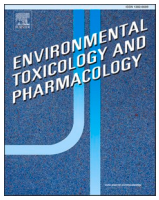
Environmental Toxicology and Pharmacology 101 (2023) 104204

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap



Microplastics: Detection in human samples, cell line studies, and health impacts

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ARTICLE INFO

Edited by Paolo Pastorino

Keywords:
Microplastics
Sample preparation
Ingestion of microplastics
Human body
Human matrices
Exposure
Spectroscopic analysis

ABSTRACT

Microplastics (MPs) are in all environmental compartments, including atmosphere, terrestrial, and aquatic environments as well as in marine organisms, foods, drinking water, and indoor and outdoor environments. MPs can enter the human body through the food chain and contaminated environment. Ingestion, inhalation, and dermal contact are the routes of their entry into the human body. Recent studies reporting the detection of MPs within the human body have raised concern among the scientific community as the knowledge about human exposure is still very limited and their impact on health is not well-understood yet. In this review article, we briefly cover the reports evidencing MP detection within the human body, e.g., stool, placenta, lungs, liver, sputum, breast milk, and blood. A concise synopsis of sample preparation and analysis of such human matrices is also provided. This article also presents a summary of the effect of MPs on human cell lines and human health.

1. Introduction

In the 20th century, worldwide plastic production already reached 320 million tons per year, and over 40% was utilized as single-use packaging. This led to the generation of huge quantities of plastic waste, a significant percentage of which ended up in the seas and oceans. For example, the seabed in the Mediterranean Sea is facing a significant issue of plastic from debris gathered on the coastlines and in the ocean (Ragusa et al., 2021). Microplastics (MPs) are smaller than 5 mm in size. They originate from a wide range of sources, and their occurrence, movement, and eventual fate in the environment are all influenced by several natural variables in addition to their inherent physicochemical characteristics (C. Wang et al., 2021). MPs are not only produced by the fragmentation or deterioration of large-sized plastic items (secondary MPs) but also synthesized commercially (primary MPs) for many applications.

Microplastics (MPs) also contaminated freshwater, atmospheric, and terrestrial environment and they have also been detected in seafood, sea salt, tea bags, bottled drinks, and in many other foods. In addition, they are detected in the gastrointestinal tract of marine animals. Since they are foreign entities inside the tissues, they can induce immunoreactions (Ragusa et al., 2021). Small size but large surface area promotes their chemical interactions with physiological fluids and tissues. MPs are of particular concern due to their persistence, bioaccumulation in the animals and human food chain, potential toxicity, and ability to act as pathogen and pollutant vectors (da Silva Brito et al., 2022; Wright and Kelly, 2017). Indeed, hundreds of reports highlight the presence of MPs' in aquatic and other organisms. For example, fish can ingest MPs accidentally or intentionally as they appear like natural food items and are very small in size (Crawford and Quinn, 2017). The ingestion of MPs in fish is reported in approximately 150 species of freshwater and marine systems (Jabeen et al., 2017). MPs can cause physical damage to fish and

Abbreviations: MPs, Microplastics; NPs, Nanoplastics; Py-GC-MS, Pyrolysis-gas chromatography-mass spectrometry; ATR, Attenuated total reflectance; LDIR, Laser direct infrared; PBS, Polybutylene succinate; PE, Polyethylene; PP, Polypropylene; PA, Polyamide; PVC, Polyvinylchloride; PVA, Polyethylene vinyl acetate; PS, Polystyrene; PET, Polyethyleneterepththalate; PU, Polyurethane; PVAc, polyvinyl propionate/acetate; PLGA, Poly(lactic-co-glycolic acid); PMMA, Polymethylmethacrylate; PC, Polycarbonate; POM, Polyoxymethylene; HDPE, High density polyethylene; LDPE, Low density polyethylene; CPE, Chlorinated polyethylene; ABS, Acrylonitrile butadiene styrene; PES, Polyester; NC, Nitrocellulose; Nylon-EVA, Nylon-ethylene-vinyl acetate; PM, Particulate matter; PCB, Polychlorinated biphenyls.

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https://doi.org/10.1016/j.etap.2023.104204
Received 20 March 2023; Received in revised form 15 June 2023; Accepted 18 June 2023
Available online 28 June 2023
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transport other hazardous substances (Wang et al., 2020).

The main entry points for MPs into the human body are ingestion, inhalation, and dermal contact. With an estimated intake of 39–52 thousand MPs per person annually, ingestion is regarded as the primary pathway (Ragusa et al., 2022). Humans are exposed to MPs directly through the atmosphere, drinking water, and sea salt or indirectly through the food chain. After MP consumption, only MPs that are tiny enough or that have formed a biocompatible surface "corona" pass through intestinal mucus to reach the intestinal cells. Then, internalization of MPs takes place. After intestinal absorption, MPs can enter to circulatory system and then can deposit in organs like gut, liver, kidney etc. Inhalation is another route and according to estimates a person can consume 272 MPs per day through this route. MPs deposited in lungs can cause inflammation. The smaller MPs can enter into the circulation or lymphatic system by macrophages. This can cause further respiratory complications. While dermal contact with MPs is not a major concern, it is important to note that MPs with a size smaller than 100 nm have the potential to cross the skin barrier (Gautam et al., 2022b). Although the tiniest particles (nanoplastics, NPs) may really infiltrate cells, cause the inflammatory response, and interfere with normal cellular activity, it is yet unknown if the amount of MPs present in human organs is sufficient to have health-damaging consequences (Sorci and Loiseau, 2022). After being consumed, MPs can cross cell membranes and travel to other body parts, where they may trigger certain cellular functions. Therefore, the health risk posed by the internalization and accumulation of MPs is a significant concern, as demonstrated by several studies indicating negative consequences in animal models (da Silva Brito et al., 2022), marine creatures (Wang et al., 2020), and human cell lines (Gautam et al., 2022a; Yong et al., 2020). Recent research has shown that exposure to MPs negatively affects male fertility and sperm quality, thus posing a threat to successful conception (D'Angelo and Meccariello, 2021). The health impacts of MPs on humans are not well-understood, but many studies on animals indicate their translocation to different organs, leading to adverse effects (Ramsperger et al., 2023). However, the key factors contributing to their toxicity are physical and chemical properties, concentrations, and microbial films (Sajid et al., 2023). Fig. 1 provides prospective pathways and routes of exposure to MPs/NPs and potential toxic effects on humans.

Due to the abundance of MPs in the environment and their potential toxicity to humans, it is of great significance to assess their presence in different human organs, tissues, or matrices. Due to the severity of the problem, reports are emerging on detecting MPs in real human samples (Basri K et al., 2021; Kutralam-Muniasamy et al., 2023). Indeed, in many of the studies, MPs have been detected in various samples, including stool, placenta, liver, lungs, sputum, and washes of the skin, face, and head. This review provides an overview of these studies, briefly covering methods, outcomes, and reported correlations. This article also provides a summary on the effects of MPs on human cell lines and on the health impact of MPs.

2. Sample collection and preparation

Human biological samples were collected using different procedures mainly dictated by the nature of the samples. The collection settings varied between clinical and non-clinical environments, depending on the samples. Various measures were taken in all the studies to avoid MP contamination during the sample collection.

The placenta samples were collected under clinical settings. One study involved six healthy women with uneventful pregnancies and vaginal delivery, which were selected after following certain exclusion criteria (Ragusa et al., 2021): this study involved use of cotton gloves, cotton towels, metal clippers and scalpels, etc. during the sample collection as a measure to avoid MP contamination. Another study involved two women having cesarean delivery with singleton pregnancies and breech presentation and considered the use of negative controls and screening of all the materials used during the cesarean delivery as a part of quality control during sample collection (Braun et al., 2021).

In some studies (Wibowo et al., 2021; Luqman et al., 2021; Ho et al., 2022; Zhang et al., 2021), feces were collected under non-clinical

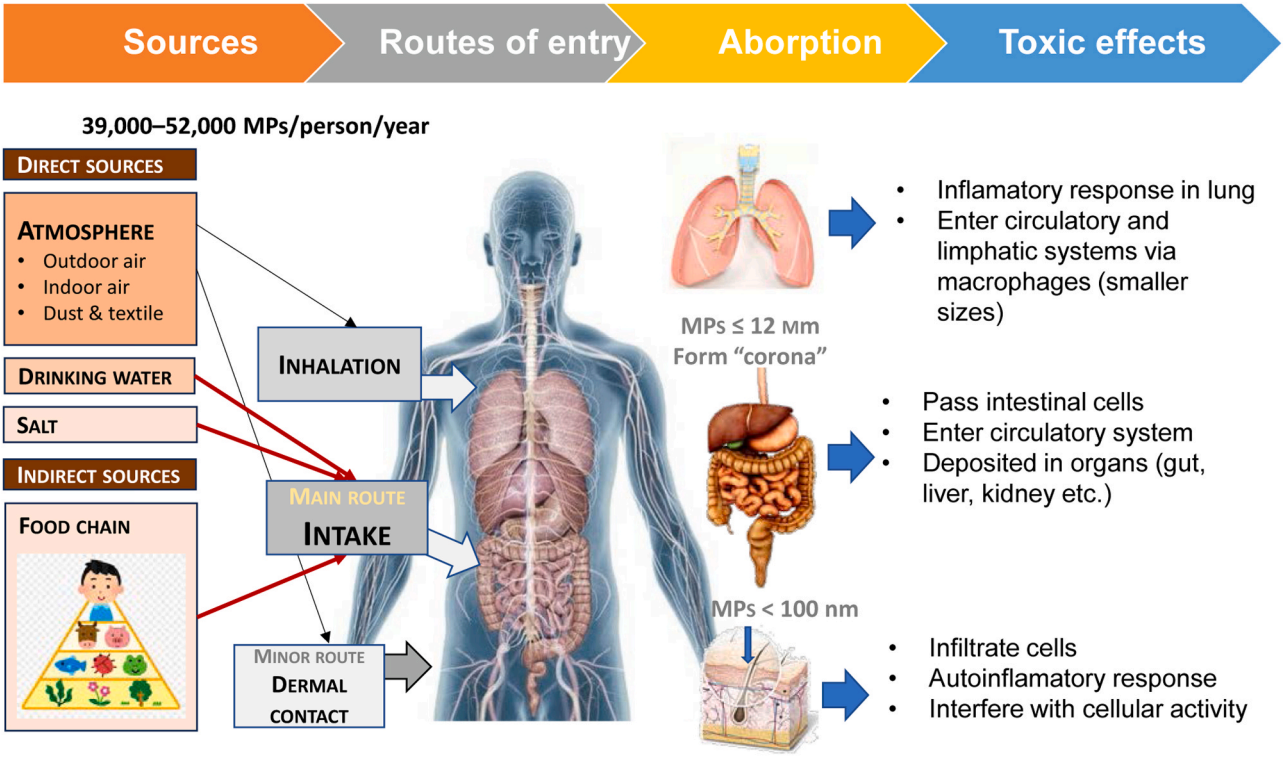

Fig. 1. Potential pathways and routes of exposure to MPs/NPs and potential toxic effects on humans.

settings. Dietary intakes were monitored for a certain period before sample collection. The participants were trained to collect the samples to avoid contamination. The use of plastic containers was avoided, instead glass container or wooden sticks were employed for sample collection. Besides, procedural blanks were employed for quality control. Only, in one study reported by Ho et al. (2022) who collected feces from healthy people and those suffering from IBD, the samples were collected in clinical settings even through the participants were also trained. There is a relatively larger number of studies using feces because they are easy to collect and do not involve any invasive procedures for sample collection.

The samples such as lung or liver, or colon tissues were obtained using clinical/surgical procedures (Jenner et al., 2022). For example, a colectomy specimen was obtained from patients undergoing colectomy, employing measures to avoid any contact between the sample and plastic materials during the surgery. The samples were stored in formalin or glass petri dish enclosed filter paper, both were monitored for MPs contamination before use (Ibrahim et al., 2021). The lung tissues were also obtained during the autopsy performed to determine the cause of the death (Amato-Lourenço et al., 2021).

Among the body fluids, saliva, sputum, blood, broncho alveolar lavage fluid (BALF), and breast milk were studied for the presence of MPs (Baeza-Martínez et al., 2022; Huang et al., 2022; Leslie et al., 2022; Ragusa et al., 2022). Saliva, sputum, and breast milk are easy to obtain as they do not involve any surgical or invasive procedures. The blood samples were obtained by venipuncture. The BALF samples were obtained using a minimally invasive approach. Again, the measures were taken to minimize the contamination during the sample collection. In other cases, the collection of samples after washing hand and face, head hair, and saliva followed a well-defined protocol to make samples comparable.

After sample collection, the major step is sample preparation. Table 1 summarizes the sample preparation and analysis used to detect MPs in samples collected from the human body. It mainly involves digestion of other matter and separation of MPs. Wet digestion, enzymatic digestion, or density separation are among sample preparation methods used for MPs in biological samples. Several approaches can be used alone or in combination.

3. Methods for analysis of MPs in biological samples

The utilization of established methodologies for MP detection and characterization is essential given the concern around MPs' effects on animal and human health.

The techniques that have been utilized for analysis of MPs in human samples fall into three categories:

i. Microscopy (stereo microscopes, optical microscopes, scanning electron microscope (SEM), fluorescence microscopes after Red Nile Stainning)
ii. Spectroscopy (Raman, FTIR)
iii. Separation (Chromatography coupled with mass spectrometric detection i.e. pyrolysis gas chromatography.mass sepctrometry (py–GC–MS), high performance liquid chromatography with tandem mass spectrometry (HPLC–MS/MS))

The microscopes are used to identify the particle size and shape —MPs > 0.1 mm can be observed with the naked eyes, MPs < 0.1 mm and > 1 μm can be seen by stereo or optical microscopes, and MPs < 1 μm can be visualized by electron microscopes. Other particles (e.g. cellulose, ceramics, etc) may be misidentified as MPs. Optical microscope (mostly stereomicroscopes) are the most commonly reported for an initial visual and physical characterization of MPs from human samples (Wibowo et al., 2021; Abbasi and Turner, 2021; Huang et al., 2022; Ragusa et al., 2021; Amato-Lourenço et al., 2021; Ibrahim et al., 2021), probably because it is a robust and inexpensive system that is available in almost all laboratories. Alternatively or complementarily,

Table 1
Brief description of sample treatment and analysis techniques used for detection of MPs in human matrices.

Matrix	Sample pretreatment	Analysis	Ref.
Filtered washes of the hand and face skin, head hair and saliva	**For hand, face or hair samples that appeared turbid**: • Reduction of volume by heating • Removal of organic matter by adding 35 mL of 35% H_2O_2 for 2–10 days. • Elimination of residual H_2O_2 heating • Addition of 50 mL of $ZnCl_2$ (1.6 g/cm^3), shaking for 5 min, and allowed to settle for 90 min • Centrifugation of remaining supernatants and vacuum filtration through 2 μm filter paper. **Samples without visible contamination: Vacuum filtration** • Filters were air-dried at room temperature in a glass cabinet for few days and then, transferred to Petri dishes for counting.	Binocular microscopy at up to 200 × magnification Polarized light microscopy Fluorescence microscopy μRaman Spectra 400–800 cm^{-1} Size determined ≥ 10 μm	(Abbasi and Turner, 2021)
Urine	• Digestion with KOH 10% at a ratio sample/solution of 1:2 (v/v) for 48 h at 40 °C • Filter through 1.2 μm pore size glass fiber filter	Binocular microscopy using × 10 and x100 objectives μRaman Spectral range 200–1800 cm^{-1} Size determined 4–15 μm	(Pironti et al., 2023)
BALF 44 healthy volunteers	• Rinse with bidestillated water and drying overnight at 60 °C	Binocular microscopy SEM-EDX μFTIR –Transmission mode Spectral range 550–4000 cm^{-1} Size determined > 50 μm	(Baeza-Martínez et al., 2022)
Sputum	• Digestion with nitric acid • Density separation with $ZnCl_2$ (1.7–1.8 g/ cm^3) • Filtration in a silver membrane • Rinsing and soaking in ethanol • Drying	Optical microscope μFTIR -Reflection Size determined > 0.1 mm LDIR imaging -Reflection Size determined 20–500 μm	(Huang et al., 2022)

(continued on next page)

Table 1 (*continued*)

Matrix	Sample pretreatment	Analysis	Ref.
Breast milk	• Digestion 10% KOH, ratio sample /solution 1:10 (w/v) at 40 °C for 48 h • Filtration on glass fiber membrane • Drying in a Petri dish	μRaman Spectral range 200–1800 cm^{-1} Size determined 2–12 μm	(Ragusa et al., 2022)
Blood	• Denaturing of proteins at 60°C for 1 h • Digesting proteins with Proteinase K and $CaCl_2$at 50°C for 2 h • Filtration and rinsing with 30% H_2O_2 solution	Double shot Py–GC–MS (First 100 −300 °C at 50°C/min, second 600 ° C) MPs total mass determined	(Leslie et al., 2022)
Placentas Breast milk Meconium Infant feces	• Digestion with HNO_3 for 48 h at room temperature, then for 3 h at 95 °C • Filtration to 13 μm stain steel sieve and rinse with water and ethanol	LDIR imaging -Reflection Size determined 20–500 μm	(Liu et al., 2023; Liu et al., 2022)
Feces	• Treatment with H_2O_2 • Keeping samples for 2 weeks to reduce the amount of natural stool constituents • Separation of solids into 2 fractions 0.05–0.5 and > 0.5 mm • Additional treatment of 0.05–0.5 mm fractions using chemical/ enzymatic procedures	• Fractions > 0.5 mm by ATR-FTIR • 0.05–0.5 mm fractions by FTIR imaging • Transmission mode	(Schwabl et al., 2019)
Feces	• Digestion of 3 g sample with 25 mL of 30% H_2O_2 for 20 days • Freeze drying • Sieving of particles > 5 mm • Filtration	μFTIR Reflection mode Spectra 4000–750 cm^{-1} 4000–750 cm^{-1} Size determined 20–800 μm	(Zhang et al., 2021)
Feces	• Digestion using Fenton's reagent • Filtration • Digestion with HNO_3 • Dilution and filtration	Raman Spectra 500–2750 cm^{-1} Size determined not reported	(Luqman et al., 2021)
Feces	• Digestion using Fenton's reagent • Filtration • Incubation with 65% HNO_3 at 50 °C for 30 min	μRaman Spectra 200–1050 nm Space resolution < 0.5 μm Size determined 1.7–393.8 μm	(Yan et al., 2022)
Feces	• Dilution and filtration • Drying • Digestion using Fenton's reagent • Filtration	Raman Spectra 500–2750 cm^{-1} Size determined not reported	(Wibowo et al., 2021)
Human placenta (n = 17)	• Digestion with 10% KOH solution in a ratio with the sample of 1:30 (w/v) for 72 h at 50 °C and 120 rpm • Filtration to 10 μm stain steel sieve an rinse with water and ethanol	LDIR imaging –Reflection Spectra 900–1800 cm^{-1} Size determined 20–500 μm	(Zhu et al., 2023)
Human placenta and meconium	• Sieving through a 50 μm stainless steel sieve • Rinsing out into a beaker with H_2O_2 30% at 25 °C keeping it ca. 5 weeks for meconium and 7 weeks for placenta. • Sieving residuals using a 50 μm filter and placing carefully into NaOH 0.05 M at 25 °C • Transfer onto a 50 μm stainless steel sieve and flushed out with ultrapure water into a pre-cleaned beaker. • Vacuum filtration using membrane • Placing filters in lidded Petri dishes and dried at 60 °C overnight	FTIR imaging system Transmittance mode Spectra 4000–1250 cm^{-1} Spectral resolution of 16 cm^{-1} Pixel size 25 μm Size determined > 50 μm	(Braun et al., 2021)
Human placenta	• Digestion with 10% KOH solution in a ratio with the sample of 1:8 (w/v) • Sealing and incubating the containers at room temperature for 7 days • Filtration of digestates • Drying filter papers and storing in glass Petri dishes.	Binocular microscopy using × 10 and x100 objectives μRaman Spectral range 160–2000 cm^{-1} Size determined 5–10 μm	(Ragusa et al., 2021)
Pulmonary tissue samples	• Digestion of tissues using enzymatic mixture	Binocular microscopy using × 10 and x100 objectives μRaman Spatial resolution of	(Amato-Lourenço et al., 2021)

(*continued on next page*)

Table 1 (*continued*)

Matrix	Sample pretreatment	Analysis	Ref.
	Corolase® 7089 for 12 h at 60 °C • Density separation by $ZnCl_2$ solution (1.5 g cm^{-3}) • Filtration by silver membrane • Drying of membrane filters	0.5 μm Size determined Particles < 5.5 μm Fibres from 8.12 to 16.8 μm	
Lung tissues	• Digestion with 100 mL of 30% H_2O_2 at 50ºC for ca. 11 days • Filtration onto Al_2O_3 membranes	μFTIR – transmittance mode Spectral range of 4000–1250 cm^{-1}, High spectral resolution 8 cm^{-1} Size determined 20 – 500 μm	(Jenner et al., 2022)
Human colectomy specimens	• Digestion: 10% KOH at 60 °C for 7–10 h. • Filtration cellulose filter • Drying at room temperature for 2 days	Stereomicroscope (hot needle test) μFTIR -ATR mode Spectral range of 4000–400 cm^{-1} SEM - EDX Size determined not reported	(Ibrahim et al., 2021)
Liver tissues, kidney, and spleen	• Digestion with 10% KOH and NaClO 2:1 ratio at 40 ºC for 72 h • Filtration silver filter • Second digestion with 30% H_2O_2 • Filtration silver filter	Fluorescent microscopy and Red Nile staining μRaman Size determine 3.3–30.1 μm	(Horvatits et al., 2022)
Saphene vein tissue	• Digestion with 200 mL H_2O_2 (30%) for 168 h at 65 ºC and 85 rpm • Filtration with AlO_3 filters of 0.02 μm	μFTIR – transmittance mode Spectral range of 4000–1250 cm^{-1} Spectral resolution 8 cm^{-1} Size determined > 5 μm	(Rotchell et al., 2023)

fluorescence microscopy after Red Nile staining of the samples (Abbasi and Turner, 2021; Horvatits et al., 2022) and polarized light microscopy (Abbasi and Turner, 2021) provide additional confirmation of the identity of the MPs but without identifying their chemical composition. Due to limitations in the particle size that can penetrate human tissues, MPs have to be identified within the smallest size range (< 10 μm). Although electron microscopy would be the technique of choice for detecting MPs at this size, it has only been reported to identify MPs in intestinal tissue within human matrices (Ibrahim et al., 2021).

Chemical confirmation of the type of polymer is also required (Picó and Barceló, 2021). The characterization is generally performed with the spectroscopic techniques, especially Raman and FTIR. In both techniques, a spectrum of the sample is obtained that allows the identification of the type of MPs by comparison in the existing library of spectra for this purpose. Off-line Raman spectroscopy has been directly applied in two cases to determine microplastics in feces (Luqman et al., 2021; Wibowo et al., 2021). However, in neither case is the determinable particle size indicated. FTIR spectroscopes can also be directly applied to determine the biggest size MPs (> 100 μm) using the attenuated total reflection (ATR) mode of the FTIR as also was reported for feces (Schwabl et al., 2019). In the ATR mode, IR radiation passes through an internal reflection element crystal material, whose surface is in direct contact with the MP.

However, the hyphenation of visualization and spectroscopic techniques in the same instrument is the best option. Optical microscopes can be combined to Raman and FTIR based instruments as (i) micro version of Raman and FTIR (μRaman or μFTIR) (ii) imaging Raman or FTIR. The latest micro version of Raman and FTIR can perform visual sorting as well as spectra recording of MPs. The latest advances include focal plane array or laser FTIR (LDIR) detectors can take image of the whole filter where MPs are collected and then characterize them particle by particle with little to no analytical bias (Huang et al., 2022; Picó and Barceló, 2021; Schwabl et al., 2019; Zhu et al., 2023; Liu et al., 2023; Liu et al., 2022). μRaman, which enables to characterize MPs morphology as well as their chemical composition (both polymer matrices and pigments), have been extensively used to analyze human biological samples because due to the enormous potential for light scattering, μRaman enables the investigation of MPs as tiny as 2 μm directly on filtering membranes (Pironti et al., 2023; Ragusa et al., 2022; Yan et al., 2022; Abbasi and Turner, 2021; Ragusa et al., 2021; Amato-Lourenço et al., 2021; Horvatits et al., 2022). μRaman attain the examination of much smaller particles than μFTIR (an advantage for human tissues), but the regions that can be investigated and thus the amount of sample that can be utilized are extremely small. μFTIR is another good method for checking composition of MPs (Rotchell et al. (2023); Zhang et al. (2021); Huang et al., 2022; Braun et al., 2021; Jenner et al. (2022); Ibrahim et al., 2021. The technique can be used in reflectance, transmittance and ATR mode. The most used is the transmittance mode, but this mode limits the wavenumber range possible for spectral capture to 4000–1250 cm^{-1}, as it needs infrared-transparent filter substrates constructed of non-polymer materials (such as anodic alumina matrix membrane). Apart from these hyphenation between optical microscopy and spectroscopy, the combination of SEM to energy dispersive X-ray spectroscopy (SEM-EDX) provides a quick nondestructive determination of the elemental composition of the sample useful to record surface characteristics and chemical composition of MPs (Ibrahim et al., 2021). This is a particularly effective technique for examining the chemical makeup of MPs since it provides thorough details on the elements and their spatial distribution within the sample, as well as the presence of inorganic additives employed in their production (Baeza--Martínez et al., 2022). This approach, however, cannot be used to calculate the mass of MPs present in the sample but the number of MPs (Braun et al., 2021).

Chromatographic methods have been used for qualitative and quantitative analysis of the main types of polymers in the biological samples. Such methods rely on the high identification power of mass spectrometry. Such methods rely on high identification power of mass spectrometry. However, in the case of human tissues, there is only one example where double shot Py–GC–MS is applied. The GC–MS determination started for any volatile compounds, as they thermally desorb between 100 and 300 °C. Then, the non-volatile compounds were pyrolyzed at 600 ºC and the resulting molecules determined by GC–MS. Py–GC–MS technique provides the mass concentrations of MPs, thus, the technique is well suited to develop studies on mass balance modeling, pharmacokinetics and comparisons among individuals in a population. More and more laboratories are using also other techniques, such as thermal desorption mass spectrometry to detect and measure the mass of polymers, although it has not yet been applied to biological matrices (Ivleva, 2021).

Approaches applied to MPs determination vary widely from microscopy to GC-MS. Fig. 2 summarizes features and limitations of each technique as a summary that could serve as an outline to selected the best technique for each particular study. Particle counting and polymer mass measurement are complementing methods (Leslie et al., 2022). The latest detailed reviews of techniques and methods for the chemical analysis of MPs and NPs show that technologies are still developing, and in the future it may be possible to measure particle number and mass simultaneously (Ivleva, 2021). However, to capture all the information, for the time being, a combination of approaches is needed.

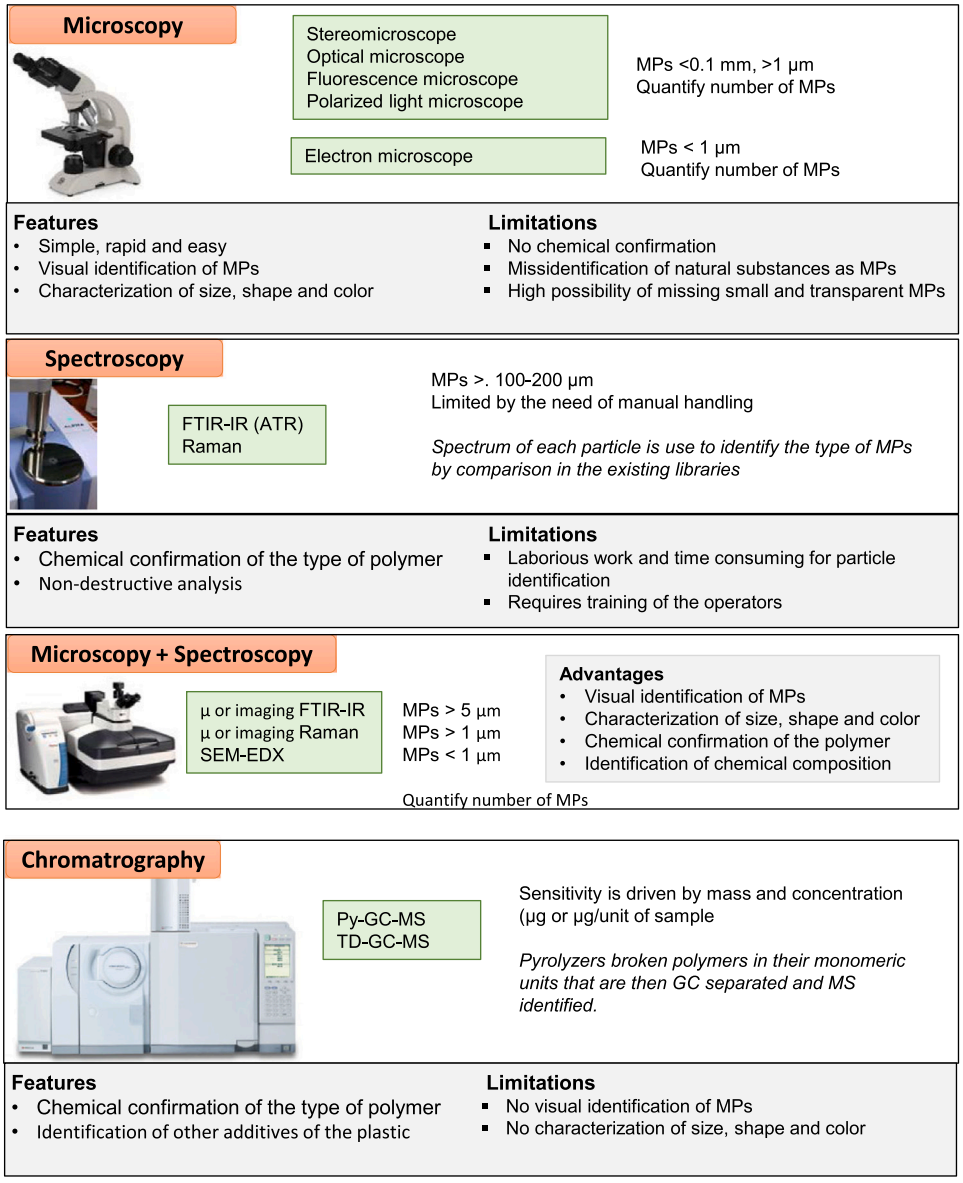

Fig. 2. Features and limitations of the techniques used to characterize and quantify MPs in human samples.

4. Detection of MPs in human samples

In the last few years, several studies have reported the presence of MPs in various kinds of samples collected from the human body providing growing evidence that MPs can enter the human body. Fig. 3 offers a global scheme of the different steps followed to perform these studies according to analytical methodology previously discussed.

Here, we present a brief overview of the studies where the presence of MPs in human matrices was investigated highlighting findings and the important outcomes. Since particles smaller than 150 µm may pass through the gastrointestinal epithelium in mammals, the absorption of MPs and NPs in humans, is plausible. However, only 0.3% of these particles are anticipated to be absorbed. MPs, like other foreign substances, can infiltrate tissues through several yet poorly characterized active and passive transport modes. Mainly, particles $\leq$ 10 µm would be able to access everywhere in the human body and pass through cellular membranes, barriers. The MPs may accumulate and cause localized toxicity by triggering and/or increasing immunological responses, weakening the body's defenses against infections, and changing energy reserves. (Ragusa et al., 2021). Table 2 outlines the most relevant polymers found in MPs as well as their characteristics when available on size, shape and color. Table 3 provides the most important findings of all the studies involving the detection of MPs in human matrix samples. Studies are still scarce and disperse as far as matrices are concerned in order to draw relevant conclusions on the type of polymer or matrix.

4.1. *Biological fluids*

Breast milk is the sole food for infants during the first 6 months of life. Breast milk is also a vehicle for the elimination of polar and especially nonpolar substances including environmental pollutants. Many chemicals have been identified so far, including organochlorine pesticides, polychlorinated biphenyls (PCBs), and metals (Lehmann et al., 2018). Their presence has shown to severely affect the infant's subsequent development.

MPs were also detected in women's breast milk in a study that recruited pregnant women without complications. Out of 34 samples, 26 showed positive results. The most prevalent MPs, with sizes ranging from 2 to 12 µm, were made of polyethylene (PE), polyvinylchloride (PVC), and polypropylene (PP). In the study, women were asked to keep

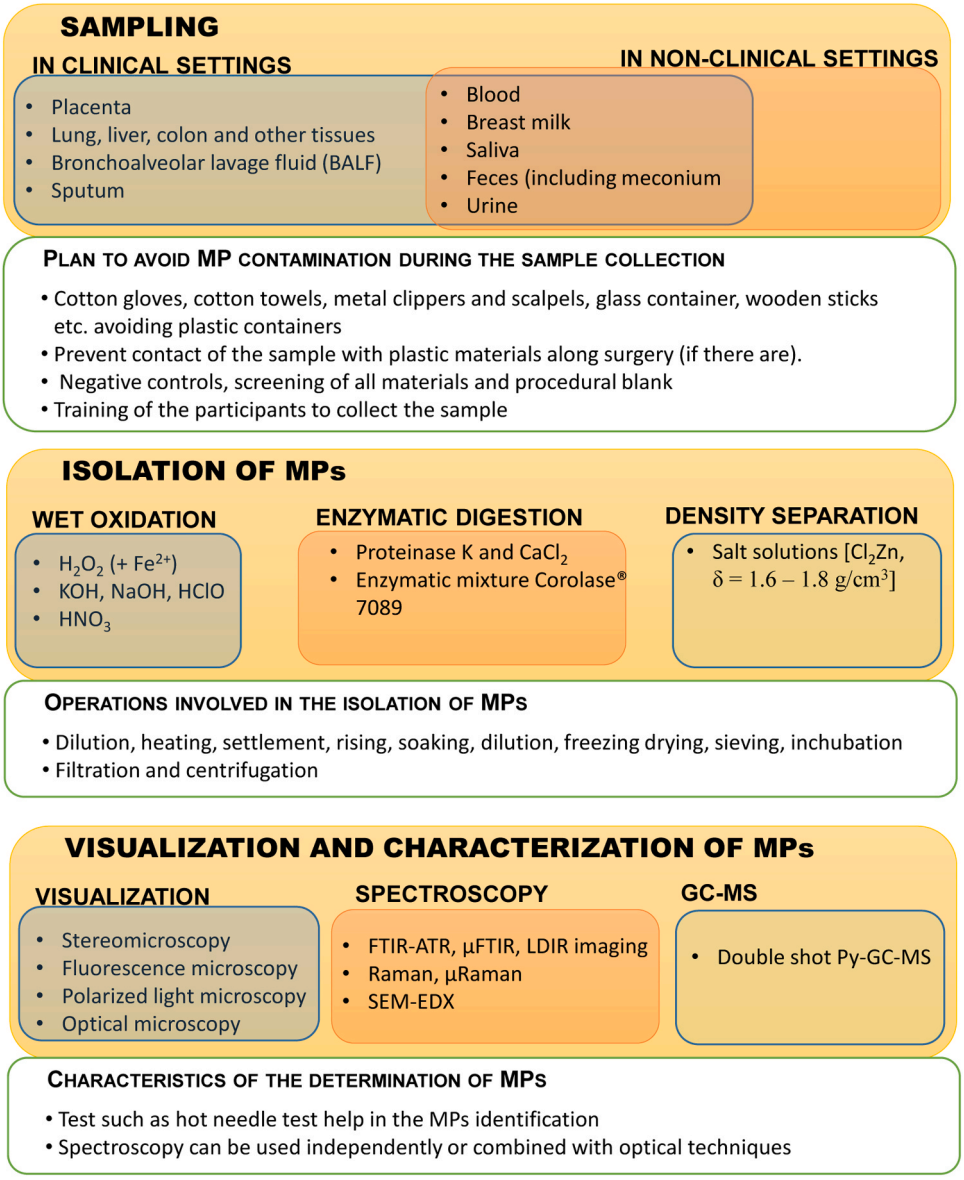

Fig. 3. Scheme of the different steps and procedures followed in the studies on MPs in human matrices.

the record of their food intake 7 days before and after the delivery data but authors could not find a statistical relation of the MPs in breast milk with age, use of personal care products, fish consumption, beverages, and food in plastic packaging (Ragusa et al., 2022).

Interestingly, Liu et al. (2023) investigated the presence of MPs in mother-infant pairs by analyzing placentas and meconium at birth and human milk or infant formula and infant feces at 6 months after delivery. The study also attempts to establish the source of exposure by engaging mothers to fill out two questionnaires with data on plastic use and dietary patterns. Polyurethane (PU) was the predominant chemical composition of MPs in breast milk and infant formula, accounting for 53.18%, 49.33%, respectively.

Blood accounts for 6–7% of body weight, irrigates organs and carries oxygen and nutrients. It can even transfer contaminants such as MPs to other organs and tissues. Once transfer, the fate of MPs in the human body will depend on their elimination pathway (e.g. by renal filtration or biliary excretion or deposition in the liver, spleen or other organs) as well as on the particle's size, shape, surface chemistry, and charge that marks its interactions with biological systems. Blood, being a main transport route and a matrix easy to obtain safely and without using plastic material, is very suitable for biomonitoring of MPs in the human organism. Furthermore, in a recent study, plastics were detected and quantified in whole human blood collected from 22 healthy volunteers. After extraction, the plastics were analyzed by double shot pyrolysis gas chromatography-mass spectrometer. This study included several quality controls measures, including duplicate samples, sampling and procedural blanks, and spiking recoveries. Polyethylenterepthalate (PET), PE, polymers of styrene, polymethylmethacrylate (PMMA) were detected and quantified. The sum of the quantifiable concentration of plastic particles in blood was 1.6 μg/mL and 17 out of 22 donors carried a quantifiable mass of plastics in their blood. The maximum concentration of PET analyzed in a blood sample was 2.4 μg/mL. For polystyrene (PS) this was 4.8 μg/mL, and for PE this was 7.1 μg/mL. This study did not involve particle counting like other studies. Instead, it determined mass concentrations of plastics by developing an analytical method for commonly used plastics (Leslie et al., 2022).

Recently, Pironti et al. (2023) evaluated for the first time the presence of MPs in urine samples collected from six volunteers (3 men and 3 women) from different cities in southern Italy. The analysis identified four fragments of pigmented microplastics (size 4–15 μm), with

Table 2
Summary of most prevalent polymers in MPs found human samples and their relevant characteristics.

MPs	Important findings	Ref.
Alkyd varnish	• In *sputum samples* as fibers and fragments < 500 μm	(Huang et al., 2022)
CPE	• In *sputum samples* as fibers and fragments < 500 μm	(Huang et al., 2022)
Nylon-EVA	• Identified in *saphene vein tissues* (20% of samples) irregular-shaped fragment/film, and mean particle length 119.59 ± 226.82 μm and mean particle width 41.27 ± 62.80 μm	(Rotchell et al., 2023)
PA	• Dominant in *placenta, breast milk, meconium* and *infant feces* in sizes mostly between 20 and 50 μm • Abundant PA (8.9–12.4%) in an study in *feces* that compares healthy individuals and IBD patients. • Present in 50% of *colectomy specimens* mostly as filaments of fibers	(Ibrahim et al., 2021; Liu et al., 2023; Liu et al., 2022; Yan et al., 2022)
PBS	• Dominant in *placenta samples* (10.2% of samples) at size < 200 μm and as fragments	(Zhu et al., 2023)
PC	• Detected in 90% of *colectomy specimens* mostly filaments or fibers	(Ibrahim et al., 2021)
PE	• Abundant in *hand-face skin*, *head hair* and *saliva* as fibers of < 100 μm • *Lung tissue:* One study identified PE as particles < 5.5 μm in size, and fibres ranged from 8.12 to 16.8 μm. Other detected it as prevalent (10%) as fragments and fibers at 50 μm size. • Prevalent in *placenta*, 14.55% of the samples at size < 100 μm as fragments • In *urine samples* was identified once as irregular fragment 4 μm red • Described in *breast milk* as particles ranging 3–12 μm colored. • Detected in *blood* (23% of the samples) • Identified in > 95% of *stool samples* in a size 50–500 μm in one study. Also prevalent in others as HDPE.	(Abbasi and Turner, 2021; Amato-Lourenço et al., 2021; Jenner et al., 2022; Leslie et al., 2022; Luqman et al., 2021; Pironti et al., 2023; Ragusa et al., 2022; Schwabl et al., 2019; Wibowo et al., 2021; Zhu et al., 2023)
PET	• Abundant in *hand-face skin, head hair* and *saliva* as fibers of < 100 μm • Described in *breast milk* range as particles 3–12 μm colored. • Most abundant in *blood* (>LOQ values in 50% of samples) • *Stools (several studies reported):* In one detected in 100% of stool samples in a size 50–500 μm. Remarked in several others as prevalent. In other that compares healthy individuals and IBD patients is the most abundant 22.3–34.0%. • In *lung tissue* was detected as prevalent (18% of the samples) as fragments and fibers at 50 μm • Detected in 80% *cirrhotic liver tissues* at a size 4–30 μm	(Abbasi and Turner, 2021; Horvatits et al., 2022; Jenner et al., 2022; Leslie et al., 2022; Ragusa et al., 2022; Schwabl et al., 2019; Wibowo et al., 2021; Yan et al., 2022)
PMMA	• Detected in 5% of *blood samples*	(Leslie et al., 2022)
PP	• In hand-face skin, head hair and saliva as fibers of < 100 μm • In *human lung tissue* in study detected as particles < 5.5 μm in size, and fibres ranged from 8.12 to 16.8 μm. Other study identifies it as most abundant (23% of the samples) as fibers and fragments at sizes ca. 50 μm • In *urine samples* most abundant as irregular fragments > 5 μm and < 15 μm of blue, green and blue/grey color • In *breast milk* range 3–12 μm colored. • Detected in a range of 95.8–100% of *stool samples* in a size 20–800 μm in two studies. In other studies considered also most prevalent. • Detected as prevalent in *placenta* and *meconium* at size < 10 μm • Present in 50% of *colectomy specimens* mostly as filaments of fibers	(Abbasi and Turner, 2021; Amato-Lourenço et al., 2021; Braun et al., 2021; Ibrahim et al., 2021; Jenner et al., 2022; Pironti et al., 2023; Ragusa et al., 2022; Ragusa et al., 2021; Schwabl et al., 2019; Zhang et al., 2021; Wibowo et al., 2021)
PS	• In *sputum samples*, is in 21.63% < 500 μm • In *BALF samples*, present in 19.05% of the samples (2nd most abundant) • In *human blood*, PS (36%) • Detected in > 95% of *stool samples* in a size 50–500 μm • Detected as prevalent in *placenta* and *meconium* at size < 10 μm • Detected in 100% of *cirrhotic liver tissues* 4–30 μm	(Braun et al., 2021; Horvatits et al., 2022; Huang et al., 2022; Leslie et al., 2022; Baeza-Martínez et al., 2022; Schwabl et al., 2019)
PU	• In *sputum samples*, the most abundant (33.95%) < 500 μm • Dominant in *placenta, breast milk, meconium* and *infant feces* in sizes mostly between 20 and 50 μm	(Huang et al., 2022; Liu et al., 2023; Liu et al., 2022)
PVA	• In *urine samples* one irregular fragment ~15 μm transparent	(Pironti et al., 2023)
PVAc	• Identified in *saphene vein tissues* (20% of samples) irregular-shaped fragment/film, and mean particle length 119.59 ± 226.82 μm and mean particle width 41.27 ± 62.80 μm	(Rotchell et al., 2023)
PVC	• In *urine samples* one sphere ~7 μm brown • Most abundant in *placenta samples* (43.27% of samples) at size < 200 μm and as fragments • Detected in *cirrhotic liver tissues* 4–30 μm	(Horvatits et al., 2022; Pironti et al., 2023; Zhu et al., 2023)
Rayon/Viscose	• In *BALF samples*, most abundant polymer (40.48%) in the shape of fibers and color	(Baeza-Martínez et al., 2022)
Resin	• In *lung tissue* prevalent (15% of samples) as fragment and fibers < 100 μm • Identified in *saphene vein tissues* alkyd resin (45% of samples) irregular-shaped fragment/film, and mean particle length 119.59 ± 226.82 μm and mean particle width 41.27 ± 62.80 μm	(Jenner et al., 2022; Rotchell et al., 2023)

irregular shapes, consisted of polyethylene vinyl acetate (PVA), PVC, PP and PE. These results, although preliminary, support the assumption that MPs passes through the gastrointestinal tract and are eliminated from the human body.

In other noteworthy study, the content of microplastics in saliva was compared with that of hand and facial skin washes and head hair of individuals (n = 2000) after a 24-hour exposure period and counted and, in a selected number of cases, microscopically characterized for

Table 3
Summary of important findings of studies involving detection of MPs in human samples according to the tissue.

Sample (n)	Important findings	Ref.
Filtered washes of the hand and face skin, head hair and saliva of individuals (n = 2000) after an exposure period of 24 h	• Over 16,000 MPs were recorded, with head hair returning the most and saliva the least • PE-PET and PP fibers of < 100 μm were the most abundant type of MP	(Abbasi and Turner, 2021)
Urine (n = 6, 3 men and 3 woman)	• Preliminary results • 4 pigmented microplastic fragments (4–15 μm size), with irregular shapes, of PVA, PVC, PP, and PE	(Pironti et al., 2023)
BALF (n = 44, European citizens)	• MPs were detected in BALF samples from the human lower airway • Main shape was microfiber (95%) • The most abundant compounds were rayon and polyester	(Baeza-Martínez et al. (2022)
Human sputum (n = 22)	• 21 types of MPs were detected in sputum samples • PU was most prevelent followed by PES, CPE, and alkyd varnish	(Huang et al., 2022)
Human breast milk (n = 34)	• Out of 34 samples, 26 tested positive for MPs • The size range of detected MPs was 3–12 μm • Most of the MPs were pigmented (blue and orange/yellow colors prevalent) • PE, PVC, and PP were the most abundant among the detected MPs	(Ragusa et al., 2022)
Human blood from healthy volunteers (n = 22)	• PET, PE, polymers of styrene, PMMA were detected and quantified • Quantifiable concentration of plastic particles in blood was 1.6 μg/mL • 77% (17 out of 22) of donors carried a quantifiable mass of plastics in their blood • The maximum concentration of PET analyzed in a blood sample was 2.4 μg/mL, for PS this was 4.8 μg/mL, for PE this was 7.1 μg/mL	(Leslie et al., 2022)
Placentas, breast milk, meconium and infant feces	• Investigated the presence of microplastics (MPs) in mother-infant pairs, and the exposure sources. • Sixteen types of MPs were identified (PA and PU were dominant) • Most of the MPs (>74%) were 20–50 μm in size.	(Liu et al., 2023; Liu et al., 2022)
Stool (n = 8)	• No MPs detected in fractions > 0.5 mm • 20 MP particles/10 g stool (median) of size 50–500 μm • 8/8 samples tested positive for MPs • 9/10 plastic types detected overall • 3–7 different plastic types per sample	(Schwabl et al., 2019)
Stool (n = 26) young male students	• PP and PET were found in 100% of samples • PP, PET, PS and PE were found in > 95% of samples • 23/26 (95.8%) participants tested positive for MPs • The abundance of MPs range from 1 particle/g to 36 particles/g (size 20–800 μm) • 1–10 different plastics types of MPs per sample • PP was the most abundant (95.8%) of fecal samples • A moderate correlation was observed between packaged water and beverage intake and MP abundance in feces	(Zhang et al., 2021)
Feces of heathy (50) and IBD patients (52)	• MP concentration in IBD patients was significantly higher than that in healthy people • In total, 15 types of MPs were detected in feces • PET (22.3–34.0%) and PA (8.9–12.4%) were dominant • A positive correlation exists between the concentration of fecal MPs and the severity of IBD	(Yan et al., 2022)
Human stools collected from fisherman community (n = 11)	• MPs were detected in more than 50% of stool sample • HDPE was the most prevalent	(Luqman et al., 2021)
Human stool samples (n = 11)	• MPs were detected in 7 out of 11 samples • PP was the most abundant detected in 4 positive samples • Detected MPs included HDPE, PP, PS, PET • MPs were also detected in commonly used food and toothpaste by the participants	(Wibowo et al., 2021)
Human placenta	• MPs were found in all placental portions: maternal, fetal and amniochorial membranes	(Ragusa et al., 2021)
Human placenta	• MPs detected in all placenta samples • PVC, PP, and PBS were the dominant polymer types of MPs • MP particles < 100 μm and fragment MPs (<200 μm) were dominant	(Zhu et al., 2023)
Human placenta and fetal meconium	• Human placenta and meconium samples were screened positive for PE, PP, PS, and PU, of which only the latter one was also detected as airborne fallout in the operating room—thus representing potential contamination	(Braun et al., 2021)
Pulmonary tissue (20 non-smoking adult individuals)	• In total, 31 synthetic polymer particles and fibers were observed in 13 of the 20 autopsied decedents, of which 87.5% were particles (all fragments) and 12.5% were	(Amato-Lourenço et al., 2021)

(continued on next page)

Table 3 (*continued*)

Sample (n)	Important findings	Ref.
	fibers (length to width ratio > 3) • PP was the most frequent polymer (35.1%), followed by PE (24.3%); cotton (16.2%); PVC and cellulose acetate (5.4%); and PA, PE co-PP, PS, PS-co-PVC, and PU (2.7%)	
Lung tissues (13)	• In total, 39 MPs were identified within 11 of the 13 lung tissue samples • Of the MPs detected, 12 polymer types were identified with PP (23%), PET (18%) and resin (15%) the most abundant	(Jenner et al., 2022)
Human colectomy specimens (n = 11)	• MPs were detected in all 11 specimens with an average of 331 particles/individual specimen or 28.1 ± 15.4 particles/g tissue • 96.1% of particles were filaments or fibers, and 73.1% of all filaments were transparent • Out of 40 random filaments from 10 specimens, 90% were PC, 50% were PA and 40% were PP	(Ibrahim et al., 2021)
11 liver, 3 kidney and 3 spleen samples from patients with liver cirrhosis (n = 6) and healthy individuals (n = 5)	• MPs were not found in any of the liver, kidney, or spleen samples from patients who did not have underlying liver disease • In contrast, cirrhotic liver tissues tested positive for MP concentrations • Six different MP polymers, with sizes ranging from 4 to 30 μm	(Horvatits et al., 2022)
Saphene vein tissues	• 20 MP particles consisting of five MP polymer types were identified within 4 of the 5 vein tissue samples. • Number of MPs was similar to those reported in blank • MPs composition was different between vein tissues and blank • MPs abundance was similar to those reported in lung and digestive tract	(Rotchell et al., 2023)

shape and size. In total, more than 16,000 MPs were recorded in the study, with hair providing the most particles (> 7000, or, on average, > 3.5 MPs per individual per day), saliva the least (about 650, or an average of 0.33 MPs per individual), and MPs approximately twice as much in men as in women (Abbasi and Turner, 2021).

Measurement of microplastics in respiratory fluids can give an idea of inhalation exposure. Baeza-Martinez et al. (2022) investigated the presence of MPs in the human lower airways of 44 adult European citizens, using bronchoalveolar lavage fluid (BALF), and their relationship with the patients' lifestyle and physiological parameters. The results indicate that the majority of MPs were microfibres (97.06%) with a mean size of 1.73 ± 0.15 mm and a mean concentration of 9.18 ± 2.45 elements/100 mL BALF. Involuntarily inhalation of MPs was also assessed in a study that analyzed sputum of 22 participants suffering from different respiratory illnesses. This method covered MPs in the range of 20–500 μm. The findings revealed that there were 21 different types of MPs, with PU as the most prevalent, followed by polyester (PES), chlorinated polyethylene (CPE), and alkyd varnish. Most aspirated MPs found had a size of < 500 μm. The prevalence of MPs in sputum was also correlated with occupation, way of life, and individual exposure to ambient particulate matter ($PM_{2.5}$ in this investigation). This study found a direct relation between high concentrations of MPs in dust and human sputum. The high abundance of PS and PU in sputum might be related to the frequent use of face masks during COVID-19. However, this relationship could not be found in a two-week investigation, which might be due to the small sample size. The authors noted several limitations in their study, including the possibility that MPs found in the mouth cavity and saliva could contribute to sputum samples, potentially leading to inaccurate estimations of the quantity of MPs entering the respiratory system (Huang et al., 2022).

4.2. Stool

Contrary to what you might think, stool is not only a waste, but their microscopic, chemical, immunological and microbiological test can diagnose various infections, malabsorption syndromes and inflammatory bowel diseases without the need for laboratory analysis (Kasırga, 2019). Stool samples have also been investigated to assess the presence of MPs and establish their involuntary intake. Since all substances that have not been absorbed in the gastrointestinal tract are eliminated in the feces, there is no limitation on the size of MPs that can be found.

A study that analyzes 8 human feces of healthy volunteers detected the presences of 9 out of 10 plastic types. About 20 MPs (50–500 μm in size) belonging to 3–7 different types were detected per sample. PP and PET were detected in all the samples, while PP, PET, PS, and PE were detected in 95% of samples (Schwabl et al., 2019). Other study performed in 26 healthy young male (18–25 years), 23 out of 24 samples showed the presence of MPs 1 particle/g to 36 particles/g (size 20–800 μm). PP was detected in 95.8% of samples. Similar to the previous study, the three most abundant types of MPs were PP, PET, and PS. However, this study had limitations as it was conducted in China and included only male participants. In contrast, the previous study had a smaller sample size but a more diverse participant pool (Zhang et al., 2021).

Interestingly, MPs were analyzed in the feces of patients with inflammatory bowel disease (IBD) and healthy people. The results showed that fecal MP concentration in IBD patients (41.8 items/g dry matter, dm) was much higher than that in healthy people (28.0 items/g dm). Among the 15 types of MPs detected, PET and polyamide (PA) were the most abundant. This study presented a positive correlation between the concentration of MPs and the severity of IBD. Combining results from a questionnaire survey with properties of fecal MPs, it was determined that dust exposure and plastic packaging of water and food were significant sources of human exposure to MPs. Additionally, the positive link between fecal MPs and IBD status raises the possibility that MP exposure is connected to the disease's progression or that MP retention is made worse by IBD. The major strength of this study was its sample size (Yan et al., 2022). Figs. 2 and 3 provide the details of MP shapes and types detected in healthy and IBD patients. .

Two studies investigated the detection of MPs in human stools from fisherman and farming communities located in Indonesia. In the fisherman community in the coastal area of Surabaya, Indonesia, MPs were detected in more than 50% of samples analyzed with a concentration range of 3.33–13.99 μg/g of feces. In total, six types of MPs were detected, including high density polyethylene(HDPE), low density polyethylene (LDPE), linear LDPE, PP, PS, and PET. HDPE was the most ubiquitous, with an average concentration of 9.195 μg/g. The interesting aspect of this study was that it also investigated the presence of MPs in food, water, toothpaste regularly consumed by people participating in it. PP was most abundant in food, LDPE in drinking water, and high concentrations of HDPE were detected in toothpaste (Luqman et al., 2021). In the other study, also located in Indonesia, MPs were detected in 7 out of 11 stool samples collected from farming communities in rural

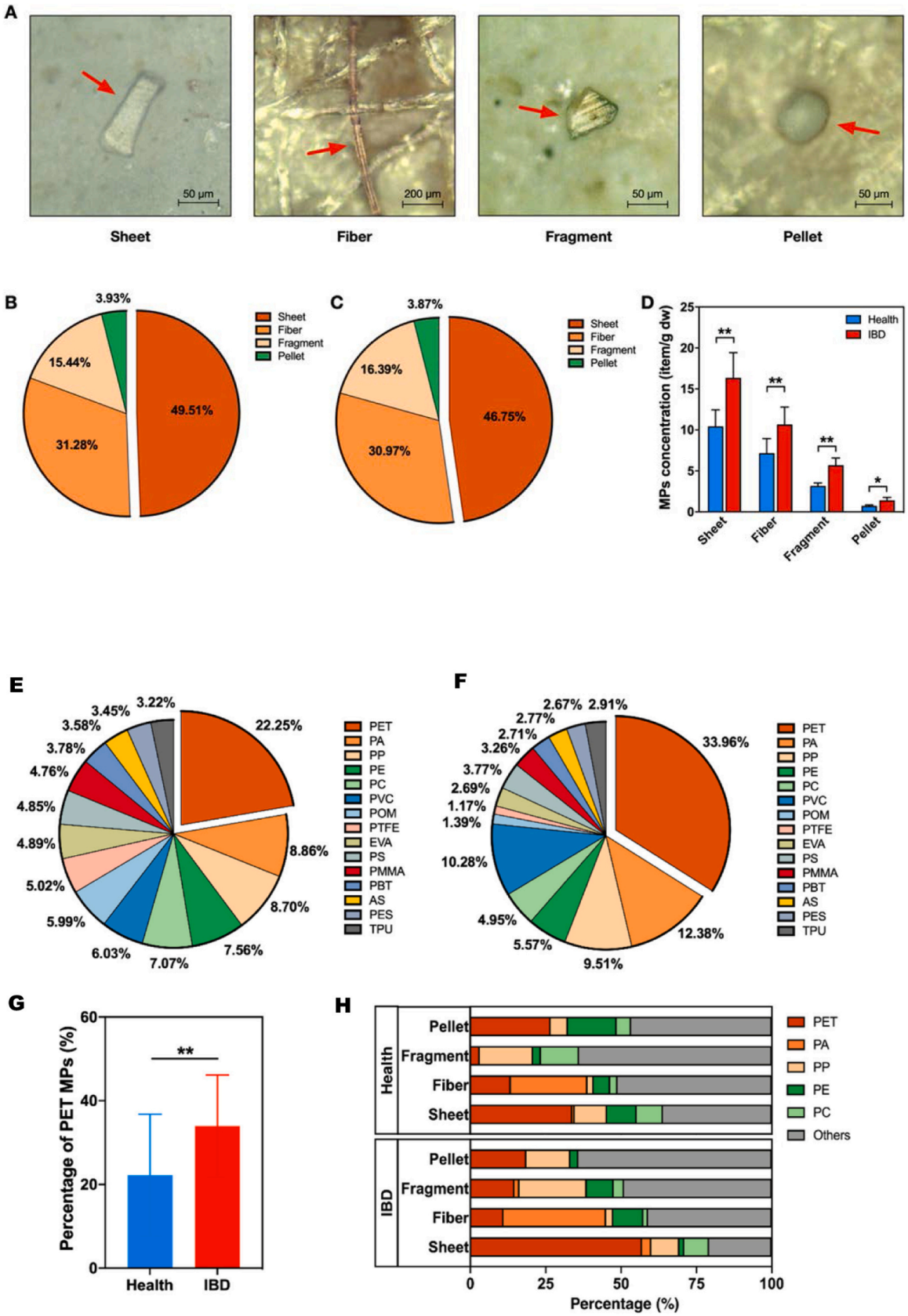

Fig. 4. Different shapes of MPs detected in feces from the participants (A) Percentage of different shapes of MPs in the feces from the healthy (B) and IBD (C) participants and the concentrations of different shapes of MPs (D). *$p < 0.05$; * *$p < 0.01$; and * **$p < 0.001$ as well as Relative abundance of different polymer types of MPs in feces from healthy (E) and IBD (F) participants. Comparison of the relative abundance of PET MPs in feces from healthy and IBD participants (G). Polymer distribution of each MP shape detected in the feces (H). * *$p < 0.01$. Reprinted from (Yan et al., 2022) with permission. Copyrights (2022) American Chemical Society.

areas. PP was the most abundant (detected in 4 positive samples), and its mean concentration was 10.19 μg/g of feces. MP types detected include HDPE, PP, PS, and PET. MPs were also detected in the foods and toothpaste commonly consumed by the subjects of this study (Wibowo et al., 2021). Both studies demonstrate the presence of MPs in human feces as result of ingesting plastics from various sources.

MPs have also been detected in meconium and infant feces as an indication of the prenatal exposure to MPs (Liu et al., 2023; Liu et al., 2022); Braun et al. (2021)). These studies compared the amount and characteristic of the MPs found in these stools to the MPs found in placentas and will be discussed latter on.

4.3. Tissues

The placenta is an organ that grows in the uterus during pregnancy. It provides oxygen and nutrients to a growing infant. Additionally, it purges waste materials from the blood of the baby. The newborn's umbilical cord grows from the placenta, which is attached to the uterus wall. Typically, the organ is affixed to the front, rear, side, or top of the uterus. The transfer of MPs in mothers and fetuses, is incompletely understood because of the limited amount of data on their presence in the human placenta.

There are recent studies that demonstrate the presence of MPs in this matrix. In one, the presence of MPs was assessed in six human placentas from women with uneventful pregnancies. In total, 12 MP fragments all pigmented (ranging in size from 5 to 10 μm) with spheric or irregular shapes were found in 4 placentas (5 on the fetal side, 4 on the maternal side, and 3 in the chorioamniotic membranes). Three of them were identified as stained PP while the remaining 9 as a thermoplastic polymer. The size range of MPs detected is compatible with transport through the bloodstream, passage across membranes and distribution in cells, posing a risk to the growing fetus. MPs in this organ could interact with the immune system and alter the recognition of the fetus as non-foreign corpse (Ragusa et al., 2021).

More recently, Zhu et al. (2023) evaluated the presence and characteristics of MPs in 17 placentas showing that MPs were in all placenta samples, with an abundance of 2.70 ± 2.65 particles/g and a range of 0.28–9.55 particles/g. The MPs were mainly composed of PVC (43.27%), PP (14.55%), and polybutylene succinate (PBS) (10.90%) with a size ranging from 20.34 to 307.29 μm, and most (80.29%) were smaller than 100 μm.

Placenta, meconium and/or infant feces were examined as part of the studies to investigate the potential transfer of MPs from placentas to the fetus. Braun et al. (2021) analyzed the ten most common types of MP (size >50 μm) in the placenta of two mothers who delivered by cesarean section with singleton pregnancies and breech presentation and in the meconium of the newborns. Contrary to theoretical estimates, PP, PE and PU were detected in placenta and PP and PE in meconium. Although a rigorous contamination control procedure was followed, there were possibilities for potential contamination of the samples, so that screening for MPs in clinical settings remains a challenging task. Consequently, research results should generally be treated with caution. Liu et al. (2023) in other study correlating MPs in placenta and meconium reported that PA, PU, PE, and PET were the most abundant types of MPs derived from both mothers and infants. Also, PP in placenta was positively associated with total MPs, PA, and PE in meconium. Although placentas and meconium had similar MPs composition, our data showed that the number of PA and the total number of MPs were observably higher in meconium compared to those in placentas. This study also analyzed MPs in breast milk, infant formula and infant feces after six months from the birth demonstrating that the most prevalent MPs in placenta, meconium and infant feces was PA, which accounted for 50.09%, 60.22%, 49.67%, respectively. The predominant MPs in breast milk and infant formula was PU, accounting for 53.18%, 49.33%, respectively.

There are growing evidence on the humans' exposure to MPs through the food chain. For example, recently, a study investigated the existence of MPs, particle properties, and polymeric composition in human colectomy specimens. Due to the common practice of colectomies in individuals with colorectal malignancies, these samples were easily accessible for investigation. Out of 11, nine subjects had colorectal cancer, while two had normal colon. MPs were detected in all the samples with an average count of 331 per individual or 28.1 ± 15.4 particles per g of colon tissue. The particles were in the shape of filament or fiber (Ibrahim et al., 2021). The duration of MPs in the human digestive tract following food exposure, as well as their potential health effects, including the risk of malignancies, still remain uncertain. However, there is already strong evidence that marine species that ingest MPs experience negative gastrointestinal effects. For instance, it has demonstrated that MPs in the gastrointestinal tissue of zebrafish produce inflammation and oxidative stress (Cole et al., 2011; Qiao et al., 2019).

Inhalation as a route of exposure for MPs is supported by historical studies conducted in occupational settings that showed respiratory symptoms and illness in workers in flocculent, vinyl chloride and synthetic textile manufacturing industries (Prata, 2018). MPs are present in the air and may be inhaled by humans. However, whether MPs can enter and remain in the lungs because of environmental exposure is unknown yet.

To shed some light on this, a study of Amato-Lourenço et al. (2022) determined the presence of MPs in human lung tissues obtained at autopsies. This study detected polymeric particles and fibers in 13 out of 20 samples. The size of the particles was < 5.5 μm, and fibers ranged from 8.12 to 16.8 μm. PP and PE were the most frequently detected polymers. This study confirmed that the respiratory system is an exposure route, and the lungs can accumulate MPs in humans. The size of the detected MPs indicates that they can reach the bronchial-alveolar regions through inertial impaction and sedimentation mechanisms. Although inhalation was the most plausible route of exposure, there is a possibility that some MP particles may have entered the body by systemic translocation and entered the lungs, as other studies indicated their presence in the placenta. Microphotographs and Raman spectra of the main types of MPs found in lung tissues is shown in Fig. 5.

Jenner et al. (2022) also confirmed the presence of MPs in all regions of the lungs by μFTIR analysis. Thirty-nine MPs were identified in 11 of the 13 lung tissue samples analyzed from 12 different types, although PP and PET fibers were the most abundant. These authors hypothesized that depending on the aerodynamic diameter and respiratory defenses, MPs can accumulate over time, as they are made of durable materials that are unlikely to degrade within the lungs.

Cirrhosis of the liver is the 11th most common cause of mortality and a clinically significant condition. Up to 1.5 billion cases of chronic liver disease are thought to exist globally, regardless of severity or stage. Viral hepatitis (HBV/HCV), alcoholic liver disease, and increasingly non-alcoholic fatty liver disease are the leading causes of liver cirrhosis globally.

There is an interesting study, which was designed to establish whether MPs accumulate in peripheral organs, especially the liver, and whether liver cirrhosis favors this process. Liver, kidney, and spleen samples from patients without underlying liver disease were negative for MPs. In contrast, significant MP concentrations were detected in cirrhotic liver tissues. Six different MPs ranging in size from 4 to 30 μm were identified. The identified MPs were PS, PVC and PET, PMMA, polyoxymethylene (POM) and PP. Due to chemical digestion, the exact locations of MPs cannot be determined. This study indicated a clear relationship between chronic liver disease and MP accumulation in the liver. However, still, there is ambiguity about whether MPs accumulation is a cause or consequence of liver disease (Horvatits et al., 2022).

Recently, blood vessels as biological barrier that can be crossed by MPs was also investigated analysing digested human saphenous vein tissue samples (Rotchell et al., 2023). In total, five MP polymer types were identified within 4 of the 5 vein tissue samples with 29.28 ± 34.88

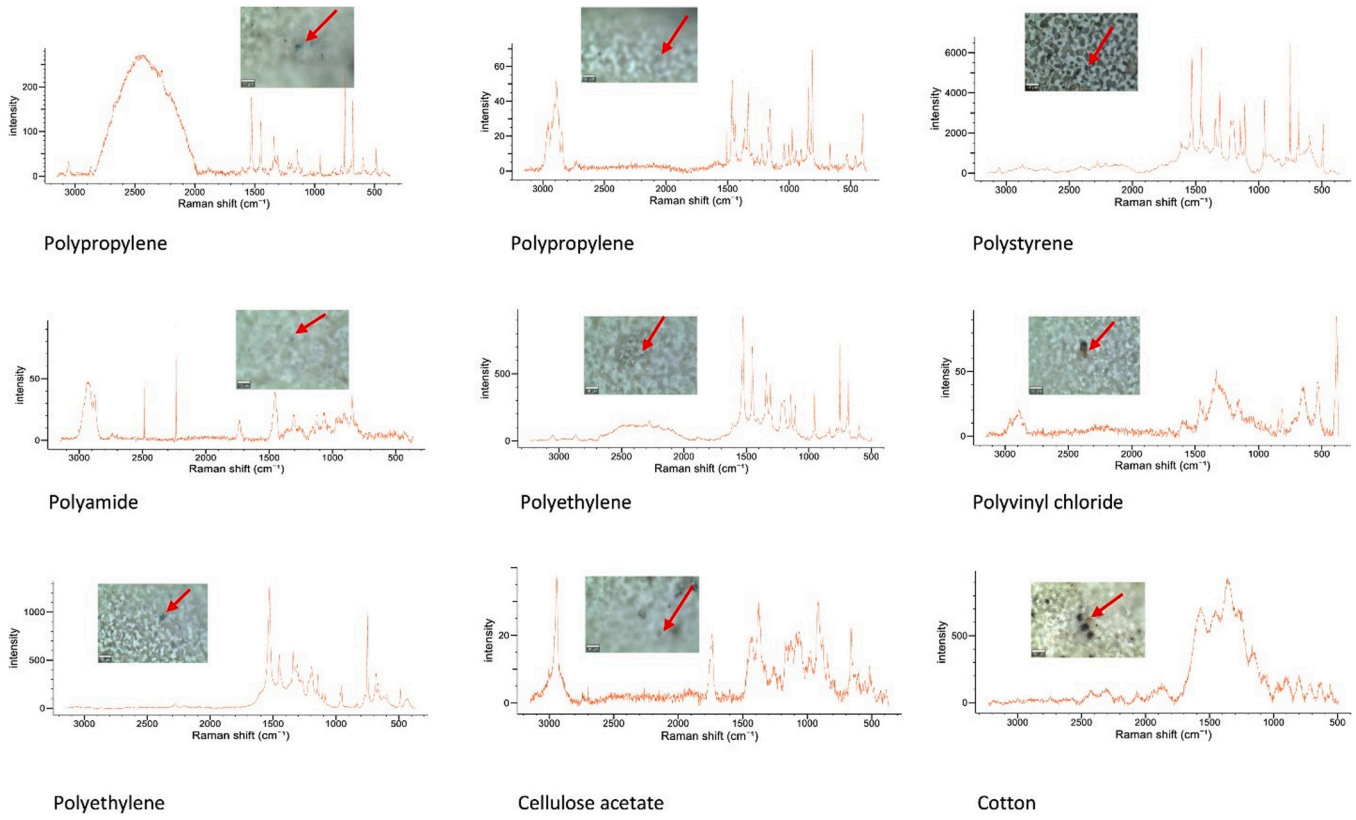

Fig. 5. Microphotographs and Raman spectra of the main types of MPs found in lung tissues. Bar scale – 10 μm. Reprinted from (Amato-Lourenço et al., 2021) with permission. Copyrights (2021) Elsevier B.V.

MP/g of tissue (expressed as 14.99 ± 17.18 MP/g after background subtraction adjustments). This mostly irregularly shaped MPs were composed of five polymers including alkyd resin (45%), poly(vinyl propionate/acetate (PVAc, 20%) and nylon-ethylene-vinyl acetate bond coat (nylon-EVA, 20%). While the number of particles found is equal to those of the blank, the composition of these particles –polytetrafluoroethylene, PP, PET and polyfumaronitrile:styrene – is very different from those of the tissues. The levels observed are similar or higher than those previously mentioned for colon and lung tissues (Ibrahim et al., 2021; Amato-Lourenço et al., 2022; Jenner et al., 2022). The size ranges are similar, although the shape characteristics and polymer types are different. All the information presented highlights the need for further studies.

5. Human cell line, simulation, in vitro studies and impact of MPs on human health

Toxics effects of MPs on the physiology and behavior of marine invertebrates have been extensively documented (Grote et al., 2023; Yong et al., 2020). Similar effects have also been observed in larger marine vertebrates such as fish (Wang et al., 2020; Crawford and Quinn, 2017; Jabeen et al., 2017). Furthermore, recent studies using mouse models have reported potential effects of MPs on mammalian gut microbiota, as well as cellular and metabolic toxicity in the host (Grote et al., 2023; Yong et al., 2020). However, the pathophysiological consequences of acute and chronic exposure to MPs in mammalian systems, particularly in humans, are not yet fully understood. Various studies have been conducted to evaluate the toxicity of MPs on human cell lines, various models, and in vitro investigations. Here, we present a brief overview of the studies involving most common MPs such as PE, PS, PVC, etc.

The primary focus of models and in vitro studies has been to examine the potential interactions between MPs and different biomacromolecules, such as lipids and enzymes, following ingestion into the gastrointestinal tract. In order to assess their impact on lipid digestion, various types of MPs including PS, PE, PVC, PET, and poly(lactic-co-glycolic acid) (PLGA) were examined using an in vitro gastrointestinal digestion model. All five types of MPs tested resulted in a decrease in lipid digestion within the in vitro gastrointestinal system. Among these MPs, PS-MPs exhibited the highest level of inhibition at 12.7%. Notably, increasing the concentration of PS-MPs led to a reduction in lipid digestion, regardless of the size of the PS particles (50 nm, 1 μm, 10 μm) (Tan et al., 2020). Furthermore, the in vitro gastrointestinal models have been used to study whether the presence of MPs can alter the microbiota. Interestingly, PET-MPs affect human gut microbiota communities during simulated gastrointestinal digestion (Tamargo et al., 2022).

One of the main pathways of human exposure to MPs is ingestion and absorption through the gastrointestinal tract. The effect of pristine and in vitro digested (to simulate the pass through the gastrointestinal tract) PS-MPs was investigated on two human intestinal cell lines (Caco-2 and NCM 460). Following a 24-hour exposure, significant internalization of 0.1 and 1 μm PS MPs was observed in both cell types. A comprehensive analysis of cell viability, reactive oxygen species (ROS) levels, and nutrient absorption/metabolism, conducted in a dose- and time-dependent manner, revealed no adverse effects. While PS-MPs were found to disrupt redox homeostasis in NCM 460 cells, they did not exert the same effect on Caco-2 cells. In vitro experiments suggest that the ingestion of PS-MPs poses a low acute cytotoxic risk to human gastrointestinal health. (Ma et al., 2022). Adverse effects of 0.1 μm and 5 μm PS-MPs were also explored in Caco-2 cells. Both PS-MP sizes demonstrated low toxicity on cell viability and oxidative stress. However, 5 μm PS-MPs caused higher mitochondrial depolarization than 0.1 μm PS-MPs. 0.1 μm PS-MPs generated higher inhibition of ABC transporter than 5 μm PS-MPs (Wu et al., 2019). Furthermore, the potential effects of PS-MPs on human colonic epithelial cell CCD841CoN and small intestinal epithelial cell HIEC-6 were examined. The 0.1–5 μm PS showed

low toxicity to CCD841CoN and HIEC-6 cells. Nano PS enters cells more easily than micro PS. The intake was related to exposure duration. The membrane damage generated by PS MPs was substantially more severe than that caused by PS-NPs. 5 μm PS can result in mitochondrial depolarization (Zhang et al., 2022).

Other less significant exposure routes, such as inhalation have also been studied through cell lines. Exposure of human lung cells to PS-MPs substantially reduced cell proliferation and triggered morphological changes. The experiments were conducted using 1 and 10 μm diameter MPs and the cultured human alveolar A549 cells. Although cell proliferation reduced but little cytotoxicity was observed (Goodman et al., 2021).

MPs are found in drinking water and after chlorine disinfection their chlorinated form may be the most accessible one. An interesting study evaluate this type of MPs by treating PS-MPs with chlorine to simulate the reactions that take place during the treatment of water and compared the toxicity of chlorinated MPs with pristine ones in gastric epithelial (GES-1) cells as a representative human cell model, which might be the primary point of contact for ingested MPs. This treatment did not change the size of MPs but enhanced their surface roughness as well as the formation of carbon chlorine bonds and the generation of persistent free radicals. In GES-1 cells, chlorinated PS-MPs significantly reduced cell proliferation, altered cellular shape, damaged cell membrane integrity, triggered an inflammatory response in the cells, and caused them to undergo apoptosis (Qin et al., 2022).

The effects of two different sized (30.5 ± 10.5 and 6.2 ± 2.0 μm) PE-MPs was extensively assessed on six different human cells involved in the processes of absorption and distribution (Caco-2, lung epithelial A549 and HaCaT keratinocyte cell lines), and both the innate (U937 macrophage and THP-1 dendritic cell lines) and adaptive (Jurkat T cell line) immune responses. Cell viability, intracellularROS, nitric oxide (NO), and cytokines were measured. PE-MPs had no significant impact on cell viability, but a slight decrease was noted in Caco-2 and A549 cells at 1000 μg/mL of both small and large-sized MPs. Furthermore, both small and large-sized PE-MPs induced increased NO production in all cell lines and upregulated ROS generation in THP-1, Jurkat, and U937 immune cell lines. A pro-inflammatory cytokine response was realized in HaCaT keratinocyte cells when cultured with PE-MPs whereas the opposite effect was found in THP-1 and U937 cells except with THP-1 cells cultured with large size MPs (Gautam et al., 2022b).

After being absorbed, MPs have the potential to be transported through the circulatory system and subsequently accumulate in various organs, including the kidney, gut, and liver. Thus, the effects on several blood and the immune system cell lines have been widely reported for several MPs. The cytotoxicity of PVC-MPs on human and fish blood lymphocytes was evaluated as an advantageous ex vivo model for accelerated human toxicity studies. The results indicated human lymphocytes are more sensitive to toxic effects of PVC-MP than fish lymphocytes. This cytotoxicity was associated with intracellular ROS generation, lysosomal membrane injury, mitochondrial membrane potential collapse, depletion of glutathione, and lipid peroxidation (Salimi et al., 2022). The toxicity of PVC-MPs on human serum albumin (HSA) was also evaluated. Fluorescence of HSA was quenched. Electrostatic interactions were involved in formation of PVC-HSA complex. The secondary structure of HSA experienced a decrease in α-helix. The backbone of HSA went through a microenvironmental alteration (Ju et al., 2020). The genotoxic and cytotoxic effects of PE-MPs on human peripheral blood lymphocytes were investigated using the cytokinesis-block micronucleus assay, a comprehensive cytogenetic method. The study demonstrated that MPs led to an increase in micronucleus frequency, nucleoplasmic bridge frequency, and nuclear bud frequency, indicating genotoxicity (Çobanoğlu et al., 2021). The effects of size, concentration, and particle shape of PVC and acrylonitrile butadiene styrene (ABS) MPs on cytotoxicity were investigated in various cell types including immune cells [peripheral blood mononuclear cells (PBMCs), human mast cells (HMCs-1), and red blood cells (RBCs)], normal cells (HDFs), and cancer cells (HeLa cells). It was observed that larger PVC particles had a tendency to induce the release of interleukin 6 (IL-6) and tumor necrosis factor-α (TNF-α). On the other hand, smaller ABS particles induced the release of IL-6 at high concentrations, while larger ABS particles tended to induce the release of TNF-α at all concentrations. These findings suggest that microplastics have the ability to elicit immune responses. (Han et al., 2020). The hemolytic potential of PP particles of various sizes upon direct contact with RBCs, as well as their ability to stimulate the immune system and potentially induce hypersensitivity reactions by increasing cytokine and histamine levels in PBMCs, Raw 264.7 mouse macrophage cell line, and HMC-1 cell lines, has also been investigated. Pro-inflammatory cytokines such IL-6, TNF alpha, and histamine can be induced by PP particles, resulting in a local immunological response. Pro-inflammatory cytokines are stimulated by PP particles in a size- and concentration-dependent manner (Hwang et al., 2019).

Moreover, MPs exhibit a "Trojan Horse" effect by sorbing and transporting various environmental pollutants. Thus, MPs with sorbed pollutants could induce more harmful effects than pristine MPs. To demonstrate this, single and combined effects of PE-MPs and two PCBs was evaluated in the human hepatoma cell line HepG2. The assessment involved cell viability and untargeted lipidomic analysis. The results indicated that MPs did not induce significant cell lethality at any tested concentration range, while PCBs showed hormetic behavior. The lipidomic analysis revealed significant changes in glycerophospholipids and glycerolipids with single PCB exposures. Conversely, the major change observed in MPs' single exposure was a substantial increase in triglyceride content. Combined exposures demonstrated that MPs could cause even more harmful effects than those resulting solely from desorption of previously sorbed toxic pollutants (Menéndez-Pedriza et al., 2022).

PS-MPs were examined in human kidney proximal tubular epithelial cells (HK-2 cells) and male C57BL/6 mice to assess their effects. Analysis of kidney cells revealed elevated levels of mitochondrial ROS and the mitochondrial protein Bad after the uptake of PS-MPs at different doses. Furthermore, exposure to PS-MPs led to increased endoplasmic reticulum stress and indicators of inflammation in the cells (Wang et al., 2021).

A 3D model of the human forebrain cortical spheroids, which mimics the early development of the human cerebral cortex, has been developed to better understand the potential impacts of PS-MPs on the human brain. The short-term exposure showed the promoted proliferation and high gene expression of Nestin, PAX6, ATF4, HOXB4 and SOD2. For long-term exposure, reduced cell viability was observed (Hua et al., 2022).

In short, MPs and NPs are up taken by cells based on particle size, surface chemistry, cell type, and biomolecular corona. MPs and NPs have different cytotoxicities for gastrointestinal, lung, immune, nerve cells, and blood components. MPs and NPs cause membrane damage, oxidative stress, activation of inflammatory factors, genotoxicity, apoptosis, and disruption of energy homeostasis and metabolism. Adhering pollutants and plastic leachates are also cytotoxic (Shi et al., 2021).

6. Conclusion and future research directions

Most of the studies (not all) are country or community specific, a broader study may include participants from all parts of the world. At the moment, no standardized procedures are in place for collection of various kinds of human biological samples. Thus, researchers have devised their own procedures that emphasize on minimizing contamination of MPs following strategies such as use of plastic-free equipment, containers, and using different control and blank samples. The presence of standard procedures is essential to cope difficulties in sample collection. Furthermore, most studies involving MPs analysis in biological samples are limited to a few samples and the representativeness

of such studies remains an unanswered question. Future studies should involve the analysis of larger number of samples to ensure representativeness and thus to visualize a broader picture.

The above comment also applied to biological sample preparation. The general steps involved are digestion and filtration. So far, there is no standardized way for type, concentration, and volume of reagents. Digestion time and temperature may affect the efficiency of sample preparation. After-digestion steps, such as, filtration or washing can also influence the analysis of MPs. A comparison of different digestion approaches will be important. Besides, a uniform procedure for sample preparation of same kind of samples should be prepared, it will provide better comparisons among various studies. The use of duplicates and spike samples can be helpful for quality control assurance through reproducibility and recovery results. This might be followed with biological samples. Furthermore, complementarity between different detection systems need to be emphasized.

One major challenge is cross-contamination of samples during collection, processing, or analysis. The researchers have devised plastic free protocols while dealing with biological samples. Despite all the precautions, this factor impacted findings in many studies. The use of filtered ventilation system in the labs is important to minimize airborne contamination. The use of blanks and their proper evaluation can have great significance in this regard.

MPs have been detected in various human samples such as placenta, stool, colon, lung, sputum, liver, breast milk, and blood samples. Although some correlations were observed –the concentration of MPs was higher in the stools of patients suffering from IBD than in those of healthy individuals and in the case of liver tissues, MPs were only detected in cirrhosis patients– they are still not established due to several limitations. As very little is known about the toxicity of MPs to humans, more research should systematically address this subject. The studies reporting the detection of MPs in the human body are very few and, in many cases, involve a limited number of samples; thus, future studies should include a more representative sample size. The area of MPs detection in humans is still at an initial phase and more studies are required to get a fully understandable picture. While the presence of MPs in human matrices is a cause for concern, the full extent of the consequences is not yet well understood.

CRediT authorship contribution statement

Damià Barceló: Methodology, Conceptualization, Resources, Investigation, Writing, Supervision, Project administration, Funding acquisition. **Yolanda Picó:** Writing – original draft, Visualization, Writing – review & editing. **Ahmed H. Alfarhan:** Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

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

Data Availability

Data will be made available on request.

Acknowledgments

The financial support from the project number (RSP-2023R11) King Saud University, Riyadh, Saudi Arabia.

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