S.A). All glassware underwent thorough manual cleaning, before a dishwasher cycle using distilled water and then a manual three rinse wash with triple filtered MilliQ water. All equipment and reagents were always covered with foil lids and a small opening made when pouring. Additionally, when filtering digested samples, glassware and the sides of the filtration kit were rinsed three times with triple filtered MilliQ water to avoid sample particle loss. All work was conducted in a thoroughly cleaned fume cupboard with power 'off' and shield down to minimise unfiltered air flow (28) and particle suspension (29). Each tissue sample was processed individually to prevent cross contamination. Plastic equipment was avoided, glass petri dishes, a cotton laboratory coat, and a new set of nitrile gloves for each sample processing step. Tissue preparation and particle analysis was conducted at times of low activity, no room ventilation and μFTIR conducted in a single person room with no windows. Finally, work was conducted by a single researcher for standardisation. To ensure no particles were contaminating the Anodisc filters from the manufacturing process of the discs used, three random filters were chosen and observed under the μFTIR, in which no particles were present.

2.5 Statistical analysis

Tests for homogeneity and significance were performed on unadjusted MP values using SPSS. All data were determined not normally distributed with a Shapiro-Wilk test and either a Kruskal-Wallis or Mann-Whitney U test applied.

3. Results

3.1 MP abundance levels detected in human lung tissue samples

A total of 39 MPs were identified within 11 of the 13 human lung tissue samples. An overall unadjusted mean of 3.00±2.55 MPs per sample (range 0-8 MPs) were identified within human lung tissue samples, significantly higher levels (p=0.001) compared with 0.53±1.07 MP per sample detected in the combined blanks. When considering the mass of the tissue sample, without accounting for background contamination, a mean of 1.42±1.50 MP/g was detected (Table 1). After subtracting background contamination, this value becomes 0.69±0.84 MP/g (Table 1). An unadjusted mean of 2.09±1.54 MP/g of tissue was identified in male (n=6) and 0.36±0.50 MP/g of tissue in female (n=5) samples (adjusted to 0.91±0.95 MP/g and 0.33±0.52 MP/g respectively after subtracting background contamination). All male samples contained at least one MP particle, whilst two of the five female samples did not. The data was not normally distributed *(p*=0.013), and a Mann-Whitney U test revealed tissue samples from male patients had significantly higher levels of MP/g compared to females (*p*=0.019). A detailed description of the characterisation of background MP contamination (procedural and laboratory blanks) can be found in the supplemental information (Table S1).

Table 1. Patient and tissue sample information alongside the number of MPs identified within samples by μFTIR spectroscopy. Polymer types and particle characteristics are included, and three different contamination adjustments to display results in units of MP/g of tissue. Abbreviations; PAN=polyacrylonitrile, PE=polyethylene, PES=polyester, PET=polyethylene terephthalate, PMMA=Polymethylmethacrylate, PP=polypropylene, PS=polystyrene, PTFE=polytetrafluoroethylene, PUR=polyurethane, Resin=alkyd/ epoxy/ hydrocarbon, SEBS=styrene-ethylene-butylene co-polymer, TPE=thermoplastic elastomer. R=right lung, L=left lung, Low=lower region of the lung, mid=middle/lingular region of the lung, up=upper region of the lung

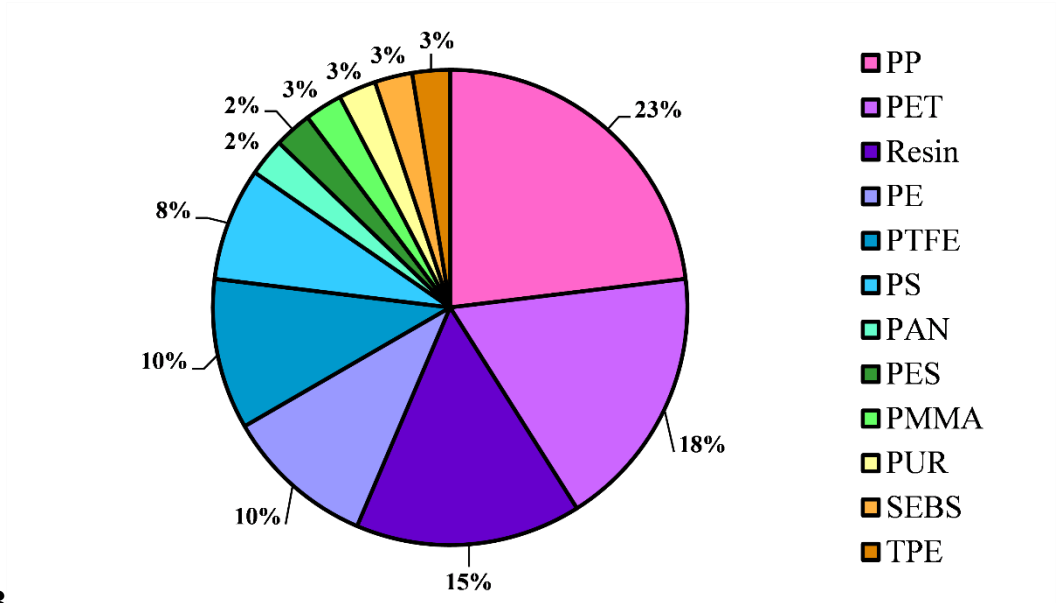
ID	Sex	Lung region	Tissue (g)	MP total	MP polymer	Length, width (µm)	Shape	MP/g †	MP/g ††	MP/g †††
1.1	M	R, Low	2.02	8	PET PP PP PP PP PS PTFE PTFE	88, 10 55, 28 39,18 420, 9 27, 10 89, 71 100, 29 92, 88	Fibre Fragment Fragment Fibre Fragment Fragment Fibre Film	3.96	2.97	1.94 based on PP only
1.2		R, Up	0.79	2	PP TPE	109, 18 66, 19	Fibre Fibre	2.53	0.00	
2.1	M	R, Low	0.80	3	PP PP PTFE	40, 22 144, 65 26, 20	Fragment Fragment Fragment	3.75	1.25	
2.2		L, Low	0.84	3	PS PTFE Resin	14, 14 96, 5 19, 13	Fragment Fibre Fragment	3.57	1.19	
3.1	M	R, Up	13.33	5	PE PE PET PP SEBS	224, 9 29, 17 202, 6 101, 17 83, 18	Fibre Fragment Fibre Fibre Film	0.38	0.23	
4.1	M	R, Up	1.53	2	PS Resin	60, 44 12, 9	Fragment Fragment	1.31	0.65	
5.1	F	L, Lin	1.37	0	none	none		0.00	0.00	
6.1	M	R, Mid	3.98	2	PE Resin	17, 10 20, 15	Fragment Fragment	0.50	0.25	
7.1	F	R, Up	8.29	1	PES	40, 22	Fragment	0.12	0.00	
8.1	F	L, Low	5.90	7	PAN PE PET PET PP Resin Resin	1112, 9 28, 20 443, 13 452, 12 160, 46 101, 9 261, 22	Fibre Fragment Fibre Fibre Fragment Fibre Fibre	1.19	1.19	
9.1	M	R, Mid	6.84	5	PET PET PMMA PUR Resin	897, 10 2475, 12 96, 76 155, 16 14, 4	Fibre Fibre Fragment Fibre Fibre	0.73	0.73	
10.1	F	R, Up	2.12	1	PET	275, 12	Fibre	0.47	0.47	
11.1	F	R, Up	7.60	0	none	none		0.00	0.00	
Mean±SD								1.42± 1.5	0.69± 0.84	

† †† †††	Total MPs detected with no account taken for MPs found in controls Total MPs in sample minus total MPs identified in controls (regardless of polymer type) (Supplementary information) MP contamination levels after LoD/ LoQ method [25], if meeting the threshold (Supplementary information)

3.2 MP particle characterisation from human lung tissue samples

A total of 12 polymer types were identified in the tissue samples, as detailed in Fig 1 A. PP (9, 23%) and PET (7, 18%) were the most abundant (Fig 1A). All MPs identified within tissue samples were fibre (19, 49%), fragment (17, 43%), or film (3, 8%), (Fig 1B, Fig 2). MP particles identified within the tissue samples had a mean particle length of 223.10±436.16 µm (range 12-2475 µm), and a mean particle width of 22.21±20.32 µm (range 4-88 µm) (Fig 3A).

A



B

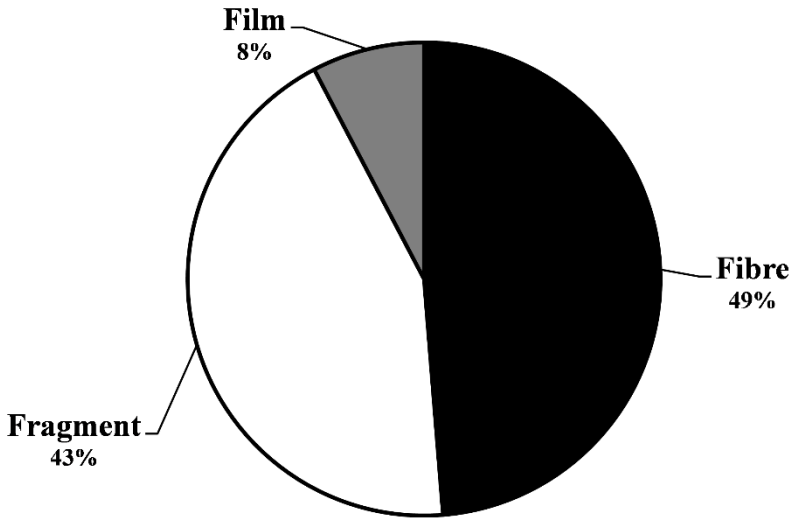


Figure 1. Polymer types (A) and shapes (B) of the MPs identified within lung tissue samples.

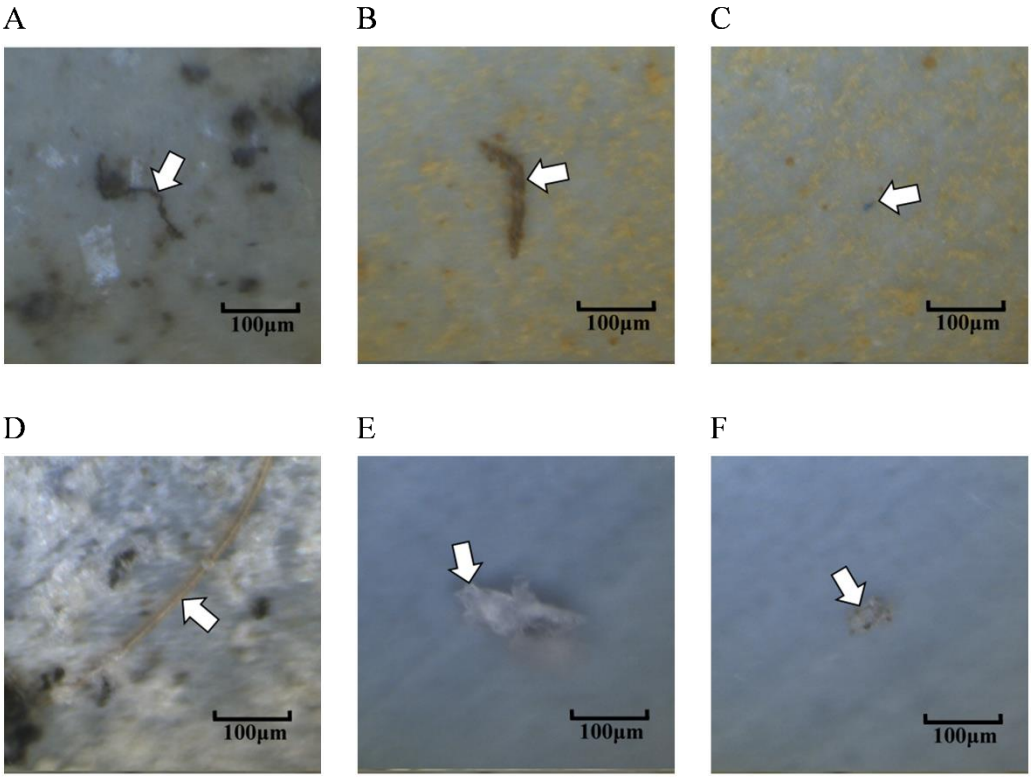


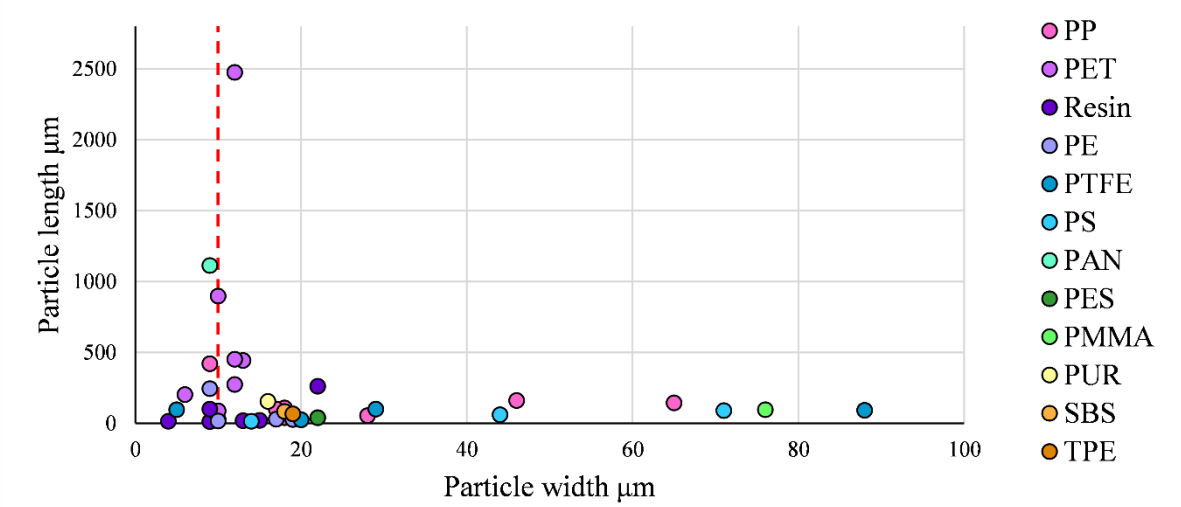
Figure 2. Images of MPs identified from human lung tissue samples. A, B, C and D= (A=PET) (B=PUR) (C=Resin) (D=PAN). E and F=MPs identified within blanks. (E=PS) (F=PP). Corresponding spectra included in Fig. S2.

3.3 Characterisation of background MP contamination (procedural and laboratory blanks)

Considering all the blank samples, the mean background MP contamination rate detected was 0.53±1.07 MP per blank. Particles identified within 'procedural blanks' had a mean MP contamination rate of 2.00±2.83 MP per sample (range 0-4), for batch 1, whereby four MPs were identified on one filter: PE, PE/PP, PS, and a resin particle. No MPs were detected on the second filter for batch 1 (Table S1). No particles were identified within 'procedural blanks' from batch 2 of tissue samples on either of the two procedural blank filters (Table S1). Particles detected from 'laboratory blanks' (n=13) had an overall mean MP contamination rate of 0.38±0.65 MP per sample (range 0-2). This comprised one PET, PP, PS, PTFE and PVA particle from the 13 laboratory control filters (Table S1). The average length of MPs detected within the combined blank samples was 105.22±92.82 µm (range 23-315 µm), and an average width of 34.44 ± 22.61 µm (range 15-73 µm). The shapes of MPs identified in the combined blank samples were either fragment (6, 67%), fibre (2, 22%), or film (1, 11%).

In addition to MP particles, non-MP ‘natural polymer’ particles were detected on the sample filters. Combining non-MP procedural and laboratory blank results 9.04±4.84 non-MP particles per sample were detected, comprised of cellulose and zein.

A



B

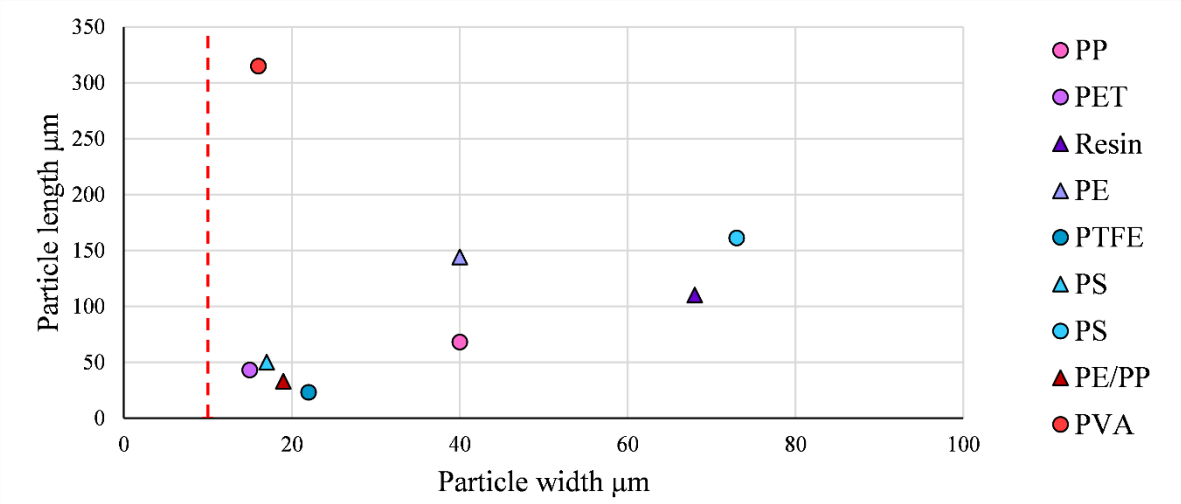


Figure 3. Polymer size dimensions and type of each MP identified within (A) human lung tissue samples and (B) ‘procedural blank’ (triangles) and ‘laboratory blank’ (circles) samples. Red line represents the assumed inhalable size limit regardless of density.

3.4 Background MP contamination adjustments

Using adjustments, to account for the combined procedural and blank contamination levels detected, decreases the level of MPs identified within tissue samples depending on the approach

used (Table 1). After blank subtraction adjustments, the total MPs identified within tissue samples have a mean of 0.69±0.84 MP/g of tissue. Subtraction adjusted MP levels in human lung tissues were statistically significant compared to blank data (Mann-Whitney U test, p=0.043). Only one lung tissue sample (sample 1.1) fit the criteria for using a LOD and LOQ calculation, showing 1.94 MP/g, above the quantification threshold. The polymer type detected above this threshold was PP. MPs above the LOD, that can be detected within lung tissue samples, but not quantified, were PE, PET, PP, PTFE and resin.

3.5 MP distribution within human samples by lung region

MPs were identified within all regions of the lung (Fig 4 and Table S2). An unadjusted mean of 0.80±0.96 MP/g was identified within the upper region (adjusted to 0.23±0.28 MP/g after background subtraction), 0.41±0.37 MP/g within the middle/lingular region (adjusted to 0.33±0.37 MP/g) and 3.12±1.30 MP/g within the lower region (adjusted to 1.65±0.88 MP/g). Data was not normally distributed (p=0.013) and a Kruskal-Wallis test showed that the number of MPs in the lower region were significantly higher than the middle/lingular (p=0.038) and the upper region (p=0.026). Within the upper region (n=6, total mass=33.66 g), 11 MPs were identified; PE (2, 18%), PET (2, 18%), PP (2, 18%), PES (1, 9%), PS (1, 9%), resin (1, 9%), SEBS (1, 9%), TPE (1, 9%). Within the middle/lingular region (n=3, total tissue mass=12.19 g), 7 MPs were identified; PET (2, 29%), resin (2, 29%), PE (1, 14%), PMMA (1, 14%), PUR (1, 14%). Within the lower region (n=4, total tissue mass=9.56 g), 21 MPs were identified; PP (7, 33%), PTFE (4, 19%), PET (3, 14%), Resin (3, 14%), PS (2, 10%), PAN (1, 5%), PE (1, 5%) (Fig 4).

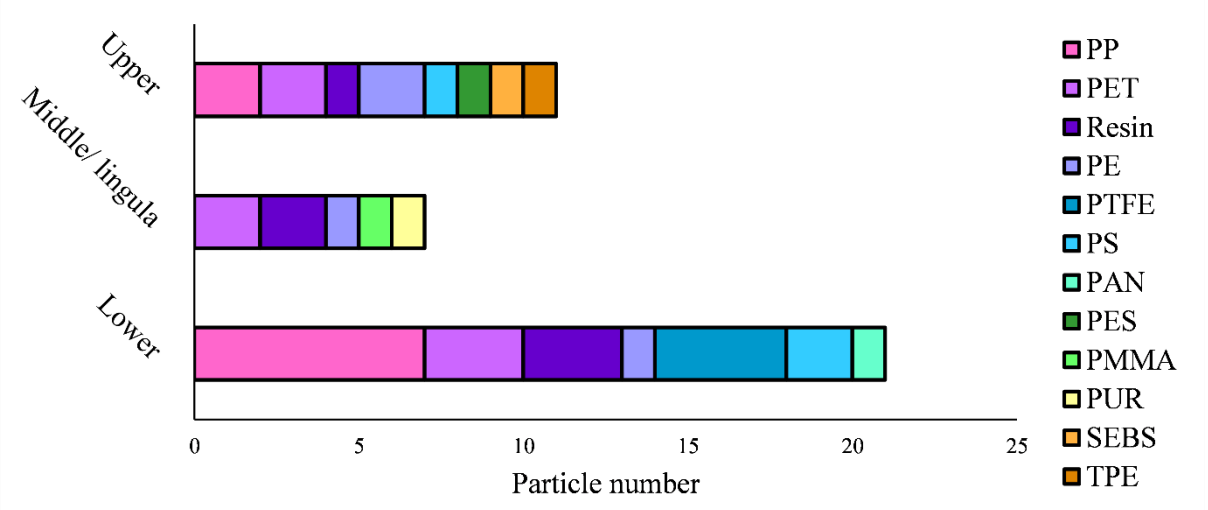


Figure 4. Particle number (total MPs detected with no account taken for MPs found in controls) and polymer type of MPs identified within human lung tissue samples, assigned to their lung region.

3.6 MP distribution within human lung tissue by individual patient

MPs were identified in 9 of the 11 patient lung samples. Multiple samples were taken from patient 1; 8 MPs in sample 1.1 and 2 MPs in sample 1.2 (Fig 5A). PP particles were identified within both samples (Fig 5B). Multiple samples were also taken from patient 2; 3 MPs in sample 2.1 and 3 MPs in sample 2.2. PTFE particles were identified within both samples, whilst multiple polymers were only identified within one patient sample (Fig 5B).

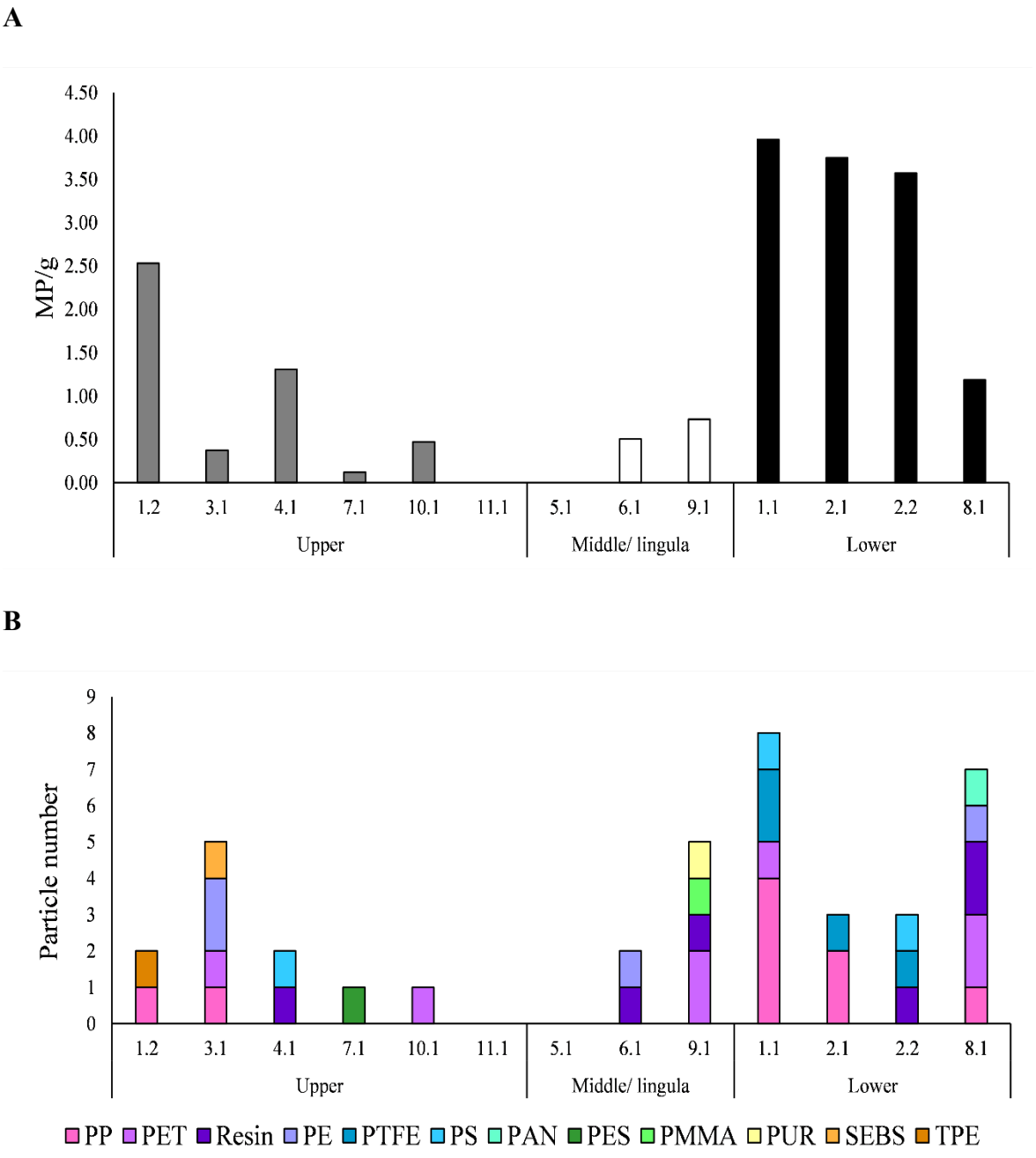


Figure 5. Number (A) and type/quantity (B) of MPs detected in each lung region for individual patients.

4. Discussion

This report provides compelling evidence of MPs within human lung tissue samples, using a robust, best practice, background contamination regime combined with μFTIR chemical composition analysis to verify the particles present. The study also highlights the importance of including and evaluating contamination adjustments within MP research, whilst providing high levels of quality assurance and control.

In total, 39 MPs were identified within 11 of the 13 lung tissue samples, with an unadjusted average of 1.42±1.50 MP/g of tissue. By subtracting any MPs detected in the corresponding blanks, an adjusted average of 0.69±0.84 MP/g tissue sample is reported. The MP levels within tissue samples were significantly higher than those identified within combined procedural/laboratory blanks. Of the MPs detected, 12 polymer types were identified with PP (23%), PET (18%), resin (15%), and PE (10%) the most abundant. It should be noted that the FTIR spectra for PET and PES (polyester) are similar and can be difficult to distinguish (30, 31), however a high match of 70% was accepted to distinguish between the MP types within this study.

MPs were identified within all regions of the lung categorised as upper (0.80±0.96 MP/g), middle/lingular (0.41±0.37 MP/g), and lower (3.12±1.30 MP/g) region. However, when a LOD and LOQ approach was applied, only one tissue sample fit the criteria, with only PP detected above the threshold levels at 1.94 MP/g (Table 1). It could be that most MPs identified were contamination, however the LOD LOQ could also be 'masking' legitimately identified MPs. The LOD LOQ adjustment approach dramatically reduced the level of quantifiable MPs identified within lung tissue samples. This quality control measure has the benefit of providing a threshold above that of a simple subtraction, allowing MPs to be reliably detected and quantified (26). Although it is an emerging technique within the MP field, it has the potential to account for polymer type as well as quantity and is commonly applied within analytical chemistry. However, samples containing low numbers of MPs, such as the human lung tissue samples reported here, commonly only have one MP particle per polymer type identified in a sample. It has been reported that when dealing with such low MP quantities within samples, the LOD LOQ technique will have more significant effects and lead to a "reduced capacity to report any MPs above the LOD or LOQ" (27). We therefore report our results in three ways; unadjusted, subtraction adjusted and LOD LOQ adjusted, but highlight the importance of the LOD LOQ technique for future studies in which MP abundance is not as low.

MPs have, to date, been detected in human samples from histological lung cancer samples (15) and cadavers (16) as well as from human placenta (32). Our findings are consistent with an early study by Pauly et al (1998) using microscopy under polarised light to identify fibres (though

without chemical characterisation validation or rigorous contamination control measures), reporting presence of fibres in 83% of nonneoplastic lung specimens (n=67/81) and in 97% of malignant lung specimens (n=32/33)(15). This study also reported that the fibres were distributed throughout all regions of the lung and were not confined to the large air spaces (15). While no formal size range is given in this early study, they reported heterogeneity with respect to fibre length, width, surface morphology and colour, with >250 μm length and ~50 μm width (15). Our findings are also in line with a recent publication by Amato-Lourenco *et al* who also found PP to be amongst the most abundant plastics identified (16). In contrast to our study, Amato-Lourenco *et al* showed that non-fibrous particles were the most abundant type of MP with sizes smaller than those seen in our study. This could partly by due to differing exposures to MP, our best practice approach used to eliminate background contamination, or the methods used to detect and characterise samples, Raman vs. μFTIR. Although Raman spectroscopy has the advantage of a lower method detection limit (~1μm), which might explain the abundance of smaller particles identified in Amato-Lourenco's study (16), it can be heavily influenced by fluorescence interference and does not detect the same polar peaks that μFTIR spectroscopy can. Additionally, Raman spectroscopy can UV degrade the particles being analysed, which could hinder potential future investigations. Thus, although both spectroscopic techniques complement each other, μFTIR has some advantages that benefit MP research (33).

Interestingly, tissue from male donors contained significantly higher levels of unadjusted MP (2.09±1.54 MP/g) compared to females (0.36±0.50 MP/g), with all samples from males containing MPs but two out of five samples from females showing no MPs. We hypothesise that this is due female airways being significantly smaller than the airways of males (34), although the relatively small sample size used herein dictates that more analyses be conducted to explore such differences further.

According to Donaldson et al (1993), only particles with a physical diameter smaller than 3 μm can enter the alveolar region of the lung (35). The alveolar duct is reported in the literature as being ~540 μm diameter and 1410 μm long (36). Particles of a size ranging from 12-2,475 μm for length and 4-88 μm for width were detected within lung samples in this study, in theory, too large to be present, yet present nonetheless.

While the fate of particles entering the lung, and their resulting biological effects in terms of inflammation responses, are well established for ultrafine particulates in the NP or PM_{10} size range (37, 38), the corresponding information is currently unavailable for the MP size range of particles observed here, highlighting a serious gap in the knowledge. There are limited recent studies giving evidence of particle sizes and deposition in the lungs. It could be that there may be a pre-conceived

assumption about the particle sizes which are inhalable and able to make it into the lower airway, but in this study, and others (15, 16) particles bigger than these are being reported, and therefore, it may be time to revisit these numbers and investigate what sizes can be inhaled. Interestingly, even after LOD and LOQ were applied, the PP identified in sample 1.1 were all above the size limit which is generally thought of as inhalable.

12 MPs ≤10 μm were identified within 7 of the 13 lung tissue samples, consisting of PET (3), resin (3), PE (2), PP (2), PTFE (1) and PAN (1) (Table 1). The smallest particle identified was 14 μm in length and 4 μm width (Fig. 2C), and identified as an 'alkyd resin', a synthetic thermoplastic used in protective coatings and paints (39). No MPs ≤10 μm were detected within blanks, surprising since the prevalence of MPs in the environment is known to increase with decreasing particle size (6-8), suggesting that the quality assurance measures undertaken eliminated these smaller particles from blanks. As these small MPs were consistently absent from blanks (Fig. 3B), it highlights the likelihood of the smaller MPs being present within lung tissue rather than from background contamination sources.

The ubiquity of MPs within the environment, results in background contamination in any study, even after strict quality control measures are applied. Blanks, or controls, are run alongside sample analysis to document the levels and types of MPs contaminating samples, either by mimicking the sample processing steps ('procedural blank'), or by opening a clean filter during sample analysis ('laboratory blank'). Rarely are procedural and laboratory blanks both applied (26). It was hypothesised in the design of this study that if MPs were present within lung tissue samples, they would be present at low levels, especially considering the detection limit of chemical verification. Thus, the importance of combining multiple procedural and laboratory blanks, is highlighted. In this study the MP characteristics identified within blanks were distinct from those identified within lung tissue samples; the main polymer abundance, size range and shape varied (Fig 3A, 3B). Human lung tissue samples were typically comprised of PP, PET and resin, with lengths ranging from 12-2475 μm and widths from 4-88 μm, and fibres being more prevalent than fragments. In contrast, MPs detected in the blanks were less abundant and comprised different particle characteristics. MPs were sized 23-315 μm and 15-73 μm for length and width, and fragments were more prevalent than fibres.

Within the MP literature, a standardised contamination adjustment technique has not been established. Therefore, this study opted to report concentrations in three commonly used ways; detailing blank results but making no adjustments (13, 40), subtraction adjustments (6, 41) and LOD LOQ adjustments (9, 27). Using no contamination adjustments, 1.42±1.50 MP/g of lung tissue was observed. While this method is common practice, it likely includes any contamination within

the samples. The subtraction adjustment decreases the lung tissue MP final mean value to 0.69±0.84 MP/g and accounts for any potential background contamination but is not specific in terms of taking into account particle characteristics. The LOD LOQ adjustment approach dramatically reduces the levels of MPs identified within the study to 0.15±0.54 MP/g using a polymer specific approach, but could be seen to 'mask' low levels of MPs. Ultimately this study highlights the need for data adjustments to account for background contamination, but alongside an evaluation into which adjustment is the best approach. Irrespective of the adjustments, low levels of MPs are present within lung tissue samples, providing evidence to support MP inhalation as a route of exposure to humans.

Airborne MPs are globally ubiquitous and especially prevalent indoors where humans spend many hours a day, such as the home (7, 9, 12, 13) and the office (7, 13). Humans are thus continuously exposed to atmospheric MPs, with inhalation estimates ranging from 6-272 MP/day (12, 19, 42). It is the smallest and least dense MP and NP particles that are the most cause for concern regarding respiratory health, as these MPs are most likely to deposit within the lungs based on aerodynamic diameter (19). In contrast to NPs, MP particles in the full micro-size range (10 μm-5 mm) have yet to be considered in terms of health implications and potential impacts, perhaps not having been a priority compared with the smaller, ultrafine particles. The results herein indicate that the larger micro-size range are detected within human lung samples, suggesting that these have been overlooked (as being considered too large to enter lungs). MPs, like all macroplastics, are designed to be resilient, with the addition of dyes, and additives that dictate their properties (2). It had previously been suggested that inhaled MPs are likely to bio-persist and possibly accumulate within a lung environment (20), showing resilience to degradation by synthetic extracellular lung fluid after 180 days (22). After deposition within the lung, mechanisms of toxicity are unknown but particle properties such as small size, density, concentration, shape, monomer type, chemical leachates and environmental adsorbents (e.g. bacteria, heavy metals and polyaromatic hydrocarbons) have all been suggested as potential contributors to cytotoxicity (19, 20). Inflammation (43), ROS and oxidative stress (44), physical damage from particle shape, frustrated phagocytosis (35), are currently suggested cellular responses to MP exposure.

In summary, this study is the first to report MPs within human lung tissue samples, using μFTIR spectroscopy. The abundance of MPs within samples, significantly above that of blanks, supports human inhalation as a route of environmental exposure. MPs with dimensions as small as 4 μm but also, surprisingly, >2 mm were identified within all lung region samples, with the majority being fibrous and fragmented. The knowledge that MPs are present in human lung tissues can now

direct future cytotoxicity research to investigate any health implications associated with MP inhalation.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. It was funded by a PhD scholarship in the “Human Health and Emerging Environmental Contaminants” cluster funded by the University of Hull.

References

1. Hartmann NB, Hüffer T, Thompson RC, Hassellöv M, Verschoor A, Daugaard AE, et al. Are We Speaking the Same Language? Recommendations for a Definition and Categorization Framework for Plastic Debris. Environ Sci Technol. 2019;53(3):1039-47.
2. GESAMP. Sources, fate and effects of microplastics in the marine environment: A global assessment. London UK: The Joint Group of Experts on Scientific Aspects of Marine Environmental Protection, Working Group 40. 2015.
3. Wang J, Liu X, Li Y, Powell T, Wang X, Wang G, Zhang P. Microplastics as contaminants in the soil environment: A mini-review. Sci Total Environ. 2019;691:848-57.
4. Danopoulos E, Jenner LC, Twiddy M, Rotchell JM. Microplastic Contamination of Seafood Intended for Human Consumption: A Systematic Review and Meta-Analysis. Environ Health Persp. 2020;128(12).
5. Danopoulos E, Twiddy M, Rotchell JM. Microplastic contamination of drinking water: A systematic review. Plos One. 2020;15(7).
6. Allen S, Allen D, Phoenix VR, Le Roux G, Jiménez PD, Simonneau A, et al. Atmospheric transport and deposition of microplastics in a remote mountain catchment (vol 12, pg 339, 2019). Nat Geosci. 2019;12(8):679-.
7. Dris R, Gasperi J, Mirande C, Mandin C, Guerrouache M, Langlois V, Tassin B. A first overview of textile fibers, including microplastics, in indoor and outdoor environments. Environ Pollut. 2017;221:453-8.
8. Cai LQ, Wang JD, Peng JP, Tan Z, Zhan ZW, Tan XL, Chen QQ. Characteristic of microplastics in the atmospheric fallout from Dongguan city, China: preliminary research and first evidence. Environ Sci Pollut R. 2017;24(32):24928-35.
9. Jenner LC, Sadofsky LR, Danopoulos E, Rotchell JM. Household indoor microplastics within the Humber region (United Kingdom): Quantification and chemical characterisation of particles present. Atmos Environ. 2021;259.
10. Wright SL, Levermore JM, Kelly FJ. Raman Spectral Imaging for the Detection of Inhalable Microplastics in Ambient Particulate Matter Samples. Environ Sci Technol. 2019;53(15):8947-56.
11. Liu K, Wang XH, Fang T, Xu P, Zhu LX, Li DJ. Source and potential risk assessment of suspended atmospheric microplastics in Shanghai. Sci Total Environ. 2019;675:462-71.
12. Vianello A, Jensen RL, Liu L, Vollertsen J. Simulating human exposure to indoor airborne microplastics using a Breathing Thermal Manikin. Sci Rep-Uk. 2019;9.
13. Zhang Q, Zhao YP, Du FN, Cai HW, Wang GH, Shi HH. Microplastic Fallout in Different Indoor Environments. Environ Sci Technol. 2020;54(11):6530-9.
14. Prata JC, da Costa JP, Lopes I, Duarte AC, Rocha-Santos T. Environmental exposure to microplastics: An overview on possible human health effects. Sci Total Environ. 2020;702.
15. Pauly JL, Stegmeier SJ, Allaart HA, Cheney RT, Zhang PJ, Mayer AG, Streck RJ. Inhaled cellulosic and plastic fibers found in human lung tissue. Cancer Epidemiol Biomarkers Prev. 1998;7(5):419-28.

16. Amato-Lourenco LF, Carvalho-Oliveira R, Junior GR, Dos Santos Galvao L, Ando RA, Mauad T. Presence of airborne microplastics in human lung tissue. J Hazard Mater. 2021;416:126124.
17. Eriksen M, Mason S, Wilson S, Box C, Zellers A, Edwards W, et al. Microplastic pollution in the surface waters of the Laurentian Great Lakes. Mar Pollut Bull. 2013;77(1-2):177-82.
18. Hidalgo-Ruz V, Gutow L, Thompson RC, Thiel M. Microplastics in the marine environment: a review of the methods used for identification and quantification. Environ Sci Technol. 2012;46(6):3060-75.
19. Prata JC. Airborne microplastics: Consequences to human health? Environ Pollut. 2018;234:115-26.
20. Wright SL, Kelly FJ. Plastic and Human Health: A Micro Issue? Environ Sci Technol. 2017;51(12):6634-47.
21. Li YW, Shao LY, Wang WH, Zhang MY, Feng XL, Li WJ, Zhang DZ. Airborne fiber particles: Types, size and concentration observed in Beijing. Sci Total Environ. 2020;705.
22. Law BDB, W. B.; Hesterberg, T. W. Solubility of Polymeric Organic Fibers and Manmade Vitreous Fibers in Gambles Solution. Inhalation Toxicology. 1990;2(4):321-39.
23. Munno K, Helm PA, Jackson DA, Rochman C, Sims A. Impacts of temperature and selected chemical digestion methods on microplastic particles. Environ Toxicol Chem. 2018;37(1):91-8.
24. Free CM, Jensen OP, Mason SA, Eriksen M, Williamson NJ, Boldgiv B. High-levels of microplastic pollution in a large, remote, mountain lake. Mar Pollut Bull. 2014;85(1):156-63.
25. Cowger W, Booth AM, Hamilton BM, Thaysen C, Primpke S, Munno K, et al. Reporting Guidelines to Increase the Reproducibility and Comparability of Research on Microplastics. Appl Spectrosc. 2020;74(9):1066-77.
26. Brander SM, Renick VC, Foley MM, Steele C, Woo M, Lusher A, et al. Sampling and Quality Assurance and Quality Control: A Guide for Scientists Investigating the Occurrence of Microplastics Across Matrices. Appl Spectrosc. 2020;74(9):1099-125.
27. Horton AA, Cross RK, Read DS, Jurgens MD, Ball HL, Svendsen C, et al. Semi-automated analysis of microplastics in complex wastewater samples. Environ Pollut. 2021;268(Pt A):115841.
28. Wesch C, Elert AM, Worner M, Braun U, Klein R, Paulus M. Assuring quality in microplastic monitoring: About the value of clean-air devices as essentials for verified data. Sci Rep. 2017;7(1):5424.
29. Wright SL, Ulke J, Font A, Chan KLA, Kelly FJ. Atmospheric microplastic deposition in an urban environment and an evaluation of transport. Environ Int. 2020;136.
30. Primpke S, Wirth M, Lorenz C, Gerdts G. Reference database design for the automated analysis of microplastic samples based on Fourier transform infrared (FTIR) spectroscopy. Anal Bioanal Chem. 2018;410(21):5131-41.
31. Veerasingam S, Ranjani M, Venkatachalapathy R, Bagaev A, Mukhanov V, Litvinyuk D, et al. Contributions of Fourier transform infrared spectroscopy in microplastic pollution research: A review. Crit Rev Env Sci Tec. 2021;51(22):2681-743.
32. Ragusa A, Svelato A, Santacroce C, Catalano P, Notarstefano V, Carnevali O, et al. Plasticenta: First evidence of microplastics in human placenta. Environ Int. 2021;146:106274.
33. Silva AB, Bastos AS, Justino CIL, da Costa JP, Duarte AC, Rocha-Santos TAP. Microplastics in the environment: Challenges in analytical chemistry - A review. Anal Chim Acta. 2018;1017:1-19.
34. Dominelli PB, Ripoll JG, Cross TJ, Baker SE, Wiggins CC, Welch BT, Joyner MJ. Sex differences in large conducting airway anatomy. J Appl Physiol (1985). 2018;125(3):960-5.
35. Donaldson K, Brown RC, Brown GM. New perspectives on basic mechanisms in lung disease. 5. Respirable industrial fibres: mechanisms of pathogenicity. Thorax. 1993;48(4):390-5.
36. Horsfield K, Dart G, Olson DE, Filley GF, Cumming G. Models of the human bronchial tree. J Appl Physiol. 1971;31(2):207-17.
37. Oberdorster G, Ferin J, Lehnert BE. Correlation between particle size, in vivo particle persistence, and lung injury. Environ Health Perspect. 1994;102 Suppl 5(Suppl 5):173-9.

38. Kreyling WG, Semmler-Behnke M, Moller W. Ultrafine particle-lung interactions: does size matter? J Aerosol Med. 2006;19(1):74-83.
39. Database PP. Alkyd Resins [Available from: https://polymerdatabase.com/polymer%20classes/Alkyd%20Resin.html.
40. Liu K, Wang XH, Wei N, Song ZY, Li DJ. Accurate quantification and transport estimation of suspended atmospheric microplastics in megacities: Implications for human health. Environ Int. 2019;132.
41. Gaston E, Woo M, Steele C, Sukumaran S, Anderson S. Microplastics Differ Between Indoor and Outdoor Air Masses: Insights from Multiple Microscopy Methodologies. Appl Spectrosc. 2020;74(9):1079-98.
42. Domenech J, Marcos R. Pathways of human exposure to microplastics, and estimation of the total burden. Curr Opin Food Sci. 2021;39:144-51.
43. Porter DW, Castranova V, Robinson VA, Hubbs AF, Mercer RR, Scabilloni J, et al. Acute inflammatory reaction in rats after intratracheal instillation of material collected from a nylon flocking plant. J Toxicol Environ Health A. 1999;57(1):25-45.
44. Schirinzi GF, Perez-Pomeda I, Sanchis J, Rossini C, Farre M, Barcelo D. Cytotoxic effects of commonly used nanomaterials and microplastics on cerebral and epithelial human cells. Environ Res. 2017;159:579-87.

5 Chapter 5: General Discussion

The aims of each publication and findings will be briefly stated, in order to not repeat the information presented within the publications. Their impact, problems and limitations within each study, relevant post-publication knowledge and suggested future work will be discussed.

Each publication will now be referred to by their corresponding chapter within the Thesis:

- **Chapter 2**; Household indoor microplastics within the Humber region (United Kingdom): Quantification and chemical characterisation of particles present
- **Chapter 3**; Outdoor Atmospheric Microplastics within the Humber Region (United Kingdom): Quantification and Chemical Characterisation of Deposited Particles Present
- **Chapter 4**; Detection of microplastics in human lung tissue using μFTIR spectroscopy

Chapter 2 discussion

There was a considerably low number of AMP investigations, stating the concentrations, types and properties of MP particles within an indoor setting. This key problem influenced the major aim of Chapter 2; to reinforce this limited knowledge by stating the concentration levels within the home, a location considered 'intimate' and 'regular', and very relatable to the general population. Additionally, the purpose of Chapter 2 was to describe the MP particles present within households, in terms of their size, shape and polymer type. Particle information would, in turn, describe which MP types humans are exposed to most. It was hoped that MPs relevant to daily human exposure would be highlighted and inform future toxicity investigations, especially concerning MP type and shape.

Prior to Chapter 2 publication, literature unanimously reported the presence of MPs within indoor air (1-4). It was therefore hypothesised that MPs would indeed be found ubiquitously within these UK household samples. However, a major problem within Chapter 2, was the mean concentration rate of 1414 MP m^{-2} day^{-1} is difficult to compare to other studies. For instance, Zhang *et al.* 2020 stated a similarly passively sampled dataset, with a mean concentration of 1500-9900 MP m^{-2} day^{-1}, but included some artificial particles within MP data (3). Whilst this is not uncommon, due to a lack of standardised definitions for MPs, it results in concentrations being difficult to reliably compare. Problems distinguishing between natural and artificial polymers (5), as demonstrated within Dris *et al.* 2017, explains why cellulose, cellulose acetate, cellophane, and rayon etc. were excluded from Chapter 2 MPs data (6). Post publication, concentration rates using passive sampling have continued to highlight prevalence within indoor homes; 3095 MP fibres m^{-2} day^{-1} within Australia (7), 78,839 – 96, 367 MP m^{-2} day^{-1} within China (8) and 3735 ± 1343 MP m^{-2} day^{-1} within the UK (9). Indoor concentration rates are included with the aim of 'cautious' comparison, but a strong consideration of

varying methodologies and MP definitions is stressed. However, it is clear that all publications continue to support a higher concentration of MPs within an indoor environment, compared to outdoors.

The dominance of fibrous AMPs (1, 3, 10), and fragmented (2, 11), as well as an increase in particle number with decrease in particle size within all studies, allowed the same hypotheses to be formed within Chapter 2. Post publication, these particle trends are continuing to present within indoor literature (7, 8, 12). Each future research article is now building upon the knowledge of past publications and confirming these particle traits improves the reliability of human exposure knowledge. By applying SOPs, where possible, for instance defining fibre and fragment based on aspect ratio (2, 7), publications can become more comparable in the future.

Chapter 2 set out to investigate households in order to gain an understanding into regular human exposure, however, a limitation of this paper is the inability to gain respirable MP concentration rates due to the passively sampled nature of the methodology. In order to gain respirable data, the volume intake of sampled air is needed, alongside MP number. However, the importance of this study remains, in that, Chapter 2 provided a cost-effective and easily replicable study into household MPs, allowing the particle properties to be obtained. At the time of publication the large sample size and duration of the study was an improvement to the field, and would not have been possible with the application of an active pump device. This improvement in sample size continues, to date (7-9). An active sampler was acquired with a future aim of actively sampling homes simultaneously alongside passive, in order to investigate sampling differences and obtaining more relevant human respiratory information. However the Covid-19 pandemic restricted this type of household interaction, and the study was terminated (13). Interestingly, the first publication investigating this combined sampling approach has recently been published (9), and it is now suggested here that future investigations into indoor MPs utilise this approach, where time and funding permit.

The omnipresence of PET MPs within Chapter 2 samples, as well as PP and PA, is complementary to both the field of AMPs and human exposure. The dominance of fibrous particles, and these types of synthetic polymer types suggests a textile source, and these are some of the most mass produced synthetic polymers within this industry, as well as the plastics industry as a whole (14, 15). This is supported when comparing other common MP types identified prior to Chapter 2 publication were; PES, Acrylic and PP (3); PP PA and PE (1), PES, PE and PA (2) and PS, PET, PE (4). Post-publication indoor MP studies also report; PA, PUR, PE and PET within China (8), and PE, PES, PET, PA within Australia (7), and PET, PVC, PP and PE within the UK (9). The commonly reoccurring reports of PET, PP, PA and PE, as well as all other plastic types identified, can guide future toxicity studies into

the consequences of exposure to these synthetic polymers, this improves the environmental relevance of toxicity investigations, which has been lacking.

A limitation within Chapter 2 is the methodology applied when selecting particles for chemical analysis. Particles were observed with a stereomicroscope and transferred via tweezers and needle tools to the diamond compression cell. This in retrospect could have favoured larger particles, as smaller were more difficult to manipulate. The relevance here, is that it is hypothesised that the smallest of MPs would be the most toxic upon human inhalation. However, due to the high number of particles taken to analysis, the technique for picking up particles improved dramatically, with particles being able to 'stick' to the tools and transfer fairly easily, with little particle loss. Nevertheless, it is a stated limitation for Chapter 2, and thus direct analysis under an infrared transparent or reflective filter is suggested for future experiments, if cost permits.

Overall, Chapter 2 provided a useful insight into AMP prevalence and properties, pushing the boundaries of study duration, sample size, quality control, detailed methodology reporting, high number of particles chemically analysed and a first introduction to a LOD/ LOQ approach to quality control within AMP research. All of these factors can improve future investigations, providing a higher quality of data available and allowing data to become more harmonised. The knowledge of PET, PP and PA fibrous particles, and fragmented to a lesser degree, emphasises their importance in terms of human exposure.

Chapter 3 Discussion

Following on from the previous household investigation, Chapter 3 aimed to passively sample different outdoor locations within the urbanised city of Kingston Upon Hull. As well as this, one of these sites was monitored longitudinally. Although not as limited as indoor literature, outdoor AMP knowledge was also lacking prior to Chapter 3 publication. The key aim within Chapter 3 was to define AMP concentration rates and particle characteristics, within different sampling regions, in the hope of gaining more insight into how specific areas of varying human activity influence human exposure. Additionally, it was the aim of this Chapter, to apply different methodology, in which particles could be analysed directly upon the laboratory filter, with the hope of aiding the analysis of smaller inhalable MPs, a problem encountered within Chapter 2.

The phenomenon of increasing particle concentration with decreasing particle size was predicted, as well as their ubiquitous nature throughout samples. The concentration of MPs identified within this study and literature are now briefly stated here, but an emphasis on caution is specified due to the varying methodologies applied, similar to Chapter 2. Urbanised locations have been previously reported to have high MP concentrations (6, 10, 16, 17). A mean concentration rate for the site 1 (A63) sampling site was reported 3055 ± 5072 MP m^{-2} day^{-1}, which greatly exceeds these. Within the city of London, UK, a concentration of 771 ± 167 MP m^{-2} day^{-1} was stated. The city of London has a higher total population compared to Kingston upon Hull, thus it could be predicted for concentrations to represent this, however, when focusing on local population density rather than total, Kingston upon Hull has a higher density than London (within the areas sampled) (18). Thus, local population density is a suggested future parameter to include within future sampling methodology. Additionally, it could be suggested that the sampling height affected the concentration rate within London, due to a rooftop sampling height being applied (~50m), and MPs being reported to have a lower concentration, vertically (19). Within Chapter 3, a sampling height similar to respirable adult breathing was applied, with the aim of this specific height being most representative of human exposure. Finally, varying rates throughout UK cities could be caused by fluctuating concentrations over different months, only captured by longitudinal investigation. Wright *et al.* 2020 was only sampled for 1 month (16). This is demonstrated within Chapter 3, where the concentration rate for the 2-week snapshot investigation at the A63 site was reported as 603 MP m^{-2} day^{-1} as opposed to the annual rate previously stated. Overall, Chapter 2 reports a longitudinal concentration higher than indoor Chapter 2 levels, suggesting particular sampling hotspots can rival that of indoor locations, however, differing methodologies, again, make it difficult to draw conclusions from data. Future investigations need to be conducted to build upon the limited concentration data available, specifically for ‘hotspots’.

A strength of this outdoor publication was the investigation into multiple location types within an urbanised setting. No publications had investigated this number of location types prior to Chapter 3 publication. Specifically focussing on different types of human activity it was hoped that a better understanding of MP exposure would be gained. Unfortunately, the 2-week snapshot investigation is a limitation, as results are less reliable in such a short time-frame. Roadside locations such as site 2, 3 and 4 were higher in concentration compared to the residential setting, supporting vehicular suspension of MPs as well as human activity (20). However, it was expected that during Covid-19 'lockdown' there would be a lower MP prevalence due to a sudden lack of activity, but this was not the case, especially for the first month. The lack of meteorological data could explain the fluctuations observed within Chapter 3, however, when looking the wider picture; the initial aim of the investigation was to identify MPs likely relevant to human exposure, rather than sources and transport of environmental MPs. It would have been extremely time consuming collecting meteorological data alongside the large number of particle chemical analyses applied. Thus more research into urbanised sources and suspension is suggested, such as the recent HYSPLIT and FLEXPART transport models (21). However, roadside, commercial and industrial sampling locations do have a high abundance of MPs and seem relevant to human exposure, based on the 2-week sampling.

Interestingly, unlike all prior publications, film-like particles were identified in great amounts within Chapter 3. This was extremely unexpected within urbanised locations, as studies predict textile and fragmented plastic product sources. It could be that there was an abundance of specific sources nearby that result in film like particle production, for example, plastic degradation and carrier bag remnants (22), however it is unclear as to why these would be so abundant within these samples, both within snapshot and longitudinal results, and not reported within other studies. It could be that the settling velocity of film particles is smaller than that of other particles, and that transport from other locations has occurred (23). More research into the region of Kingston upon Hull is essential to fully understand the picture here, because film like particles have not been extensively researched within AMP studies (24). The knowledge that humans are exposed to high amounts of film like particles compliments the field in its own right, as it has not yet been stated before, and could therefore be a neglected area of research. Fragmented particles were also prevalent, to a lesser extent in the longitudinal study, and more-so within the snapshot study. This suggests fragmented sources originating from other locations such as industrial settings in which a variety of polymer types were identified.

The prevalence of PE does suggest plastic product degradation from thin plastics such as carrier bags, that would produce film like particles. However resin particles were also abundant, as well as Nylon, and PET. Such a variety of polymer types (total 25) supports the outdoor environment being a much more complex compartment, influenced by countless factors, especially compared to an indoor

setting. Consequentially, much more research is needed to understand each synthetic polymer type concentration, source, and characteristics. However, the synthetic polymer types stated do concur with MPs literature (20), regardless of shape, and thus human exposure knowledge is gained.

In terms of methodology, Chapter 3 had the limitation of a subset of particles undergoing analysis, and the assumption of accurate data after extrapolation to represent the filter, and again to represent the units of m^{-2} day^{-1}. In retrospect, the complex samples could have been simplified via density separation, allowing more relevant particles to be analysed at a greater proportion of analysed filter. However the high number of particles taken to FTIR analysis greatly improves the validity of this study, as well as the use of field, procedural and laboratory blanks – ultimately accounting for contamination at every stage of investigation. Again, a LOD/ LOQ approach was applied in order to ensure data did not represent contamination. Building upon the limitation of Chapter 2, this method applied Anodisc filter analysis in order to directly characterise and identify particles upon the filter, avoiding the manipulation of particles altogether. This allowed the ease of smaller particle identification and improves methodology within literature, and post-publication this filter has now been discussed in more depth, showing specific advantages alongside limitation (25).

Ultimately, a lot more 'unknowns' are stated within this chapter, in terms of where the specific MPs have been sourced from, and why there are so many fluctuating levels and inconsistent shapes compared to other studies. However, Chapter 3 benefits the field by increasing knowledge, highlighting films, fragments, PE, Nylon, Resin and PET as relevant to human exposure. The publication also introduces the possibility of scientific research combining with local councils in order to gain sampling location information, permission for placing sampling equipment in a variety of locations and also gaining information relating to safe locations in which the samplers are likely not to be vandalised. All of these factors improve the quality of the investigation.

Chapter 4 Discussion

There was a focus within this Chapter, on the analysis of human lung tissue samples. The primary aim was to identify the presence, or absence, of MPs within lung tissue samples. In doing so, it was hoped that results would complement the field of MP inhalation by providing evidence to support, or oppose, MP inhalation as an exposure route for humans. Parallel to this aim, Chapter 4 strived to apply strict quality control assurances in order to provide a valid dataset. Additionally, if any MPs were identified within samples, they could be characterised in terms their particle size, shape and polymer type, with the aim of gaining as much information relevant to human exposure, as possible. Furthermore, MPs identified within human lung tissue samples could be compared to prior Chapter results, thus observing whether the MPs present within the air represent those found within the lungs, if at all.

The ubiquitous presence of AMPs was stated prior to Chapter 4 publication, yet, the concerns regarding human inhalation were speculative, and building (26). Prior to publication, fibrous particles had been 'observed' within human lung tissue samples (27), and more recently identified, via Raman Spectroscopy (28). It was not expected to identify MPs within human lung tissue samples, due to the size restrictive physiology of the lungs and the limitations of the chemical analysis tool applied (29, 30). Yet, Chapter 4 is one of the first publications reporting the presence of MPs within human lung tissue, and at a concentration above that of background contamination adjustments.

A total of 13 lung tissue samples were analysed within Chapter 4, and 20 within Amato-Laurenço *et al*. 2021 (28). A mean lung tissue mass of 4.26g and 3.28g per sample was stated, respectfully. The sample size, and mass of lung tissue analysed, is considered small, and results gained from both publications indicate that more research is necessary, in order to draw firm conclusions. However, serving as the first publications to chemically and physically identify MPs within human lung tissue, specific particle properties that humans are exposed to, can now be highlighted in terms of their relevance to human health.

Amato-Lourenço *et al.* 2021 identified one single MP particle within their 2 procedural blanks, indicating a low contamination rate alongside positive control blanks (28). Chapter 4, however, identified 9 particles within 17 procedural/ laboratory blanks. This difference in contamination is likely due to the larger number of blanks conducted here, important in increasing the validating of this study. However, taking into account the low contamination rate of Amato-Lourenço *et al.* 2021, it seems necessary to compare MP abundance within human lung tissue, after control adjustments; 0.69 ± 0.84 MP/g within chapter 4, as opposed to 0.56 MP/g within Amato-Lourenço *et al.* 2021 (28). Between the two publications, a similar abundance of MPs is present, yet the differing methodology

approaches, again, makes it difficult to draw firm conclusions. Within both publications, PP is highlighted as relevant to human exposure.

Chapter 4 identified 39 MPs within human lung tissue samples, consisting of 12 synthetic polymer types; PP, PET and Resin being most abundant. Comparatively, PP and PE were predominantly identified via μRaman (28). The common identification of PP particles within lung tissue is complemented by atmospheric abundance (1, 6, 11), as well as their identification within Chapter 2 and 3 samples. PP and PE are the most mass produced plastics, globally (15). It could be assumed that a high manufacture production has a 'knock on' effect on product use, leading to high release into the atmosphere, and consequentially resulting in a higher likelihood of human inhalation. Resin, additionally identified within the lungs of Patients, within Chapter 4, was a common plastic type to a lesser degree. Resin represented a high proportion of particle fallout within Chapter 3, suggesting outdoor sources such as; industrial and roadsides, are important in terms of MP inhalation.

Whilst fibre and fragmented particles were both abundant within Chapter 4 lung tissue samples, Amato-Lourenço *et al.* 2021 reported fragmented more concentrated (88%) (28). Both studies applied similar length to width ratio standard operating procedures for categorising particles into shapes, thus avoiding bias. Therefore this particle shape difference shows the variations that can be observed within different sample sets, and thus the necessity for larger sample sizes. However, the abundance of fragment and fibres within lungs, and the environment (1, 2, 6, 10) suggests these are relevant to future toxicity investigations. Film particle were identified low within lung tissue, and although uniquely highlighted in abundance within Chapter 3, it could be considered that these forms are less likely inhaled, perhaps due to their particle properties or their likelihood to stay airborne (23).

The major unexpected result from Chapter 4 is the size ranges of particles identified within the lungs. Particles with a diameter <3 μm are expected to be identified within the lower lungs, and MPs >10 μm thought to be size excluded within the lungs due to impaction and removal. Already stated within Chapter 4 is the prevalence of larger particles, compared to these parameters, initially suggesting an influence of contamination, however strict quality control and assurances were adhered to. Pauly *et al.* 1998 did highlight larger particles within all regions of human lung samples, but the results were lacking detail via chemical analysis, and thus more lung tissue analysis is vital in order to understand the prevalence of larger MPs within human lungs (27). Amato-Lourenço *et al.* 2021, using a lower size detection limit, exclusively identified particles smaller than 5.5 μm which indeed seems more applicable to inhalation. Nevertheless the presence of these larger particles above contamination levels raises questions (28). Interestingly, post publication, larger particles have since been reported within lung tissue (31, 32), supporting the validity of Chapter 4. Additionally, imaging of a cellulose fibre embedded within human lung tissue, with a size range of 887 μm and 24 μm supports the ability

of larger fibres to penetrate the lungs via inhalation (32). This also emphasises the need for future work to investigate the toxicity of natural/ artificial particles within atmospheric samples, due to their ubiquity (33), regardless of their inclusion within the MP definition.

One study reported 14.19 ± 14.57 MP/g of human lung tissue, a level greater than Chapter 4, as well as greater than human tissue samples from other regions of the body (31). Contrastingly the PP and Resin (Chapter 4) and PP and PE (28), were not in abundance, but instead PVC, PA, PET and PBS were highlighted as abundant within human lungs (31). PES was in abundance within Chen *et al.* 2022 (32), as well as BALF samples indicating synthetic polymers; PAN (34), PE PET PP (35), PP PE PES (36).

The presence of commonly identified plastic particles present within the lungs; PP, PE, PET, PES etc., as well as fragment and fibre shape abundance, provides the foundations for relevant human toxicity research to be built upon. Preliminary investigations into associations of MP presence within the lungs and cancer have been reported, as well as the suggestion of age increasing the accumulation of MPs within the lungs, and the suggestion of larger particles being able to escape mucociliary clearance (32). Additionally, the potential for polymer type, size, shape, adsorbed pollutant, biofilm and leachates entering the lungs is now of direct importance to human health.

Conclusion

Future Research Priorities and Directions

A harmonised approach

It is now clear that in order for the field of AMPs to progress, standardised definitions of MPs need to be achieved. Their size, traditionally 1 μm – 5 mM, is an essential factor, yet, studies that accept this size range, more often than not, cannot analyse the smallest MPs due to analysis constraints, as seen within the publications presented within this thesis. This is a hurdle that needs to be overcome in order for publications to become truly comparable. As well as size, there should also be a consensus regarding whether natural and artificial particles can be classified as a MP. Experts within the AMP field could therefore combine opinions to form an agreed definition, as seen within the aquatic compartment (37). However, in the meantime, it is of great importance that clear and transparent reporting of MP definitions, and the analysis limitations within each publication, are stated within future publications.

A harmonised approach also applies to sampling conditions and methodologies, and is a key priority. The use of quality controls, filter substrates, density separation, microscopic and chemical analysis protocols etc., all differ greatly within research. Data comparisons can only be reliable after achieving standardisation, and this, in turn, permits the formation and application of SOPs. Whilst these SOPs

are still developing, using similar sampling devices, such as an identical passive sampler that is cheap and easy to use, allows the capture of particles in a standardised manner. Additionally, an example of a standardised method emerging within the field is the use of the 3:1 length to width ratio rule, when categorising MPs as fibres. The particle 'tweezer' methodology method applied during Chapter 2 was inefficient and lacked standardisation, yet trialling this approach led to the development of the Anodisc approach, applied within Chapter 3 and 4. Thus, these publications are indeed improving the field, and leading to novel approaches that have since been applied to other research (38). The developments published within the AMP field continue, and should be promoted.

Improving analysis

The analysis of AMP samples has progressed from little, or no chemical analysis, to publications requiring chemical verification in order to be accepted. This is a great improvement within the field, and progress continues by ensuring a large number, or proportion, of particles are taken to analysis. Recent advances have led to fully automated analysis, through FPA FTIR analysis and Laser Direct Infrared Imaging (LDIR) (39). The removal of human bias and error will complement the field and reduce the time consumed within the laboratory, but regardless, there is a call for new analysis techniques that reliably characterise particles < 1 μm. A possible combined approach, utilising multiple chemical verification tools could be applied, providing a holistic analysis of multiple particle size ranges. The limitations of one approach, could essentially be complemented by another. Procedural and laboratory controls were not regularly applied, or reported, within research, however, higher quality investigations and reporting of contamination adjustments should take place. Chapter 2 was the first introduction of the LOD/ LOQ technique within the AMP field, and was effectively applied to Chapter 3 and 4. In order to continue progressing, it is recommended that future research apply this stringent contamination adjustment, thus improving validity. It could also be investigated as to whether other characteristics of AMPs could be used within LOD/ LOQ adjustments, such as size and shape, rather than polymer type. Finally, of great importance for future work, is the necessity for longitudinal sampling and large sample sizes, due to the fluctuations of AMPs observed within Chapters 2 and 3.

Future AMP research

There are countless ways in which the AMP field can continue, as the foundations are only just being established. As already stated, achieving SOPs is key, but multiple other 'branches' of research can greatly impact the limited knowledge. Prior to this thesis, most sampling investigations into AMPs were of an environmental focus, and not particularly focussed on areas most relevant to human exposure. It is now necessary for additional research to take place in sampling locations most relevant to humans, as observed within Chapter 2 and 3. By investigating locations such as schools, hospitals,

transport, common work spaces, shopping centres, and other outdoor locations, a better understanding of human exposure can be gained, rather than relying solely on households and outdoor hotspots. It is clear that AMPs are ubiquitous, but as concentration is driven by human activity, it is paramount that human exposure is understood, fully. In doing so, demographics such as age, pre-existing medical conditions, smoking, gender and others, can be assessed to determine who is most at risk of inhaling AMPs.

The mounting evidence supports MPs are indeed present within lung tissue, thus larger sample sizes are necessary to build upon the quality of current human tissue analysis. Conclusions regarding MP inhalation and specific demographics can be drawn, with greater validity, when sample size is increased. For instance, Chapter 4 would benefit greatly from an increase in human lung tissue samples, especially when investigating whether MP accumulation within the lungs occurs over time, thus age potentially being a risk factor of MP retention. An interesting perspective concerning human exposure to MPs is the potential to analyse full body cadavers for a holistic assessment on which tissues can be penetrated, and determining what MP characteristics are hazardous within what tissue. To date, multiple organ analysis suggests MP translocation (40), but full cadaver analysis would provide great insight into human exposure. Future *in-vitro* and *in-vivo* investigations can capitalise on the growing knowledge regarding the types and characteristics of AMPs that humans are regularly exposed to, and steer away from unrealistic concentrations, shapes and sizes, and apply exposures of relevance. For this to take place, the production of MPs within the laboratory, that mimic AMPs captured within samples, must be created.

Advice and future policies

Ultimately, there is considerable pressure to reduce the plastic consumed, worldwide. This is achieved by avoiding plastic products that are not essential, re-using plastic that is, and disposing of plastic waste appropriately. The formation of policies, regular environmental monitoring of AMPs, educating the population on AMP presence and exposure, and further plastic research can facilitate this.

The U.K. government aims to “eliminate avoidable plastic waste by end of 2042”, with initiatives such as deposit return schemes, simplifying household recycling, working alongside packaging providers, banning certain single use plastic products, and imposing a fee on plastic products containing <30% recycled plastic (41). Research, including the publications presented within this thesis, can direct and influence future policies. Additional policies can potentially be agreed, that can regularly monitor AMPs, in a similar fashion to environmental pollutants and air quality assessments. Chapter 3 is an example of how collaborating with a local authority can positively benefit AMP research. Filters containing particulate matter, collected by local councils, can be analysed and

potentially correlated to other monitored pollutant levels. A future network could be created in which Council filters, that would otherwise be disposed of, are analysed in a standardised manner, nationwide, and the data can subsequently be combined to observe a holistic picture of AMP pollution and air quality. Local councils also present the benefit of local knowledge, resources, and contacts to boost education within the region, as well as access to specific sampling sites of interest.

Education is key to ensuring plastic use and waste is reduced, allowing a public understanding of the importance of responsible plastic use. Involving education within schools, workshops and even advertisements can ease the strain on the circularity of the plastic lifecycle. AMPs is a novel field, and thus it can be assumed that the public is less aware of plastics within the air we breathe, than for instance, compared to MPs within the oceans. But combining research into the health effects of AMP exposure, alongside environmental impacts, ensures a dual approach, captivating as much interest as possible from the population. Education can impact AMP prevalence and exposure, by informing the population of the importance of choices when purchasing clothes, furnishings and other products, allowing informed decisions into their health and triggering positive behavioural changes (42).

It is essential that AMP research continues, and hurdles are addressed and tackled. Research into bio-plastics can alter the trajectory of the plastics industry. Bio-plastics such as Polylactic acid (PLA), derived from plant material, can avoid fossil fuel use and provide an alternative to plastic use (42). However, properties are yet to mimic the desirable qualities of petrochemical plastics, and costs to produce bio-plastics remain high. Thus promoting future research in this field is key. Additionally future research into the successful breakdown of synthetic plastics using macro-organisms, for instance caterpillars of wax moths, *and* microbial agents (42), has the potential to influence the circularity of the plastic lifecycle.

‘One Health’

‘One Health’ is an approach to understanding the impact of a balanced ecosystem, involving the environment, animals and the people within it. The usefulness of this approach was highlighted during the Covid-19 pandemic, in which there was a close connection between humans and their environment (43). This approach applies to the MP field, because it is clear that there are interconnecting pathways associated with the presence of MPs within the environment, organisms and humans, as well as transport between these. As MPs are ubiquitous on land, water, air and within the food and drink we consume, it is clear that they are impacting every level of ecosystems. Thus in order to understand the impact of MPs, a holistic approach is essential. This thesis bridges the gap between environmental MPs and human health, providing the much needed link between AMPs and human inhalation. A future emphasis on this ‘One Health’ approach can positively impact the knowledge of MPs as a whole, as AMP can complement environmental research, and vice versa.

Collaborating with all sector experts, communicating, sharing knowledge, co-ordinating research, forming research communities and fully understanding the impact of any publications, can greatly impact many sectors (44). The unique methodologies and QA/ QC applied within the publications presented here, may benefit many sectors outside of AMP research. The human population is increasing, air quality declining, and intensive farming and pollution put pressure on the shared environment that should be maintained (44), therefore research has started to encompass this 'One Health' approach, ensuring the development of future research (45).

Conclusion

Overall, the thesis greatly contributes to the field of AMPs and human respiratory health. The three publications apply a quality above that of other available research, directing future work to achieve a similar standard. In particular, this research has been key in developing unique methodologies to accurately process AMP and tissue samples, whilst ensuring the highest QA/ QC. Additionally, utilising Anodisc filters to directly analyse particles, upon the FTIR, with minimal manual manipulation provides a novel method to combat the size limitations, and potential contamination, associated with AMP research.

All data supports AMPs being abundant within environmental samples, especially within areas of high human activity and indoors. PET, PP, PA MPs were identified within homes, and PE, Resin, Nylon and PET within outdoor MP 'hotspots'. All of these MPs have also been identified within human lung tissue analyses, within this thesis and other available publications. The environmental monitoring of this 'microplastic cocktail' within indoor and outdoor air presents a good opportunity to understand what MPs we are likely exposed to regularly. The identification of these specific synthetic polymer types, as well as fibrous and fragmented particle shapes being most abundant, and smaller particles being most prevalent, can allow human toxicity research to be better guided and focus more on MPs relevant to human exposure. Ultimately, the thesis supports the human inhalation of MPs, via the analysis of human lung tissue samples. The importance of understanding the consequences of this inhalation is now of great importance.

References

1. Dris R, Gasperi J, Mirande C, Mandin C, Guerrouache M, Langlois V, Tassin B. A first overview of textile fibers, including microplastics, in indoor and outdoor environments. Environ Pollut. 2017;221:453-8.
2. Vianello A, Jensen RL, Liu L, Vollertsen J. Simulating human exposure to indoor airborne microplastics using a Breathing Thermal Manikin. Sci Rep-Uk. 2019;9.
3. Zhang Q, Zhao YP, Du FN, Cai HW, Wang GH, Shi HH. Microplastic Fallout in Different Indoor Environments. Environ Sci Technol. 2020;54(11):6530-9.
4. Gaston E, Woo M, Steele C, Sukumaran S, Anderson S. Microplastics Differ Between Indoor and Outdoor Air Masses: Insights from Multiple Microscopy Methodologies. Appl Spectrosc. 2020;74(9):1079-98.

5. Cai H, Du F, Li L, Li B, Li J, Shi H. A practical approach based on FT-IR spectroscopy for identification of semi-synthetic and natural celluloses in microplastic investigation. Sci Total Environ. 2019;669:692-701.
6. Cai LQ, Wang JD, Peng JP, Tan Z, Zhan ZW, Tan XL, Chen QQ. Characteristic of microplastics in the atmospheric fallout from Dongguan city, China: preliminary research and first evidence. Environ Sci Pollut R. 2017;24(32):24928-35.
7. Soltani NS, Taylor MP, Wilson SP. Quantification and exposure assessment of microplastics in Australian indoor house dust. Environ Pollut. 2021;283:117064.
8. Cui J, Chen C, Gan Q, Wang T, Li W, Zeng W, et al. Indoor microplastics and bacteria in the atmospheric fallout in urban homes. Sci Total Environ. 2022;852:158233.
9. Ageel HK, Harrad S, Abdallah MA. Microplastics in indoor air from Birmingham, UK: Implications for inhalation exposure☆. Environ Pollut. 2024;362.
10. Dris R, Gasperi J, Saad M, Mirande C, Tassin B. Synthetic fibers in atmospheric fallout: A source of microplastics in the environment? Mar Pollut Bull. 2016;104(1-2):290-3.
11. Allen S, Allen D, Phoenix VR, Le Roux G, Jiménez PD, Simonneau A, et al. Atmospheric transport and deposition of microplastics in a remote mountain catchment (vol 12, pg 339, 2019). Nat Geosci. 2019;12(8):679-.
12. Amato-Lourenco LF, Dos Santos Galvao L, Wiebeck H, Carvalho-Oliveira R, Mauad T. Atmospheric microplastic fallout in outdoor and indoor environments in Sao Paulo megacity. Sci Total Environ. 2022;821:153450.
13. Government If. Timeline of UK government coronavirus lockdowns and restrictions. 2022.
14. Chen XM, H. H.; Wan, Y.; Marriam, I.; Tebyetekerwa, M. Circular Economy and Sustainability of the Clothing and Textile Industry. Materials Circular Economy. 2021;3.
15. Europe P. Plastics - the Facts 2022 2022 [Available from: https://plasticseurope.org/knowledge-hub/plastics-the-facts-2022/.
16. Wright SL, Ulke J, Font A, Chan KLA, Kelly FJ. Atmospheric microplastic deposition in an urban environment and an evaluation of transport. Environ Int. 2020;136.
17. Klein M, Fischer EK. Microplastic abundance in atmospheric deposition within the Metropolitan area of Hamburg, Germany. Sci Total Environ. 2019;685:96-103.
18. Statistics OfN. Census 2021 2021 [Available from: https://www.ons.gov.uk/census/maps/choropleth/population/population-density/population-density/persons-per-square-kilometre.
19. Liu K, Wang XH, Fang T, Xu P, Zhu LX, Li DJ. Source and potential risk assessment of suspended atmospheric microplastics in Shanghai. Sci Total Environ. 2019;675:462-71.
20. O'Brien S, Rauert C, Ribeiro F, Okoffo ED, Burrows SD, O'Brien JW, et al. There's something in the air: A review of sources, prevalence and behaviour of microplastics in the atmosphere. Sci Total Environ. 2023;874:162193.
21. Nafea TH, Chan FKS, Xu YY, Wang CJ, Wang XY, Zhao WL, et al. Microplastics Aloft: A comprehensive exploration of sources, transport, variations, interactions and their implications on human health in the atmospheric realm. Earth-Sci Rev. 2024;255.
22. Iniguez ME, Conesa JA, Fullana A. Microplastics in Spanish Table Salt. Sci Rep. 2017;7(1):8620.
23. Preston CA, Neuman CLM, Aherne J. Effects of Shape and Size on Microplastic Atmospheric Settling Velocity. Environ Sci Technol. 2023;57(32):11937-47.
24. Zhang YL, Kang SC, Allen S, Allen D, Gao TG, Sillanpää M. Atmospheric microplastics: A review on current status and perspectives. Earth-Sci Rev. 2020;203.
25. Liu Y, Prikler B, Bordos G, Lorenz C, Vollertsen J. Does microplastic analysis method affect our understanding of microplastics in the environment? Sci Total Environ. 2023;902:166513.
26. Prata JC. Airborne microplastics: Consequences to human health? Environ Pollut. 2018;234:115-26.

27. Pauly JL, Stegmeier SJ, Allaart HA, Cheney RT, Zhang PJ, Mayer AG, Streck RJ. Inhaled cellulosic and plastic fibers found in human lung tissue. Cancer Epidemiol Biomarkers Prev. 1998;7(5):419-28.
28. Amato-Lourenco LF, Carvalho-Oliveira R, Junior GR, Dos Santos Galvao L, Ando RA, Mauad T. Presence of airborne microplastics in human lung tissue. J Hazard Mater. 2021;416:126124.
29. Donaldson K, Brown RC, Brown GM. New perspectives on basic mechanisms in lung disease. 5. Respirable industrial fibres: mechanisms of pathogenicity. Thorax. 1993;48(4):390-5.
30. Gasperi J, Wright SL, Dris R, Collard F, Mandin C, Guerrouache M, et al. Microplastics in air: Are we breathing it in? Curr Opin Env Sci Hl. 2018;1:1-5.
31. Zhu L, Kang Y, Ma M, Wu Z, Zhang L, Hu R, et al. Tissue accumulation of microplastics and potential health risks in human. Sci Total Environ. 2024;915:170004.
32. Chen QQ, Gao JN, Yu HR, Su H, Yang Y, Cao YJ, et al. An emerging role of microplastics in the etiology of lung ground glass nodules. Environmental Sciences Europe. 2022;34(1).
33. Stanton T, Johnson M, Nathanail P, MacNaughtan W, Gomes RL. Freshwater and airborne textile fibre populations are dominated by 'natural', not microplastic, fibres. Sci Total Environ. 2019;666:377-89.
34. Baeza-Martinez C, Olmos S, Gonzalez-Pleiter M, Lopez-Castellanos J, Garcia-Pachon E, Masia-Canuto M, et al. First evidence of microplastics isolated in European citizens' lower airway. J Hazard Mater. 2022;438:129439.
35. Qiu L, Lu W, Tu C, Li X, Zhang H, Wang S, et al. Evidence of Microplastics in Bronchoalveolar Lavage Fluid among Never-Smokers: A Prospective Case Series. Environ Sci Technol. 2023;57(6):2435-44.
36. Chen C, Liu F, Quan S, Chen L, Shen A, Jiao A, et al. Microplastics in the Bronchoalveolar Lavage Fluid of Chinese Children: Associations with Age, City Development, and Disease Features. Environ Sci Technol. 2023;57(34):12594-601.
37. Ho KT, Bjorkland R, Burgess RM. Comparing the definitions of microplastics based on size range: Scientific and policy implications. Mar Pollut Bull. 2024;207:116907.
38. S VLL, Liddle CR, Atherall CA, Chapman E, Watkins M, S DJC, Rotchell JM. Microplastics in human blood: Polymer types, concentrations and characterisation using muFTIR. Environ Int. 2024;188:108751.
39. Morera-Gómez YAG-V, M.; Pescoso-Torres, B. M.; Helguera-Pedraza, Y.; García-Chamero, A.; Alonso-Morejón, A.; Bernard, N.; Alonso-Hernández, C. M. Advancing data processing for microplastics characterization: Laser direct infrared (LDIR) analysis of atmospheric deposition in Cienfuegos, Cuba. Atmospheric Pollution Research. 2025;102715.
40. Roslan NS, Lee YY, Ibrahim YS, Tuan Anuar S, Yusof K, Lai LA, Brentnall T. Detection of microplastics in human tissues and organs: A scoping review. J Glob Health. 2024;14:04179.
41. Smith L. Plastic waste research briefing: UK Parliament House of Commons Library; 2024 [Available from: https://commonslibrary.parliament.uk/research-briefings/cbp-8515/.
42. Munhoz DR, Harkes P, Beriot N, Larreta J, Basurko OC. Microplastics: A Review of Policies and Responses. Microplastics-Basel. 2023;2(1):1-26.
43. WHO. World Health Organization; [Available from: https://www.who.int/europe/initiatives/one-health.
44. CDC. About One Health 2025 [Available from: https://www.cdc.gov/one-health/about/index.html.
45. Prata JC, da Costa JP, Lopes I, Andrady AL, Duarte AC, Rocha-Santos T. A One Health perspective of the impacts of microplastics on animal, human and environmental health. Sci Total Environ. 2021;777:146094.

Supplemental Material

Chapter 2

Supporting Information Cover Sheet

Authors: Lauren C. Jenner, Laura R. Sadofsky, Evangelos Danopoulos, and Jeanette M. Rotchell.

Manuscript Title: Household indoor microplastics: quantification and chemical characterisation of particles present.

Summary: 13 pages: 2 Figures, 5 Tables, 1 Method, and 1 Survey Questionnaire

Figure S1

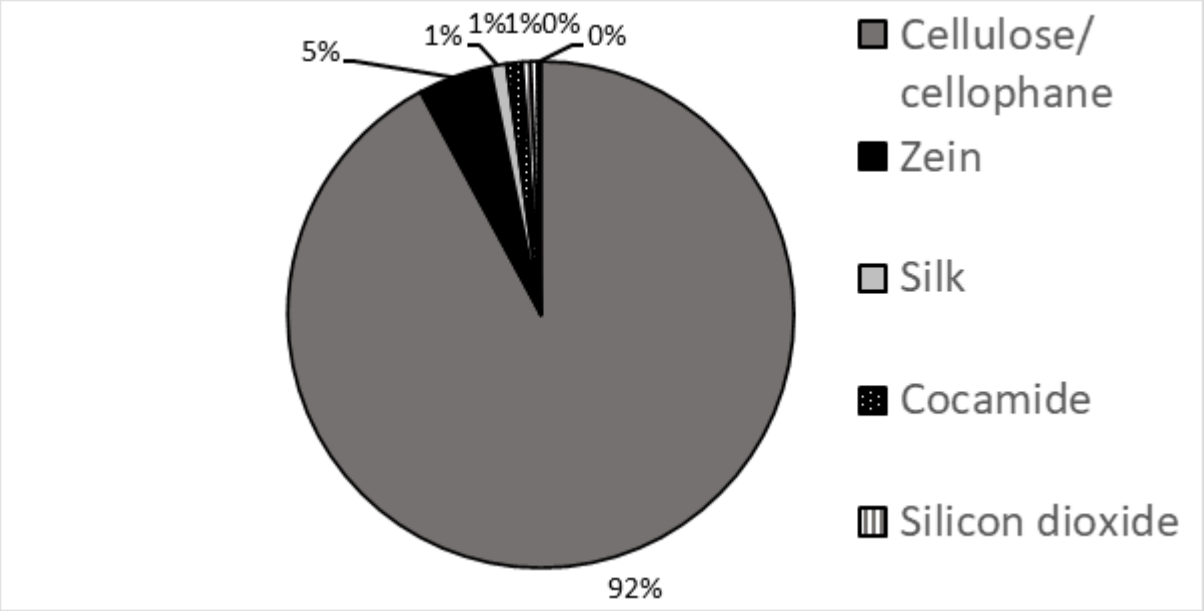


Figure S1. Pie chart displaying non-MP particle types alongside their abundance.

Figure S2

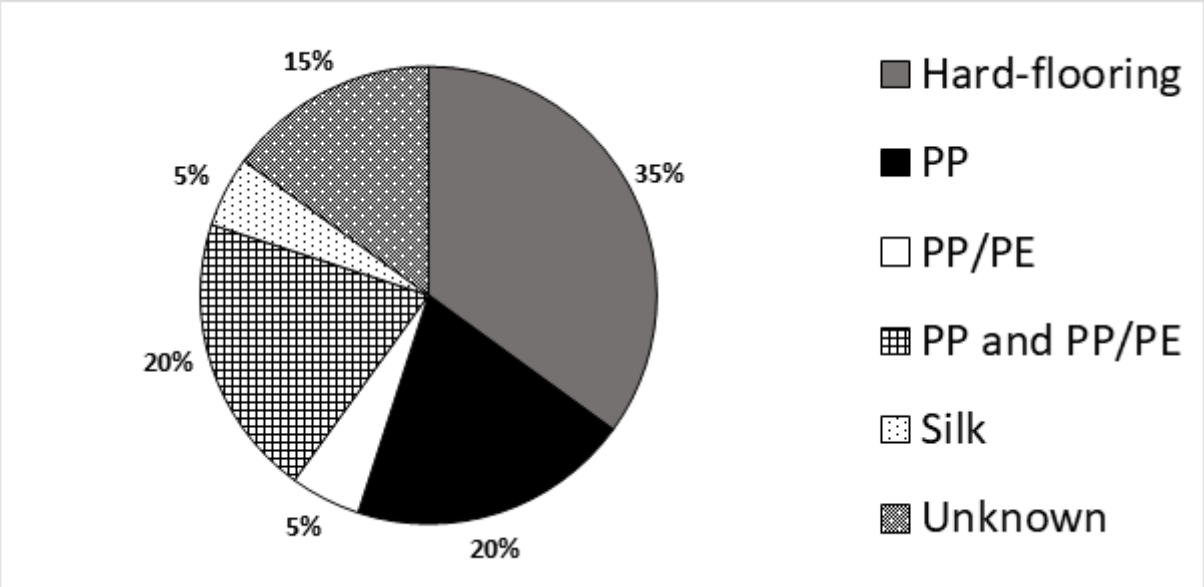


Figure S2. Pie chart displaying the flooring/carpet compositions for the households investigated.

Chapter 3

atmosphere

MDPI

Supplementary Materials

Outdoor Atmospheric Microplastics within the Humber Region (United Kingdom): Quantification and Chemical Characterisation of Deposited Particles Present

Lauren C. Jenner, Laura R. Sadofsky, Evangelos Danopoulos, Emma Chapman, David White, Rebecca L. Jenkins and Jeanette M. Rotchell

Methods 1. SM1 Calculation used to determine the LOD/LOQ for each MP polymer

[A] = MP polymer quantity in analysed proportion of a sample	A = 2
[B] = MP polymer quantity in whole sample ([A]*8) **	B = 16 (2*8)
[C] = Mean MP polymer quantity in blanks	C = 0.2±0.4
[D] = Blank correction ([B]-[C])	D =15.80 (16-0.2)
[LOD] = 3*SD of [C] or 1.1 (whichever value is higher)	[LOD] = 1.2 (3*0.4)
[LOQ] = 10* SD of [C] or 3.3 (whichever value is higher)	[LOQ] = 4 (10*0.4)

Blank corrected results above the LOQ will be included in final concentrations**:** [D] > LOQ included in results

** For the two samples in which one quarter of the Anodisc was analysed by FTIR = ([A]*16).

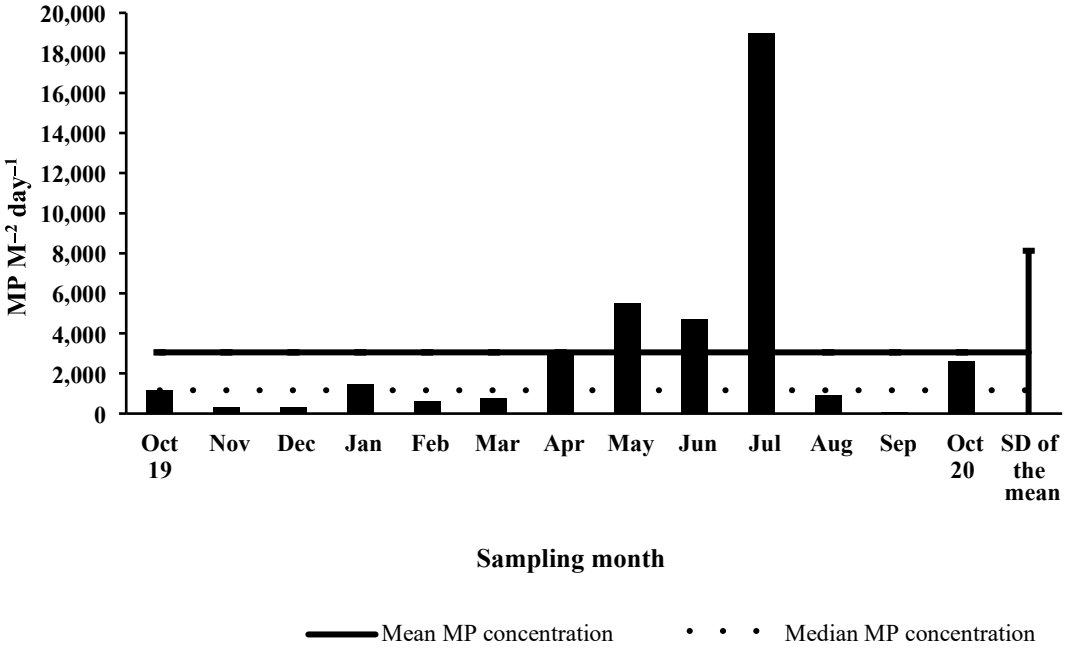
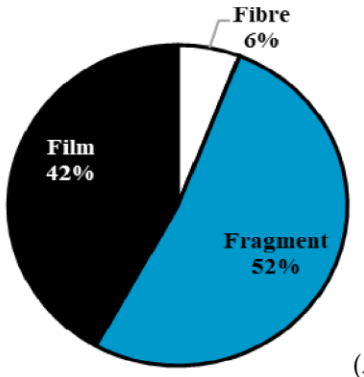
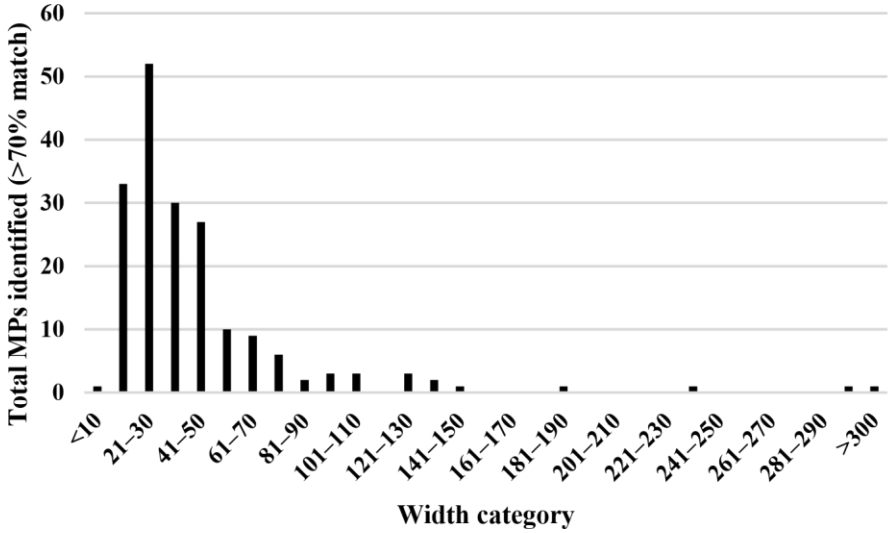


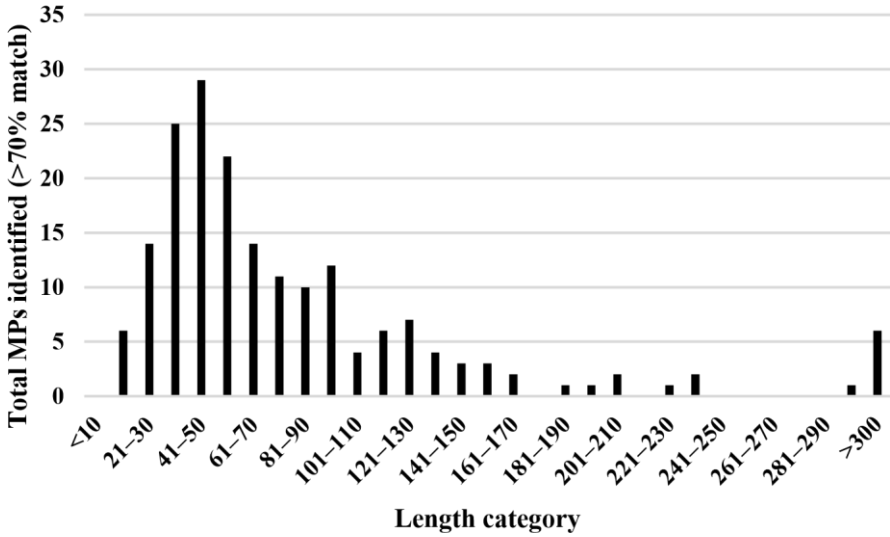
Figure S1. Chart showing the MP levels (MP m^{-2} day^{-1}) during each 2-week sampling period per month, for the 13-month investigation of Site 1. The solid bar indicates the mean value. Dotted line indicates the median value. April and May coincided with an official SARS-CoV lockdown.



(A)



(B)



(C)

Figure S2. MP characteristics observed in the atmospheric samples obtained from all sites, from a single 2-week sampling period. (**A**) shape, (**B**) length, and (**C**), width.

Table S1. The background level of MPs detected within procedural and laboratory blanks for the 13-month sampling at Site 1 and the 2-week investigation of 5 sampling sites. No MPs were detected in any of the 5 field blanks.

Procedural blanks	MPs identified	MP polymer type
1 (Site 1)	6	Nylon (2) PET (1) PP/ PE (1) PS (1) Resin (1)
2 (Site 1)	1	PE (1)
3 (Site 2, 3, 4, 5)	4	PES (1) PET (1) PP (1) TPE (1)
4 (Site 2, 3, 4, 5)	1	PES (1)

Laboratory blanks	MPs identified	MP polymer type
Site 1 Oct-19	0	-
Site 1 Nov	0	-
Site 1 Dec	0	-
Site 1 Jan	4	PP/ PE (1) PPA (1) PVP (2)
Site 1 Feb	0	-
Site 1 Mar	4	Co-polymer (1) Nylon (3)
Site 1 Apr	6	Nylon (1) PS (4) PP/ PE (1)
Site 1 May	1	Co-polymer (1)
Site 1 Jun	5	Co-polymer (1) PPA (1) PS (2) PVC (1)
Site 1 Jul	1	PP/ PE
Site 1 Aug	0	-
Site 1 Sep	0	-
Site 1 Oct-20	0	-
Site 2 Feb	0	-
Site 3 Feb	1	Nylon (1)
Site 4 Feb	0	-
Site 5 Feb	5	PES (1) PET (1) PP (2) TPE (1)

Chapter 4

Detection of microplastics in human lung tissue

Jenner et al.

Supplemental information.

Two Figures:

Figure S1. Overview of the procedural and laboratory blanks used to determine background contamination.

Figure S2. μFTIR spectral images of MP particles identified within human lung tissue samples. A, B, C and D = (A=PET) (B=PUR) (C=Resin) (D=PAN). E and F = MPs identified within blanks. (E=PS) (F=PP).

Two Tables:

Table S1. Background levels of MP contamination detected in each sample and blank.

Table S2. MP results for lung region and gender, displayed in three formats; unadjusted raw data, subtraction adjusted data and LOD LOQ adjusted data.

One Methods:

Methods S1. Contamination adjustment methods

Fig. S1

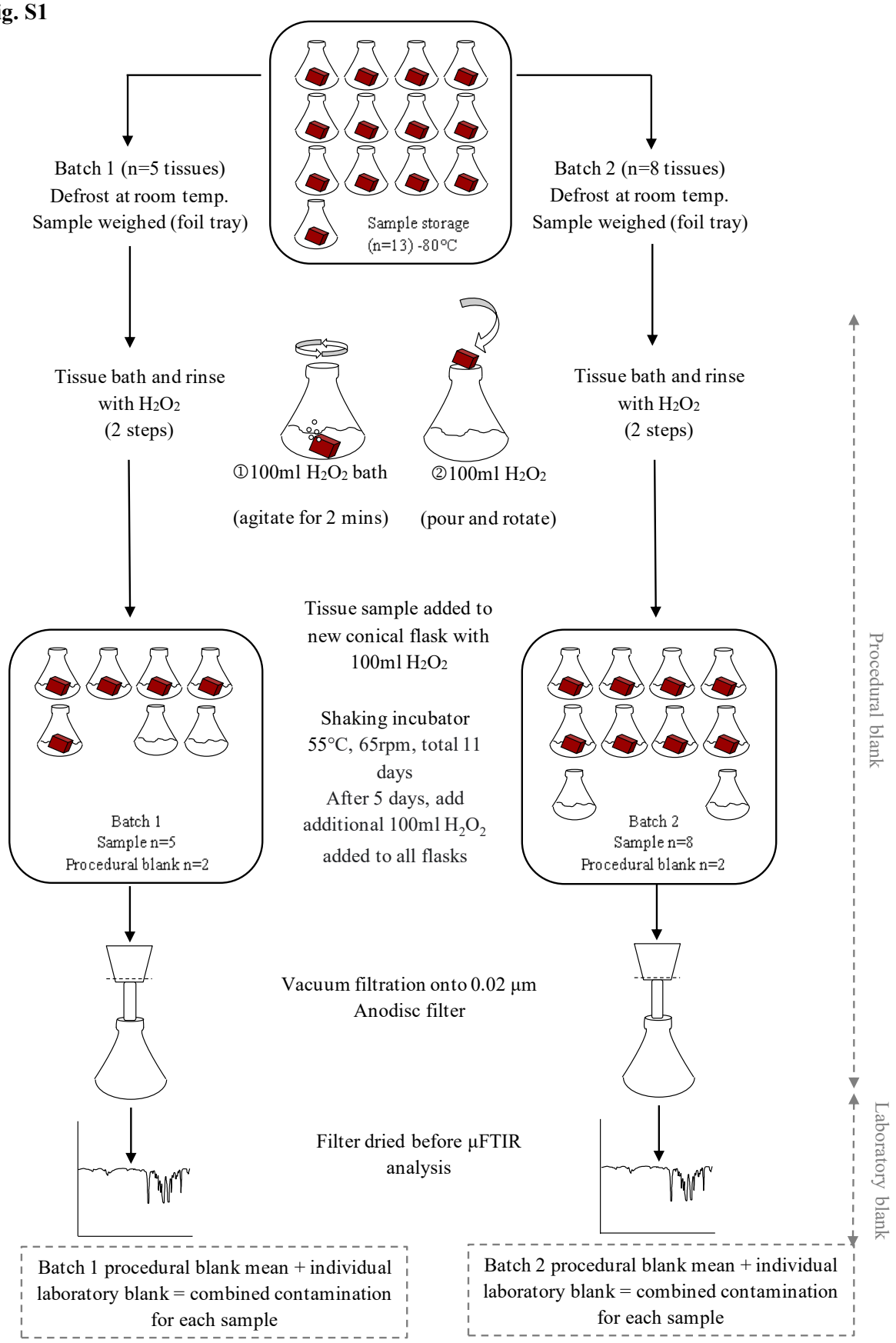
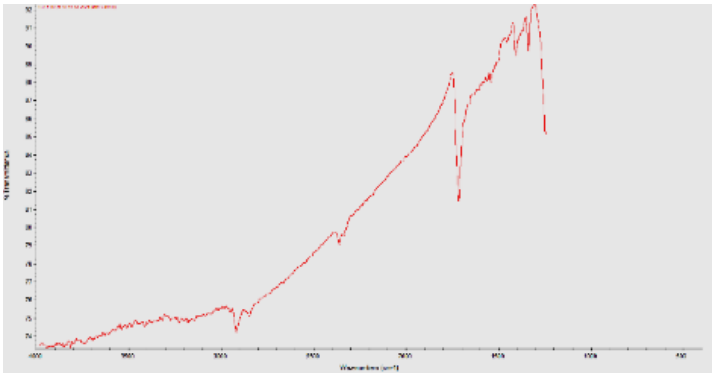
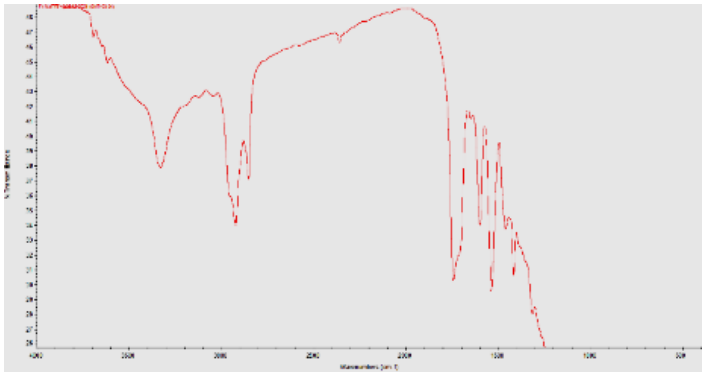

Batch 1 (n=5 tissues)
Defrost at room temp.
Sample weighed (foil tray)
Sample storage
(n=13) -80°C
Batch 2 (n=8 tissues)
Defrost at room temp.
Sample weighed (foil tray)
Tissue bath and rinse with H2O2 (2 steps)
①100ml H2O2 bath
(agitate for 2 mins)
②100ml H2O2
(pour and rotate)
Tissue bath and rinse with H2O2 (2 steps)
Tissue sample added to new conical flask with 100ml H2O2
Shaking incubator 55°C, 65rpm, total 11 days
After 5 days, add additional 100ml H2O2 added to all flasks
Batch 1
Sample n=5
Procedural blank n=2
Batch 2
Sample n=8
Procedural blank n=2
Vacuum filtration onto 0.02 µm Anodisc filter
Filter dried before µFTIR analysis
Procedural blank
Laboratory blank
Batch 1 procedural blank mean + individual laboratory blank = combined contamination for each sample
Batch 2 procedural blank mean + individual laboratory blank = combined contamination for each sample

Fig. S2

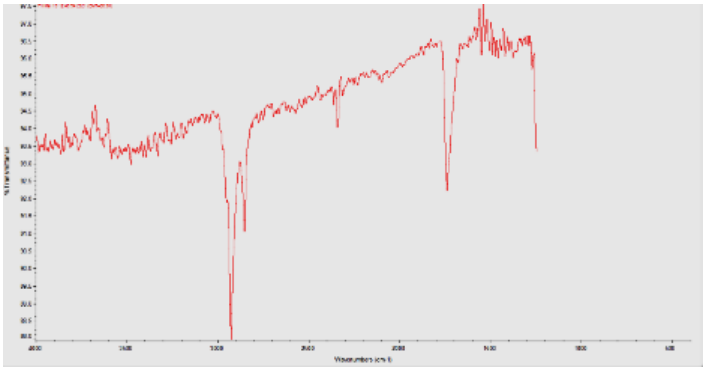
A



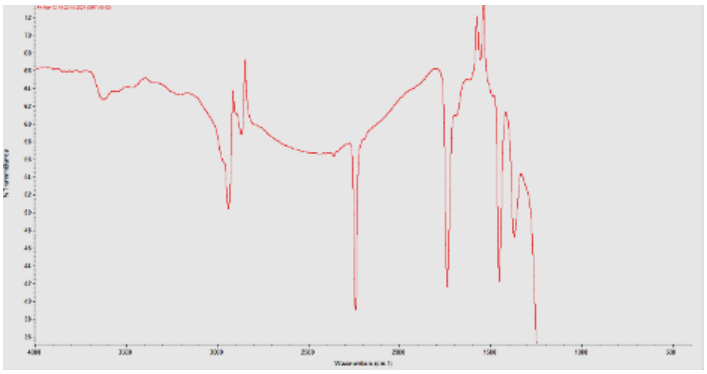
B



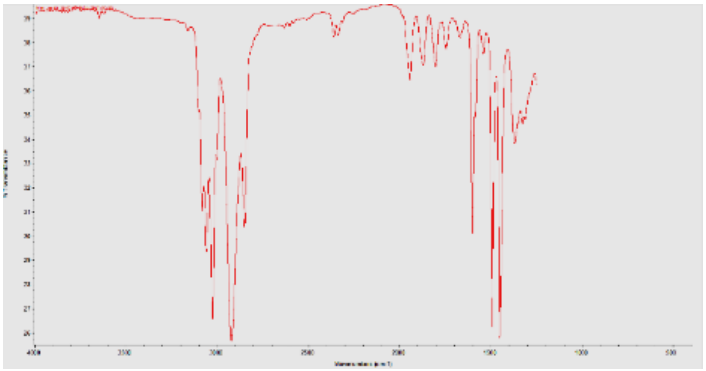
C



D



E



F

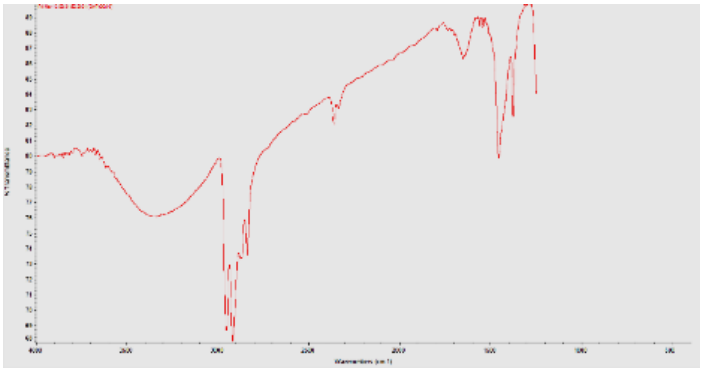


Table S1

Procedural blank	**No. of MPs**	**MP polymer**	**Length, width (μm)**	**Shape**
1	4	1 x PE 1 x PE/PP 1 x PS 1 x Res	144, 40 33, 19 50, 17 110, 68	Fibre Fragment Fragment Fragment
2	0	none detected	none detected	none detected
3	0	none detected	none detected	none detected
4	0	none detected	none detected	none detected
Laboratory blank		**MP polymer**	**Length, width (mm)**	**Shape**
1.1	0	none detected	none detected	none detected
1.2	2	1 x PS 1 x PTFE	161, 73 23, 22	Fragment Fragment
2.1	0	none detected	none detected	none detected
2.2	0	none detected	none detected	none detected
3.1	0	none detected	none detected	none detected
4.1	1	1 x PVA	315, 16	Fibre
5.1	0	none detected	none detected	none detected
6.1	1	1 x PET	43, 15	Film
7.1	1	1x PP	68, 40	Fragment
8.1	0	none detected	none detected	none detected
9.1	0	none detected	none detected	none detected
10.1	0	none detected	none detected	none detected
11.1	0	none detected	none detected	none detected
Total:	9			
Mean ±SD:	0.53±1.07			

Table S2

Category	Unadjusted MP/g of tissue	Subtraction adjusted MP/g of tissue	LOD LOQ adjusted MP/g of tissue
Upper lung tissue samples	0.80±0.96	0.23±0.28	0.00±0.00
Middle (left lingular) lung tissue samples	0.41±0.37	0.33±0.37	0.00±0.00
Lower lung tissue samples	3.12±1.30	1.65±0.88	0.49±0.97
Male samples	2.09±1.54	0.91±0.95	0.24±0.69
Female samples	0.36±0.50	0.33±0.52	0.00±0.00

Methods SM1:

Contamination adjustment approach 1: Subtraction

For each sample, the MPs identified within combined blanks were minimised from the total MPs identified within a sample.

E.g.

[A] Batch 1 procedural blank average MP	= 0.00
[B] Sample 4.1 laboratory blank MP	= 1.00
[C] Combined blank ([A]+[B])	= 1.00
[D] MPs identified in sample 4.1	= 2.00
[E] Subtraction adjustment ([D]-[C])	= 1.00
[F] Sample 4.1 tissue mass (g)	= 1.53
[G] MP/g ([E]/[F])	= 0.65

Contamination adjustment approach 2; Limits of Detection and Quantification

The LOD LOQ adjustment applies a subtraction adjustment before a threshold for detection and quantification is calculated, but importantly takes into account polymer type.

[A] PE quantity identified in sample 3.1	= 2.00
[B] Mean ± SD of PE identified within entire combined blanks	= 0.19 ± 0.25
[C] Blank correction ([A]-[B])	= 1.81
[D] LOD (3xSD of [B]) or (1.1) – whichever value is higher	= 1.1
[E] LOQ (10xSD of [B]) or 3.3 – whichever value is higher	= 3.3

PE particles identified within sample 3.1 can only be detected if [C] > [D]
PE particles identified within sample 3.1 can only be quantified if [C] > [E]

Within sample 3.1, PE particles can be detected but not reliably quantified and therefore PE particles identified here are not included in the data or overall MP/g of human tissue values.