MICROPLASTICS

Second edition *(Updated May 2025)*

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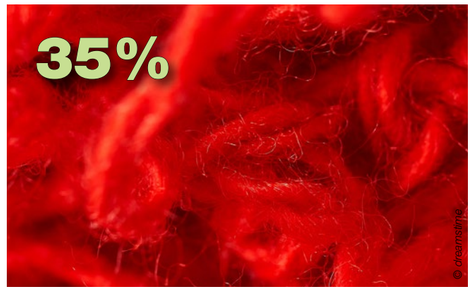
What are microplastics?

Where are they coming from?

A study by the International Union for Conservation of Nature (IUCN)[1] identified the main sources of Microplastics and divided them into 7 main categories:

Synthetic textiles



Synthetic textiles are the single greatest contributors to engineered microplastics in the ocean, accounting for 35 percent of the total volume; indeed washing synthetic textiles frees engineered microplastics through abrasion and shedding of fibers from the fabrics. This is due to the mechanical and chemical stresses that fabrics undergo during the washing process in a laundry machine.
Browne et al.[2] showed that a single garment can release more than 1900 microplastics (<1mm) in each washing cycle and as there are more than 840 million washing machines globally[3] it is clear why synthetic textiles are the main source of microplastics.

Tires

Today, about 24% of a tire consists of synthetic rubber, a plastic polymer, and 19% natural rubber. Microplastics form a matrix of the synthetic polymers, giving the tire rigidity and providing traction. The rest of the tire is metal and other compounds. Tires erode through heat and friction from contact with the road. The wind and rain spread the tire dust and wash it off the road. It enters tributaries, lakes and eventually the oceans.

City Dust

City dust, which accounts for 24 percent of microplastics in the oceans, comes from a variety of sources. While each is a small contributor, it adds up in a populated area. City dust includes losses from the abrasion of objects like synthetic soles of footwear, synthetic cooking ustensils and of infrastructure like household dust, artificial turf, harbors and marina building coatings. It also includes particles from blasting, abrasives, weathering of plastic materials and use of detergents.

Road Marking

Crews apply road markings while building and maintaining roadways. Particularly in Europe these markings include polymer tapes and paints. These are thermoplastics that become soft and flexible at warmer temperatures, allowing weathering or abrasion by vehicles to turn them into microplastics.

1. Boucher, J. and Friot D. (2017). Primary Microplastics in the Oceans: A Global Evaluation of Sources. Gland, Switzerland: IUCN. 43pp.
2. Browne et al. ENVIRONMENT SCIENCE & TECHNOLOGY 21 p.9175 2011
3. F. Salvador Cesa et al. SCIENCE OF THE TOTAL ENVIRONMENT 598 p.1116 2017

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Marine Coatings

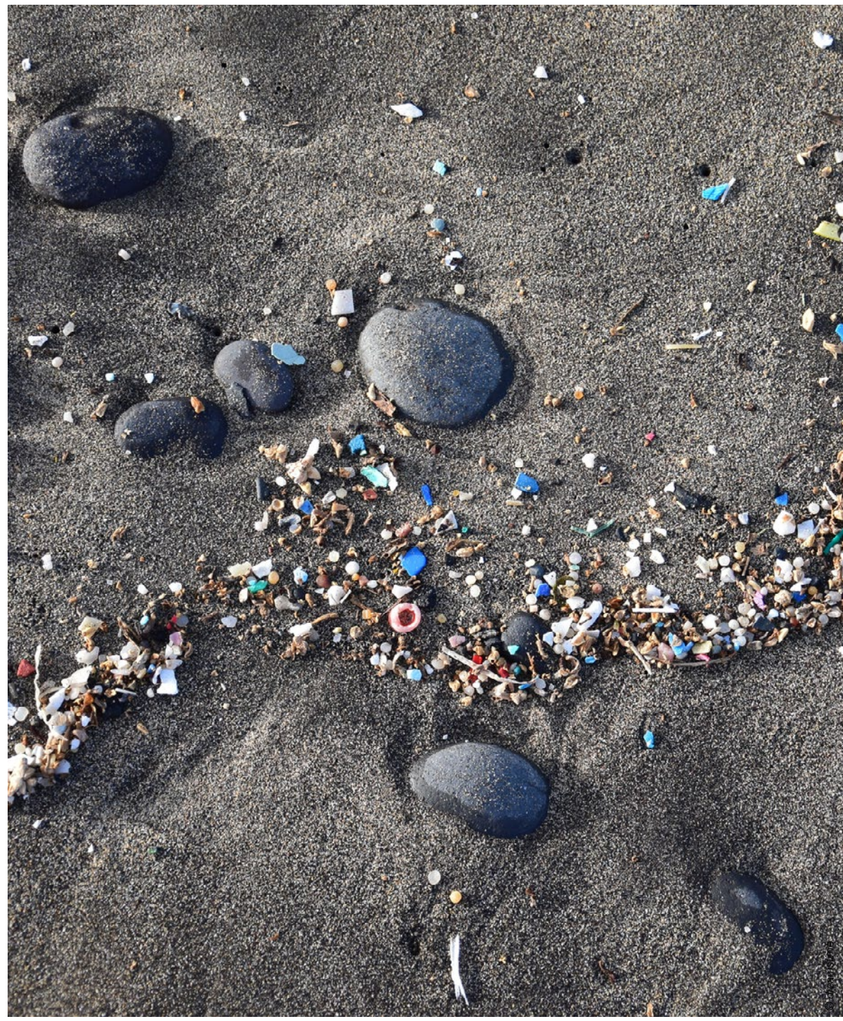
Operators apply marine coatings to all parts of seagoing vessels for protection. Coating's developers use several types of plastics for marine coatings, most commonly polyurethane and epoxy coatings, vinyl and lacquers. Weathering and spills during application, maintenance and disposal of these coatings cause the release of primary microplastics.

Plastic Pellets

Manufacturers often produce primary plastic as small pellets or powders. These producers then transport the pellets to plastic transformers that make end products. Pellets can inadvertently spill into the environment during manufacturing, processing, transport and recycling. Plastic pellets make up 0.3 percent of the ocean's primary microplastics.

Personal Care Products

Many personal care and cosmetic products contain a type of engineered microplastic known as microbeads. The products include scrubbing agents, shower gels and creams.



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What are microplastics?

Definition

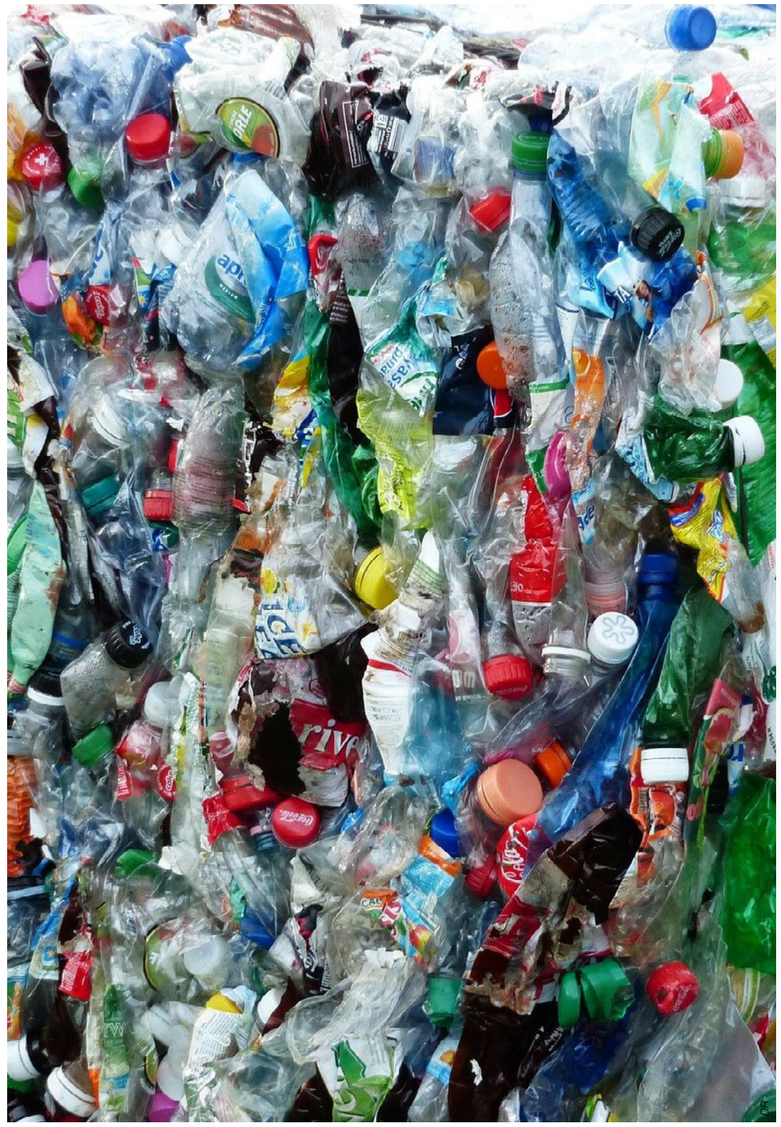
The term microplastic was coined only in 2004 in a paper published by Thompson et all[1] in Science. In this pioneering work they observed the presence of microplastics for the first time in sediment coming from a UK beach close to Plymouth and their subsequent tests found microplastics in 17 other beaches. microplastics remained mainly an academic topic up to 2018 when the presence of microplastics was observed in bottled water[2] and human stools[3] raising a huge interest from the media.

Nowadays a universally agreed and official definition of "Microplastic" is still missing even if there is general agreement on what this term refers to within the relevant communities (Researchers, media etc.: **Microplastics are small pieces of plastic made from synthetic polymers**.

The National Oceanic and Atmospheric Administration, NOAA, defined in 2009 (Arthur et all[4]) an upper size limit in 2009: **"Piece of plastic particles smaller than 5 mm"**.

In 2015 the Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP[5]), added a lower limit, including for the first time, nanoplastics (down to 1 nm): **Microplastics are particles in the size range 1 nm to < 5 mm**.

In our view, the definition which it summarizes all the others and provides an additional constraint around fibers (which are one of the main sources of microplastics in marine environments, see "where are they coming from?" section) is the one used by the European Chemical Agency in their Annex XV Restriction Report on Intentionally added Microplastics of August 2019[6].



'Microplastic' means a material consisting of solid polymer-containing particles, to which additives or other substances may have been added, and where ≥ 1% w/w of particles have (i) all dimensions 1nm ≤ x ≤ 5 mm, or (ii), for fibres, a length of 3 nm ≤ x ≤ 15 mm and length to diameter ratio of >3. Polymers that occur in nature that have not been chemically modified (other than by hydrolysis) are excluded, as are polymers that are (bio)degradable.

1. Thompson et al., SCIENCE, 304 p.838 2004
2. Mason et al., FRONTIERS IN CHEMISTRY, 6 (article 407) p.1 2018
3. Schwabl et al., ANNAL INTERNAL MEDICINE, 171(7) p.453 2019
4. Arthur, C., J. Baker and H. Bamford (eds). 2009. Proceedings of the International Research Workshop on the Occurrence, Effects and Fate of Microplastic Marine Debris. Sept 9-11, 2008. NOAA Technical Memorandum NOS-OR&R-30.
5. GESAMP (2015). (Kershaw, P. J., ed.). (IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection). Rep. Stud. GESAMP No. 90, 96 p.
6. European Chemical Agency - Annex XV Restriction Report on "Intentionally added Microplastic"

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What are microplastics?

We understand the definition of microplastics as small pieces of solid polymer particles etc., but it is important to make a step forward and identify which are the most common types of plastics produced globally[1].

Polyolefins (PP and PE based plastics) represent more than 50% of the global production (2015 data) as they have several advantages such as low production costs, good chemical/physical resistance, etc.; advantages that can turn into downsides when considering their lifecycle because they also degrade very slowly and can survive in the environment as microplastics for centuries, being one of the main components of city dust.

An additional differentiation of microplastics widely used by the community, introduced first by Cole et al. in 2011[2], is the separation between:

Primary microplastics & Secondary microplastics.

Primary microplastics are directly released into the environment as small pieces of plastic. These are intentionally engineered particles, like those found in some consumer and industrial products. Cosmetics, for example, have used microplastics as abrasives and textiles use it for durability.

Secondary microplastics are the result of the degradation of large plastic waste, like plastic bags and bottles, into smaller plastic fragments when exposed to our environment.

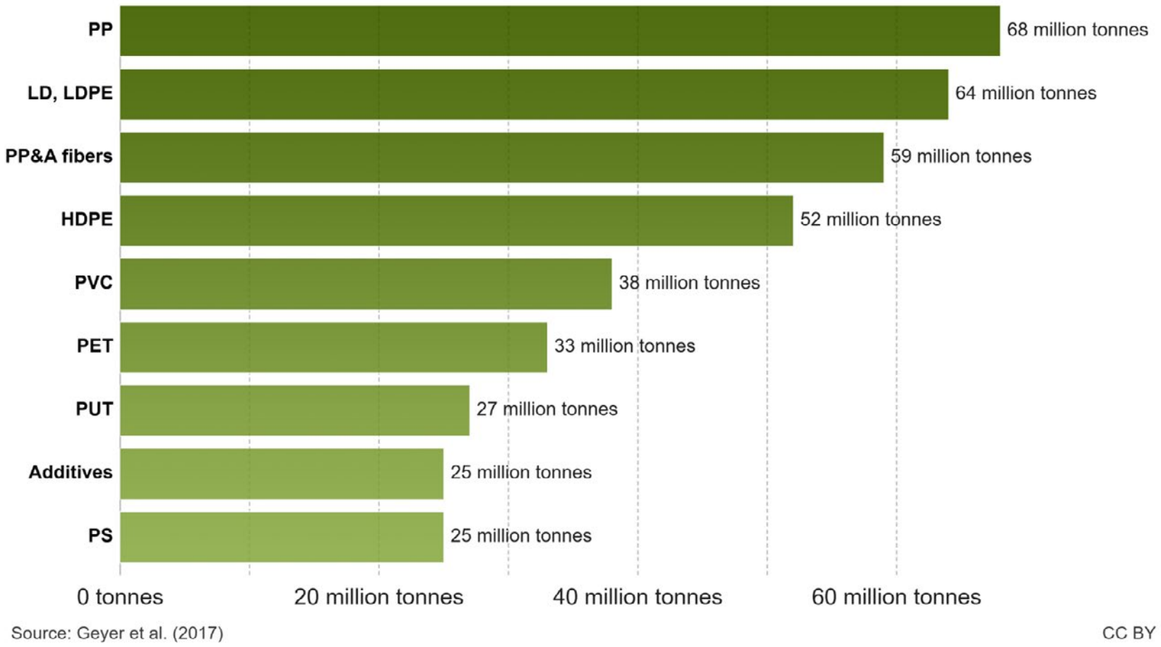

Fig. 1. Primary plastic production by polymer type, 2015
Global primary plastic production by polymer type, measured in tonnes per year. Polymer types are as follows: LDPE (Low-density polyethylene); HDPE (High-density polyethylene); PP (Polypropylene); PS (Polystyrene); PVC (Polyvinyl chloride); PET (Polyethylene terephthalate); PUT (Polyurethanes); and PP&A fibers (Polyphthalamide fibers).

1. Hannah Ritchie (2018) - "Plastic Pollution". Published online at OurWorldInData.org
2. Cole et al., MARINE POLLUTION BULLETIN 62 p2588 2011

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What are microplastics?

Why are they a concern?

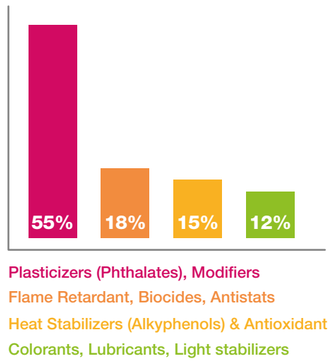
Microplastics are considered a great concern due to several reasons:

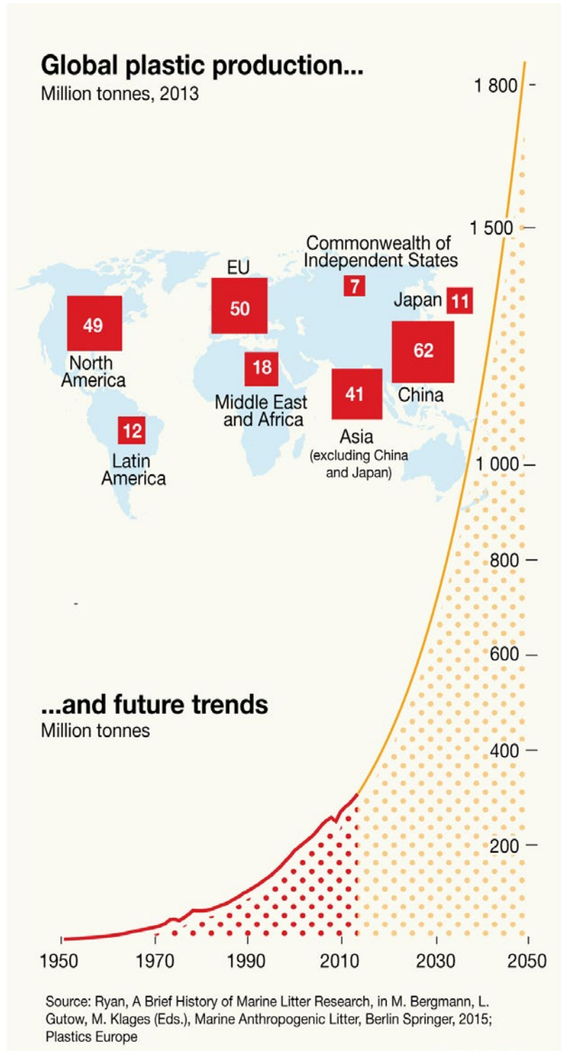
- **Plastic production** is increasing year over year and their degradation process is very slow. Plastics can remain in the environment, particularly the marine environment, for centuries*.

- Microplastics on average contain 4% by weight[1] of other substances whose **human toxicity is well-known**, including:
  - Organics such as some Persistent Organic Pollutants (POPs), Polychlorinated Biphenyls (PCBs), Polycyclic Aromatic Hydrocarbons (PAHs), Phthalates etc.
  - Inorganics such as Titanium dioxide, Barium oxide etc.
  - Remaining monomers

Microplastics can absorb and be an aggregation center for these types of substances dissolved in water due their higher chemical affinity with respect to water (higher hydrophobicity), increasing their load and potential toxicity.

The plastic additives industry represents 10% (≅ 58 billion dollars) of the overall plastics value with plasticizers, modifiers and flame retardant being the main produced.





Expected global plastics production up to 2050

* A high number of industries are strongly depended by plastics and several of their innovation were not achievable without them. The issue is not the plastics but their recycling process, waste management and human behavior.

Fig. 2. Trends in global plastic production. The projected exponential increase is the result of predictions based on increasing population and resulting demand and forecasting from the known curve

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Why are they a concern?

Microplastics have been found in a huge number of species among all groups of wildlife (over 557 species[2]) and in several types of food (salts[4], fish[5], beer, honey[6], tap and mineral water[7], for example.
The consumption of these foods can transfer microplastics and their additives into the human body.

DR

Exposure to microplastics in laboratory environment has demonstrated their potential toxicity, causing serious effects to marine animals[3] such as mortality, reduced feeding rate, body mass, and metabolic rate, decreased fertilization and larval abnormalities, neurotoxicity and others.

Recent studies have shown the presence of microplastics in human bodies:

- Schawbl et al.[8] in 2019 found microplastics in human stools, the number of samples was only 8 but each sample had a median of 20 plastic particles ranging from 50 to 500 µm in size. Nine polymer types were identified with polypropylene and polyethylene terephthalate the most abundant. The study of Schawbl demonstrates that microplastics can find a way through the human gut and potentially may move to the circulatory system,

- Ragusa et al.[9] detected plastic fragments in placenta samples collected from six patients with uneventful pregnancies, All the particles were less than 10 µm in size. The presence of microplastics in the placenta shows that they can reach the circulatory system and be transported to different organs.

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Why are they a concern?

Notwithstanding the potential risk associated with microplastics it is difficult to predict their toxicity for human health due to the lack of studies providing in vivo data on the absorption of microplastics. Moreover. the few in vitro studies show that particle uptake by the human body (Lusher et al.[10] and references cited within) is expected to be limited and strongly linked to the size of the particles.

Only microplastics below 150 µm may translocate from the gut epithelium and a small portion of them, with sizes below 5 µm (nanoplastics being the more dangerous), may penetrate into other organs as demonstrated by the work of Ragusa.

Considering this, it is crucial to focus on the analytical techniques which allow the identification and characterization of the smallest particles.These include Raman Microscopy, SEM, AFM etc.

DR

1. Bouwmeester *et al*. ENVIRONMENT SCIENCE & TECHNOLOGY 49 p.8932 2015
2. Kühn, S., Rebolledo, E. L. B., & van Franeker, J. A. (2015). Deleterious effects of litter on marine life. In Marine Anthropogenic Litter (pp. 75-116). Springer, Cham.
3. Barboza *et al*., 2018. MARINE POLLUTION BULLETIN 133 p.336 2018
4. Yang *et al*., ENVIRONMENT SCIENCE & TECHNOLOGY 49 p.13622 2015
5. Rochman *et al*., SCIENTIFIC REPORTS 5 p.1 2015
6. Liebezeit *et al*., FOOD ADDITIVES & CONTAMINANTS: Part A 30 p.2136 2013
7. Mason *et al*., FRONTIERS IN CHEMISTRY, 6 (article 407) p.1 2018
8. Schwabl *et al.*, ANNAL INTERNAL MEDICINE, 171(7) p.453 2019
9. Ragusa *et al*., ENVIRONMENT INTERNATIONAL 146 p.1 2021
10. Lusher *et al*., FAO Fisheries and Aquaculture Technical Paper. No. 615. Rome, Italy. 2017

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What are microplastics?

Regulatory landscape around microplastics

Although the impact of microplastics on the environment and the health of living organisms remains under investigation, there are already existing regulations addressing microplastics. These regulations aim to:

- **Reduce the release of microplastics into the environment where it is feasible through legislative measures**
- **Standardize analytical methods for measuring microplastic concentration**
- **Implement systematic monitoring of microplastic concentrations.**

To reduce the microplastics pollution

Single-use plastics

In 2018, the United Nations Environment Programme (UNEP) published a global review of national laws and regulations regarding legal limits for single-use plastics and microplastics [1]. The review mapped the status of legislation in 191 countries. The findings showed that restrictions or taxes had been adopted on:

- Plastic bags in 127 countries
- Single-use plastics in 27 countries
- Microbeads in 9 countries

The latest updates:
Since 2018, the situation has evolved, and many more countries have introduced national legislation.

[1] Legal limits on single-use plastics and microplastics | UNEP - UN Environment Programme, 2018
[2] Commission Regulation (EU) 2023/2055

What are microplastics?

Regulatory landscape around microplastics

Restriction on intentionally added microplastics has been adopted by EU

Since October 17, the **European Union's** (EU) Regulation, known as the **"microplastics restriction,"** has been in effect [2]. This regulation bans synthetic polymer microparticles in concentrations of ≥ 0.01% by weight, either on their own or in mixtures. The following products containing such particles are considered:

- Glitter,
- Facial scrubs and other types of cosmetics,
- Detergents
- Waxes, polishes and air fresheners,
- Certain fertilisers, plant protection products and seeds treated with them, biocides,
- Other agricultural and horticultural products other than those listed above
- Certain medical devices covered by Regulation (EU) 2017/745
- Granular infill for use in synthetic sports surfaces (such as rubber substrate for artificial turf sports surfaces).

Exceptions to the ban include polymers that are:

- A result from a natural polymerization process
- Bio-degradable
- Highly-soluble (greater than 2 g/L)
- Carbon-free
- Permanently incorporated into a solid matrix
- Physically modified during use so they no longer fit the microplastics definition

There is no exhaustive list of affected cosmetic ingredients; each substance must be assessed individually to determine if it qualifies as a synthetic polymer microparticle (SPM).

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What about unintentionally released microplastics?

In October 2023, the EU Commission proposed measures to prevent microplastic pollution from the **unintentional release of plastic pellets**. The proposal aims to ensure that all operators handling pellets in the EU take necessary precautionary measures. The proposal encompasses best practices for handling, obligatory certifications, self-declarations, and the development of a standardized method for estimating pellet loss. The proposal will be discussed by the European Parliament and the Council. Both EU-based and international economic operators, must comply with the requirements set out in this regulation within 18 months of its entry into force.

Limiting microplastic release from other sources, such as paints, tires, synthetic textiles, and geotextiles, presents greater challenges since it necessitates significant modifications to products or shifts in consumer behavior. Currently, these issues are being tackled through the **Ecodesign for Sustainable Products Regulation** and the **EURO 7 Regulation proposal**.

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The efforts to standardize the analytical methods for measuring microplastics concentration have been performed by several technical committees included in the International Organization for Standardization (ISO) and European Committee for Standardization (CEN).

Below you will find the information on the documents which were already published or are expected to be published soon.

Standardization of the analytical methods:

ISO 24187:2023

Principles for the analysis of microplastics present in the environment

This document outlines principles for analyzing microplastics in various environmental matrices, covering particle size classification, sampling apparatus, sample preparation, and determining representative sample quantities. It sets minimum requirements and gives general guidelines until specific standards for the different case situations are available.

Prepared by Technical Committee ISO/TC 61, Plastics, Subcommittee SC 14, Environmental aspects, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 249, Plastics.

ISO 4484:2023

Textiles and textile products — Microplastics from textile sources

This document addresses and standardizes the analysis of microplastics from textile sources. It consists of 3 parts
Part 1: Determination of material loss from fabrics during washing
Part 2: Qualitative and quantitative analysis of microplastics
Part 3: Measurement of collected material mass released from textile end products by domestic washing methods

Prepared by Technical Committee ISO/TC 38, Textiles, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC248, Textiles and textile products

ISO/DIS 16094 (not published yet)

Water quality — Analysis of microplastic in water

This document is expected to be published in 2025. It aims to provide a detailed description of the typical workflow, method validation, and quality control requirements for microplastics analysis in water with low level of total suspended solids. This project is divided into three parts:
Part 1: General principles (no exact name is available at the time of publication of this booklet)
Part 2: Vibrational spectroscopy methods for waters with low content of suspended solids, including drinking water
Part 3: Thermo-analytical methods for water with low content of suspended solids including drinking water.

The three parts are proceeding separately; part 2 and 3 have reached the DIS stage (Draft International Standard).

Prepared by Technical Committee ISO/TC 147, Water quality, Subcommittee SC 2, Physical, chemical and biochemical methods

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Towards regular monitoring

In March 2024, the **Drinking Water Directive (EU) 2020/2184**, which previously mentioned microplastics as emerging contaminants that needed to be monitored, was supplemented with guidelines for measuring microplastic concentrations. The updated document recommends to use vibrational spectroscopy for systematic microplastics monitoring (https://environment.ec.europa.eu/publications/delegated-act-measure-microplastics-water_en).
The frequency of monitoring will depend on the presence of microplastics in tap water and may be adapted by the member states in function of established risk assesment and measured concentration of microplastics.

In the USA, California has been a leader in this area, passing legislation to study and address microplastic pollution in drinking water and the marine environment. Various federal agencies, including the Environmental Protection Agency (EPA) and the National Oceanic and Atmospheric Administration (NOAA), conduct research and monitoring related to microplastics. These efforts can provide insight into future regulations and strategies for addressing microplastic pollution.

In Europe, the Marine Strategy Framework Directive has initiated monitoring along coastlines, on the surface of the sea, and in seabed sediment.

Drinking Water Directive, Groundwater Directive, and the Environmental Quality Standards Directive establish a legislative foundation for future systematic microplastics monitoring under the overall Water Framework Directive. The proposed revisions to the Urban Wastewater Treatment Directive include the implementation of monitoring at the inlets and outlets of urban wastewater treatment plants and in sludge.

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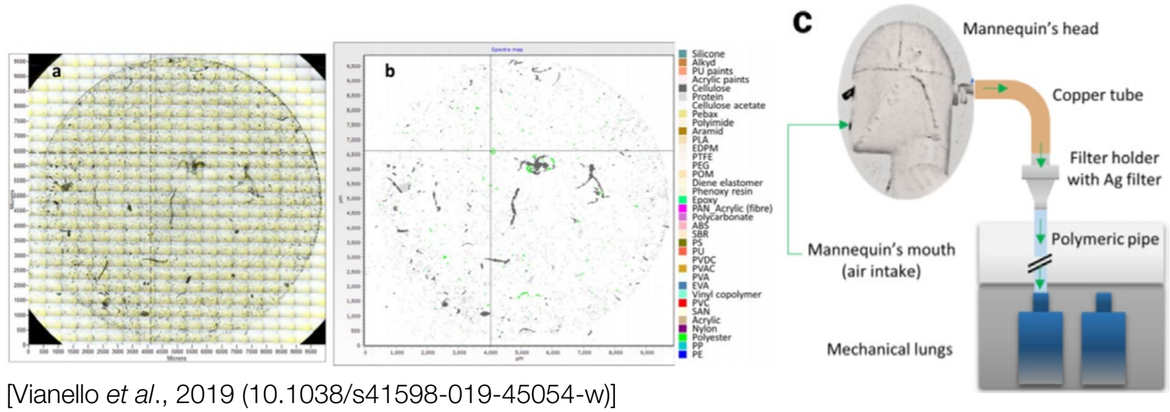
Humans and Plastic

Microplastic and humans: proofs of exposure and hypothesis of danger

Considering the increasing concern about the human and animal exposure to Microplastics we decided to have a dedicated chapter on this topic and as first and introductory contribution we asked Valentina Notarstefano to write a short review to explain the most common routes of human exposures to microplastics and show the evidence of their accumulation and translocation in the human tissues. Valentina is a post-doc researcher at the Department of Life and Environmental Sciences at the Polytechnic University of the Marche (Ancona, Italy) and is working in the team of Prof. Elisabetta Giorgini (head of the Laboratory of Vibrational Spectroscopy). Valentina has focused her research on the use of Infrared and Raman spectroscopy to study biological systems such as cells, tissues, fluids and is providing in this review a clear picture of the actual state of the art about Humans&Microplastic without reaching hasty conclusion.



[Vianello *et al*., 2019 (10.1038/s41598-019-45054-w)]

Valentina Notarstefano

Postdoc at Università Politecnica delle Marche - DiSVA - Ancona, Marche, Italy

The presence of microplastics in the environment has been widely documented and their ubiquitous nature makes the human exposure inevitable. The three so-called exposure routes are ingestion, inhalation and dermal contact. However, we have to pinpoint that dermal absorption is a minor exposure route, since only particles smaller than 100 nanometers can likely pass the dermal barrier [1].

Ingestion is considered the principal exposure route, with an estimated intake of about 39 to 52 thousand ingested particles per person per year. These particles can be ingested from contaminated food, like fish and mussels, but also from table salt, sugar, honey, milk and bottled water. Moreover, food can be contaminated from our use of plastic packaging and plastic kitchen utensils [1].

Besides ingestions, it has been demonstrated that microplastics also contaminate the air we breathe. These microplastics mainly derive from synthetic textiles, but also from the abrasion of plastic materials. In particular, it has been estimated that outdoor concentrations range from 0.3 to 1.5 microplastics per cubic meter, while the indoor ones can also reach 56.5 microplastics per cubic meter. Moreover, a study demonstrated that a person can inhale up to 272 particles per day, with a tangible possibility that these particles reach alveoli and enter the bloodstream, obviously according to their dimensions [1–3].

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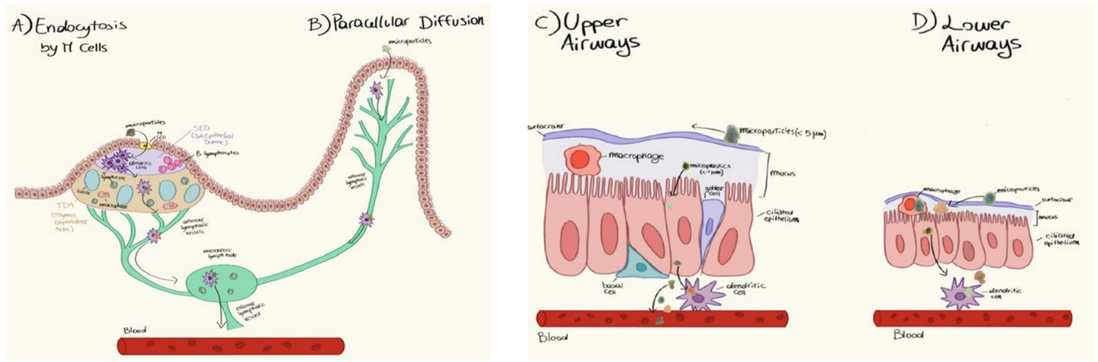
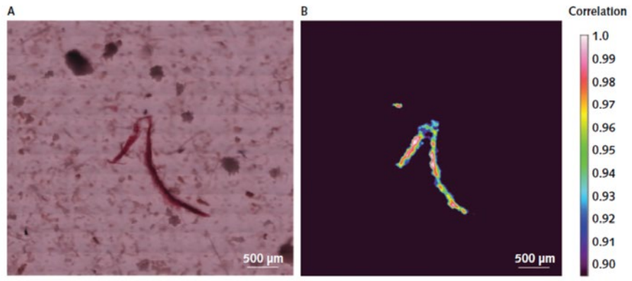
Once the routes of exposure have been identified, researchers focused on elucidating what happens to the internalized particles. Obviously, the fate of the inhaled or ingested microplastics strongly depends on their features, first of all their dimensions, that cannot exceed 10-15 microns, making it possible for the particles to travel in the bloodstream. In fact, the interest towards the toxic effects of microplastics in terms for example of oxidative stress, inflammation, and immune response is everyday greater, however these phenomena can only happen after the passing of microplastics through cellular membranes and their accumulation within tissues. Some hypotheses have been suggested to explain the penetration of microplastics through human tissues, until their arrival into the bloodstream from which, hence, they can potentially reach numerous body districts [4,5]. 1) endocytosis by the M cells (which are mucosal cells of the intestine, placed next to the lymphoid nodules called Peyer's Patched, with the role of modulating the immune response); the M cells act by endocytosing solid particles and transferring them to the dendritic cells; when these soldi particles are microplastics, we know that they cannot be destroyed by the action of the lytic enzymes and hence they would be transported by the dendritic cells to the lymphatic stream and then to the blood stream. 2) paracellular diffusion: microplastics may also penetrate the organism by passing the intestinal lumen if there are points where the junctions are more loose; this possibility arises when there are inflammation states, for example; even in this case, the dendritic cells would transfer microplastics to the lymphatic and then blood streams. 3) at the level of the Upper airways, the mucus layer is thick and allows a successful clearance of the foreign bodies; moreover, the mechanical movement of the ciliated epithelium prevents particles from spreading through the epithelium and reach the circulation. (D) conversely, at the level of the Lower airways, the mucus layer is thinner, thus facilitating the diffusion of particles which have reached the respiratory tract. Once penetrated, the MPs can spread into the general circulation by cellular uptake or diffusion.



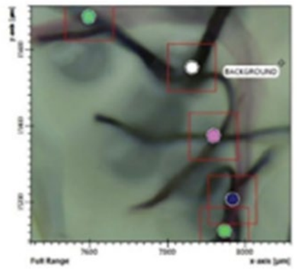
[Ragusa *et al*., 2021 (10.1016/j.envint.2020.106274)]

The proofs of human exposure are growing in literature. The first study in this sense is from 2019: the authors reported the presence of MPs in human stool. This is not a proper evidence of accumulation in tissues, but for the first time the researchers demonstrated that ingestion really is an exposure route for humans [6].



[Schwabl *et al*., 2019 (10.7326/M19-0618)]

A step forward has been made by another research group, who found MPs in human colectomy samples, ultimately proving that not only MPs are ingested and excreted, but also a part of the mis also internalized through the intestinal tissue [7].



[Ibrahim *et al*., 2021 (10.1002/jgh3.12457)]

Then, MPs have been found in lung tissues by exploiting Raman spectroscopy: this result confirms that inhalation is an exposure route and that inhaled particles can accumulate in human tissues [8]. Recently, this study has been confirmed by another one, quite similar, but performed by exploiting IR spectroscopy [9]. In 2021, some Italian researchers found microplastics in human placenta samples, by using Raman microspectroscopy; in particular, 12 MPs were found in 4 out of the 6 analysed placenta samples [5]. We obviously have to mention a very recent paper where researchers report the presence of MPs bigger than 700 nanometers in blood, with an average concentration of about 1.6 micrograms per ml. The main identified polymers where polyethylene, polyethylene terephthalate and various styrene-based polymers. Obviously, this result is crucial, since it indirectly confirms all the others, proving that MPs really reach the bloodstream, by which then they reach numerous other body districts. For this study, authors did not exploit a vibrational spectroscopy, pyrolysis double shot - GC/MS [10]. Finally, the same Italian team who found microplastics in human placenta recently published their discovery of microplastics in human breastmilk; in particular, microplastics ranging from 2 microns to 12 microns, mainly made of PE, PVC, and PP, were found in 26 out of the 34 analyzed samples [11].

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Humans and Plastic

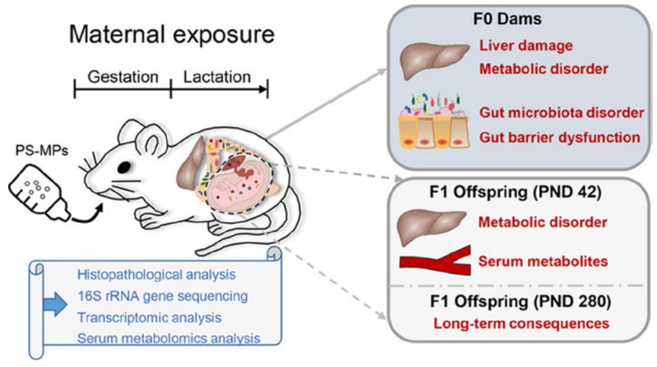
Microplastic and humans: proofs of exposure and hypothesis of danger

So far we have seen how microplastics are able to enter the human organism, and travel through the bloodstream, potentially accumulating in almost all districts. The next question is: once internalized and accumulated, can microplastics exert a toxic effect? Or are they just inert material? Several studies have reported clear toxic effects of various types in animal models, marine organisms and human cell lines, and these results are precious in understanding what can happen to humans. It has been reported that microplastics, in addition to translocating to other tissues, can cause for example oxidative stress, cytotoxicity, neurotoxicity, immune reaction, etc. As regards cytotoxicity and immune modulation, it has been demonstrated, for example, that PP-MPs exert a cytotoxic effect on immune and blood murine cells, mainly by inducing an increase in ROS, in a size-dependent and concentration-dependent manner [12]. Moreover, the direct contact of the polypropylene particles with the immune cells did not in itself lead to toxicity, but induced an increase in the production of cytokines and histamine.

Oxidative stress can derive from an overcrowding of antioxidant responses, generated by the high surface area of microplastics, from the release of oxidizing species adsorbed on their surfaces, such as metals, or from the inflammatory response. Microplastics have been shown to alter some biomarkers of oxidative stress and to trigger the production of reactive oxygen species. For example, the tissue uptake and accumulation of polystyrene microplastics in zebrafish were investigated in this study [13]: the authors reported that, depending on their size, microplastics accumulate in the gills, liver and intestines of fish, also inducing inflammation and accumulation of lipids in the liver, with an increase in the activities of superoxide dismutase and catalase, signals of oxidative stress. Also in mice, fluorescent polystyrene microplastics added to the water showed to accumulate in the liver, kidneys and intestine; moreover, the authors highlighted a disturbance of energy and lipid metabolism, together with oxidative stress [14].

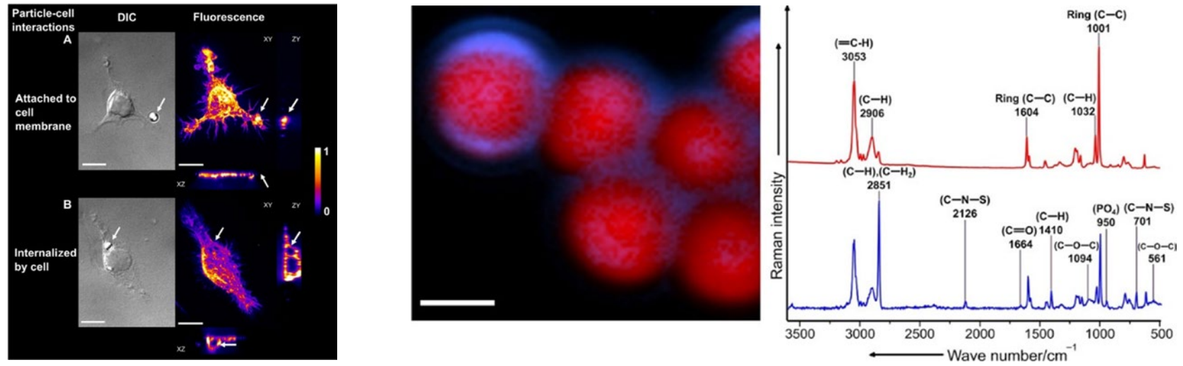
The immune system also appears to be strongly influenced by the absorption of microplastics: in this regard, microplastics act like other environmental particles which, once internalized, provoke local or systemic immune responses. However, the evidence in this area is still limited. For example, a study performed on the mussel *Mytilus galloprovincialis*, showed that the exposure of the mussels to microplastics led to the interruption of global homeostasis, with the production of immune-related proteins; the removal of microplastics showed to activate apoptotic processes and to upregulate stress-related proteins, in an attempt to compensate for the stress associated with exposure to microplastics [15]. Notably, repeated exposures to microplastics have suggested that mussels may be able to create some sort of memory about exposure to microplastics.

There is also evidence of detrimental effects on reproductive abilities. A very alarming study reported transgenerational effects due to the exposure to microplastics [16]. Rodents who were given contaminated water during gestation and lactation showed liver changes and altered intestinal microbiota; in addition, the F1 generation showed similar hepatic alterations and altered levels of metabolites in serum; negligible effects were highlighted in generation F2. Another study reported that the exposure to polystyrene microplastics is able to cause a thinning of the endometrium and severe deposition of collagen fibres in female mice, finally leading to uterine fibrosis [17].



[Luo *et al.*, 2019 (10.1021/acs.est.9b03191)]

All these works are inevitably conducted in the laboratory, under very controlled conditions and with microplastics produced for the very specific purpose. A very interesting study has highlighted how the erosion and aging of microplastics caused by their environmental exposure play a fundamental role in the internalization process in cells [18]. In fact, the researchers have identified biomolecules that form an eco-corona on the surface of the microplastic particles that appear to significantly increase the internalization of microplastics after environmental exposure.



[Ramsperger *et al.*, 2020 (10.1126/sciadv.abd1211)]

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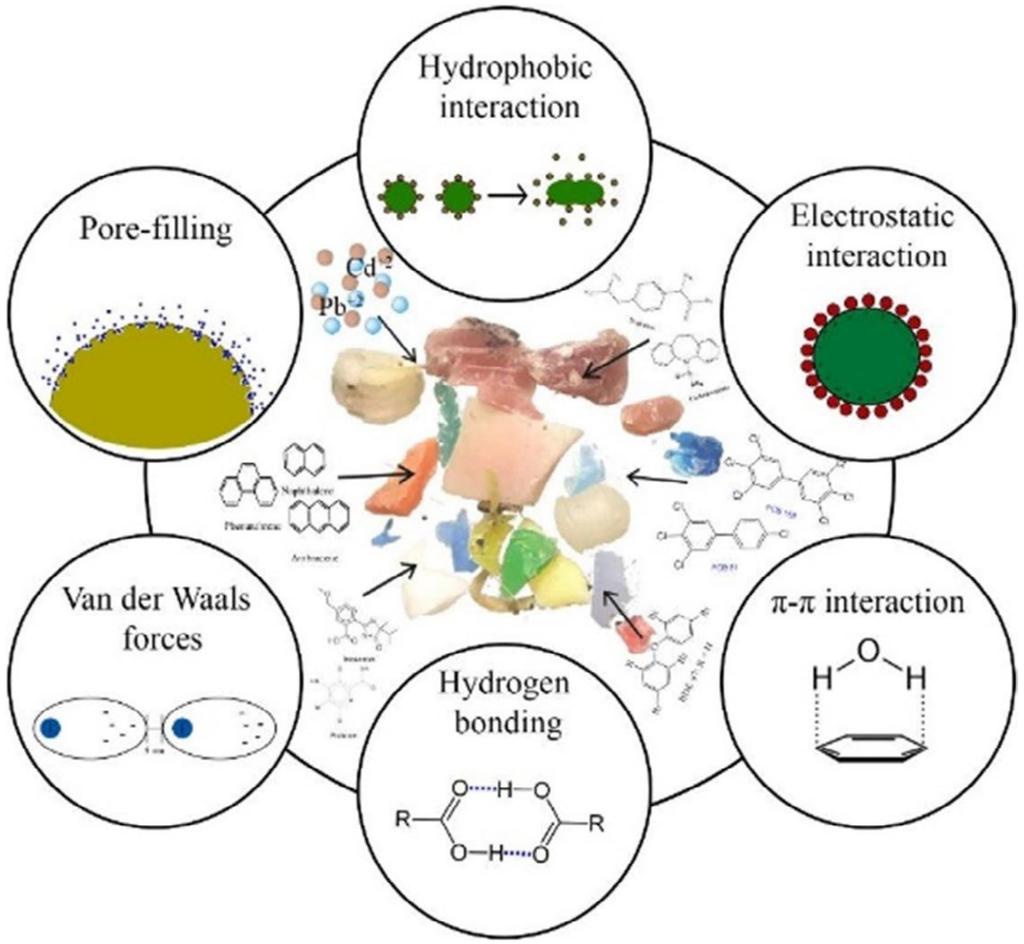
Another aspect must be taken into account when considering the interaction between microplastics and the external environment: it has been widely demonstrated that microplastics display the ability to act as carriers of other possibly toxic and dangerous chemicals, metals and microorganisms, concentrating them several orders of magnitude respect to the levels in the surrounding environment [19–21]. The research presented in the previous examples was conducted in the laboratory, under controlled conditions and with pristine microplastics, while it can be assumed that environmental exposure to microplastics also involves contact with other chemical and biological species. Microplastics may act as efficient vectors for the transport of other potentially toxic and even carcinogenic chemicals, including persistent organic pollutants (POPs) has emerged, including pyrene, benzo (a) pyrene, phenanthrene, polychlorinated bisphenyls, DDT, and polycyclic aromatic hydrocarbons. This absorption is finely regulated by various factors, both related to the polymer (type, colour, size, state of aging) and to the environment (pH, salinity, temperature). It has to be considered that the real evidence of the delivery and desorption of toxic compounds by microplastics is difficult to prove: most of the effects described for pristine microplastics and toxic chemicals are similar and it is difficult to discriminate between the two pollutants. For this reason, the debate on the vector role of microplastics is still ongoing, with favourable and unfavourable arguments: for example, it is not clear whether the absorption of POPs on the surface of microplastics makes them more or less bioavailable to the body once internalized.

[Torres *et al*., 2021 (10.1016/j.scitotenv.2020.143875)]

Furthermore, microorganisms can colonize the surface of microplastics, which act as vectors, carrying microorganisms to tissues, protecting them from the immune system and creating tissue damage that can promote infection. Microorganisms can form fully grown biofilms on the artificial substrate of microplastics, which allowed Yang and colleagues to describe microplastics as new microbial niches in the aquatic environment. For example, Vibrio bacteria are normally characterized by low concentrations in water, but several studies have highlighted their presence on the surface of microplastics with the highest concentration of all microorganisms found. Surely the microplastics that carry bacteria and viruses are more biotoxic and can trigger, among all the effects previously considered, even infections [1,22,23].



In this brief chapter, the identity of microplastics, their sources of contamination, the routes of exposure by which they contaminate animals and humans, and the reasons why they are a concern, have been investigated. In particular, the routes of human exposure, mainly ingestion and inhalation, were explained, together with the published evidence on the translocation and accumulation of microplastics in human tissues. The reported selection of the studies clearly supports the theory that microplastics are not inert particles, but have various effects once internalized: oxidative stress, cytotoxicity, altered immune responses, neurotoxicity and so on; moreover, microplastics are potential and effective vectors of other toxic chemicals and microorganisms: however, evidence of all these aspects has been partly found in marine organisms and animal models, and some results have been achieved in the laboratory, under controlled conditions, which are not always comparable to what actually happens in the environment. Based on all these considerations, it must be stated that the results presented are valuable for understanding what can happen to humans, although we must be cautious in translating this information into alarmism.

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References

1. Prata, J.C. *et al*.; SCIENCE OF TOTAL ENVIRONMENT 702 p.134455 2020
2. Dris, R *et al*.; ENVIRONMENTAL POLLUTION 221 p.453 2017
3. Vianello, A. *et al*.; SCIENCE REPORT 9 p.8670 2019
4. Alimba, C.G *et al*; JOURNAL OF HAZARDOUS MATERIAL 416 p.126143 2021.
5. Ragusa, A. *et al*.; ENVIRONMENTAL INTERNATIONAL 146 p.106274 2021 (https://www.sciencedirect.com/science/article/pii/S0160412020322297)
6. Schwabl, P. *et al*.; ANNALS OF INTERNAL MEDICINE 171 p.453 2019
7. Ibrahim, Y.S. *et al*.; JGH OPEN 5 p.116 2921 (https://onlinelibrary.wiley.com/doi/full/10.1002/jgh3.12457)
8. Amato-Lourenço, L.F. *et al*.; JOURNAL OF HAZARDOUS MATERIAL 416 p.126124 2021
9. Jenner, L.C. *et al*.; SCIENCE OF THE TOTAL ENVIRONMENT 831 p.154907 2022
10. Leslie, H.A. *et al*.; ENVIRONMENTAL INTERNATIONAL 163 p.107199 2022 (https://www.sciencedirect.com/science/article/pii/S0160412022001258?via%3Dihub).
11. Ragusa, A. *et al*.; POLYMERS (Basel) 14 p.2700 2022, 14, 2700 (https://www.mdpi.com/2073-4360/14/13/2700)
12. Hwang, J. *et al*.; SCIENCE OF THE TOTAL ENVIRONMENT 684 p.657 2019
13. Lu, Y. *et al*.; ENVIRONMENT SCIENCE & TECHNOLOGY 50 p.4054 2016
14. Deng, Y. *et al*.; SCIENCE REPORT 7 p.46687 2017
15. Détrée, C. *et al*.; IMMUNOLOGY 83 p.52 2018
16. Luo, T. *et al*.; ENVIRONMENT SCIENCE & TECHNOLOGY 53 p.10978 2019
17. Wu, H. *et al*.; ENVIRONMENT SCIENCE & TECHNOLOGY 838 p.155825 2022
18. Ramsperger, A.F.R.M. *et al*.; SCIENCE ADVANCES 6 p. 2020 (https://www.science.org/doi/10.1126/sciadv.abd1211)
19. Rodrigues, J.P. *et al*.; TRENDS IN ANALYTICAL CHEMISTRY 111 p.252 2019
20. Ricardo, I.A. *et al*.; JOURNAL OF CHEMICAL ENGINEERING 424 p.130282 2021
21. Torres, F.G. *et al*.; SCIENCE OF THE TOTAL ENVIRONMENT 757 p.143875 2021
22. Yang, Y. *et al*.; APPLIED MICROBIOLOGY AND BIOTECHNOLOGY 104 p.6501 2020
23. Wang, X. *et al*.; JOURNAL OF HAZARDOUS MATERIAL J402 p.123496 2021

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Nanoplastics

A growing interest

Nanoplastics, as discussed on chapter 1 (i.e. Microplastics) are the smallest piece of plastics and they are less than 1 micron in size. The interest on plastics below 10 microns and down to nano size range, if we consider publications in scientific peer reviewed journals, grew almost 70% year over year in the last 5 years. One of the main drivers of this growth is connected to the capability of plastics of these sizes to enter in the human body through different routes such as ingestion, inhalation and dermal contact (indeed dermal absorption is possible only for nanoplastics smaller than 100 nanometers) posing a big concern about their effect on the human's health (see chapter Humans and Plastic).

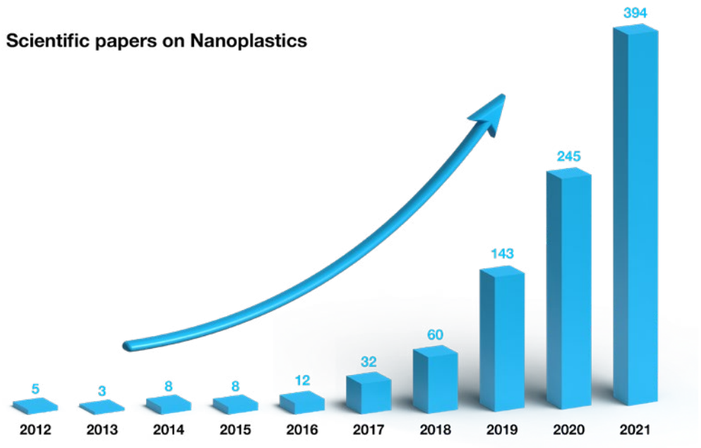

Fig.1 Scientific peer reviewed papers on Nanoplastics in the last 10 years

The growing interest on Nanoplastics can also be measured by looking at the increased number of grants financed on this topic in the last three years (see picture 2). These data are considering a limited number of all the financing entities, but they can be representative of a global trend.

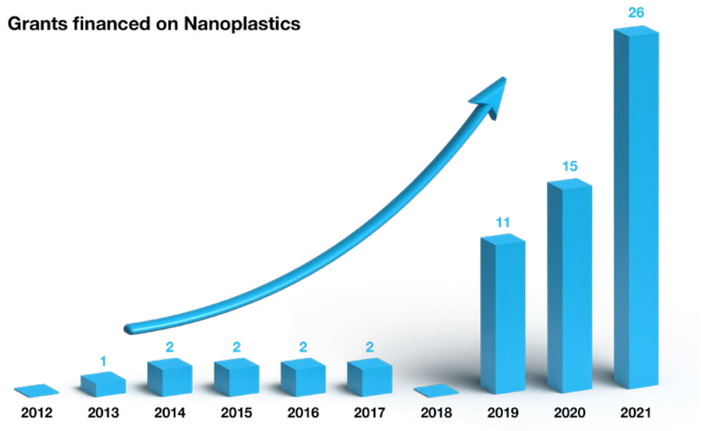

Fig. 2 Financed grants around Nanoplastics in the last 10 years

(information collected using SCITODATE engine by different sources Swiss National Science Foundation – SNSF; Deutsche Forschungsgemeinschaft – DFG; National Science Foundation – NSF; UK Research Innovation – UKRI; Community Research and Development Information Service – CORDIS; French National Research Agency – ANR; Russian Science foundation – RSF; National Institute of Health – NIH etc…)

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Nanoplastics

A growing interest

At HORIBA, we are focused on testing and optimizing various solutions for nanoplastic analysis. This led to the development of nanoGPS, for correlative microscopy.

The nanoGPS is a patented technology that enhances the correlative microscopy approach by allowing fast localization of small objects (μm^2) on large samples (cm^2), making it possible to analyze them using multiple microscopy techniques. This technology combines two software applications for navigation (navYX™) and map superimposition (graphYX™), along with hardware tags. It ensures the accurate re-localization of points of interest across different modalities with micron-level precision, regardless of magnification, sample orientation, or instrument brand.

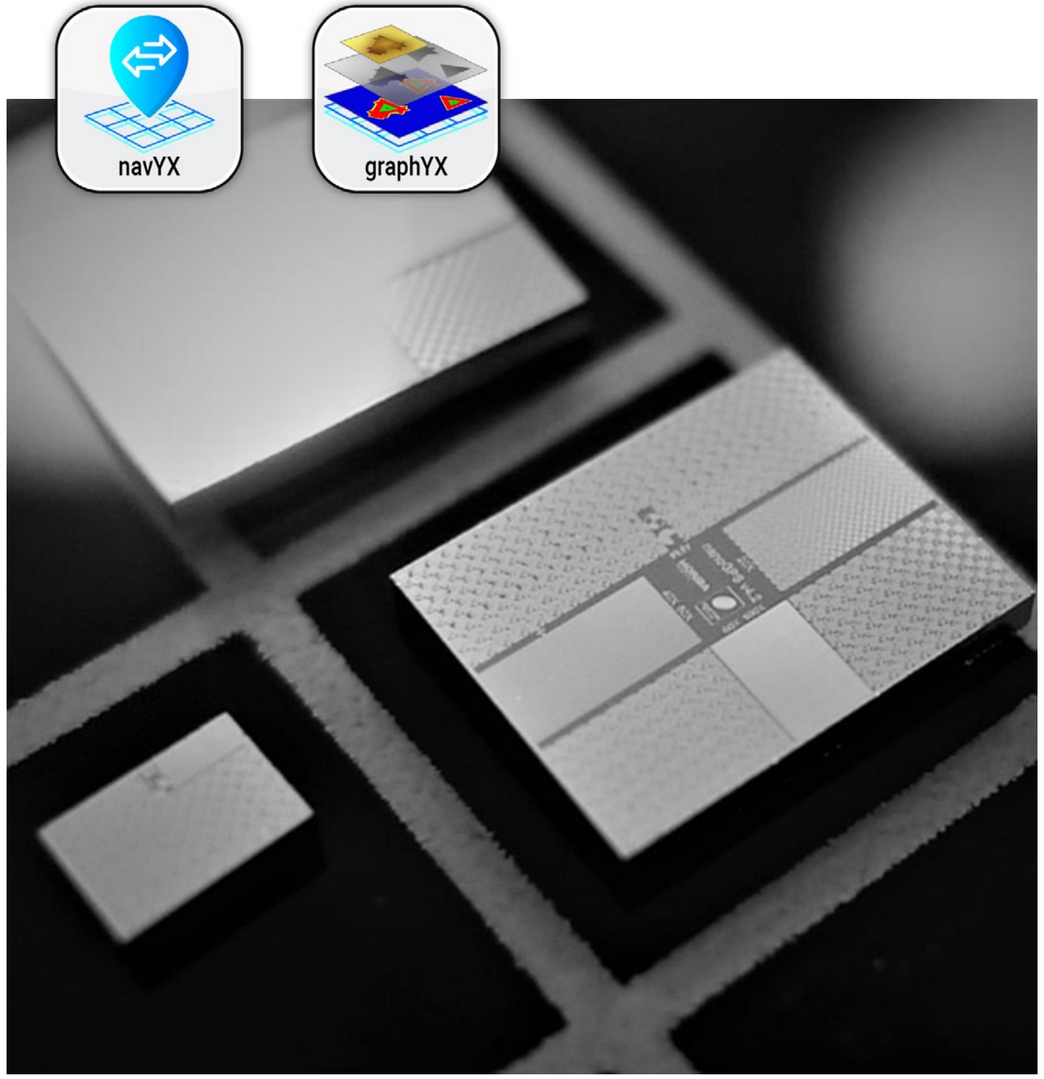
For nanoplastics, the nanoGPS and correlative approach ensure Raman analysis of plastics in the nanometer range by combining SEM and Raman microscopy:

- The SEM image helps locate the nanoplastics (which are not clearly visible under the optical microscope of a Raman instrument), revealing whether they are well-separated or aggregated.
- Raman microscopy, using nanoGPS technology for precise particle localization under the laser, then provides chemical characterization.
- Additionally, the nanoGPS tags are especially well-suited for nanoplastic characterization because they are small (1.4 x 2 mm), easy to attach to any type of filter, and compatible not only with SEM but also with Atomic Force Microscopy and Fourier Transform Infrared Microscopy.

The following pages present you the contribution by George Sarau et al., titled “Context Microscopy and Fingerprinting Spectroscopy of Micro- and Nanoplastics and Their Effects on Human Kidney Cells Using nanoGPS and ParticleFinder,” were we can see the nanoGPS technology applied to a real-life case.

A growing interest

Nanoplastics and human kidney cells



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Human kidney cell and Nanoplastics

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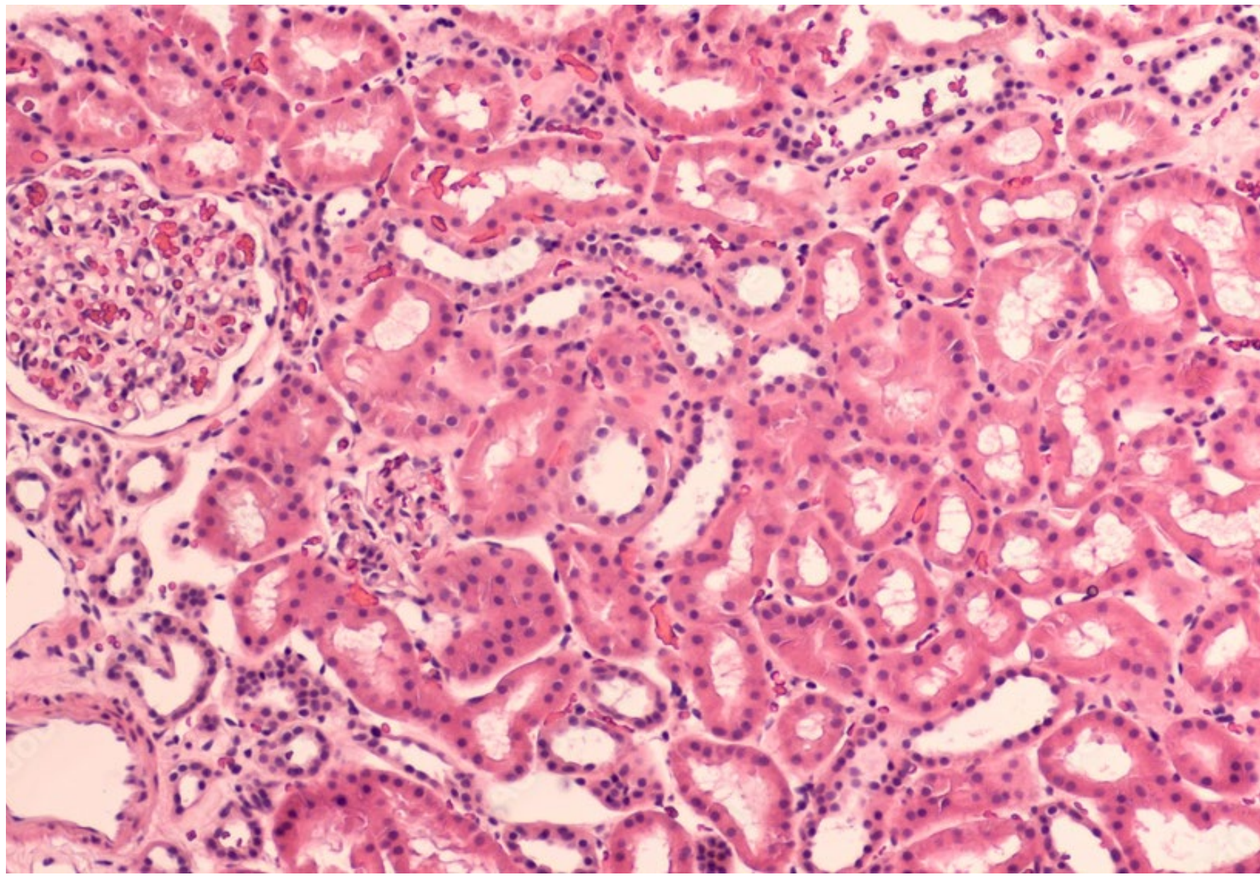
Key words: microplastic, nanoplastic, tyre wear, podocytes, kidney, nanoGPS, ParticleFinder, SEM, Raman, correlative workflow, microscopy, spectroscopy

Nowadays humans are almost continuously exposed to micro- and nanoplastics (MNPs) through food and air, but very little is known about the exposure level and impact on our health. Here, we focus on bottled mineral water and cultured human podocytes as representative kidney cells prone to accumulation of particles. It is demonstrated that identical MNPs and cells can be precisely relocalized and extensively characterized down to nanoscale in independent instruments using nanoGPS and ParticleFinder technologies developed by HORIBA. Reference particles and particles contained in mineral water were detected, enabling statistical distributions of their mean number, size, and type depending on the bottle and label materials. The primary effects of MNPs (three standards and tyre wear) on human podocytes were assessed using a cell viability test followed by correlative microscopy and spectroscopy investigations of the same cells. We observed changes in the biological features of MNP treated cells compared to non-treated controls, attributed to cell damage through surface adhesion and uptake of plastic particles. The integration of automatic relocalization and detection of identical objects in a multi-instrument workflow represents a novel analytical approach that can be applied beyond this topic.



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Introduction

Production of plastics has dramatically increased over the last decades and with it the plastic waste in the environment.[1] Plastics are nowadays used almost in all products including packaging, construction, textiles, tires, cosmetics, and so on.[2-4] The major issue is the mismanaged plastic waste that is not collected at all or improperly filtered and recycled, which significantly contaminates thea environment on a global scale through the transfer between terrestrial, river, and ocean compartments.[5] Once left in the environment, plastic debris persists and degrades continuously into smaller fragments down to micro- and nanoplastic (MNP) particles, attributed to size classes of < 5 mm and < 1 µm or ≤ 100 nm, respectively.[6,7].With time, these MNPs are assumed to develop into toxic chemical cocktails by increased adsorption of hazardous pollutants and pathogens from the environment given their larger surface areas due to fragmentation, in addition to additives and pigments added during manufacturing of plastics. Moreover, the smaller the plastic particles become (< 1.5 µm), the higher the probability to enter by ingestion and inhalation into human organs and subsequently to accumulate and leach chemicals with still unknown toxicological effects on our health.[8-10]

Microscopy- and spectroscopy-based methods are commonly used to monitor MNPs in environmental samples usually after filtering as well as in various biological matrices and organisms. The employed techniques mainly include optical microscopy with stereozoom, scanning electron microscopy (SEM) with energy dispersive X-ray spectroscopy (EDS), pyrolysis gas chromatography coupled with mass spectrometry (py-GC-MS), Fouriertransform infrared (FT-IR) and Raman microspectroscopies, each method with its benefits and drawbacks.[11-13] Recently, we showed that a correlative approach is needed to avoid overestimation of particles' size and underestimation of particles' number for clustered MNPs as well as to measure Raman without optically visualizing the plastic nanoparticles by overlapping SEM and optical images of high (< 10 nm) and low (~ 1 µm) spatial resolution, respectively. This was achieved by a correlative microscopy and spectroscopy workflow applied to identical MNP particles on large-area filters using an optical zoom microscope and a hyphenated SEM-Raman instrument (with a bright field optical objective for micro-Raman inside the SEM vacuum chamber).[14] However, such combined systems are limited with respect to the number of measurement techniques available on one instrument compared to stand-alone, method-specific instruments from different manufacturers, in which finding the same micro- and nanosized objects is still a challenge.[15-19]

In this work, the first application of a newly developed relocalization technology for a detailed characterization of MNPs and their effects on human kidney cells in independent instruments is demonstrated. This technology is based on a patented position encoder tag (from HORIBA), called nanoGPS tag, with lithographically defined patterns. These patterns are used to translate the sample coordinates corresponding to the regions of interest (ROIs) into the stage coordinates of different instruments (from HORIBA, Zeiss, Leica in this study), regardless of the sample orientation. Furthermore, the applicability of the ParticleFinder software module (from HORIBA) for automatic detection of microplastic (MP), pigment, and additive particles on large-area filters is shown. Context microscopy and fingerprinting spectroscopy approaches were applied to standard MPs, microparticle contamination of bottled mineral water, and human podocytes that were either untreated or incubated with MNPs. The podocytes exposed to MNPs were under stress and started to die gradually, indicating an overall effect of particle exposure on cell viability.

Experimental

The samples investigated in this study can be divided into three categories: reference micro-sized plastic particles, mineral water from different bottle types bought in Bavarian food stores, and human podocytes cell cultures exposed to MNPs.

Standard MP particles

Commercially available standard plastics (see Table 1) were selected to match the polymer types routinely encountered in the environment.[13,20,21] A mixture of polyethylene (PE), poly(vinyl chloride) (PVC), polyamide-Nylon 6 (PA), polystyrene (PS), and polypropylene (PP) particles were suspended in a solution (ultrapure water and sodium dodecyl sulphate (SDS)) followed by vacuum filtration through polycarbonate (PC) membrane filters (diameter 25 mm, pore size 0.4 µm) previously coated with aluminum (Al thickness 100 nm) as detailed in our previous work.[22] These reference materials were used to evaluate the nanoGPS relocalization technology (hardware and software) and its integration in a correlative microscopy and spectroscopy workflow applied to identical MNP particles (see Figure 1). The nanoGPS tag (4×5 mm²

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silicon piece) is firmly attached next to the filter, which is rigidly stretched and flattened between two metal rings fixed on a SEM holder, to avoid any thermal drift and ensure precise relocalization in different instruments. Along with the corresponding NaviGo software, the instruments' stages involved in the workflow are calibrated and the coordinates of ROIs are recorded.

Mineral water particles

Real mineral water samples packaged in reusable bottles made of poly(ethylene terephthalate) (PET), in single use PET bottles, and in glass bottles (single and reusable) were analyzed for microparticle contamination, taking also into account bottle age as well as label and cap type. Before suspension in SDS solution and vacuum filtration through Al coated PC membranes, calcium and magnesium carbonate particles were dissolved with ethylene diamine tetraacetic acid tetrasodium salt (EDTA) to reduce the number of non-plastic particles.[21] To obtain statistically relevant data given the complexity of bottled mineral water contamination including microplastic, pigment, additive, and mixed particles, we employed an automatic particle detection approach. This is based on the ParticleFinder software that transforms large-area (1 mm^2) dark field optical images obtained by stitching into grey scale images, on which particles are easily detectable using their brightness, counted, classified by size and shape, and their coordinates recorded for further micro-Raman chemical identification. Thus, the mean number of microplastic, pigmented, and additive particles (projected to 1 L sample volume), their size, and type distributions were estimated (see Figure 2, additives not included).[21,22]

Table 1: Details of the plastic particle standards used in the present study to assess the nanoGPS relocalization and the exposure of human podocytes to plastics (PVC, PA, PP). Adapted with permission from Springer Nature.[22]

Material	Type	Manufacturer	Size (µm)
Polyethylene (PE)	Clear microspheres, powder	Cospheric	1-10
			10-106
Poly (vinyl chloride) (PVC)	Powder	Pyropowders.de	< 50
Polyamide - Nylon 6 (PA)	Powder	GoodFellow	15-20 (average particle size)
Polystyrene (PS)	Polybead Micron Microspheres, 2.5% solids in water	Polysciences Inc.	1
Polypropylene (PP)	Chromatographic Grade, powder	Polysciences Inc.	25-85

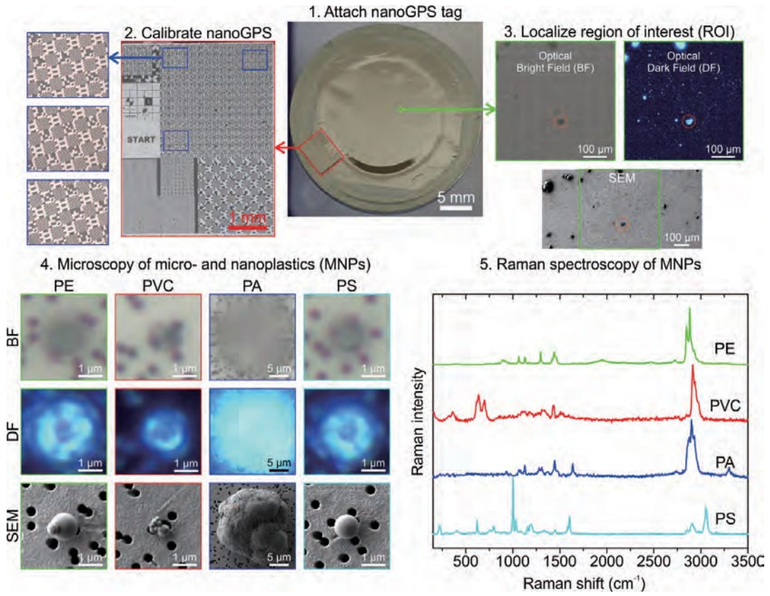

Figure 1 Correlative microscopy and spectroscopy workflow for micro- and nanoplastics on an Al coated PC membrane used to filter MNPs from water. First, a so-called nanoGPS tag is attached directly to the sample. Second, three images are recorded at random positions on a pattern (different patterns correspond to various instrument magnifications) and fed into a software that calibrates the global, stage coordinates into local, tag (sample) coordinates including sample rotation. This procedure is repeated for each instrument to be used in the workflow. Third, identical ROIs are precisely relocalized in independent instruments, regardless of the sample orientation. Fourth, the same single or agglomerated particles are imaged at optical (BF, DF) and SEM spatial resolutions to assess size, shape, number, and surface morphology of MNPs down to nanoscale. DF imaging is used to clearly distinguish MNPs from the porous structure of large-area filters. Fifth, unambiguously chemical identification by micro-Raman spectroscopy is applied. The Raman spectra are taken with permissionfrom the Society for Applied Spectroscopy.[14]

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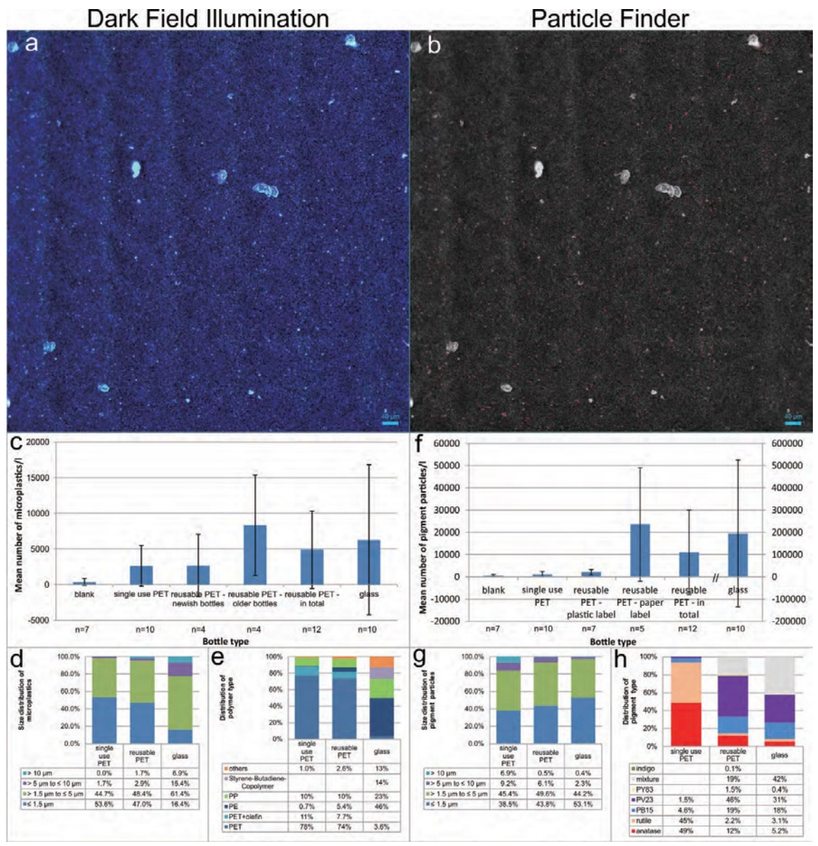

Figure 2: (a) Example of a dark field montage (1 mm²) obtained by stitching, on which particles from mineral water samples shine brighter than the pores of the Al coated PC membrane filter. (b) ParticleFinder software converts the DF image into a grey scale image used to automatically detect, classify, and measure Raman spectra of individual particles at their center, marked by red points. (c, d, e) Mean number of microplastics ± standard deviation projected to 1 L sample volume, size, and plastic type distributions function of the bottle material. (f, g, h) Mean number of pigments ± standard deviation projected to 1 L sample volume, size, and pigment type distributions function of the bottle material. Adapted with permission from Elsevier.[21]

Human podocytes exposure to MNPs

Conditionally immortalized human podocytes that contain a heat sensitive CV40T antigen were cultured as described previously.[23] Podocytes were proliferated under growth permissive conditions at 33°C and further differentiated through the inactivation of SV40 T-antigen at 37°C. After 7 days of differentiation, cells were treated with different concentrations of diluted standard plastic (PVC: 0.5, 1 mg/ml; PA: 0.5, 1 mg/ml; PP: 2.5, 5 mg/ml) and tyre wear (0.125, 0.5 mg/ml) particles for 7 h to evaluate their possible effects on the cells. In order to decrease the aggregation of particles, they were sonicated before the incubation. Following the particle treatment, cells were washed two to three times with phosphate buffered saline (PBS) and fixed for further biological, imaging, and spectroscopy assays. For this study, the podocytes were grown on the surface of silicon wafers previously coated with platinum (Pt thickness 100 nm) that were attached along with nanoGPS tags to SEM holders to avoid relative sample - tag position shifts when moving between instruments.

Analytical methods

Complementary analytical techniques present on different instruments were used to visualize and detect MNPs on filters and inside cells as well as to determine the changes in cells caused by the contact with MNPs. All measurements have been performed at room temperature. The latter point was first addressed by using a live-dead cell imaging kit based on two-color fluorescence cell viability assay (Thermo Fischer Scientific). Based on this assay, cell-permeable and cell-impermeable dyes were used for staining of live and dead/dying cells, respectively. Following the particle treatment, the live/dead cells were assigned based on the kit instruction. Fluorescent images were collected with the use of an Evos M5000 imaging microscope (Thermo Fischer Scientific) (see Figure 3).

Furthermore, we employed a confocal micro-Raman spectrometer (HORIBA LabRAM HR Evo-Nano or XploRA PLUS), operated by the LabSpec 6 software (with data analysis and ParticleFinder), equipped with bright and dark field illumination (BF, DF) objectives coupled to a camera to image MNPs and cells (~ 1 µm spatial resolution). Three lasers (532, 633, and 785 nm) focused by 50× (NA 0.75) or 100× (NA 0.9) objectives were used for Raman excitation and collection in a backscattering geometry with

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laser powers of 1.2 mW or 3.2 mW (532 nm), 11.2 mW (633 nm), and 5.3 mW (785 nm). Two gratings (300 and 600 grooves/mm) and integration times of 1- 20 s and 2x accumulations were applied. The acquired Raman spectra and maps (step size 1 µm) were analyzed to chemically identify the particles and the structural damage induced by them on the human podocytes. A SEM (Zeiss field emission Auriga, secondary electron detector) was used for a detailed morphological imaging of MNPs and cells (< 10 nm spatial resolution) at a low voltage of 1 kV to avoid modifications caused by electron scanning. The height profiles of the same cells investigated by micro-Raman and SEM were measured by a confocal imaging microscope (Leica DCM 3D), the relocalization of identical cells being realized using the nanoGPS technology (see Figure 4). Moreover, because of the superposition of Raman bands related to the plastic materials and cells, we applied a classical least squares algorithm (CLS) available in LabSpec 6 to highlight the spatial distribution of MNPs on the mapped cells (see Figure 5).

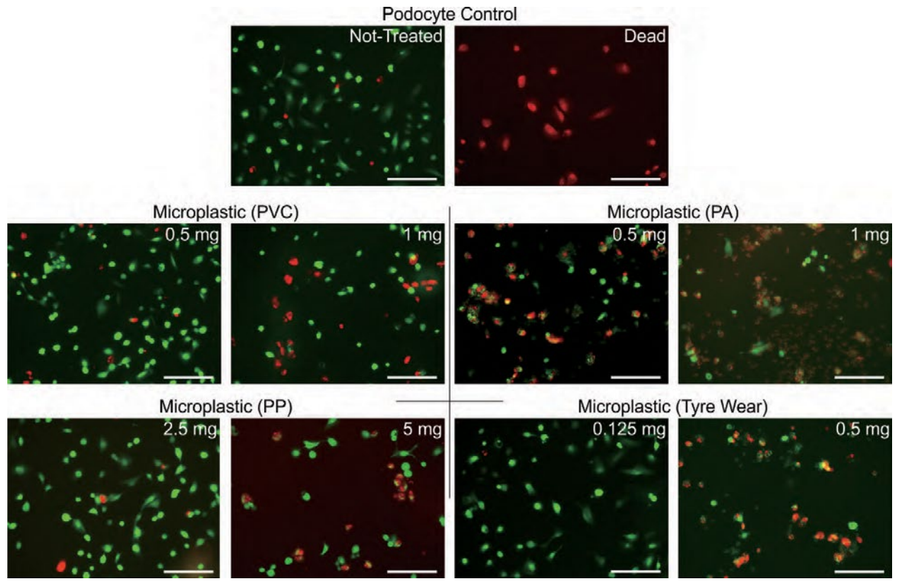

Figure 3: Fluorescence live - dead cell imaging (green - red) to assay the cytotoxicity of microplastic and tyre wear particles on podocytes, following 7 h particle exposure at relevant concentrations (mg/ml) and washing with PBS. The control cells were non-treated or intentionally killed to check the live - dead cell imaging kit. The concentrations to initiate and induce a notable impact on podocytes depends on the polymer type. During particle incubation the cells are under stress and start to die gradually. Consequently, some of the degraded cells are washed away and not assigned with colors. Some attached particles with intrinsic fluorescence are also visible. The preliminary results of this assay are yet mostly qualitative and show an overall effect of particle treatment on the cell viability. Scale bars are 300 µm.

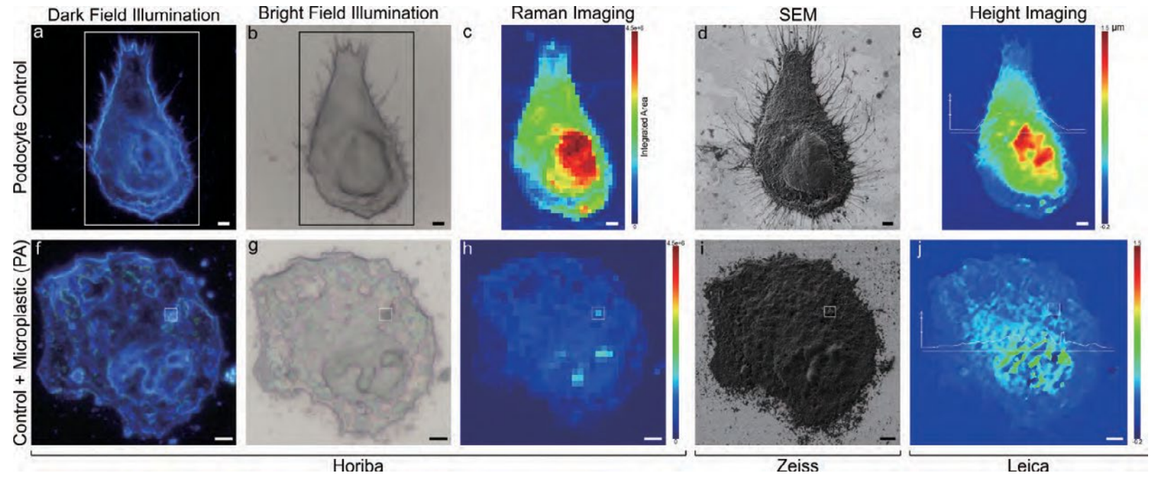

Figure 4: Correlative microscopy and spectroscopy workflow applied to podocytes untreated, control (first row) and particle treated (second row) with 1 mg/ml PA (Table 1 and Figure 3) using the nanoGPS position encoder tag (Figure 1). Two representative cells were easily relocalized and investigated in three independent instruments from different manufactures (Horiba, Zeiss, Leica) with complementary analytical techniques. First, an integrated optical microscope with dark (a, f) and bright (b, g) field illumination and micro-Raman spectrometer are used for a fast visual inspection of cells, followed by Raman imaging (c, h), showing less Raman signal for treated cells (note the same scale) that is an indication of podocytes damage after exposure to PA. Second, SEM imaging (d, i) reveals detailed surface morphology changes at nanoscale induced by the PA treatment and visualizes a PA nanoparticle (~ 30 nm), as confirmed by micro-Raman spectroscopy, delimitated by the square in the second row. Third, an interferometric profilometer is employed to measure the height profile without (e) and with (j) plastic contamination (note the same scale), PA incubated cells being flatter. Two horizontal profiles are also shown (maximum heights of ~ 1.5 µm and ~ 0.8 µm for the control and treated cell, respectively). Scale bars are 3 µm.

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Results and Discussion

nanoGPS relocalization

The nanoGPS relocalization technology for correlative microscopy and spectroscopy investigations is illustrated in Figure 1 for standard micro-sized plastic particles (Table 1), with some particles being by chance < 1 µm. First, a nanoGPS tag is rigidly mounted next to the Al coated PC membrane filter, both on a SEM holder that is moved between instruments, such that the tag and sample keep their positions relative to each other. The smaller the distance between tag and sample, the better the relocalization accuracy that can be further influenced by stage and imaging characteristics. Second, the multiscale and multimodal patterns on the tag are employed to calibrate the stage of each instrument, different feature sizes being used for distinct instrument magnifications (see SEM image of the entire tag). Three images are taken at random positions on a chosen pattern and fed along with the global, stage coordinates into the NaviGo software. In this example, images were recorded with the 10× objective of the optical microscope on the micro-Raman spectrometer. The software automatically determines the local, sample coordinates and rotation with respect to the tag. This calibration procedure is repeated for all instruments in the workflow and can be recalled anytime by recording one single image on the same pattern, independent of stage and sample rotation.

In the third step, one or more ROIs are located on the filter and their sample coordinates are saved in one instrument and retrieved in other instruments by converting sample, local into stage, global coordinates. In our case, largearea optical images acquired by stitching under BF and DF illumination are compared to a large field of view SEM image, with the same particle marked on all overview pictures. Next, MNPs can be directly relocalized and imaged at spatial resolutions of optical and electron microscopies (step four) and their spectral fingerprints determined by micro-Raman spectroscopy (step five) (PP is not shown). While on the BF and DF optical images these particles appear to be single, SEM imaging reveals that PE and PVC are cluster particles. When approaching the filter pore size, particles are barely visible in BF, but clearly noticeable in DF because they shine brighter than the pores, as seen for PVC. Moreover, SEM shows smooth surfaces with spherical and fragment-like shapes for the studied polymer particles. It should be noted that BF, DF, and Raman are usually performed before SEM; however, low-voltage

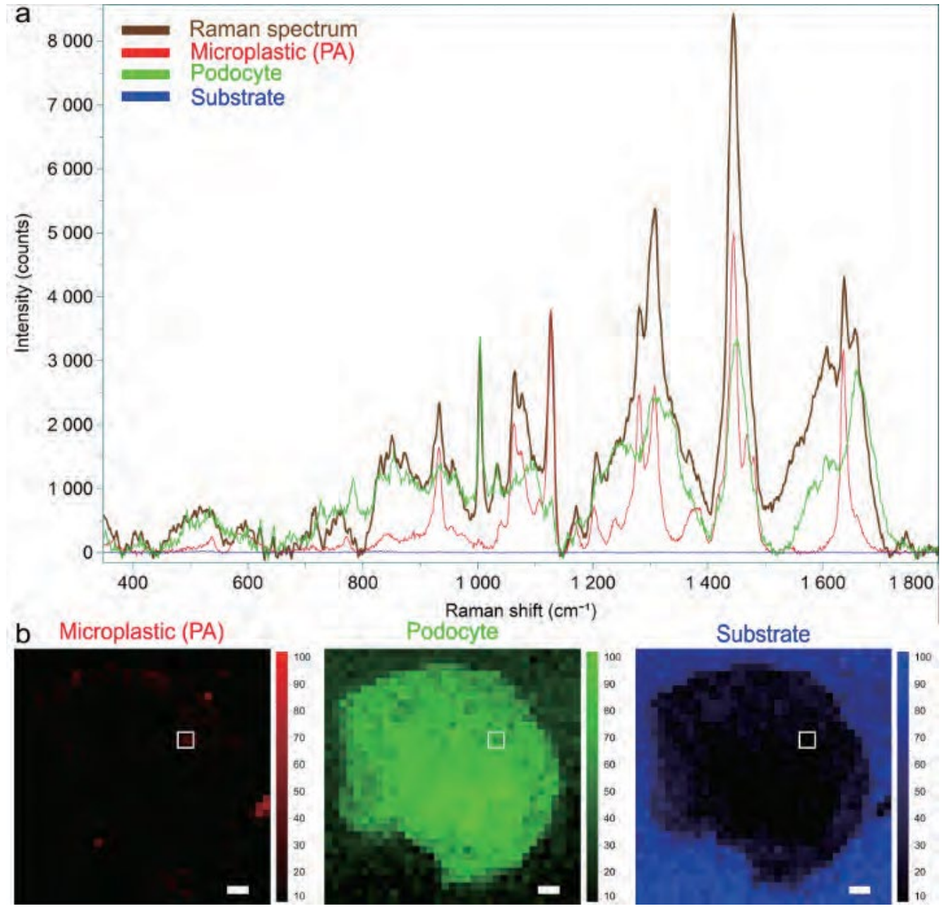

Figure 5: (a) Classical least squares (CLS) fitting is applied to decompose each measured Raman spectrum into its spectral components based on given reference spectra. (b) Separate score maps are generated for each component as illustrated for the podocyte cell treated with PA shown in Figure 4 (second row). The square indicates the position of a PA particle. Thus, despite the superimposed and complex Raman bands of cells and MNP particles, the spatial distribution of MNPs can be clearly localized. Scale bars are 3 µm.

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SEM does not damage MNPs, so that Raman after SEM is also possible.[14] All in all, nanoGPS tagging enables sample navigation and observation at different length scales in independent instruments, thus detailed morphological (size, shape, surface, number) and chemical characterization of the same micro- and nanoparticles is achievable.

ParticleFinder

The ParticleFinder software module combined with DF optical microscopy and micro-Raman spectroscopy represents another example of correlative analysis applied here to study contamination by microplastic, pigment, and additive particles in bottled mineral water. 32 samples from 21 different brands of mineral water were investigated to determine the number, size, and type of particles, the results being summarized in Figure 2.[21] DF imaging is used to scan five large-area image montages (1 mm^2) on each sample to warrant significant particle statistics. Such a montage generated by stitching (Figure 2a) is then converted into a grey scale image, on which all particles ≥ 1 µm are automatically detected and individually measured by micro-Raman (Figure 2b).

We identified varying amounts of microplastics in water from all bottle types, partly resulting in large error bars when calculating the mean particle number (Figure 2c); however, some trends are clearly visible. On average, higher number of microplastics were found in water from reusable (PET and glass) compared to single use PET bottles. Interestingly, newish, reusable PET showed less microplastics than older, reusable PET, but similar to single use PET, suggesting that the bottle age can critically affect MP contamination. Regarding the average size distribution, 90.5% of MPs were ≤ 5 µm in all bottles and ~ 50% were ≤ 1.5 µm in PET bottles (Figure 2d), these MP size classes being addressed for the first time in such samples.[21,24] The predominant polymer type detected in PET bottles was PET considered to originate from the bottle material, while some PET particles displayed olefinic or pigment spectral interferences. In glass bottles, we mainly found PE and PS attributed to abrasion of caps on the glass bottleneck as well as PS, styrenebutadiene- copolymer, and PET most likely from the machinery used for the cleaning process (Figure 2e).

In addition to microplastics, pigmented and additive particles were also detected in the analyzed mineral water samples. Large variations in the number of pigmented particles in water from different bottle and label types were observed (Figure 2f). On average, single use PET contained less pigments similar to blank samples, while reusable PET and glass bottles with printed paper labels showed higher amounts of pigments. Alike MPs, older, reusable PET displayed more pigments than newish, reusable PET and most of the pigmented particles belonged to size classes investigated for the first time, 91.5% were ≤ 5 µm and 45.1% were ≤ 1.5 µm (Figure 2g).[21,24] We found that the pigment types mainly correspond to the colors used for printing on the paper labels (Figure 2h). These pigment particles originate from the paper labels and enter into the bottles during the cleaning process.[25] Additive particles were detected in reusable PET bottles and considered to leach from the bottle material (68.6% were ≤ 5 µm and 11.7% were ≤ 1.5 µm). These results demonstrate that ParticleFinder can be used for automatic detection, classification, and Raman measurement of particles < 1.5 µm from real samples, which is very important due to toxicological reasons, since this size class is considered small enough to penetrate deeply into organs.[21,22]

Effects of MNPs on podocytes

The potential risk of plastic particles on human health is addressed in this study using human podocytes as a highly-specialized kidney cell type. Since kidneys are involved in the filtration process and do not regenerate their cells continuously, they are likely to accumulate MNPs over the lifetime.[26] We performed cell viability tests after incubation of podocytes with four different MNP types (standards PVC, PA, PP, and tyre wear) using a live-dead (green - red) cell fluorescent based kit. Representative results for relevant plastic concentrations after 7 h exposure with respect to control cells are summarized in Figure 3. The cytotoxicity response is found to depend on the polymer type, a higher concentration is needed for PP (5 mg/ml) compared to PVC, PA, and tyre wear (0.5 - 1 mg/ml) to achieve a similar cell mortality rate. Two mechanisms are proposed to explain the damage induced by the plastic particles on podocytes and finally their death. First, particles can attach on the cell surface and limit the nutrient uptake, the degree of attachment depending on particles' adhesion properties and sizes. Some particles still remained attached

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after three times washing with PBS following incubation and can be visualized based on their intrinsic fluorescence as shown in Figure 3. Second, smaller size particles can be taken up into the cells by phagocytosis as illustrated in Figure 4 for PA particles.

The correlative microscopy and spectroscopy characterization of identical cells using the nanoGPS relocalization technology is demonstrated in Figure 4, exemplary shown for PA treated cells. Two representative podocytes (control and incubated) are localized in three independent instruments and studied with complementary analytical techniques down to nanoscale resolution. Optical imaging (~ 1 µm spatial resolution) under DF (a, f) and BF (b, g) illumination show the degradation and deformation of cells after particle exposure. The structural damage is further confirmed by micro-Raman mapping (c, h), treated cells display Raman spectra with less intensity (note the same scale for the integrated area maps). High spatial resolution SEM imaging (< 10 nm) is used to assay the integrity of cell features at nanoscale, exposed cells do not regularly show normal biological features like heterogeneous surface, nucleus, and foot processes (d, i). Height profile imaging acquired with an interferometric profilometer quantifies the deformation of incubated cells that flatten with respect to control cells (e, j), with height changes from ~ 0.8 µm to ~ 1.5 µm, respectively (note the same scale). Given the complex peak structure of Raman spectra from cells and plastic particles and the large overlap between peaks, we employed a CLS fitting algorithm that decomposes each measured Raman spectrum into its spectral components and provides score distribution maps for each component as displayed in Figure 5. This enables us to spatially resolved MNPs without underlying podocyte and substrate backgrounds, which are shown separately. Taking advantage of the nanoGPS relocalization capability in a correlative workflow, the same PA particle (outlined by the square in Figure 5b and Figure 4 - second row) was imaged by SEM and found to be a nanoparticle (~ 30 nm) most likely taken up into the cell by phagocytosis (Figure 4i). All in all, these preliminary experiments indicate the negative influence of plastic particles on human podocyte cells; however, more assays are needed to account for other relevant polymers present in the environment and their separate and mixed effects on different human organs, tissues, and cells.

Conclusion

The present study introduces an efficient measurement protocol for the assessment of contamination, accumulation, and hazards related to micro- and nanoplastic particles in bottled mineral water and human kidney cells. This protocol combines context microscopy and fingerprinting spectroscopy with automated relocalization (nanoGPS) and detection (ParticleFinder) of the same MNPs and cells in separate instruments from distinct manufactures (HORIBA, Zeiss, Leica). Results on microparticle contamination (average number, size, type) in mineral water and toxicity effects of MNPs (standards PVC, PA, PP, and tyre wear) on podocytes (in-vitro) are reported. It was found that the bottle material (single use, reusable PET and glass), bottle age (older, newish reusable PET), and label print (paper, plastic) affect the distributions of microplastics, pigments, and additives. In contrast to non-treated controls, podocytes incubated with MNPs tend to lack usual cell characteristics such as heterogeneous surface, nucleus, and foot processes, confirming the potential risk of plastic particles on the viability of cells. These findings were revealed by a biological cell test supported by complementary methods involving optical (bright, dark field) and scanning electron microscopy, micro-Raman spectroscopy (with CLS spectra fitting), and height interferometric profilometry. Further work will deal with different plastic types, concentrations, and exposure times.

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* Editorial note: This content is based on HORIBA’s investigation at the year of issue unless otherwise stated.

George Sarau and Melina Yarbakht contributed equally to this work.

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References

1. L. Lebreton *et al*., PALGRAVE COMMUNICATION 5 p.1 2019 (https://doi.org/10.1057/s41599-018-0212-7)
2. R. Geyer *et al*., SCIENCE ADVANCES 3 p.1 2017 (https://www.science.org/doi/pdf/10.1126/sciadv.1700782)
3. P.J. Kole *et al*., INTERNATIONAL JOURNAL OF ENVIRONMENTAL RESEARCH AND PUBLIC HEALTH 14 p.1265 2017
4. L.M. Hernandez *et al*. ENVIRONMENT SCIENCE & TECHNOLOGY 4 p.280 2017
5. C.M. Rochman, SCIENCE 360 p.28 2018.
6. K. Munno *et al*., ANALYTICAL CHEMISTRY 92 P.2443 2020
7. L.M. Hernandez *et al*., ENVIRONMENT SCIENCE & TECHNOLOGY 52 p.12300 2019
8. S.L. Wright *et al*., ENVIRONMENT SCIENCE & TECHNOLOGY 51 p.6634 2017
9. R. Lehner *et al*., ENVIRONMENT SCIENCE & TECHNOLOGY 53 p.1748 2019
10. J.C. Prata *et al*., SCIENCE OF THE TOTAL ENVIRONMENT 702 p.134455 2020
11. W.J. Shim *et al*., ANALYTICAL METHODS 9 p.1384 2017
12. G. Renner *et al*., CURRENT OPINION IN ENVIRONMENTAL SCIENCE AND HEALTH 1 P.55 2018
13. L. Cabernard *et al*., ENVIRONMENT SCIENCE & TECHNOLOGY 52 p.13279 2018
14. G. Sarau *et al*., APPLIED SPECTROSCOPY 74 p.1155 2020
15. C. Cardell *et al*., TRENDS IN ANALYTICAL CHEMISTRY 77 P.156 2016
16. G. Sarau *et al*., PHYSICA STATUS SOLIDI B 256 p.1800454 2019
17. G. Sarau *et al*., NANOSCALE 6 p.11953 2014
18. G. Sarau *et al*., ACS APPLIED MATERIALS AND INTERFACES 9 p.10003 2017
19. G. Sarau *et al*., SOLAR ENERGY MATERIALS AND SOLAR CELLS 95 p.2264 2011
20. S.-A. Strungaru *et al*., TRENDS IN ANALYTICAL CHEMISTRY 110 p.116 2019
21. B.E. Oßmann *et al*., WATER RESEARCH 141 p.307 2018
22. B.E. Oßmann *et al*., ANALYTICAL AND BIOANALYTICAL CHEMISTRY 409 p.4099 2017
23. J. Muller-Deile *et al*., AMERICAN JOURNAL OF PHYSIOLOGY 297 p.F1656 2009
24. D. Schymanski *et al*., WATER RESEARCH 129 p.154 2018
25. B.E. Oßmann, “Determination of microparticles, in particular microplastics in beverages”. 2020. urn:nbn:de:bvb:29-opus4-132788.
26. X. Xu *et al*., JOURNAL OF AMERICA SOCIETY OF NEPHROLOGY 27 p.3739 2016

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Analysis Workflow

Typical workflow for microplastics analysis includes the following steps:

1 Sampling

Collection of a matrix where the presence of microplastics will be investigated.

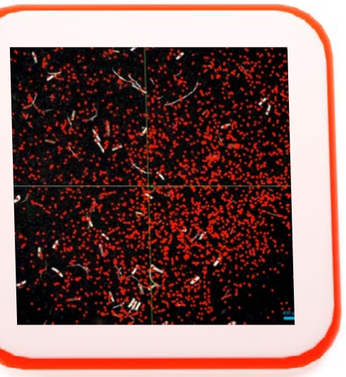
2 Pre-Treatment

Isolation of microplastics from the matrix. Size fractionation, density separation, extraction and/or chemical digestion might be considered

3 Filtration

Microplastics particles are collected on filters which must be carefully chosen based on expected size and quantity, and compatibility with the chosen analytical technique.

4 Data Acquisition



Infrared Micro-spectroscopy, Raman micro-spectroscopy, Pyrolysis–gas chromatography–mass spectrometry, etc.

5 Data Analysis & Reporting

Chemical identity of particles is reported. Particles are counted or their mass is measured.

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Analysis Workflow

Sampling

The appropriate sampling step is highly dependent on the matrix to be investigated/analyzed for the presence of microplastics. Considering the number of possible matrixes, it is tough to provide a complete picture, but we will touch the most important ones.

Water Sampling.
Most important point for water (but also valid for sediment and other matrixes) is the representativeness of the sample collected. Ocean, sea and river water samples must come both from the water surface and the water column. Several studies (review of Hidalgo-Ruz et al.[1]) have demonstrated that the water surface has a higher number of microplastic items than the water column: Microplastics ranks from 0.022 to 8,654 items m^3 at the surface and from 0.014 to 12.51 items m^3 in the column.

The most common tools for water withdrawal are manta trawls (surface water) and plankton nets (water column) (Stock et al. [2] and papers cited within); the mesh of the net can vary between 50 to 3000 µm but the most common is 300 or 330 µm. Due to the mesh size most microplastics under 300 µm are lost. A mesh size net of 80 µm has also been used but the risk of clogging is high. A flow meter is usually used to measure the amount of water flowing through them for comparative and quantitative measurements.

Plankton Net

Manta Trawls

An alternative tools are: Continuous flow centrifuge which can collect particles down to 5 µm without clogging, but with a longer sampling time (1 hour for 1 m^3 of water); Filter cascade with a fractionated pressure filtration, which guarantees fast measurement times and direct separation of the particles into size classes.

Some general guideline for water sampling, and also sediment and biota, in seas can be found in Guidance on Monitoring of Marine Litter in European Seas[3] by the Marine Strategy Framework Directive (MFSD).

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Sampling

Sediment Sampling.
For marine and freshwater sediment, the golden rule of ensuring the representativeness of the sample is still key. Some guidelines are provided in the MSFD document of the MSFD. Important are; the amount of the sample collected (often measured in volume L, weight kg or areal extension m^2, analyzed); The location and the repetition for each location. The sampling depth is also an important parameter and can vary depending on the aim of the study but in many publications the upper 5 cm or less, is where most of microplastics are concentrated, and therefore has been used for microplastics monitoring. Sediments contain more microplastics than water, ranging from 0.21 to more than 77,000 items per m^2.

Marine sediment, a part of the shoreline (beaches), can be differentiated by the location where they are collected in 3 different zones: Tideline or supralittoral, intertidal or eulittoral and sublittoral. In freshwater ecosystems the same differentiation does not apply, due to the minimal effects of tides. The tools for sediment collection are mainly mechanical, such as tweezers, table-spoons, hand picking and grabbers for deep sediment.

Biota Sampling.
It's important to define the term Biota as a common starting point: Biota is the animal and plant life of an ecosystem.

Sampling methods are highly diverse and depend on the target and type of habitat: water column, sea surface, aquaculture etc. Lusher et al.[4] wrote a wide and exhaustive review on this field, underling as the most important points: the avoidance of plastic contamination and handling of animals.

Handling stress can result in a loss, and therefore underestimation of microplastics due to gut evacuation. The safest methods of storage of the organisms, before their analysis are desiccation and freezing.

Food Sampling.
Foods is more straightforward than sediment, water and biota sampling, They are readably available thorough the commercial chain; the key sampling factors in food sampling are the number of samples and repetitions, even if a recognized protocol is still missing. Below we summarize some examples of food sampling.

Honey and Sugar.
Liebezeit et al.[5] collected mainly from Germany, 19 types of honey, both solid and liquid plus 5 types of sugar directly from the producers or supermarkets. Honey samples were filtered with a 40 µm sieve (the solid one after melting it), sugar was dissolved in deionized water and then filtered with a 0.8 µm cellulose filter.

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Sampling

Salts.
Several studies on salts have been conducted since the first one of Yang et al.[6] but the one of Kim et al.[7] in 2018 is the most exhaustive. Kim collected 39 brands of table salt from supermarkets in 17 different countries over 6 continents, Salts were selected to provide a diverse range of sources (seawater, lake water) and manufacturing methods (solar-dried, refined or un-refined). A minimum of 500 grams for each salt were tested and duplicated.

Tap water.
A recent study of Kosuth et al.[8] published in 2018 is a good example of tap water sampling. Kosuth collected 159 samples from 14 different countries. Samples were collected by running the tap water for 1 minute and then, while the water source was running, a bottle of 500 ml was filled and dumped twice before the final filling.

Bottled water.
Manson et al.[9] in 2018 conducted a study on bottled water selecting 259 bottles from 11 brands in 27 lots, including leading global brands from various bottled water producers, purchased in 9 different countries. Bottled water came in bottles of different capacities (from 0.5 L up to 2 L) and several bottles were analyzed to reach a volume close to, or above, 5 L for each lot.

1. Hidalgo-Ruz, *et al*., ENVIRONMENT SCIENCE & TECHNOLOGY 46 p.3060 2012
2. Stock *et al*., TREND IN ANALYTICAL CHEMISTRY 113 p. 84 2019
3. https://mcc.jrc.ec.europa.eu/documents/201702074014.pdf
4. Lusher *et al*., ANALYTICAL METHODS 9 p.1346 2017
5. Liebezeit *et al*., FOOD ADDITIVES & CONTAMINANTS: Part A 30 p.2136 2013
6. Yang *et al*., ENVIRONMENT SCIENCE & TECHNOLOGY 49 p.13622 2015
7. Kim *et al*., ENVIRONMENT SCIENCE & TECHNOLOGY 52 p.12819 2018
8. Kosuth, M. *et al*. PLOS ONE 13 No. e0194970 2018
9. Mason *et al*., FRONTIERS CHEMISTRY 6 p.407 2018

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Sample Preparation

Sample pre-treatment is the most important step for microplastics analysis because, if done correctly, it eliminates all types of possible organic contaminants that can affect microplastic chemical identification when using various techniques: Infrared Microscopy, Raman Microscopy, Pyrolysis Gas Chromatography/ Mass Spectrometry (GC/MS) etc. These contaminants are always present (even when analyzing bottled water) and their amount depends on the matrices analyzed.

There is extensive literature on pre-treatment protocols which vary depending by the type of matrix under investigation. There are some key aspects that must be considered independent of the analysis method :

Integrity of the microplastic
Chemical treatment can modify both chemistry and sizes of microplastics if too aggressive. These two aspects are important to determine the potential toxicity of microplastics, so treatment must be carefully chosen to avoid changing the sample.

Plastic contamination
Sample manipulation can cause additional plastic contamination from the laboratory environment. A blank, or reference, sample of just filtered deionized water is essential to understand plastic contamination and to avoid over-estimation of the microplastic content. Preparing samples under a laminar flow hood is highly recommended.

This section provides a general overview of the different methodologies and also gives detailed suggestions for some of the most common matrices. Due to the absence of standardized methods, the protocols suggested are the combination of our experience and the literature.

Apart from the organic contaminant removal protocols, additional treatments must be mentioned for sediment analysis. The first step is **physical separation** using various sieves to isolate Microplastics and inorganic materials depending on their size. The second step is **extraction**. Sediments contain other inorganic materials, such as quartz sands and silicates. These must be separated from the microplastics to avoid interference during chemical identification. Extraction is done by means of density separation, exploiting the different densities of plastic and inorganic materials; the majority of polymers possess a lower density (usually from 0.8 to 1.6 see Table 1) than the inorganic constituents of the sediment. As an example, silicates density range from 2.196 for amorphous to 2.648 for α-quartz.

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Analysis Workflow

Sample Preparation


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Commonly density separation involves 4 steps as highlighted in the review of Hanvey et al.[1]:

- Introduction of an aqueous solvent with a specific density
- Mixing for defined periods of time
- Settling, or equilibration time
- Filtering to specific size fractions

By using an aqueous solvent with a higher density than plastics, they will float on the surface allowing them to be separated from inorganic materials. It is important to vigorously mix the solution to ensure that the microplastics can separate out during the settling step. It is highly recommended to repeat these steps at least two times.

The addition of salts increases the density of the aqueous solution and varying the types of salts allows the density to be tuned to meet specific requirements. Several salts (Hamm et al.[2] and references within) have been used in literature and the most common ones are listed in the following Table.

Table 2. Density values for the most common polymers. The ones highlighted in grey represent more than 80% of global plastic production.

Polymer type	Density (gr/cm^3)
Poly(propylene), PP	*0.861*
Poly(ethylene), PE (Low to High density)	*0.854-0.96*
Poly(vinyl chloride), PVC	*1.388*
Poly(ethylene terephthalate), PET	*1.333*
Thermoplastic Polyurethane PUR	*1.23-1.35*
Polystyrene, PS	*1.052*
Polytetrafluoroethylene, PTFE or Teflon	*2.2*
Poly(amide) 6, PA6	*1,06-1,16*
Poly(vinylidene fluoride), PVDF	*1.675*
Polychloroprene, Neoprene	*1.243*
Poly(methyl acrylate), PMA	*1.224*
Poly(isobutene)	*0.864*
Poly(caprolactam)	*1.084*
Poly(Bisphenol A carbonate), PC	*1.206*
Polylactic acid, PLA	*1.248*
Poly(ethylene glycol), PEO, PEG	*1.128*
Poly(methyl methacrylate), PMMA	*1.159*
Poly(vinyl alcohol), PVOH	*1.300*
Poly(vinyl acetate), PVA PVAC	*1.190*
Poly(ethylene-vinyl acetate) PEVA	*0,92 - 0,94*

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Sample Preparation

This table also provides the cost, an important consideration for sediment analysis, and the potential safety issues correlated with the handling of some of them.

Table 3. List of salts for density separation process.

Salt		Maximum density ρ (g/cm³)	Amount (Kg)	Cost (euro)	Amount (gr/L) for Maximum ρ	Safety
Sodium Chloride	**NaCl**	1.2	1	35,9	311	no effect
Sodium Iodide	**NaI**	1.8	0,5	287	797	
Zinc Chloride	**$ZnCl_2$**	1.9	1	116	1373	
Zinc Bromide	**$ZnBr_2$**	1.7	0,5	139	1125	
Calcium Chloride	**$CaCl_2$**	1.4	0,5	25	558	
Sodium Polytungstate	**$3\ Na_2WO_4\ 9W0_3$**	3 (1.55)	0,1	216	5671 (798)	no effect

 Eye, skin and respiratory tract irritation;

 Corrosive;

 Possible burns.

Sodium Chloride is the readily available and safe solution but the maximum density achievable is only 1.2 g/cm³ which does not allow separation of high-density plastics such as PVC and PET. A minimum density of 1.5 to 1.55 is needed to recover more than 90% of the plastics.
Sodium Polytungstate is probably the best technical solution because the density can be easily tuned up to 3 g/cm³, it has a low viscosity, it can be reused and additionally it has no safety constraints, but its main drawback is the cost which is more than 2000 euros per kilograms. According to Coppock et al.[3] and considering all the aspects, we suggest as the best balanced solution Zinc Chloride; care must be taken with handling, but the price is reasonable and densities up to 1.9 g/cm³ can be reached.

After this overview of physical separation and extraction methods we can move on to organic contaminant removal protocols or the digestion step. Most of the digestion protocols envisage the use of concentrated acids and alkali solution which destroy proteins, carbohydrates and fats (the main constituents of organic residues in sediment and marine water samples and also in foods). These are the main interference agents for microplastic chemical identification using the common analytical methods e.g. Fourier Transform Infrared/ Raman Microscopy and Pyrolizer GC-MS.

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The review of Hamm et al.[2] of 2018 (and papers cited within) provides an exhaustive picture of digestion protocols and a summary of their efficiency vs. their unwanted ability to degrade Microplastics. A visual representation of this summary is depicted in figure here after.

Acid treatments are highly effective for removing organic residues reaching an efficiency above 80% in several cases, but they can easily damage microplastics preventing their chemical identification.

Alkali treatments can have different effects:

1. 30% and 35% aqueous solutions of H_2O_2 are an effective treatment but they can chemically damage some types of plastics (such as PVC and Polyamide 6-6/6 – Nuelle et al.[4]) and moreover they can also modify the shape and size of the particles. Temperature and incubating time are also important parameters to be considered, increasing them we have a positive impact on the digestion efficiency but a negative one on the particle chemistry/shape/size.

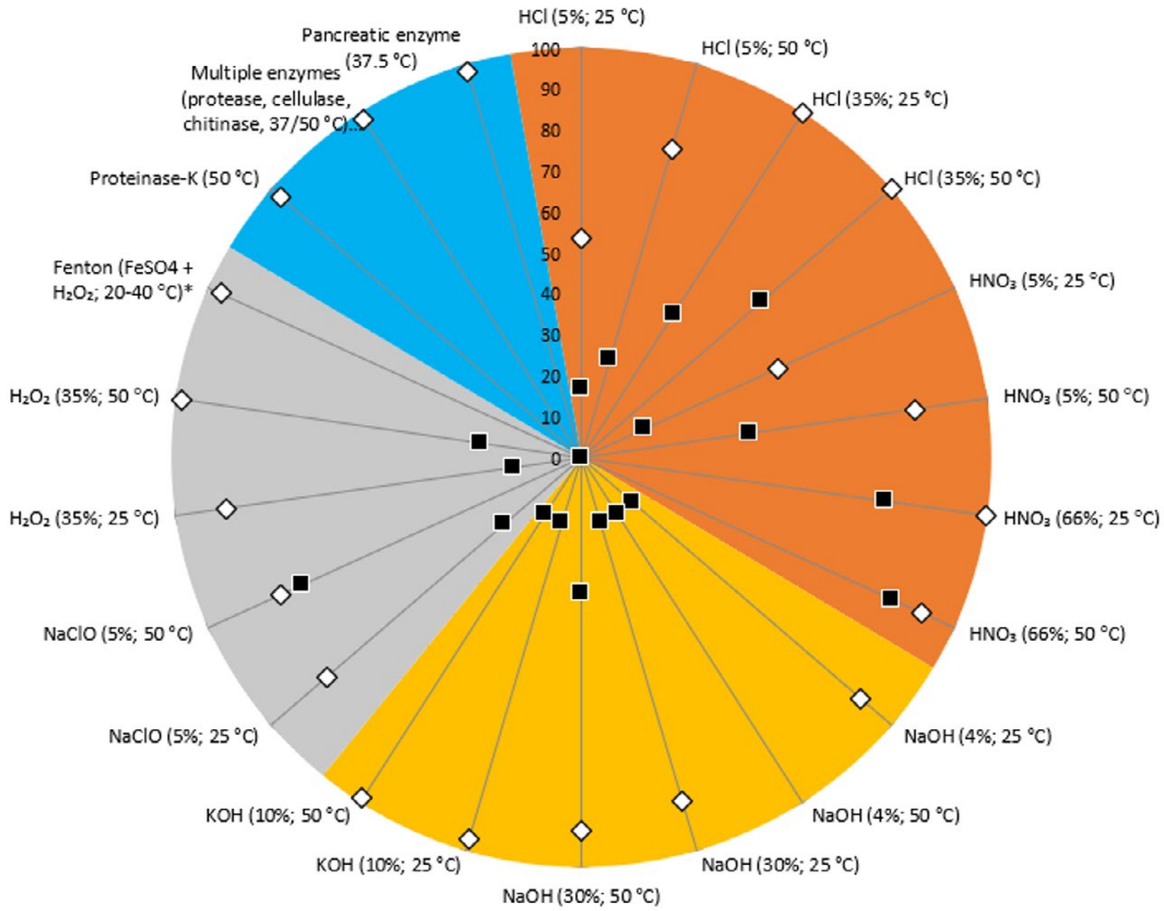

Fig. 5. (■) Max. % of Microplastic negatively affected by treatment; (◇) Effectiveness of the treatment in %. (Image provided by Claudia Lorenz, University of Aalborg)

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2. 10% aqueous solution of KOH provides better results than H_2O_2 as demonstrated by Karami et al.[5]. Karami tested this solution at different temperatures and incubation times, the table below summarizes the results.

Table 4. Treatment efficiency in function of conditions

	Temperature (°C)	Incubation Time (hours)	Efficiency / Recovery Rate
Digesting Solution 10% KOH	25	96	97.1
	40	48	98.6
	50	36	98.9
	60	24	97.61

The treatment efficiency (obtained by averaging the values for all the polymers tested) is above 97% with all combinations but at 50°C and 60°C, Karami observed some degradation of PVC, PET and Polyamide 6-6/6. The best condition balancing temperature / speed (i.e. incubation time) was at 40°C for 48 hours, where only PVC shows a recovery rate below at 93%.

Three additional digestion processes are important to mention:
Fenton's Reagent (Tagg et al.[6]); Mono-Enzymatic treatment (Cole et al.[7] used Proteinase-K, while Courtene-Jones et al.[8] used Trypsin); Basic and Universal Enzymatic Purification Protocol (BEEP-UEEP) which combines a Multi-Enzymatic treatment with an oxidizing agent (H_2O_2) and a detergent Sodium Dodecyl Sulfate (SDS), this protocol is usually employed for protein denaturation (Loder et al.[9])

Fenton's reagent is prepared by mixing solutions of 30% H_2O_2 and $FeSO_4 \cdot 7H_2O$ to reach final $FeSO_4 \cdot 7H_2O$ concentrations of: 3.33, 6.67 and 10 mg/ml. Its efficiency was demonstrated with infrared microscopy and even PVC and Polyamide didn't undergo to any modifications. The major advantages of Fenton's reagent stressed by Tagg et al. is the rapid digestion time of only around 10 minutes, much faster than Alkali treatments and the fact that it works at room temperature.

We observe only one issue connected to Fenton's reagent digestion that can affect microplastic identification by Raman microscopy (the key technique for analyzing microplastics below 5/10 microns): the presence of Iron leads to the formation of fluorescence compounds that interfere with the chemical identification of polymers by Raman.

Enzymatic treatment. Both the mono-enzymatic digestions were tested on marine biota, bivalve species for Cole and mussel for Courtene-Jones, and they show very high efficiency and no degradation of any plastics. Efficiency was 97% for Proteinase-K and 88% for Trypsin.

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As some biogenic material remains undigested, using Trypsin, Courtene-Jones et al.[8] proposed the use of an additional enzyme such as chitinase to remove the residual parts. The protocol developed by Cole[7] for bivalves differs on one aspect: the sample was homogenized with a solution of 400 mM Tris–HCl buffer, 60 mM EDTA, 105 mM NaCl and 1% SDS before adding the enzyme while Courtene-Jones[8] used a solution of Trypsin, made with deionized water, directly on the sample.

These enzymatic protocols are particularly useful for marine biota and marine sediments, their main drawback is the relatively high cost of the purified enzymes.

Basic and Universal Enzymatic Purification Protocol (BEEP-UEEP). Loder et al.[9] starts from the approach of Cole[7] and Courtene-Jones[8] but develops a complete protocol (BEEP), including a density separation step, combining multi-enzymatic digestion (Protease, Cellulase and Chinase) and oxidative treatments. Moreover, he successfully evaluated the protocol for its efficiency and applicability for infrared microscopy and for Microplastics with dimensions down to 20 µm.

UEEP is a further optimization of the BEEP protocol that widens its versatility for different environmental sample matrices (BEEP was developed first for seawater samples) by adding two additional enzymes (Lipase and Amylase). Loder[9] developed one of the most complete sample pre-treatment protocols (particularly suited for marine environments – biota, sediment and water) where all the possible interferents (such as chitin-containing materials, plant residues, planktonic organisms and cell residues) for microplastics identification are selectively attacked and, notwithstanding the use of enzymes, he was able to reduce the cost compared to Cole[7] by using technical grade enzymes.

One potential drawback of the BEEP/UEEP protocols is the incubation time needed for all the enzyme steps which bring the overall pre-treatment time to between 10-12 days. Furthermore, the presence of several steps is an additional potential source of unintended plastic contamination and/or particle loss.

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1. Hanvey *et al*., ANALYTICAL METHODS 9 p.1369 2017
2. Hamm *et al*. (2018) Microplastics in Aquatic Systems – Monitoring Methods and Biological Consequences. In: Jungblut S., Liebich V., Bode M. (eds) YOUMARES 8 – Oceans Across Boundaries: Learning from each other. Springer, Cham. https://doi.org/10.1007/978-3-319-93284-2_13
3. Coppock *et al*., ENVIRONMENTAL POLLUTION 230 p.829 2017
4. Nuelle *et al*., ENVIRONMENTAL POLLUTION 184 p.161 2014.
5. Karami *et al*. SCIENCE OF THE TOTAL ENVIRONMENT 578 p.485 2017
6. Tagg *et al*., CHEMICAL COMMUNICATION 53 p.372 2017
7. Cole *et al*. SCIENTIFIC REPORTS 4:4528 2014
8. Courtene-Jones *et al*., ANALYTICAL METHODS 9 p.1437 2017
9. Loder *et al*., ENVIRONMENTAL SCIENCE & TECHNOLOGY 51 p.14283 2017

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Following the overview of the sample pre-treatment workflows, in this section we will propose protocols for various matrices, starting with bottled and tap water. This section will be updated twice a year and new detailed protocols will be added for different matrices as a result of advances in the literature and HORIBA experience.

Bottled Tap Water.

Bottled water sample can be analyzed without any pre-treatment, but we recommend the protocol developed by Oßmann et al.[1] as the treatment is rapid and the removal of many non-plastic particles can reduce the total measurement time. The same treatment can also be used for tap water.

The method uses:

- Ethylenediaminetetraacetic salt (EDTA): EDTA is well-known to reduce the water hardness by complexing metal ions such as Ca^{2+} and Mg^{2+};
- Sodium Dodecyl Sulfate (SDS): SDS is an anionic surfactant that improves plastic suspension and provides better homogeneity.

EDTA (250 g/L solution) is added in an equimolar amount depending on the content of Calcium and Magnesium ions indicated on water bottle label.

EDTA must be left for 15 minutes. This treatment reduces the number of Calcium and Magnesium carbonate particles speeding up the full analysis time; since Raman and infrared microscopy identify plastics by analyzing each particle individually (see “Measurements Methodologies” section) removing the inorganic ones in advance reduces the overall acquisition time.

Following EDTA, 3 ml of SDS (100 g/L) is added per litre of water. After the filtration step, SDS, must be removed with a solution of 50% ethanol (ultrapure ethanol) in deionized water. This SDS step is optional.

Prior to using any solution, filtering using a 0.1 µm mesh will limit plastic contamination from the lab environment.

1. Oßmann et al., WATER RESEARCH 141 p.307 2018

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Marine water samples

Mitigating unwanted microplastic contamination

Unwanted contamination is a relevant issue affecting the quality of data related to microplastic analysis. It is hence of paramount importance to avoid or at least to try to limit contamination throughout the whole workflow.

Specifically, all glassware used must be flushed with filtered MilliQ water three times before usage! Moreover, it is good practice to cover all sample containers with a glass lid and aluminum foil. As much as possible, the sample preparation should be done in a fume hood, or better, inside a laminar bench.

During the sample preparation, always flush the 'previous' sample container three times after transferring the sample into a new container. This way, sample loss between steps will be limited. For the same reason, use the same filter(s), beaker and magnet for each of the filtration steps (keep the filter(s) and magnet in a petri dish).

Filtration times can be significantly reduced by allowing the particles to settle at least 1 hour (the more a sample stays still, the lower the filtration time will become) and by handling the sample with great care not to disturb the settled particles.

Filtered demineralized water (0.7 µm GF) and MilliQ water can be both used during the sample preparation (it is just important to use pre-filtered water). On the contrary, it is important to use Milli-Q to prepare reagents to avoid to contaminate them with either organics molecules or inorganic ions dissolved in the water (e.g. when you prepare buffer solutions). A similar approach must be adopted when adding water during the preparation of Fenton's reaction (use filtered Milli-Q water).

It is possible that, in certain steps, the particles from a sample will be stuck on the side of the beaker. To detach the particles, fill the beaker up with MilliQ (or filtered water) and sonicate for a couple of minutes.

Preparing reagents for microplastic sample preparation

Sample preparation for MP analysis is a complex procedure involving multiple steps and to use of several reagents. A brief chapter illustrates how to prepare the reagents needed for the sample processing.

5% w.v SDS solution (NaC12H25SO4) (1 L)

Sodium dodecyl sulphate is an anionic surfactant which is present in small quantities in many cosmetics and soaps. In this framework it is used to start degrading the matrix of the sample, preparing the substrate for the enzymatic treatment.

The materials, glassware and equipment needed to prepare SDS solutions is summarized in this bried list: measuring cylinder (1 L); glass bottles (1 L - glass cap); beacker (2 L); pre-muffled glass fibre filters (GF-F 0.7 µm or GF-C 1.2 µm); magnetic stirrer and stirrers (Teflon stirrers); vacuum filtering equipment (glass vacuum flask, filtering unit 47 mm diameter, funnel, clamp); vacuum pump; sodium dodecyl sulphate in pellets (better than powder); balance.

Measure 1 L of filtered Milli-Q water using the measuring cylinder and pour it into the beaker. Weigh 50 g of sodium dodecyl sulphate (SDS) and add it into the beaker together with a magnet. Place the beaker on a stirring plate and stir vigorously. When the powder is completely dissolved (and the foam has disappeared), filter the solution using a GF filter (0.7 µm or 1.2 µm) and transfer the solution in a glass bottle.

Buffer solutions for enzymatic treatment

Enzymathic treatmets is nowadays widely used in microplastic science to process environmental samples, because they proved to be effective without damaging the MPs contained in the sample. Enzymes work at their best in specific ranges of pH, so it is important to use buffer solutions when dealing with enzymes to ensure their optimal activity.

TRIS buffer (pH 8.2) for enzymatic treatment with Protease

Tris buffer is used with proteolytic enzimes, such as protease. These enzymes show their best activity with a slightly basic pH. In this specific case, the TRIS buffer has to be prepared at pH 8.2. The material, equipment and glassware needed to prepare buffer solutions is summarized in this brief list: measuring flask (1 L); glass bottles (1 L - full glass); glass fibre (GF) filters (0.7 µm or 1.2 µm pore size); tris(hydroxymethyl) aminomethane; hdrochloric acid (HCl, 37%); sodium hydroxide (NaOH); filtered demi-water or Milli-Q water; glass filtering equipment (glass vacuum conical flask, filtering unit, funnel, clamp); vacuum pump; balance.

The first step is to prepare the stock solutions. The Tris (hydroxymethyl) aminomethane (Solution A) is simply prepared by weighing 24.2 g of Tris (hydroxymethyl) aminomethane, transfer them into a measuring flask (1 L) and fill with Milli-Q to the mark. The hydrochloric acid (0.2 M HCl) (Solution B) is prepared by diluting 16.6 mL of HCl 37% in 1 L of Milli-Q water (measuring flask – 1 L). It is very important to add first most of the water and then the concentrated acid, then adjust the volume to the mark with more water. Diluting concentrate acid is very dangerous. The preparation of the actual buffer solution (1L; pH 8.2) is done as follow: add 250 mL of A and 109.5 mL of B in a 1 L measuring flask, then dilute to 1 L with Milli-Q. Mix the solution turning upside down the flask several time. Filter the solution over a 0.7 µm or 1.2 µm GF filter and transfer it to a 1 L glass bottle.

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Acetate buffer (pH 4.8) for enzymatic treatment with Cellulase

Acetate buffer is used with cellulolytic enzymes, such as cellulose or blends of cellulase. These enzymes show their best activity with a slightly acid pH. In this specific case, the acetate buffer has to be prepared at pH 4.8. The material, equipment and glassware needed to prepare buffer solutions is summarized in this brief list: measuring flask (1 L); glass bottles (1 L - full glass); glass fibre (GF) filters (0.7 µm or 1.2 µm pore size); acetic acid (CH3COOH); Sodium acetate (C2H3O2Na or C2H3O2Na * 3H2O); Filtered Milli-Q water; glass filtering equipment (glass vacuum conical flask, filtering unit, funnel, clamp); vacuum pump; balance.

The first step is to prepare the stock solutions. The 0.2 M solution of acetic acid (Solution A) is prepared by diluting 11.55 g of acetic acid (just weigh the CH3COOH on a balance) in 1 L (measuring flask). The 0.2 M sodium acetate solution is prepared by diluting 16.4 g of C2H3O2Na or 27.2 g of C2H3O2Na * 3H2O in 1 L of Milli-Q. To prepare 1 L of buffer solution (pH 4.8), add 200 mL of A and 300 mL of B in a 1 L measuring flask, then dilute to 1 L with Milli-Q. Mix the solution turning upside down the flask several time. Filter the solution over a 0.7 µm GF filter and transfer it to a 1 L glass bottle.

Preparing solutions for Fenton oxidation

Catalyzed oxidative reactions are widely used in sampling prepararion for microplastic analysis nowadays. Although there are different recipes, these reactions, called Fenton oxidation, use Iron (II) as catalyst to reduce reaction time and enhance reactivity. The material, equipment and glassware needed to to perform a Fenton reaction (AAU recipe) is summarized in this brief list: measuring flask (500 mL); glass fibre (GF) filters, 0.7 µm or 1.2 µm; iron sulphate heptahydrate (FeSO4 * 7H2O); filtered Milli-Q water; measuring cylinder (10 mL); concentrated sulphuric acid (H2SO4); glass filtering equipment (glass vacuum conical flask, filtering unit, funnel, clamp); vacuum pump.

0.1 M Iron Sulphate (FeSO4 + H2SO4)

Find a 500 mL measuring flask, fill it half way with Milli-Q water. Measure 15 g of iron sulphate heptahydrate and add it into the measuring flask, then mix the until it is completely dissolved. When all powder is dissolved remove the magnet and fix the volume to 500 mL with Milli-Q water. Transfer the 0.1 M iron solution to a glass flask and add 6 mL concentrated sulphuric acid using a small measuring cylinder. Filter the solution using GF filter (0.7 µm or 1.2 µm.

0.1 M Sodium hydroxide (NaOH)

Find a 500 mL measuring flask, fill it half with MilliQ. Measure 2 g of sodium hydroxide and add it into the measuring flask and mix it until the powder is dissolved Fix the volume to 500 mL with filtered demi-water or filtered Milli-Q water. Filter the solution through a 1.2 or 0.7 µm GF filter. Transfer the solution to a glass flask.p

Preparing high density salt solution for sample flotation

Flotation by using high density salt solution is widely used in microplastic sample preparation when extracting solid matrices to isolate less dense materials (including plastics) from the denser inorganic materials, but also to remove remaining inorganic solids and digested organic matter later on in sample prep. (also for liquid matrix samples). Different options are available when it comes to high density salt solutions. Here we report the procedure to prepare a solution of Sodium Polytungstate and a solution of Zinc Chloride (ZnCl2). The material, equipment and glassware needed to perform a Fenton reaction (AAU recipe) is summarized in this brief list: 2 L beaker; glass bottles (full glass); glass fibre (GF) filters, 0.7 µm or 1.2 µm; SPT (powder); filtered demi water or Milli-Q water; stirring plate; magnetic stirrer (Teflon); glass filtering equipment (glass vacuum conical flask, filtering unit, funnel, clamp); vacuum pump.

SPT (density 1.75 g/cm^3) (1 L)

This solution is prepared assuming that the density of water is 1 g/cm^3 at room temperature (this is a simplification). Weigh 927 g of SPT powder (Sodium polytungstate) into a 2 L beaker. Add 823 mL filtered Milli-Q water. Add a magnet to the beaker, stir the solution and wait until powder is dissolved. Filter the solution over a 0.7 or 1.2 µm GF filter, then transfer the solution in a glass bottle. Measure the density of the obtained solution by weighing 3 times 1 mL of SPT-solution on a scale (use a calibrated 1 mL micropipette). The density should be around 1.75 g/cm^3.

Preparing 50% v/v Ethanol for sample evaporation and deposition (1 L)

The last step of sample treatment for MP analysis involves a sample transfer inside a 10 mL headspace vial after mixing it with an ethanol solution (50% v/v) (39.5% w.w). The material, equipment and glassware needed to prepare a Fenton reaction (AAU recipe) is summarized in this brief list: glass measuring cylinder (1 L or 0.5 L); glass bottles (plastic lid); glass bottles (full glass) (pre-muffled at 500°C); glass fibre (GF) filters, 0.7 µm or 1.2 µm; ethanol absolute (CH3CH2OH ≥ 99.8% for HPLC); filtered demi water or filtered Milli-Q water (0.7 µm GF filtered); stirring plate; magnetic stirrer (Teflon); glass filtering equipment (glass vacuum conical flask, filtering unit, funnel, clamp); vacuum pump.

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Measure 0.5 L of ethanol absolute using a measuring cylinder. Transfer the liquid into a glass flask. Do the same with 0.5 L of Milli-Q water (or demi-water). Put a plastic lid and mix the two liquids until you obtain a homogeneous solution. Filter the solution using a GF filter (0.7 µm or 1.2 µm) and transfer the filtered solution into a pre-muffled glass bottle (glass cap).
NOTE: Absolute ethanol is used in all the steps excluding the evaporation, where HPLC grade ethanol is used instead (it prevents to have unwanted residues in the samples).

Sample preparation of marine water samples

Sonication and SDS treatment

Place the filters (the enriched filters from the AAU UFO) in a crystallizer, cover all filters with 5% SDS solution, and incubate them by placing the crystallizer on a heating plate for at least 24h. Sonicate the filters separately into enough 5% SDS solution to cover the filter for 5 min (each filter) in an additional glass crystallizer. Remove, flush and scrape the filters using enough SDS (up to 700 ml), and then pour all the liquid into a 1 L beaker. If you have access to an orbital shaking water bath place the sample into a water bath (set 50°C and 100 rpm) for at least 24 hours. If you have a stirring water bath, add stirring (glass stirrer) and a glass lid and place the sample into a water bath (set 50°C and 100 rpm) for at least 24 hours. Alternatively use a heating plate with the same settings. The water level in the water bath should be approximately the same than in the sample and this could cause the sample's beaker to float. Add a weight to the glass watch to secure the sample.
After this step, filter the content of the beaker onto a 10 µm steel filter, taking care of rinsing thoroughly with filtered demi-water (0.7 µm or 1.2 µm GF filtered).

Enzymatic treatment: Protease

The glassware and equipment required to carry out a Fenton reaction is listed here: 1 L beaker (use the same used for the previous steps); 100 mL cylinders class A (x2), 250 mL cylinder class A (x1); glass syringe (luer lock with aluminium joint needle); micropipette (1 mL) and tips; TRIS buffer (pH 8.2); Protease enzyme; Viscozyme; shaking water bath or stirring water bath.
Measure 200 mL of TRIS Buffer solution (pH 8.2), use a fraction of it to sonicate and flush the filter containing the sample (save the steel filter for the next filtration steps by placing it into a glass Petri dish). The beaker from the previous step can be used. Add the rest of the TRIS buffer, and then add 0.5 mL Protease to the sample. Place it into the water bath (set 50°C and 100 rpm) and leave it for at least 40 hours.
After this step, filter the content of the beaker onto a the same 10 µm steel filter used previously, taking care of rinsing thoroughly with filtered demi-water (0.7 µm or 1.2 µm GF filtered).

Enzymatic treatment: Cellulase, Viscozyme

The glassware and equipment required to carry out a Fenton reaction is listed here: 1L beaker (use the same used for the previous steps); 100 mL cylinders class A (x2), 250 mL cylinder class A (x1); glass syringe (luer lock with aluminium joint needle); micropipette (1 mL) and tips; Acetate buffer (pH 4.8); Cellulase enzyme blend; Viscozyme; shaking water bath or stirring water bath.
Measure 200 mL of Acetate Buffer solution (pH 4.8), use a fraction of it to sonicate and flush the filter containing the sample (save the steel filter for the next filtration steps). The beaker from the previous step can be used. Add the rest of the Acetate buffer, and then add 0.5 mL of Cellulase enzyme blend and 0.5 mL of Viscozyme.
Place the sample into a water bath (set 50°C and 100 rpm). Incubate the sample for at least 40 hours.
After this step, filter the content of the beaker onto a the same 10 µm steel filter used previously, taking care of rinsing thoroughly with filtered demi-water (0.7 µm or 1.2 µm GF filtered).

Fenton oxidation

The glassware and equipment required to carry out a Fenton reaction is listed here: 1 L beaker (use the same used for the previous steps); 100 mL cylinders class A (x2), 250 mL cylinder class A (x1); glass syringe (luer lock with aluminium joint needle); bucket containing ice (storage); large buckets to use as water/ice bath.
Measure 200 ml of Milli-Q use a fraction of it to sonicate and flush the filter containing the sample (save the steel filter for the next filtration steps by placing it into a glass Petri dish) in a 1 L beaker and fix the volume to 200 mL. Cool the sample to ca. 15-20°C and add 145 mL H2O2 (50%), 62 mL of 0.1M FeSO4 and 65 mL of 0.1M NaOH. Place the sample on an icy water bath and keep the temperature between 20-30°C for at least 4 hours (add ice in the water bath when necessary). Let it stand overnight.
(Use the ice bath to cool your sample down. If the sample gets too cold (< 20°C), then remove your sample from the ice bath and place it on the table for some time.). Keep a close eye on the oxidation, as the temperature can increase even after a couple of hours from the start of the reaction.

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Size fractionation

The glassware and equipment required to carry out a size fractionation is listed here: metal sieve (10 cm diameter; 500 µm or 300 µm mesh); glass funnel with large opening (to place the sieve) 1 L beaker (use the same used for the previous steps); 100 mL cylinders class A (x2), 250 mL cylinder class A (x1); glass syringe (luer lock with aluminium joint needle); bucket containing ice (storage); large buckets to use as water/ice bath.
MP analysis using FTIR technology requires to use different approach depending of the size range of the particles targeted. The fraction between 5 mm and 500 µm (or 300 µm) is analysed via ATR-FTIR spectroscopy, while the fraction < 500 µm (or 300 µm) is instead analysed via µFTIR-Imaging spectroscopy. Therefore, it is necessary to size-fractionate the sample at this stage of the sample preparation.
Place a 500 µm (or 300 µm) steel sieve on top of a filtration unit containing the 10 µm filter used for sample filtration in the previous steps. The sieve can be accommodated on top of a glass funnel with large opening. Pour the liquid through then thoroughly flush the previous beaker (containing the sample after Fenton reaction). Flush the particles on the sieve abundantly with Milli-Q. Remove the sieve, backflush the particles into a beaker and save them for further treatment (then proceed with step K.). The fraction < 500 µm (or 300 µm) which passed through the sieve is filtered onto the 10 µm steel filter.

Flotation in separation funnel with high density liquid (SPT or ZnCl2)

The glassware and equipment required to carry out a flotation is listed here: glass separation funnel (from 100 mL to 250 mL) with Teflon stopcock; glass lid for the funnel; lab stand and clamps to secure the funnel; 150 mL glass beaker; glass syringe (luer lock with aluminium joint needle); nitrogen/compressed air intake; silicon pipes and piping joints.
Transfer the filter enriched with the sample (< 500 µm or < 300 µm) to a pre-cleaned 150 mL beaker containing around 25 mL of SPT (or Zinc Chloride). Sonicate for three minutes. Remove the filter (or filters in case you have to use more than one) and flush it with SPT. (Use the appropriate density, check the density before usage, because it can change during storage). Save the filter for the next step by placing it into a glass Petri dish.
Transfer the liquid to the separation funnel (choose an appropriate funnel volume according to the amount of solids in the sample); flush the beaker with SPT into the funnel. Fill the funnel 3/4 with SPT. Apply air for 15 minutes from the bottom of the funnel (filtered compressed air or nitrogen) by connecting a silicon pipe to the funnel's bottom opening. Do this by slowly opening the air valve, then the funnel's stopcock. If you do not have compressed air or nitrogen available, simply shake the funnel manually for a couple of minutes (close it with a glass lid before shaking).
After bubbling/shaking the funnel, flush the inside walls with SPT. Add SPT until the level reaches the largest aperture of the separation funnel. Leave it to settle overnight. Remove the settled matter using the funnel's stopcock. Wait 30 minutes, then remove the settled matter again. Repeat this until there is nothing settling. In case a relevant amount of particulate is still present, repeat all these flotation steps once more. After removing the settled particulate, proceed with filtering the top part of the flotated sample through the 10 µm steel mesh (! Filter the top part of the liquid ↧), flush 1 L of warm (50°C) Milli-Q water, then 100 ml of EtOH through the filter. Transfer the filter containing the sample to a 150 mL beaker with 50 mL of 50% v/v ethanol and incubate overnight at 50°C to clear out any SPT residue that may be attached to the particles. Filter the liquid through a new 10 µm steel mesh, flush with 4 L cold and 1 L of warm (50°C) Milli-Q water (the large volume are used to further flush the sample to remove any SPT residue), then 100 mL of EtOH through the filter.

Evaporation

The glassware and equipment required to carry out the sample evaportation is listed here: 10 mL headspace vials; vials lids with Teflon septa; glass syringe (luer lock with aluminium joint needle); ethanol (EtOH) 50% v/v (HPLC grade); metal spatula; warm pre-filtered demi-water; evaporation bath (biotage XXXXX).
Add the filter containing the sample to a new muffled 150 ml beaker, use 50% ETOH (HPLC grade) to flush (used a glass syringe with luer-lock attachment and needles with aluminium joint) and sonicate. Use as little EtOH as possible. Add the liquid from the beaker into a 10 mL headspace vial. Fill the vial ¾ and evaporate it into an evaporation bath before adding more liquid (the evaporation bath is a water bath operation at 50 °C using a gentle stream of nitrogen directed inside each vial to speed-up the evaporation process). After every aliquot is transferred into the vial for evaporation, flush the 'pouring'-side of the beaker, so particles do not get stuck to the glass wall. When the beaker is empty, flush it three times to ensure the whole sample is transferred into the vial.
Be careful not to dry out the beaker while waiting to transfer a new aliquot to the vial; the bottom of the glass must always be covered with some 50% EtOH.
After all the sample has been transferred into the vial and dried, remove the vial from the evaporator, and add 5 mL of 50 % EtOH (HPLC grade) to the vial using a calibrated 5 ml glass pipette. Sonicate the vials for three minutes. The sample is now ready to be analysed. The known volume allows to analysed sub-samples and re-calculate the particle conc. in the whole vial.

SPECIAL THANKS to Alvise Vianello and the group of prof. Jes Vollertseen for sharing this protocol

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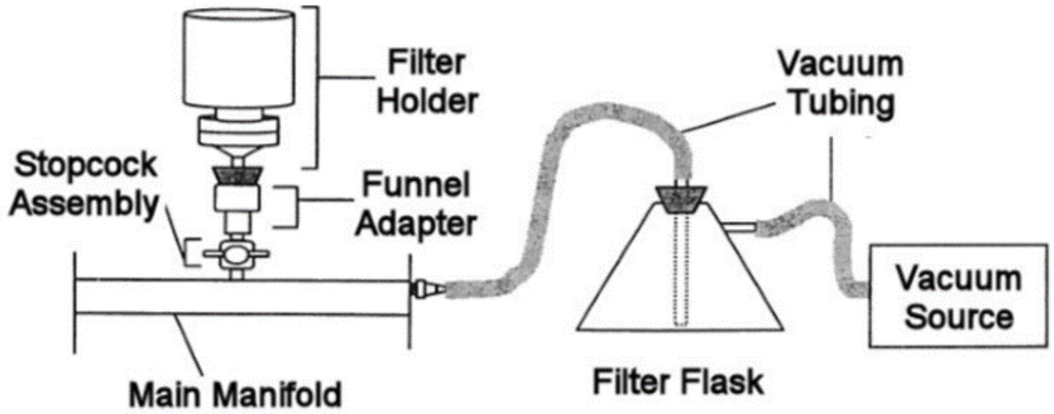
Filtration

Filtration apparatus.

There are several choices of set-up but the main point to keep in mind is to avoid, as far as possible, plastic parts since they can be an unintended source of microplastics. The HORIBA choice (the parts depicted below are offered in our “Microplastic package” see HORIBA Solution section) for the filtration apparatus is:



DR

Stainless steel manifolds: These can be selected depending on the workload but we do not include any of these in our package where only the flask with the side arm is proposed.

Filtration is the last step prior to the identification of the microplastics by the technique of choice (FTIR microscopy, Raman microscopy and optical microscopy) and two points must be addressed in this section:
Filtration apparatus and filter types.

We have selected Sterlitech (https://www.sterlitech.com/) as our preferred filtration apparatus supplier, and the parts are:

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Glass funnel (available up to 1 L, 100 ml in our package), a sintered glass support base with 13 mm available filtration area, a silicone stopper and a 1 L borosilicate glass flask with side arm.

DR

A diaphragm vacuum pump chemically resistant and completely oil-free.

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Analysis Workflow

Filtration

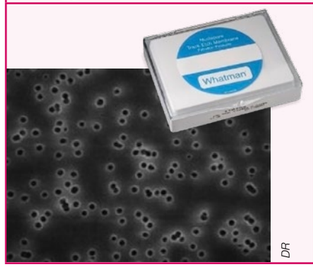
Filters.

There is a wide choice of filter/membrane and several of them have been tried and tested microplastics analysis. The three important characteristics are: filter size (13, 25, 47, 55 mm in diameter), filter material (polycarbonate, polytetrafluoroethylene PTFE, alumina, silicon etc. and pore size (0.2, 0.7, 1.6, 4.2 µm etc.). Of course, these features must be tuned depending on the microplastic sizes of interest and also on the techniques that will be used to identify them.

Our focus for filter choice is on the microscopy techniques (FTIR, Raman and optical microscopy) which are the most commonly used and seem to provide the most complete microplastics picture allowing: Chemical identification (true for Raman and FTIR), counting (number and size distribution) and quantitative estimation (number and mass).

The most commonly used filters are: Borosilicate glass fibers, Alumina, Polycarbonate (un-coated and coated with various metal layers) and Silicon. The table below summarizes the pros and cons of each of them including: **optical quality** (for microscope visualization); **mechanical resistance and handleability; interference** for microplastic chemical identification with Raman and Infrared Microscopy; and price.

Table 5. Filters pros and cons (part 1)

Filter Type		Optical Quality	Handleability	Interference	Unit Price per filter (euro)
	**Borosilicate Glass Fiber** (no binder) available with different pore sizes (lowest 0.6 µm)	Rough surface can reduce ability to identify microplastics (most significant for small particles, below 10 µm). White membrane ⇛ low contrast for transparent plastics	No issue	Possible interference signals for Raman and Infrared Microscopy.	0,25 to 14 Depending on filter size (up to 257 mm in diameter available) and grade
	**Polycarbonate Uncoated** available with different pore sizes (lowest 0.2 µm)	Flat surface. White membrane ⇛ low contrast for transparent plastics	Issue in case of Alkali tretament (KOH)	Strong interference with Raman and Infrared Microscopy. Polycarbonate shows strong bands both in Raman and Infrared. Not usable for Transmission Infrared Microscopy	0,6 to 13 Depending on filter size (up to 142 mm in diameter available)

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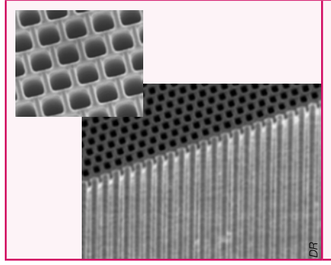
Analysis Workflow

Filtration

Table 6. Filters pros and cons (part 2)

Filter Type		Optical Quality	Handleability	Interference	Unit Price per filter (euro)
	**Polycarbonate Coated** available with different pore sizes (from 0.2 to 5 µm) and different metal coating: gold,silver	Flat surface and high relectivity and good contrast (Highly textured surface for Silver)	Issue with Alkali treatment (KOH)	Less interference than uncoated, but still present if metal is thin and for particles below 5 µm. Not useable for Transmission Infrared Microscopy	8 to 23 for silver and 18 to 30 for gold Depending on filter size (up to 47 mm in diameter available for both)
	**Alumina (Anodisc)** supported (surrounded by a polypropylene ring) and unsupported available with different pore sizes (from 0.02 to 0.2 µm)	Flat surface. White membrane low contrast for transparent plastics	Highly fragile, careful handling required	Low interference for FTIR (peak intensity change over the filter) and for Raman (broad spectral feature) - Useable for Transmission Infrared Microscopy but no signal below 1250 cm^{-1}	5 to 15 Depending on filter size (up to 47 mm in diameter available)
	**Silicon** with different pore sizes (from 1 to 18 µm)	Flat surface. High relectivity and good contrast	Easy handling, possible fragility along crystalline direction. Squareshaped (dedicated holder needed)	Raman (silicon peaks do not interfere with plastic peaks) - FTIR (possible interference from silicon oxide) - Useable in Transmission Infrared Microscopy	14 to 24 Depending on volume

Regarding polycarbonate filters one research group[1] tried different metal coatings, not commercially available, and obtained very good results with Raman microscopy and Aluminum coated polycarbonate. Aluminum can enhance Raman scattering by a factor of 4[2], thus improving detectability.

Alternatively, it is possible to use CaF_2 and/or ZnSe windows (usually with a diameter of 13 mm); these are not filters but windows widely used in Infrared and Raman microscopy. A solution of microplastics can be concentrated to few millilitres by evaporating the solvent and then it can be poured onto the window and left to dry before spectroscopic analysis.

A last important point, it is simple but is key to getting good results, is the amount of microplastics in the solution to be analyzed. The filter must not be tightly packed with material otherwise optical identification and further analysis of the particles will be complicated if they overlap. In this case just prepare a more dilute solution before filtration as was done in the literature[3].

1. Oßmann *et al*., WATER RESEARCH 141 p.307 2018
2. Kamemoto *et al*., APPLIED DPECTROSCOPY 64 p.255 2010
3. Bergmann *et al*., ENVIRONMENTAL SCIENCE & TECHNOLOGY 51 p.11000 2017

Analysis Workflow

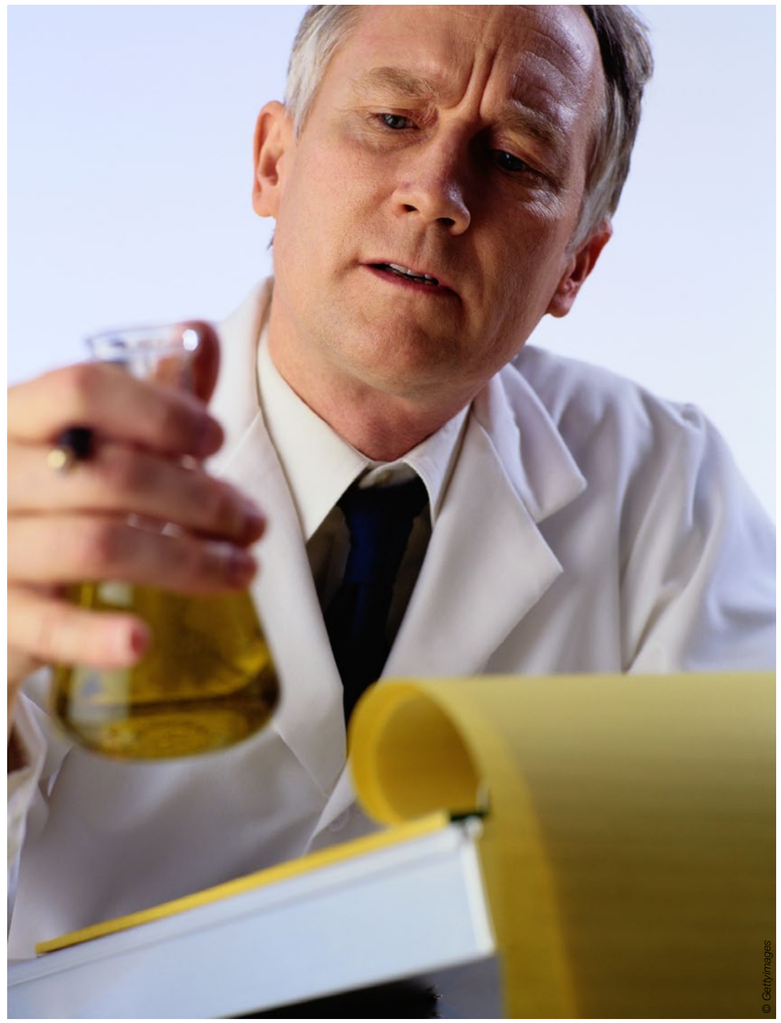
Measurement methodologies

The last step following sampling, sample preparation and filtration is identification of the microplastic using one or more different techniques.

Five main techniques are used for this purpose:

1. Fluorescent staining with Nile Red, coupled with Fluorescence microscopy
2. Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM-EDX)
3. Infrared Microscopy
4. Raman Microscopy
5. Pyrolysis Gas Chromatography Mass Spectrometry (Pyr-GCMS)

They are complementary with each other but Infrared and Raman provide a more detailed picture. Raman being more flexible (all sizes of plastics can be analyzed) since it is able to detect microplastics below 10 µm. These represent the main threat/concern for Human health (Ragusa et al.[1] observed plastic pieces below 10 µm in the placenta).



Nile Red staining/Fluorescence microscopy.
Nile Red is a fluorescent dye (see absorption curve, dashed line, and emission curve, below) widely used to localize and quantify lipids but it can also selectively bind to most plastics, allowing them to be identified by looking at the fluorescence in both the green as well as in the red.

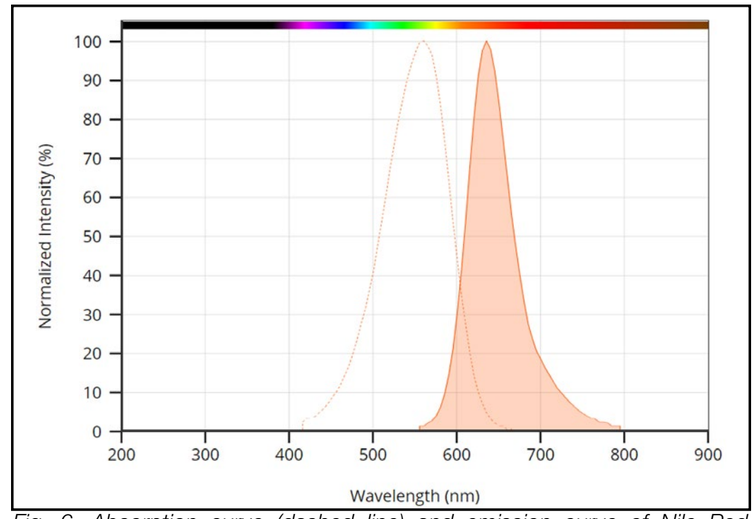


Fig. 6. Absorption curve (dashed line) and emission curve of Nile Red fluorescent dye

Since Nile Red also binds to lipids, environmental samples, careful sample preparation (complete digestion of the biogenic material[2]) is key to successful analysis, as the presence of biological residues can lead to an overestimation of the amount of microplastics.

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HORIBA

Analysis Workflow

Measurement methodologies

For non-environmental samples such as bottled/tap water[3] where digestion is not needed Nile Red can be used directly. After staining microplastics can be easily and automatically counted by looking at the green or red colored particles with a fluorescence microscope. The best approach, as demonstrated in the literature[2], is to use green / yellow fluorescence (excitation/emission 450–490/515–565 nm) as this avoids natural lipids which emit deeper into red (higher wavelength). A Nile red concentration of ranging from 0.1 and 2 µg/mL is typically used.

The advantage of this technique is speed and simplicity, the main drawbacks are the lack of chemical identification of the polymer types and the possible presence of false positives. Erni-Cassola et al.[3] validated this method by also using Raman microscopy, which notwithstanding the presence of the dye, can still be used, to chemically identify the polymer.

SEM-EDX.

Scanning Electron Microscopy stand alone allows the complete morphological characterization of the particles down to the nanometer range (which is a strong advantage in comparison to the other techniques) but it is not able to provide chemical information and samples, in almost all cases (Fries et al.[4]), must undergo to additional treatments due to the high vacuum in the test chamber and to avoid charge accumulation. Moreover, the filters suitable for Infrared and Raman microscopy cannot be used, instead the microplastics must be dried and then transferred onto double-sided adhesive carbon tabs on aluminum SEM stubs.

SEM combined with the energy dispersive X-ray spectroscopy can give additional information by providing the elemental composition of the sample. Elemental information allows:

- Inorganic and carbon-based material to be distinguished (the full digestion of organic contaminants is essential to assign carbon-based material to plastic);
- Some polymer types to be identified, such as PVC due to the presence of Chlorine[5];
- Identification of the presence of other elements[6] such as Al, Ca, Mg and Si on the plastics which can be the signature of polymer additives.

Summarizing, SEM-EDX is a technique which, unlike others, can provide detailed morphological information down to nanometer range (morphology may influence the diffusion of microplastics within the human body) but it cannot be used alone as it does not provide comprehensive chemical information.

Infrared Microscopy.

Infrared microscopy is currently the most widely used technique for microplastic analysis and all the commercial instruments are also combined with optical microscopy. In some instruments the optical microscopy is limited by the use of infrared objectives only, which cannot provide the flexibility and high magnification of standard visible objectives and this limits the identification of small particles.

Infrared microscopy is a non-destructive technique and can provide morphological information (by the analysis of the optical and/or chemical image), quantitative analysis (in terms of number of particles) and chemical identification of the microplastic (by comparing the collected infrared spectra with the ones in commercial libraries). Like Raman microscopy one of the most complete techniques. The main drawback/limitation of Infrared microscopy is its inability to identify particles smaller than 10 µm. Zhu et al.[7] in a recent review of June 2020 mentions that the smallest particle size determined with infrared microscopy is 20 µm.

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Analysis Workflow

Measurement methodologies

There are two main approaches to analyze microplastic with infrared and both start with an optical image acquisition of the filter used in the filtration step:

1. In the first approach, the particles' size, shape and their location on the filter are determined by analyzing the optical image, the location is then used to individually measure each particle by moving the infrared beam to the required location;
2. In the second approach, the particles' size and shape are determined by the analysis of the optical image and then the whole filter is chemically imaged with the infrared beam (This approach collects many spectra so needs a specialized detector such as a Focal Plane Array (FPA) or a linear array, to reduce the acquisition time as much as possible). The size and shape of the particles can also be determined by analyzing the chemical image, but accuracy can be limited by the resolution of the image.

Raman Microscopy.

Raman microscopy is the second most common technique and like Infrared it also includes standard optical microscopy. Raman instruments use visible objectives that are available with a full range of magnifications, so image quality and morphological information is uncompromised, Raman microscopy is a non-destructive, non-contact technique that provides morphological information (by analyzing the optical image), quantitative analysis (number of particles) and chemical identification of the microplastic (by comparing the collected Raman spectra with the ones in commercial libraries).

The biggest advantage of Raman microscopy compared to infrared is the ability to measure and identify particles of 1 µm[8] and below in size. This point is crucial since the biggest concern for human health seems to come from particles below 10 µm because they can migrate within our body[1].

One drawback of Raman microscopy is the interference caused by fluorescent materials such as environmental and/or plastic pigments, additives and pollutants; fluorescence which can overlap with the Raman spectrum, limiting the ability to identify the microplastic. The presence of fluorescent material does not always limit plastic identification (Enri-Cassola et al.[2] successful measured Nile red stained particles) and additionally several excitation wavelengths are available to assist avoiding fluorescence interference (the use of a near infrared excitation source, i.e. 785 nm, often limits the fluorescence signal).

The main approach to analyze microplastics with Raman microscopy is:

First step is the acquisition of an optical image of the filter from which the particles' size, shape and location are determined; second step, is to move the laser beam to each identified location, and acquire a Raman spectrum of each particle.

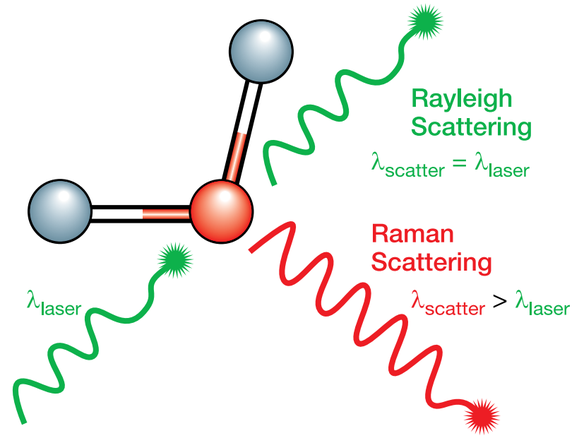

Fig. 7. Raman principle

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Analysis Workflow

Measurement methodologies

Pyr-GCMS.

Pyrolysis combined with Gas Chromatography Mass Spectrometry can determine the chemical composition of the microplastic by analyzing their pyrolysis products (Pyrograms). Similar to Infrared and Raman, which use spectral libraries for chemical identification, the pyrograms obtained are compared with reference ones of known polymers. The chemical identification is not as detailed as for vibrational spectroscopy techniques, in particular, the polymer[9] sub-type (such as Low density vs. High density polyethylene) cannot be discriminated and, in case of complex matrices, the identification can be misleading.

The main advantages of Pyr-GCMS are the quantitative analysis of Microplastic in terms of weight per polymer type for polymers which exceed the quantification detection threshold, and the low amount of material needed (5 µg can be enough) although this small quantity may not be representative for complex environmental matrices.

The drawbacks of Pyr-GCMS are:

- Destructive technique: Samples cannot be re-analyzed;
- Lack of information on particle morphology: size and shape, which are well known to influence the risk assessment of microplastics;

Table 7. In the table, we have summarized the main advantages and disadvantages of the different techniques.

Technique	Nile Red & Fluorescence Microscopy	Scanning Electron Microscopy & Energy Dispersive X-ray Spectroscopy (SEM-EDX)	Infrared Microscopy	Raman Microscopy	Pyrolysis Gas Chromatography Mass Spectrometry (Pyr-GCMS)
Pros	Fast and Simple Low-cost Morphological information Quantitative analysis (Number of Particles)	Particle down to nanometer size Discrimination between Inorganic and carbon-based material Elemental analysis	Non destructive Morphological information Chemical identification Quantitative analysis (Number of Particles) and Quantitative per polymer type	Non contact and non destructive Morphological information Chemical identification Quantitative analysis (Number of Particles) and Quantitative per polymer type Particles down to 1 micron and below	Quantitative analysis (weight of particles) Partial Chemical identification
Cons	False positives No chemical identification No Quantitative analysis per polymer type	Additional preparation needed No chemical identification High cost	Sensitive to particle dimension (bigger particles cannot be analyzed in transmission) Smaller particles (<10 micron) cannot be analyzed	Interference by fluorescent material	No Morphological information Destructive

1. Ragusa *et al*., ENVIRONMENT INTERNATIONAL 146 p.1 2021
2. Erni-Cassola *et al*., ENVIRONMENTAL SCIENCE & TECHNOLOGY 51 p.13641 2017
3. Mason *et al*., FRONTIERS IN CHEMISTRY, 6 (article 407) p.1 2018
4. Fries *et al*., ENVIRONMENTAL SCIENCE PROCESSES & IMPACTS 15 p.1949 2013
5. Wang *et al*., SCIENCE OF THE TOTAL ENVIRONMENT 603-604 p.616 2017b
6. Dehghani *et al*., ENVIRONMENTAL SCIENCE POLLUTION RESEARCH 24 p.20360 2017
7. Zhu *et al*. ANALYTICAL METHODS 12 p.2944 2020
8. Oßmann *et al*., WATER RESEARCH 141 p.307 2018
9. Dehaut *et al*., ENVIRONMENTAL POLLUTION 215 p.223 2016a

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HORIBA

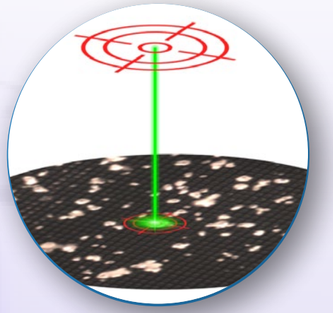
HORIBA Solution

HORIBA Solution
Filtration appartus
Raman microscope

MVAPlus, ViewSharp, Navsharp, VRM
ParticleFInder
Reference standard sample

After considering the needs and challenges of those involved in microplastics analysis, HORIBA has developed a comprehensive solution that spans from sample preparation to results reporting:

This ebook keeps you updated on new trends, sample preparation protocols, upcoming regulations, etc.

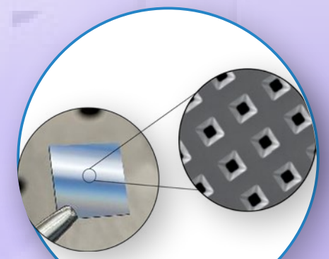


Video Raman Matching Tool with GPS-like technology to accurately locate particles on the filter

Reference Microplastics Materials to validate the entire analytical workflow

Raman Microscopes

Filtration Apparatus



Filters & Filter Holders

ParticleFinder™: Fully automated particle analysis software that enables:

- Viewing and locating particles in optical images
- Characterization by size/shape
- Raman analysis
- Reporting results with statistics on shape, size, etc.

IDFinder™: Automated tool for spectra identification and management of spectral libraries. ST Japan and HORIBA spectral libraries, offering a starter or full package option (10,000 or 21,000 spectra, respectively) are available.

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HORIBA Solution

Filtration apparatus

Included in the starter kit are:

- 100 ml Glass funnel

- Silicone stopper

- Silicon gaskets

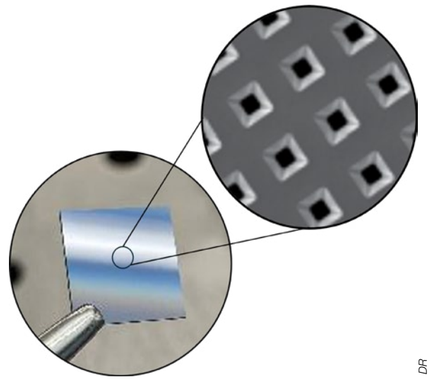
- 1L Borosilicate glass flask

- Sintered glass support with 13 mm filtration area

- Diaphragm vacuum pump

DR

We offer a dedicated holder designed specifically for square-shaped filters. There are two holders, each optimized for different filter thicknesses, and each holder accommodates up to 3 filters (see picture below). This design allows for efficient system use, enabling analysis of up to 3 filters without requiring the user to be present to change the samples.



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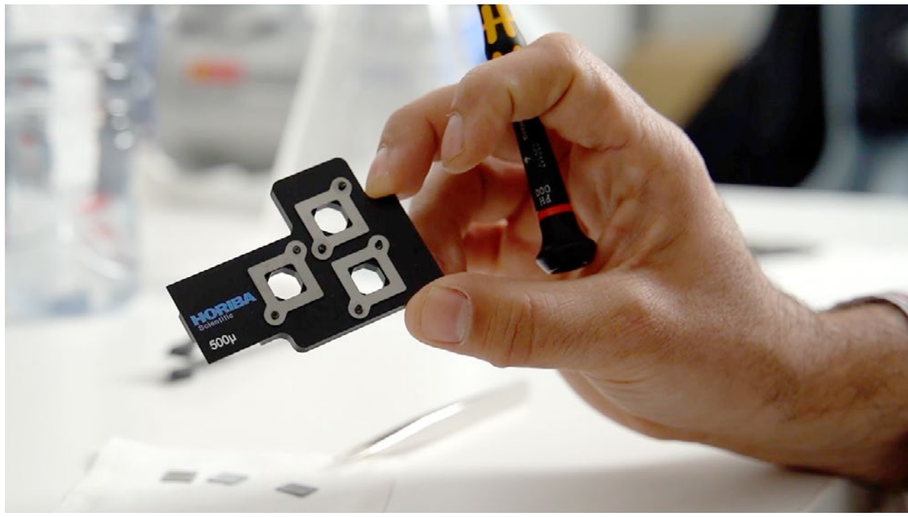

Why Silicon Filters?

Silicon filters offer several distinct advantages for particle analysis:

1. **Surface Properties:** Silicon filters are flat and reflective, which provides excellent contrast for particles on the filter surface. This property significantly aids in the automated localization and analysis of particles.
2. **Raman Spectroscopy Compatibility:** The Raman signal of silicon does not overlap with that of polymers, facilitating the automatic identification of microplastics.
3. **Reusability:** These filters can potentially be reused after thorough cleaning, enhancing their cost-effectiveness.

For the starter filtration kit, we provide square-shaped filters (10x10 mm) manufactured by SmartMembranes (http://www.smartmembranes.de/en/). The kit includes 25 filters with three different pore sizes: 1 µm (5 filters), 2.5 µm (10 filters), and 5-6 µm (10 filters).

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HORIBA Solution

Raman Microscopes:

Two platforms are available: XploRA™ PLUS and LabRAM Soleil™.

Both Raman microscopes can be equipped with all necessary features for microplastics analysis:

- 2 lasers (532 nm and 785 nm)
- 5X, 20X, 50X LWD (Long Working Distance), and 100X-LWD objectives
- Dark-field illumination is highly recommended for at least the 20X and 50X-LWD objectives to facilitate automatic particle localization on filters.
- Standard detector (CCD – Charge Coupled Device)
- LabSpec 6 software with all necessary options for microplastics analysis

Compared to XploRA™, LabRAM Soleil™ offers more flexibility in fine-tuning acquisition parameters, resulting in higher speed and analytical throughput. The possibility to use more lasers and finely adjust laser power enables analysis of the most challenging samples.

LabRAM Soleil™ is an ideal solution for various applications, including fast chemical imaging.

XploRA™ PLUS

DR

LabRAM Soleil™

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HORIBA Solution

MVAPlus

Multivariate Analysis module includes a number of multivariates (chemometric) methods, providing additional tools for data analysis such as PLS (Partial Least Square), CLS (Classical Least Square), PCA (Principal Component Analysis), MCR (Multivariate Curve Resolution) and Cluster Analysis.

ViewSharp™ and NavSharp™

ViewSharp and NavSharp which provide a clear view of the sample's surface and guarantees the highest focal quality in Raman Images (autofocusing during Raman collection) and particle images, provides a topography image and allows 3D chemical visualization.

Video Raman Matching (VRM) with nano-GPS Technology

Video Raman Matching is the perfect tool to reliably move to your particles even when transitioning from low to high magnification (do the mosaic with a 10x and measure the particles confidently with higher magnification). It allows also a perfect correlation between the chemical information and the visible image. VRM technology is based on GPS technology, a patented tag allows locations to be identified and to accurately position the sample and /or particles. This technology is the HORIBA gateway to Correlative Microscopy.

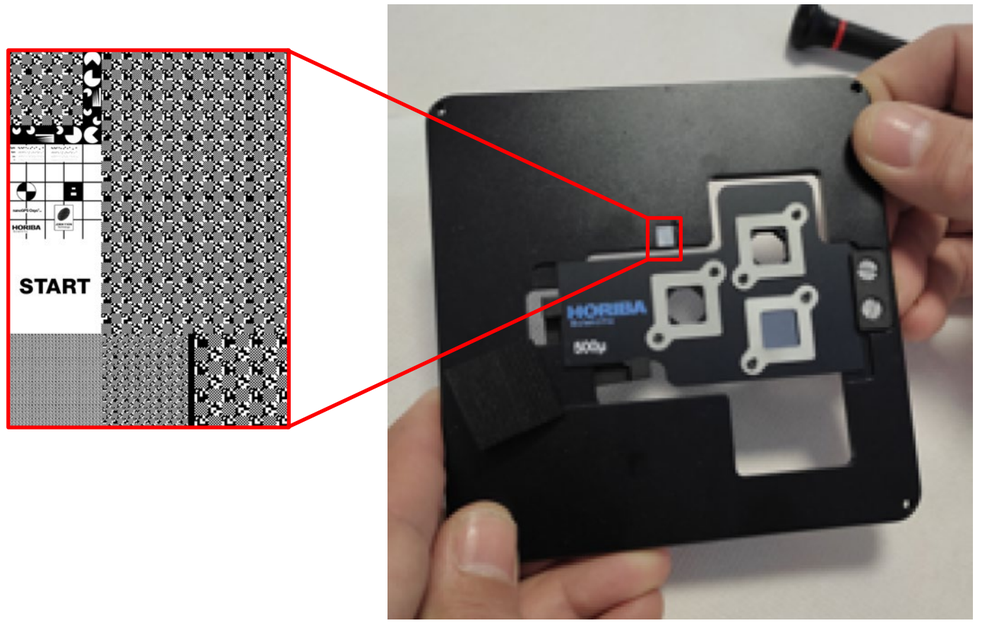

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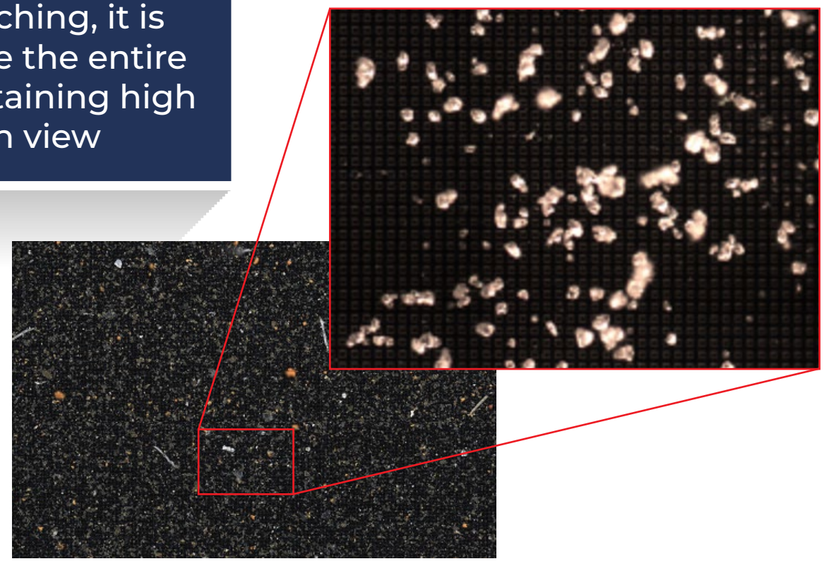
Fig. 8. Video Raman Matching (VRM)

NEW ParticleFinder™

ParticleFinder™ is software for automated particle analysis. It provides a step-by-step routine to locate, characterize, and chemically identify thousands of particles.

1 Image Acquisition

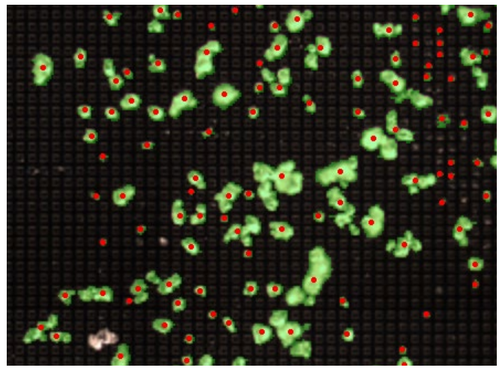
Due to image stitching, it is possible to visualize the entire sample while maintaining high magnification view



2 Particles Detection

Raman mapping of the entire filter can be very time-consuming.
To make the analysis faster and more efficient, only the particles should be analyzed.

Particles are detected in the image due to their contrast with the filter surface. In this example, particles are deposited on a silicon filter and illuminated with a dark field objective, resulting in high contrast with the filter surface. This high contrast facilitates their automatic localization. The green mask indicates the particle surfaces, while the red spots mark the locations where the laser beam will be directed for Raman analysis.



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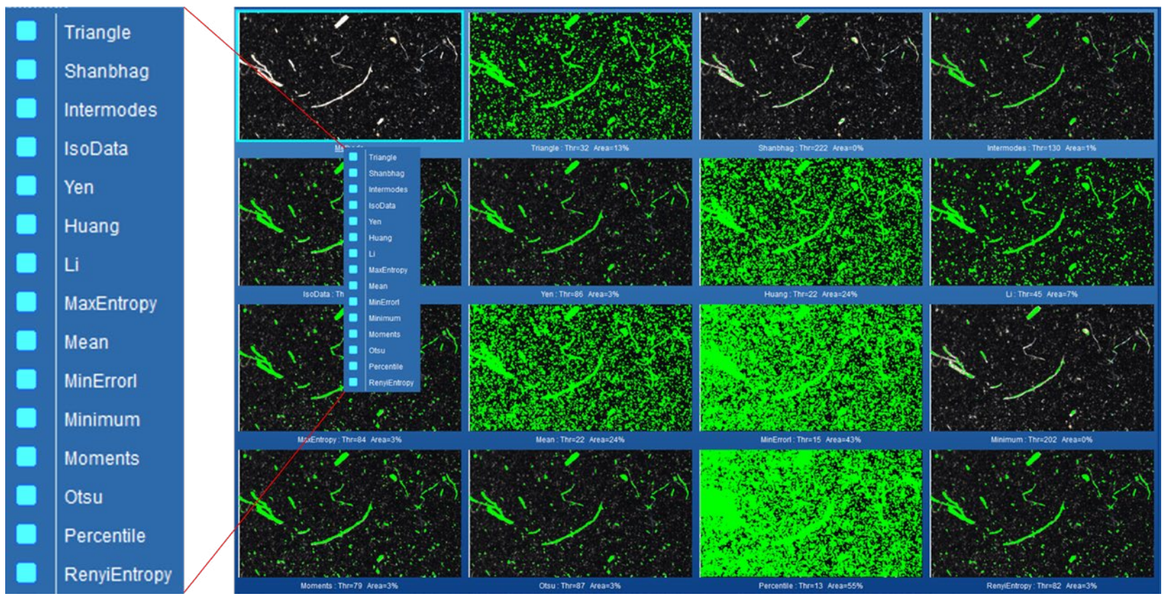
HORIBA Solution

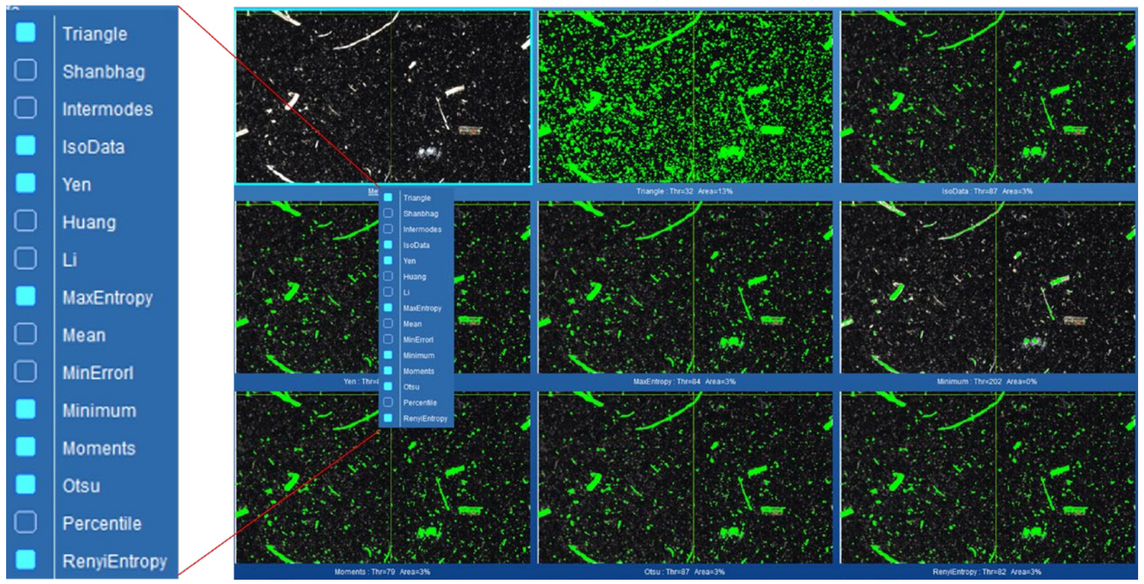
The Auto-thresholding provides unbeatable flexibility; indeed, it allows to select between several algorithms

➠ Up to sixteen are available.

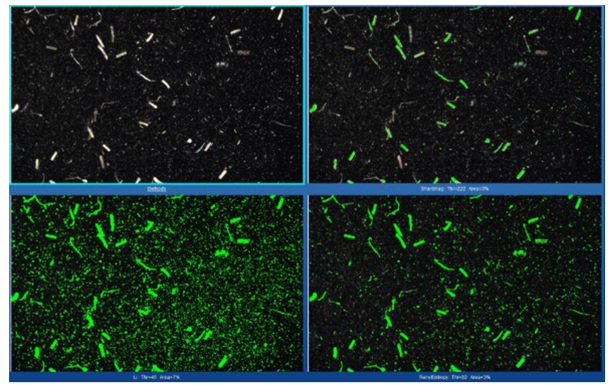
Each of these algorithms will automatically set the range of the grey scale intensity distribution on the histogram based on the video image and the algorithm sensitivity.

The choice of the best algorithm does not require any expertise since a visual comparison allows to easily identify the one most suitable for the selection of the particles and/or the fibers of interest.





The number of algorithms to be visually compared is not limited: all 16 or 8 or only 3 can be displayed.



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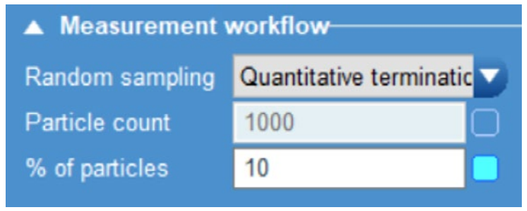
HORIBA Solution

HORIBA Solution
Filtration appartus
Raman microscope

MVAPlus, ViewSharp, NavSharp, VRM
ParticleFInder
Reference standard sample

To further reduce analysis time, a statistical approach can be applied, analyzing a certain percentage of particles in "random" mode.



Raman Analysis
Spectrum of each selected particle is recorded
3

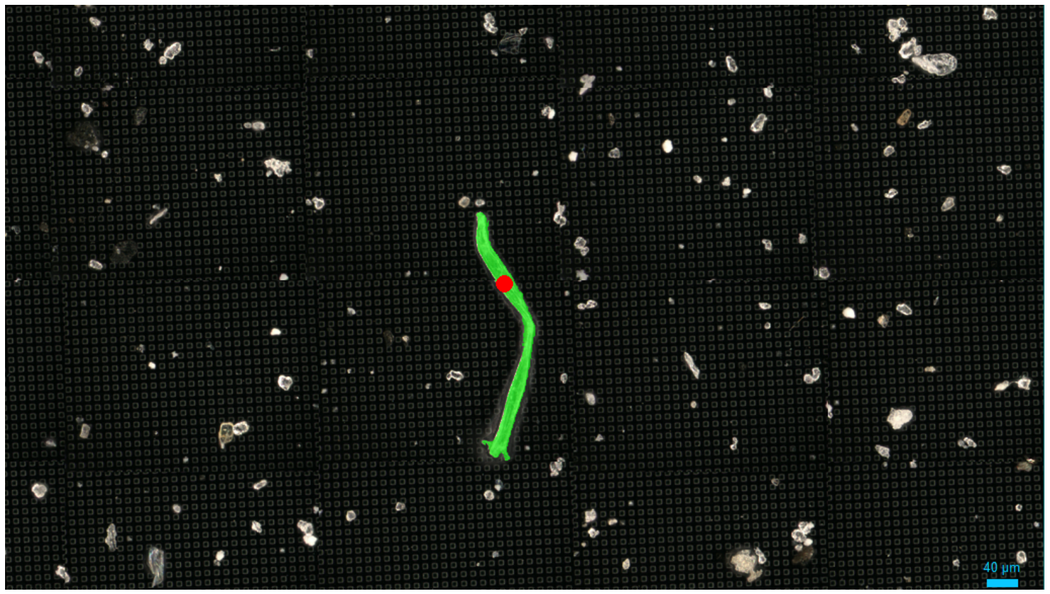
Steps 1, 2, and 3 can be performed in "Static" or "Dynamic" mode.

In Static mode, the full image of the filter is acquired, particle detection parameters are adjusted by the operator, and the Raman analysis starts. **This mode is ideal for fast analysis.**

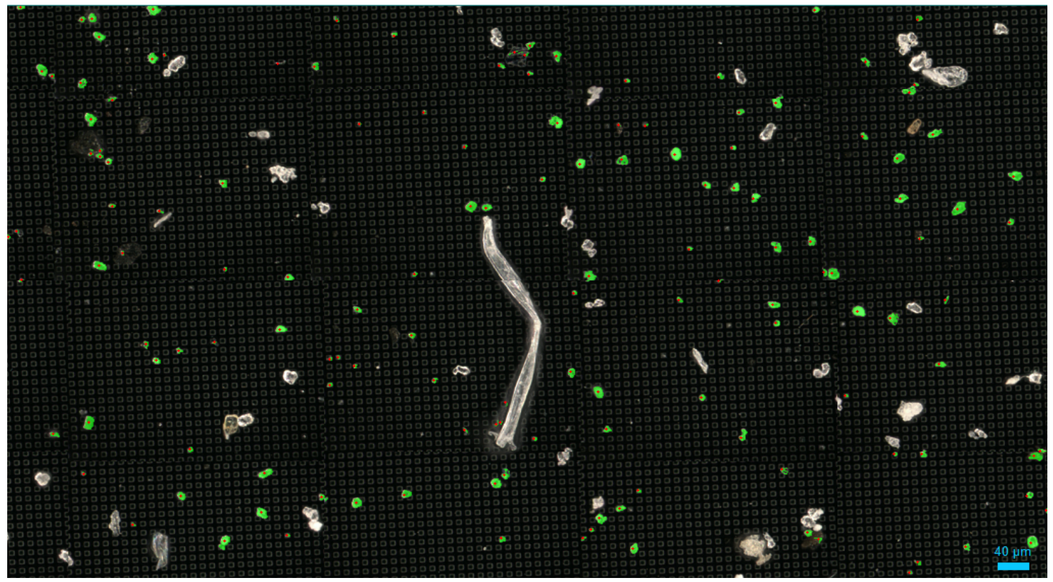
In Dynamic mode, image and spectra acquisition are performed sequentially in small zones of the filter. This approach prevents the risk of particle displacement due to external factors and ensures high precision in positioning even the smallest particles, down to 1 micron. **This mode is recommended for highly precise analysis of the smallest particles.**

Particles can be selected for Raman analysis based on their size and/or shape.

Each particle can be identified by collecting a single spectrum, or several spectra, and averaging them.



For example, only fibers are selected for the analysis using morphological filters



Here, particles with a Feret diameter between 5 and 20 microns are selected for Raman analysis

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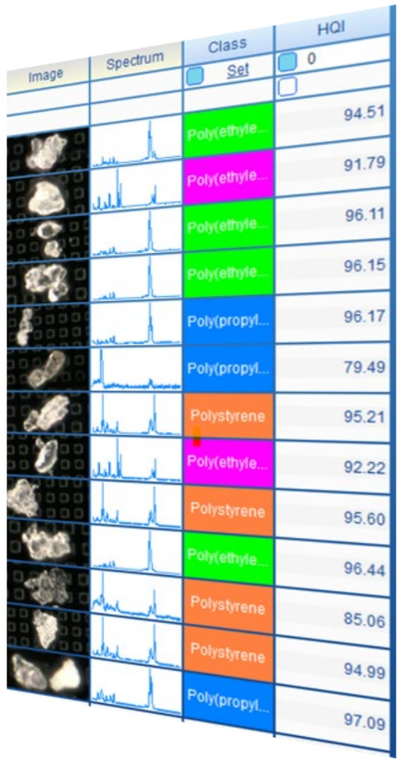
4 Spectra Identification

Each spectrum is identified by comparing it to the spectral library

IDFinder

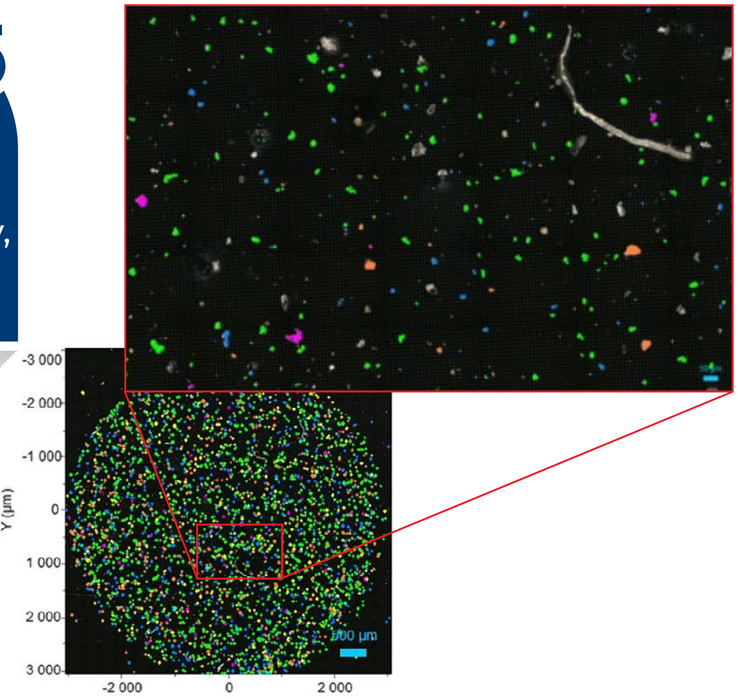
IDFinder™ is a tool for managing spectral libraries and automatically identifying thousands of spectra. Each spectrum is compared with a dedicated spectral library using the Hit Quality Index (HQI). The component with the highest HQI is recognized as the chemical identity of the particle.

It is important to customize the library for routine analysis by including typical spectra, not only of microplastics but also of other substances commonly present in samples due to incomplete digestion of organic and/or inorganic matter (e.g., minerals, cellulosic fibers, proteins, etc).

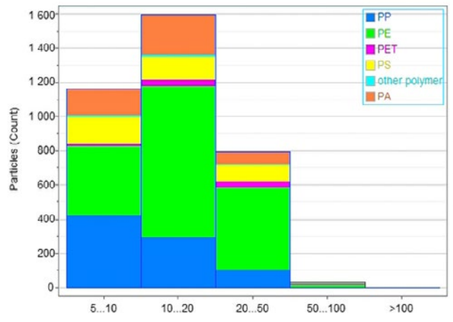


5 Reporting

Particles are counted and classified by their chemical identity, size, shape, and color



Results summary can be presented in the form of histogram or table



ID	Ferret diameter					
	5 – 10 µm	10 – 20 µm	20 – 50 µm	50 – 100 µm	>100 µm	Sum
PP	417	291	101	0	0	809
PE	405	887	482	14	1	1789
PET	15	34	36	5	0	90
PS	156	136	98	7	1	398
PA	161	236	78	4	0	479
Other polymers	10	16	4	1	0	31
Other compounds	334	211	166	20	3	734

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Reference standard sample

In order to validate the entire analytical workflow, a set of referenc materials should be used. Standards, in an easy-to-use tablet forma containing a defined number of polymer particles of specified sizes are included in the HORIBA package*. The irregular shapes of th particles mimic microplastics found in nature. The low concentratio of particles in the tablets is specifically designed for the validation c spectroscopy-based particle-counting methods. These standard have been successfully tested in interlaboratory studies.

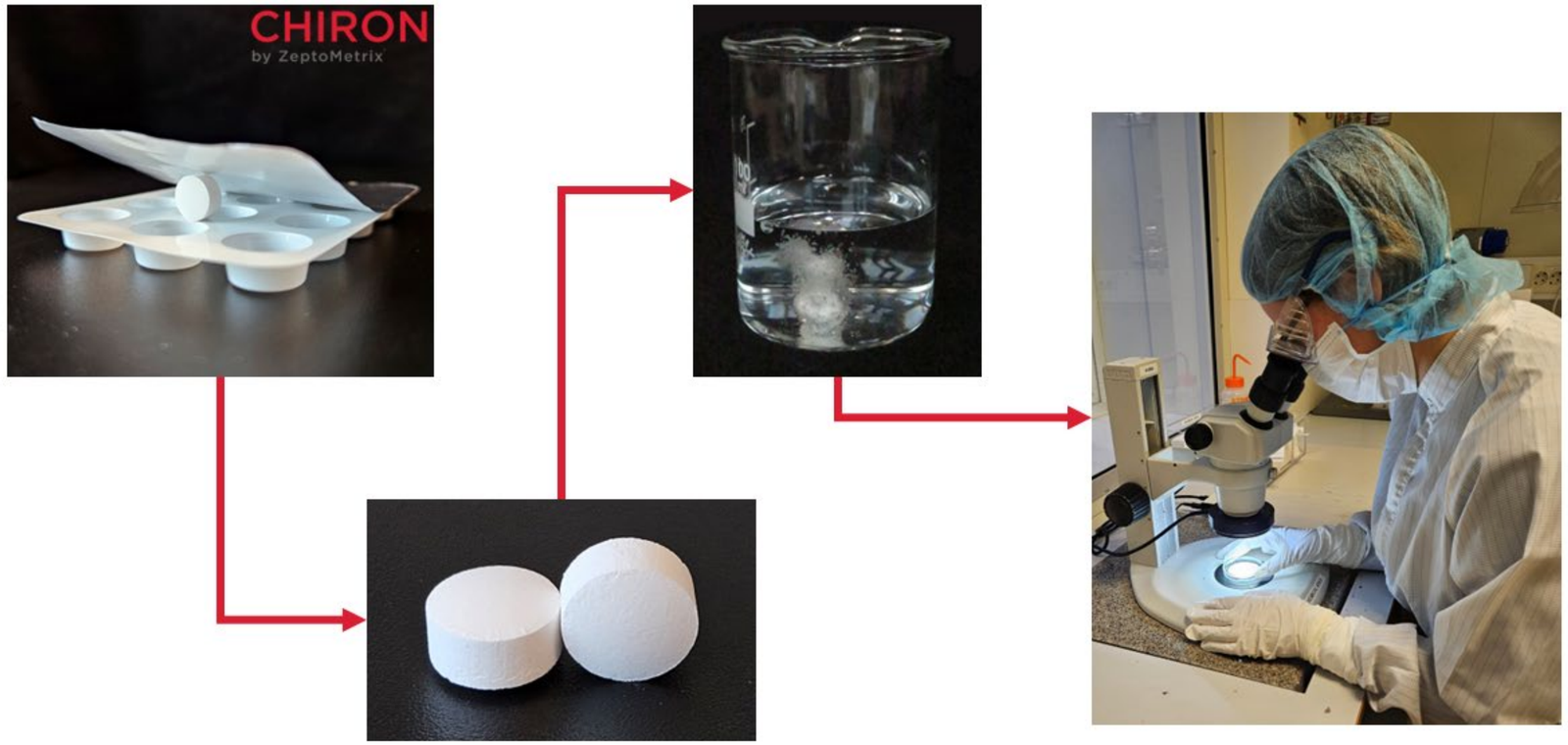

Fig. 9. Reference standard sample preparation

DR

*Martínez-Francés, E., van Bavel, B., Hurley, R. et al. Innovativ reference materials for method validation in microplastic analysi including interlaboratory comparison exercises. Anal Bioanal Cher 415, 2907–2919 (2023). https://doi.org/10.1007/s00216-023 04636-4

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Assessing human exposure through Raman Micro-Spectroscopy

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Every month, HORIBA Raman XPerience newsletter reviews scientific articles on various applications of Raman spectroscopy. The September 2024 issue focused on microplastics analysis and the assessment of human exposure. This edition features examples of studies measuring microplastics concentrations in air, drinking water, and food. Each article highlights the importance of Raman spectroscopy in identifying and quantifying microplastics, showcasing its precision and relevance in environmental monitoring and public health research.

Every Breath You Take: High Concentration of Breathable Microplastics in Indoor Environments

L. Maurizi, L. Simon-Sanchez, A. Vianello, A.H. Nielsen, J. Vollertsen

Inhalation is the most evident source of exposure to microplastics. The smallest, low-weight particles that tend to float in the air have a higher probability of being inhaled. In this very recent publication (June 2024), Raman micro-spectroscopy was employed to assess the concentration of indoor airborne microplastics >1 µm in indoor environments under different levels of human activity.

Sampling was conducted by actively pumping air through a Si membrane, which was then analyzed using Raman spectroscopy. **A XploRA Nano confocal Raman microscope and ParticleFinder™ software** were used for automated particle analysis. This publication provides detailed infomation about the instrumental settings and explains the functionality of ParticleFinder™, which allowed for the **automatic characterization of thousands of particles** on the filter.

The results revealed a concentration of microplastics between 58 and 684 MPs per cubic meter, depending not only on the type and level of human activity but also on the surface area and air circulation of the investigated locations. The authors estimated a human microplastics (MPs) intake from indoor air of 3,415 ± 2,881 MPs per day. A total of 15 polymers were identified, with polyamide (PA) clearly dominating the polymer composition.

It should be noted that **this work presents a good example of quality control that must be applied while performing microplastics analysis**. The authors presented the limit of quantification (LOQ) for each type of polymer based on multiple procedural blanks analyses. This approach is crucial to distinguish between microplastics contamination due to sample preparation (consumables, clothing, etc.) and microplastics collected from the sample itself.

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Assessing human exposure through Raman Micro-Spectroscopy

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The Majority of Potable Water Microplastics Are Smaller Than the 20 µm EU Methodology Limit for Consumable Water Quality

O. Hagelskjær, F. Hagelskjær, H. Margenat, N. Yakovenko, J.E. Sonke, G. Le Roux

In this publication, the authors studied the presence of microplastic particles down to 1 micron in bottled and tap water. First, 4.5 L of the tested sample was subjected to chemical digestion with H_2O_2 and HCl to reduce the quantity of organic matter and mineral deposits and then filtered on a filter suitable for Raman analysis.

LabRAM Soleil™ Raman microscope and **ParticleFinder™** software were used for **automatic particle detection and analysis**. The **ViewSharp™ option**, which permits precise optical focus for each particle, was applied, guaranteeing high precision in Raman analysis.

Even though the authors mention some perspectives for improving sample preparation and quality control procedures, they demonstrated that Raman microspectroscopy is a reliable tool for detecting the smallest particles.

According to the findings, the majority of particles identified as microplastics were in the range of 1-20 µm. Thus, **authors suggest an amendment to the guidance published in the EU directive 2020/2184 regarding water intended for human consumption. More precisely, the authors recommend including the fraction of particles below 20 microns in systematic monitoring and explain that the minimum sampling volume should be reduced from 1 m³ to several liters**. This suggestion is in accordance with both realistic human daily/weekly consumption and the sensitivity of the methodology for particle detection and analysis.

Detection and Characterization of Small-Sized Microplastics (≥ 5 µm) in Milk Products

P. A. Da Costa Filho, D. Andrey, B. Eriksen, R. P. Peixoto, B. M. Carreres, M. E. Ambühl, J. B. Descarrega, S. Dubascoux, P. Zbinden, A.Panchaud, E. Poitevin

To understand potential exposure to microplastic pollution via ingestion, monitoring drinking water alone is not enough. Measuring the concentration of microplastics in different food products, especially those subjected to industrial production, is essential.

In this article published in 2021, the authors used Raman microscopy to study the presence of microplastic particles in milk-based products. Enzymatic and chemical digestion were used to dissolve the organic matrix and have been validated for 5 types of polymers. The liquid phase was then filtered through a Si filter and analyzed with a confocal **micro-Raman LabRAM HR Evolution**. Instead of a particle-by-particle approach, **Raman imaging** was used to scan the surface of the filter and identify polymeric particles. This approach overcomes the difficulties related to the agglomeration of particles of different natures and thus offers high precision in terms of particle counting in complex matrices.

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Microplastics analysis in food: assessing human exposure through Raman micro-spectroscopy

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Abstract

Sugar, a widely consumed dietary ingredient, is present in numerous food products, including desserts and beverages. Its production involves several stages—extraction, purification, crystallization, and drying—each presenting potential contamination risks from plastic-based equipment and packaging. In this work, we demonstrate the typical workflow for microplastics analysis using Raman microspectroscopy.

Introduction

In 2022 the World Health Organization (WHO) published a report highlighting growing concerns about human exposure to microplastics through dietary intake and inhalation. While current data on exposure and potential health effects remain limited, WHO emphasized the urgent need for continued research, particularly focused on the smallest microplastic particles (<10 µm), and called for the development of standardized methods to generate reliable data on human exposure.

Among the available analytical techniques, Raman micro-spectroscopy stands out as the only reference method capable of providing comprehensive information on microplastic particles in this critical size range [1]. Raman analysis enables determination of particle count, chemical composition, and size distribution, making it an indispensable tool for advancing microplastics research and risk assessment.

In this application note, we demonstrate how Raman spectroscopy can be effectively used to detect and characterize microplastics in food matrices. Using sugar as a model sample, we present a step-by-step workflow for sample preparation, Raman analysis, and data interpretation to highlight the potential of this technique for routine monitoring of microplastics in food products.

In this application, you will learn:

- How to prepare samples for microplastics analysis using HORIBA's filtration kit, optimized for maximizing recovery of small particles.
- How to analyze thousands of particles in a fully automated workflow using the latest generation Raman microscope, LabRAM Soleil™, combined with the powerful ParticleFinder™ software for particle detection and spectral acquisition.
- How to rapidly and reliably identify thousands of Raman spectra using the integrated IDFinder™ module, part of the LabSpec 6 software suite, which streamlines the particles classification based on dedicated spectral database.
- How to compile, interpret, and report results with confidence — quantifying the presence of microplastics in a sample and distinguishing between microplastics originating from the sample and those introduced during the sample preparation process.

This comprehensive workflow provides a robust approach to detect and characterize microplastics in food matrices, supporting the development of harmonized protocols for future routine monitoring and regulatory studies.

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Instrument and methods

Samples preparation

For this study, 5 grams of white sugar obtained from a local supermarket were dissolved in 500 mL of hot Milli-Q water to ensure complete dissolution of the sugar crystals. The resulting solution was then filtered through a silicon (Si) filter with a pore size of 5 µm using the HORIBA filtration kit (Figure 1). This filtration step concentrated any non-soluble particles present in the sugar sample onto the filter surface, facilitating subsequent Raman analysis.

Figure 1. Filtration kit: glass funnel, glass support base, silicone stopper, glass flask, vacuum pump and Si filters (5 µm porosity).

To ensure data accuracy and account for potential contamination during sample preparation, analytical blanks were prepared by filtering 500 mL of hot Milli-Q water through the same type of 5 µm Si filters. These blanks served as controls to detect any environmental contamination introduced during the sample preparation process.

The choice of Si filters is a key element of this analytical workflow. Their flat reflective surface facilitates automated particle detection and imaging during Raman analysis (see section Raman Analysis). In addition, silicon generates minimal and well-defined Raman signals, reducing background interference and avoiding overlap with the characteristic Raman fingerprints of polymer particles. This property significantly enhances the accuracy of microplastic identification, even in complex food matrices.

Raman analysis

The Raman analysis was performed using the LabRAM Soleil™ Raman microscope (Figure 2). For particle analysis, including automated Raman spectra acquisition, particle counting, and size characterization, ParticleFinder™ software, a module in LabSpec 6, was used.

Dark-field illumination was employed to enhance the optical contrast of the particles on the Si filter, facilitating their automatic localization with ParticleFinder. Once the particles were located in the image, the Raman spectrum of each particle was recorded using a 532 nm laser.

The filter was analyzed in a so-called "Dynamic mode," meaning that the following analytical sequence was automatically repeated for small zones of the filter, including several fields of view of the optical objective:

1. Image acquisition
2. Automatic particle localization
3. Raman spectra recording

This approach is well-suited for large filters, minimizing the risk of particle displacement during the analysis due to external factors, and ensuring high precision in localization and particle size characterization.

Figure 2. LabRAM Soleil Raman microscope

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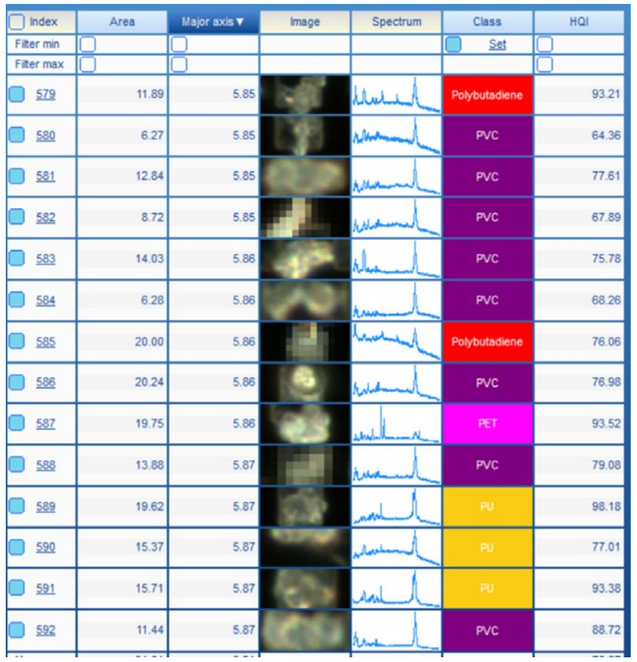
Data treatment

Apart from baseline correction, no additional processing was applied to the spectra. The spectra identification was performed using IDFinder™ software (part of the LabSpec 6 suite). The spectra were compared with a dedicated spectral library, and a matching score called the Hit Quality Index (HQI) was assigned to each spectrum. Pearson's correlation was used to calculate the HQI (Figure 3). The compound with the highest HQI was considered the chemical identity of the particle. The minimum acceptable HQI score for automatic particle identification was set at 60%.



Index	Area	Major axis ▼	Image	Spectrum	Class	HQI
Filter min					Set	
Filter max						
579	11.89	5.85			Polybutadiene	93.21
580	6.27	5.85			PVC	64.36
581	12.84	5.85			PVC	77.61
582	8.72	5.85			PVC	67.89
583	14.03	5.86			PVC	75.78
584	6.28	5.86			PVC	68.26
585	20.00	5.86			Polybutadiene	76.06
586	20.24	5.86			PVC	76.98
587	19.75	5.86			PET	93.52
588	13.88	5.87			PVC	79.08
589	19.62	5.87			PU	98.18
590	15.37	5.87			PU	77.01
591	15.71	5.87			PU	93.38
592	11.44	5.87			PVC	88.72

Figure 3. Extract of the results table: each spectrum is compared with dedicated spectral library, the component with the highest matching score (Hit Quality Index, HQI) is mentioned in a column "Class".

Table 1. List of most abundant polymers included in basic spectral library for microplastics analysis.

Polymer	Abbreviation
Polyethylene	PE
Polypropylene	PP
Polyethylene terephthalate	PET
Polycarbonate	PC
Polystyrene	PS
Polytetrafluoroethylene	PTFE
Polyvinyl chloride	PVC
Polyamide	PA
Polymethyl methacrylate	PMMA
Polyurethane	PU

The spectral library was customized to include spectra of the 10 most abundant polymers (listed in Table 1) and common non-plastic materials (such as Si, amorphous carbon, cellulose, proteins, $CaCO_3$, TiO_2, etc.). It should be noted that the above-mentioned list forms the basic content of the spectral library adapted for microplastics analysis, which can be supplemented with other organic and inorganic compounds expected to be present in the samples.

Results and Discussion

Filters for Raman analysis

Figure 4 presents optical images of the filters corresponding to the sugar sample and the analytical blanks. A clear visual difference can be observed: significantly more particles were detected in the sugar sample compared to the blank, indicating the presence of insoluble residues not attributable to environmental contamination during sample preparation. It should be noted that although sugar is soluble in water, matrix residues can still remain on the filter. Therefore, for successful analysis of individual particles using Raman spectroscopy, the sampling mass should be adjusted to avoid particle overlap while maintaining a representative quantity of the sample for analysis. For more complex food matrices that are not water-soluble, additional sample preparation steps—such as chemical digestion and/or density separation—may be required prior to filtration on a silicon (Si) filter (see examples of sample preparation here [2] and in HORIBA microplastics e-book).

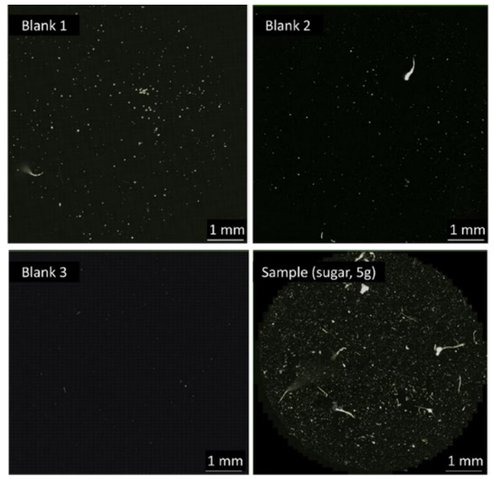


Figure 4. Optical images of analyzed filters.

Particles identification

The results of particle identification and counting are summarized in Figure 5 (a). For clarity, particles identified as cellulose, minerals, proteins, fatty acids, or amorphous carbon were grouped into a single category labeled "non-plastics." Most of the outlier spectra—defined as those with a hit quality index (HQI) below 60%—either lacked a clear Raman signal due to fluorescence interference or displayed only the characteristic Raman peak of the silicon (Si) filter.

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Figure 5 (b) shows the size distribution of the detected microplastic particles. The data reveal that the majority of microplastics detected in the sugar sample are smaller than 20 µm, highlighting the importance of using Raman spectroscopy for the detection of small-sized particles that are typically below the detection limit of other techniques.

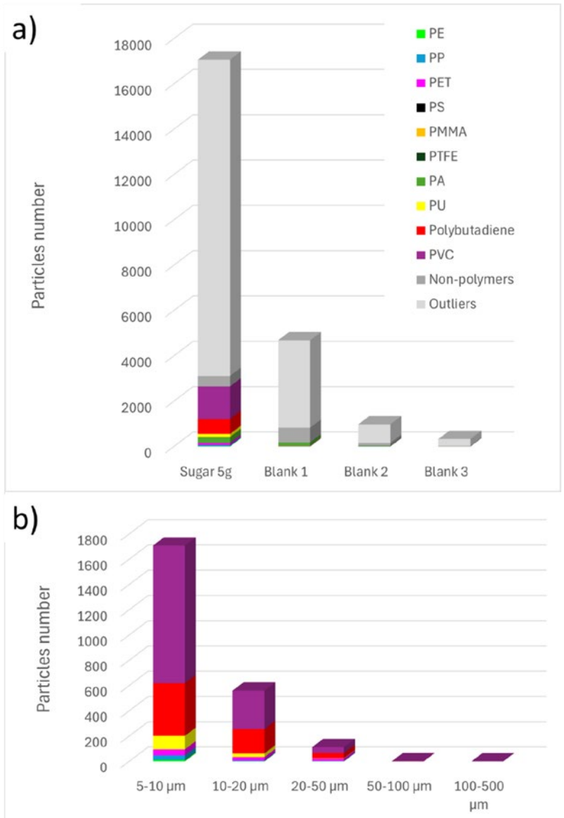

Figure 5. Results of Raman analysis for the particles with Feret diameter > 5 µm: a) Particles number in sugar sample and in the blanks; b) Microplastics size distribution in Sugar sample. Values > LOD are considered in this figure.

Besides the standard spectra included in the microplastics analysis library, an additional spectrum was detected in the sugar sample. This spectrum was identified as a mixture of acrylonitrile-butadiene copolymer and titanium dioxide (TiO_2) using the ST Japan and HORIBA spectral libraries (Figure 6). To facilitate automated identification and counting of similar particles, this spectrum was added to the reference library and labeled as “Polybutadiene” for simplicity in further analysis.

Table 2. Limit of detection (LOD) of microplastics particles and results of sugar sample. LOD was calculated per type of polymer using formula LOD = Average of blanks + 3 St Dev of blanks. Values in red are above the limit of detection.

Polymer	LOD Particles number	Sugar Particles number
Polyethylene	2	**14**
Polypropylene	14	**45**
Polyethylene terephthalate	20	**93**
Polystyrene	4	2
Polyamide	289	**262**
Polyvinyl chloride	6	**1442**
Polyurethane	2	**139**
Polytetrafluoroethylene	1	1
Polymethyl methacrylate	1	0
Polycarbonate	1	0
Polybutadiene	2	**651**

Results reporting and blanks management

To evaluate the significance of the detected microplastics, a limit of detection (LOD) was calculated for each polymer using the following formula:

$$\text{LOD (polymer)} = \text{Average (blanks)} + 3 \cdot \text{Standard Deviation (blanks)}$$

Where Average (blanks) is the mean number of microparticles of a specific polymer detected in the blank samples, and Standard Deviation (blanks) is the variability for that polymer across blank measurements.

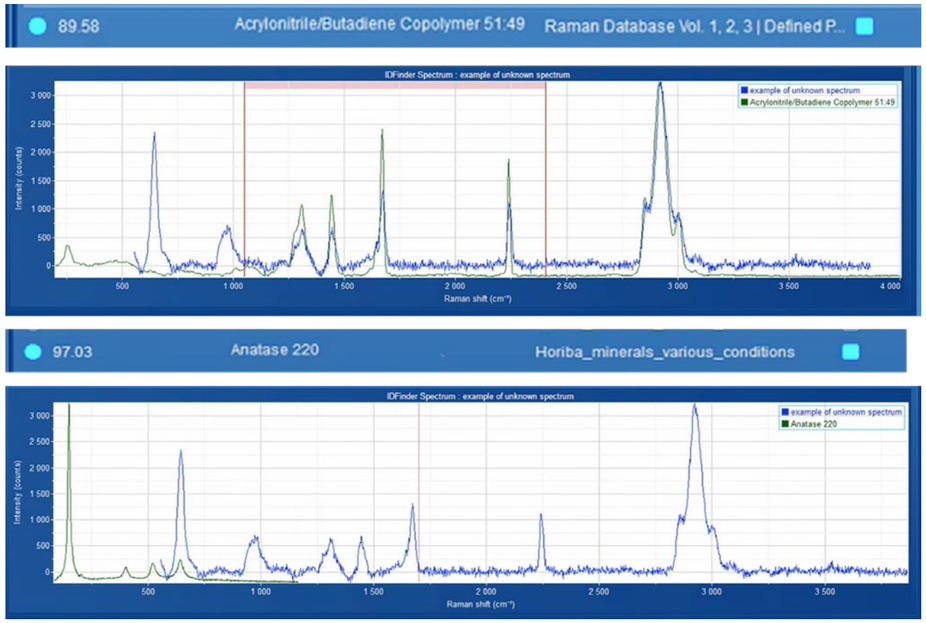

Figure 6. Identification of an unknown spectrum using the ID Finder™ software. The recorded unknown spectrum (blue) was compared against entries from the ST Japan and HORIBA spectral libraries (green). The best match was identified as a combination of acrylonitrile-butadiene copolymer and titanium dioxide (anatase).

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To confirm the presence of a given polymer in the sugar sample, the number of detected particles was compared to the LOD for that polymer. If the number of particles exceeded the LOD, the polymer was considered present in the sample at a statistically significant level, above background contamination. Table 2 presents the calculated LOD values for each polymer and the corresponding particle counts in the sugar sample. Based on this comparison, the presence of PE (polyethylene), PP (polypropylene), PET (polyethylene terephthalate), PVC (polyvinyl chloride), PU (polyurethane), and Polybutadiene is confirmed. Among these, PVC, PU, and Polybutadiene were the most abundant. The presence of PA (polyamide) remains uncertain due to similar levels being observed in the blank, suggesting possible contamination during handling or filtration.

Conclusions

This application note illustrates the capabilities of Raman micro-spectroscopy for the detection, identification, and quantification of microplastics in food products, using white sugar as a test case. The applied workflow—combining HORIBA's filtration kit, LabRAM Soleil™ Raman microscope, and automated analysis with ParticleFinder™ and IDFinder™—proved effective for characterizing microplastics down to the <20 µm range.

Several polymer types were identified in the sugar sample, including PVC, PU, and Polybutadiene, with the majority of particles measuring below 20 µm. The consistent presence of these polymers raises important questions regarding their origin. Potential sources may include food processing equipment (e.g., conveyor belts, seals, or packaging), environmental contamination during production or transport, or additives and processing aids used in sugar refining.

These findings underscore the need for continued research into the pathways through which microplastics enter the food chain. In particular, the identification of sub-20 µm particles, which are of growing concern due to potential human health impacts, aligns with WHO's 2022 recommendations to improve data on human exposure and to develop standardized analytical methods.

We encourage researchers to expand studies on various food matrices and explore the toxicological relevance of different polymer types and sizes. At the same time, food manufacturers and suppliers are urged to begin routine monitoring of their raw materials and production environments to ensure the highest quality standards and anticipate future regulatory requirements.

The methodology presented here offers a robust and scalable solution to support these efforts and contribute to a better understanding of microplastics exposure through diet.

References

1. N. P. Ivleva, "Chemical Analysis of Microplastics and Nanoplastics: Challenges, Advanced Methods, and Perspectives," Chemical Reviews, vol. 121, no. 19, pp. 11886-11936, 2021.
2. P. A. Da Costa Filho, D. Andrey, B. Eriksen, R. Peixoto, B. Carreres, M. E. Ambühl, J. B. Descarrega, S. Dubascoux, P. Zbinden, A. Panchaud and E. Poitevin, "Detection and characterization of small-sized microplastics (≥ 5 µm) in milk products," Sci Rep., vol. 11, no. 1, p. 24046, 2021.
3. World Health Organization, "Dietary and inhalation exposure to nano- and microplastic particles and potential implications for human health," Geneva, 2022.

Analysis of microplastics in hand sanitizers using ParticleFinder™

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Abstract

Since October 2023, the European Union has enforced restrictions on the intentional use of microplastics in products such as cosmetics and self-care items. Using the example of hand sanitizer, this application note demonstrates how to evaluate whether the product contains synthetic polymer microparticles covered by this restriction. Three different hand sanitizers from various countries were analyzed, demonstrating the presence of microplastics measuring from 20 to 100 microns in one of them.

HORIBA provides a full solution for microplastics analysis, including high-performance Raman microscopes, a filtration kit, ParticleFinder™ software for automatic particle analysis, and IDFinder™ software for the automatic identification of multiple spectra.

Introduction

Since October 2023, the European Union has enforced restrictions on the intentional use of microplastics in certain products, including cosmetics and self-care items [1]. According to these restrictions, particles considered as microplastics must be excluded from products if they meet the following criteria:

Consist of synthetic polymers
Solid
Insoluble in water
Not biodegradable
Measure less than 5 mm

In the case of microplastic beads used as exfoliating components, the regulation is straightforward and strict: they should no longer be included in formulations. However, the situation is less clear for other ingredients consisting of synthetic polymers. While they may be classified as microplastics in their pristine form, significant changes can occur once they are added to the hydroalcoholic solvents of liquid formulations. Therefore, it is essential to evaluate their fate after the final usage of the finished product [2].

In this application note, we demonstrate the classical approach for microplastics analysis applied to hand sanitizers, which have become part of our daily routine since the COVID-19 crisis. The objective is to simulate the use of hand sanitizers and evaluate whether particles considered as microplastics remain on our hands after its using. Raman microspectroscopy is recognized as one of the reference methods for microplastics analysis, providing detailed information on the chemical identity, size, morphology, and number of particles in a sample [3]. In this application note, we demonstrate the typical workflow for microplastics analysis, from sample preparation to data treatment, using the full solution provided by HORIBA.

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The workflow involves several key components:

- Sample Preparation: Utilizing HORIBA's filtration kit, samples are prepared for analysis by filtering and isolating microplastic particles.
- Raman Microscopy: The latest generation of Raman microscopes, LabRAM Soleil™, is used for high-resolution imaging and spectral analysis.
- Automated Particle Analysis: Dedicated software, ParticleFinder™ and IDFinder™, facilitates fully automated analysis of particles, including their identification and characterization.

This integrated approach ensures precise and efficient analysis of microplastics, making it an invaluable tool for researchers and industry professionals concerned with the presence of microplastics in various products.
Considering this, it is crucial to focus on the analytical techniques which allow the identification and characterization of the smallest particles, such as Raman Microscopy.

Raman microscopy is a non-destructive, non-contact technique that provides:

- Full morphological information for each particle through the analysis of the optical image (diameter, ellipse ratio, area…);
- Quantitative analysis (number of particles);
- Chemical identification of each particle (by exploiting a dedicated microplastic database library).

Hand sanitizers are usually presented as a gel. Therefore, a sample filtration is required. For this reason, HORIBA developed an easy-to-use filtration kit specifically for this application. Allying this with the powerful particle analysis tool ParticleFinder™, and with the comprehensive spectral identification library KnowItAll®, we get a complete and simple procedure to study and differentiate the components of hand sanitizers.

Instrument and methods

Samples preparation

Three hand sanitizers, referred to as Sample 1, Sample 2, and Sample 3, originating from different countries, were selected for microplastic analysis. A volume ranging from 25 to 50 ml of each sample was diluted in ethanol and filtered through Silicon (Si) filters (SMART MEMBRANES, www.smartmembranes.com) with varying porosities using HORIBA's filtration kit. The exact sampling volume, ethanol quantity, and filter porosity are detailed in Table 1.

For the blank filtration, the same filtration conditions were replicated; however, instead of adding a sample, 50 ml of distilled water was used.

Table 1: Filtration conditions for each sample and blank.

Sample	Blank	1	2	3
Volume of sample (ml)	50	50	25	50
Volume of Ethanol (ml)	50	50	75	50
Filter pore diameter (µm)	5	5	10	10

Figure 1: Filtration apparatus: glass funnel, glass support base, silicone stopper, glass flask, and vacuum pump.

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Raman analysis

The Raman analysis was performed using the LabRAM Soleil™ Raman microscope. For particle analysis, including automated Raman spectra acquisition, particle counting, and size characterization, ParticleFinder™ software was utilized.

Dark-field illumination was employed to enhance the optical contrast of the particles on the Si filter, facilitating their automatic localization with ParticleFinder™. Once the particles were located in the image, the Raman spectrum of each particle was recorded using a 785 nm laser. Only particles with a circle equivalent diameter between 20 and 100 µm were considered for Raman analysis to enable fast scanning.

Figure 2: LabRAM Soleil Raman microscope

The filter was analyzed in a so-called “Dynamic mode,” meaning that the following analytical sequence was automatically repeated for each field of view of the optical objective:

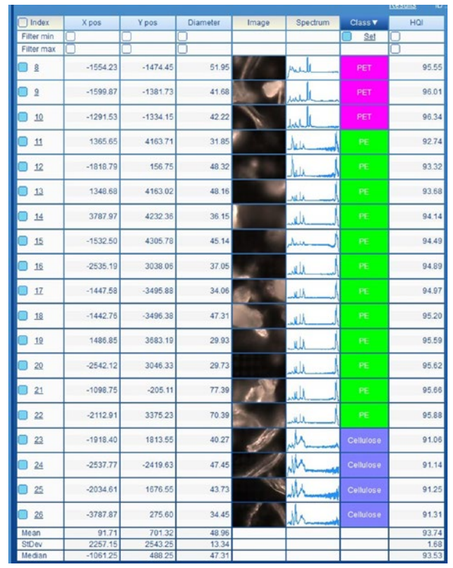
- Image acquisition
- Automatic particle localization
- Raman spectra recording

This approach is well-suited for large filters, minimizing the risk of particle displacement due to external factors and ensuring high precision in localization and particles size characterization.

Data treatment

Apart from baseline correction, no additional processing was applied to the spectra. The spectra identification was performed using IDFinder™ software. The spectra were compared with a dedicated spectral library, and a matching score called the Hit Quality Index (HQI) was assigned to each spectrum. Pearson’s correlation was used to calculate the HQI (Figure 3). The compound with the highest HQI was considered the chemical identity of the particle. The minimum acceptable score for automatic particle identification was set at 65%.

The spectral library was customized to include spectra of the 10 most abundant polymers (listed in Table 2) and common non-plastic materials (such as Si, amorphous carbon, cellulose, proteins, $CaCO_3$, and TiO_2, etc.). It should be noted that the above-mentioned list forms the basic content of the spectral library adapted for microplastics analysis, which can be supplemented with other organic and inorganic compounds expected to be present in the samples.



Index	X pos	Y pos	Diameter	Image	Spectrum	Class	HQI
Filter min						Sel	
Filter max							
8	-1554.23	-1474.45	51.95			PET	95.55
9	-1599.87	-1381.73	41.68			PET	96.01
10	-1291.53	-1334.15	42.22			PET	96.34
11	1365.65	4163.71	31.85			PE	92.74
12	-1818.79	156.75	48.32			PE	93.32
13	1348.68	4163.02	48.16			PE	93.68
14	3787.97	4232.36	36.15			PE	94.14
15	-1532.50	4305.78	45.14			PE	94.49
16	-2535.19	3038.06	37.05			PE	94.69
17	-1447.58	-3495.88	34.06			PE	94.97
18	-1442.76	-3496.38	47.31			PE	95.20
19	1486.85	3683.19	29.93			PE	95.59
20	-2542.12	3046.33	29.73			PE	95.62
21	-1098.75	-205.11	77.39			PE	95.66
22	-2112.91	3375.23	70.39			PE	95.88
23	-1918.40	1813.55	40.27			Cellulose	91.06
24	-2537.77	-2419.63	47.45			Cellulose	91.14
25	-2034.61	1676.55	43.73			Cellulose	91.25
26	-3787.87	275.60	34.45			Cellulose	91.31
Mean	91.71	701.32	48.96				93.74
StDev	2257.15	2543.25	13.34				1.65
Median	-1061.25	488.25	47.31				93.53

Figure 3: Information about each particle is resumed in a table. Each spectrum is compared with dedicated spectral library, the component with the highest matching score (Hit Quality Index, HQI) is mentioned in a column “Class”.

Table 2: List of most abundant polymers included in basic spectral library for microplastics analysis.

Polymer	Abbreviation
Polyethylene	PE
Polypropylene	PP
Polyethylene terephthalate	PET
Polycarbonate	PC
Polystyrene	PS
Polytetrafluoroethylene	PTFE
Polyvinyl chloride	PVC
Polyamide	PA
Polymethyl methacrylate	PMMA
Polyurethane	PU

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Results

Figure 4 presents optical images of three filters corresponding to the analysed samples and a blank filtration. Compared to the blank sample, significantly more particles were detected in the analyzed samples, with fibers visible in Samples 2 and 3.

For Raman analysis, only particles with a circle equivalent diameter between 20 and 100 microns were selected. The results of particle identification and counting are presented in Figure 5. Compared to the blank filtration, Samples 2 and 3 exhibit a similar order of magnitude in the number of plastic particles. Tens of particles of Polyethylene (PE), Polypropylene (PP), and a few particles of Polyethylene Terephthalate (PET), Polystyrene (PS), and Polytetrafluoroethylene (PTFE) were detected in both the blank and these two samples. This may be explained by slight microplastic contamination during sample preparation. For example, PE and PP were the major materials of an ethanol wash bottle, so the presence of tens of particles of these polymers was expected.

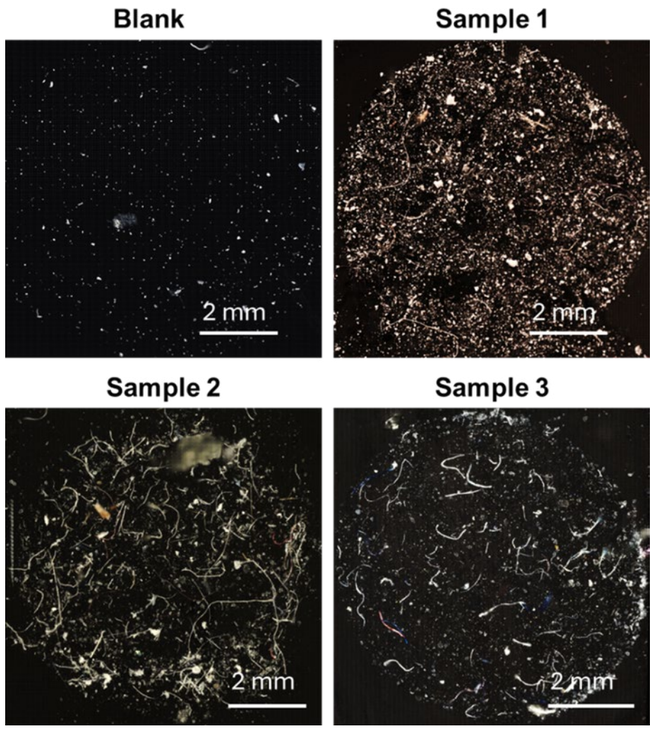

Figure 4: Optical images of analysed filters.

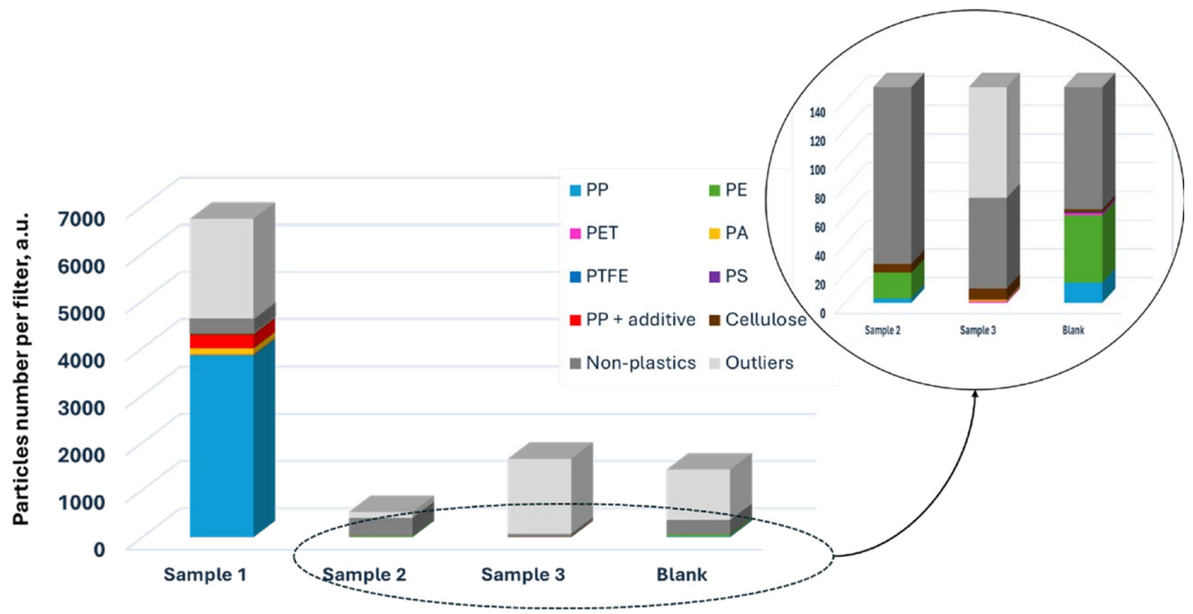

Figure 5: Results of Raman analysis for the particles measuring 20 – 100 µm.

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However, the presence of thousands of PP particles in Sample 1 cannot be explained by external contamination; it is attributed to the presence of these particles in the hand sanitizer itself. Most spectra identified as PP had a high matching score with Polypropylene reference spectra. However, they also showed a high score with PP-acrylic acid polymer or another polymer with a similar skeletal structure to PP, as shown in Figure 6. For simplicity, these particles were labeled as PP. Several hundred particles had spectra presenting signatures of both PP and some fatty alcohol molecules (Figure 7). These molecules may be additives to the plastic or components of the hand sanitizer itself. Thus, these particles are indicated as PP + additive in Figure 5. It should be noted that this identification may not be exact, so information about the chemical composition of the cosmetic product itself can help in data interpretation.

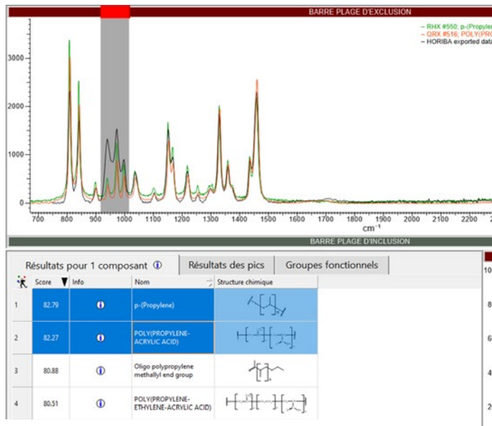

Figure 6: Matching experimental spectrum (black) identified as PP with KnowItAll Database spectra. Green and red spectra correspond to Polypropylene and Polypropylene-acrylic acid. Both spectra are similar to each other are reveal similar matching score of 82% with experimental spectrum.

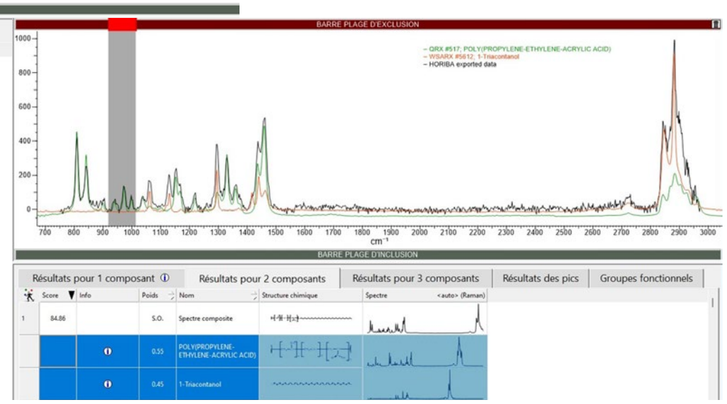

Figure 7: Matching experimental spectrum (black) identified as "PP+additive" with KnowItAll Database spectra. Green and red spectra correspond to Polypropylene-ethylene-acrylic acid and to 1-Triacontanol. Black spectrum can be deconvoluted as a sum of red and green spectra.

For simplicity, particles identified as starch, calcium carbonate, or amorphous carbon are grouped into one category called non-plastics. Most of the outlier spectra did not have a Raman signal, which was either masked by fluorescence or indicated only a silicon (Si) signal.

Therefore, the presence of microplastics was confirmed in Sample 1. As mentioned in the sample preparation section, the detected amount of microplastics corresponds to 50 ml of hand sanitizer, which represents approximately 16 single doses. Thus, approximately 400 microplastic particles in the size range of 20 to 100 µm are expected to be deposited on hands with a single dose of this hand sanitizer.

Conclusion

In this application note, we demonstrate the intuitive and automated workflow for microplastics analysis using Raman microspectroscopy with the latest generation of HORIBA Raman microscopes, LabRAM Soleil™, along with ParticleFinder™ and IDFinder™ software. This method enables the chemical identification and quantification of thousands of particles, including microplastics.

Three different hand sanitizers were analyzed, revealing the presence of hundreds of microplastic particles in the size range of 20 to 100 µm per dose in one of the samples. This analytical approach can be extended to microplastics analysis in any other sample subjected to the appropriate sample preparation procedure.

References

1. EUROPEAN COMMISSION, «COMMISSION REGULATION (EU) 2023/2055 of 25 September 2023,» https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32023R2055.
2. M. Marchetti, A. Perini, M. Zanella, F. Benetti et D. Donelli, «Study on the Fate of the Carbopol® Polymer in the Use of Hand Sanitizer Gels: An Experimental Model to Monitor Its Physical State from Product Manufacturing up to the Final Hand Rinse,» Microplastics, vol. 3, n° 13, pp. 390-404, 2024.
3. N. P. Ivleva, «Chemical Analysis of Microplastics and Nanoplastics: Challenges, Advanced Methods, and Perspectives,» Chemical Reviews, vol. 121, n° 119, pp. 11886-11936, 2021.

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Understanding the Nature of Microplastic Pollution and Identifying Environmental Impacts

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The document explores the pervasive issue of microplastic pollution, stemming from the massive global production and inadequate disposal of plastics. Microplastics, tiny particles measuring less than 5 mm, are produced directly (e.g., microbeads and industrial pellets) or form as larger plastics degrade. These particles have been detected in nearly all environments, including air, water, sediment, and biota, and are even found in food products consumed by humans. Their resilience and widespread presence make them a significant concern for environmental and human health.

Microplastics cause both physical and chemical toxicity. Physically, they accumulate in organisms, leading to health issues like respiratory problems, immune responses, and organ stress. Chemically, they leach harmful additives or absorb toxic pollutants from the environment, which can bioaccumulate and biomagnify across the food web. Research highlights their potential to disrupt ecosystems and pose risks to human health, although the full extent of their impact on humans remains under investigation.

To combat microplastic pollution, the document emphasizes the need for standardized methods to collect, extract, and analyze microplastics. Techniques like density separation, chemical digestion, and advanced spectroscopic analysis are vital for identifying their properties and origins. Innovative approaches, such as magnetic extraction and fluorescence staining, have been developed to improve precision and efficiency. Establishing uniform methodologies and spectral libraries will ensure consistency in research and data interpretation across laboratories.

Efforts to mitigate microplastic pollution require a multifaceted approach involving governments, industries, and individuals. Policies banning microbeads, promoting recycling, and encouraging sustainable materials are crucial steps. Public initiatives, such as beach cleanups and technologies that capture microfibers from washing machines, contribute to reducing pollution at the source. Ultimately, greater understanding and collaboration are essential to addressing the global challenges posed by microplastics and safeguarding ecosystems and human health.

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