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Nano Today 41 (2021) 101296

Contents lists available at ScienceDirect

Nano Today

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

Opinion

The analytical quest for sub-micron plastics in biological matrices

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ARTICLE INFO

Article history:
Received 30 June 2021
Received in revised form 27 August 2021
Accepted 13 September 2021
Available online 23 September 2021

Keywords:
Nanoplastics
Identification
Characterization
Particle extraction
Metal-doped tracking
Sample preparation

ABSTRACT

The current debate on hazards associated with sub-micron sized plastics is hampered by a lack of quantitative data on the uptake and biological fate of plastics in organisms. Analytical methods should be developed to identify, characterize, and quantify sub-micron particulate plastic in biota to understand their biological fate in terms of biodistribution, localization, bioaccumulation and clearance. Here we give a perspective on a promising workflow of sample preparation methods and techniques that could enable analysis of sub-micron plastics in biological matrices and discuss their application for biological fate studies of particulate plastic in organisms. We also expect these methods to be largely transferrable to studies considering sub-micron plastics in food, consumer products, human and some environmental compartments.

Introduction

In the last decade, researchers began to realize that sub-5 mm plastic particles are ubiquitous in the environment and that their bioavailable size fraction is likely in the range of few tenths of microns or less. The analytical capacity to measure plastics in this size range is now on the cusp of being developed to appropriately target the sub-micron range and compositional variability of particles at trace concentrations. It is therefore timely to give a perspective on what specifically is needed for analysis of sub-micron sized plastic particles in biological matrices to enable answering questions on the fate and impacts of these materials in organisms.

Plastics eventually fragment in the environment as a result of weathering processes, producing smaller debris of different sizes, shapes and chemical compositions, including microplastics (1 μm–5 mm) and sub-micron plastics (SMPs < 1 μm). For example, fragments of polyethylene, (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS), polyurethane (PUR), and polyethylene terephthalate (PET) are most commonly reported in the environment [1]. There are also engineered SMPs which are intentionally used in consumer products such as in paints [2]. These SMPs could also enter the environment upon use [3]. Intentionally produced SMPs are only a small percentage of the total diversity of SMPs in the environment, and several are being targeted for phase-out from so-called single use products, e.g., in the EU [4].

Organisms (humans, animals, and plants) present in different ecosystems may be exposed to SMPs. No documentation is available yet to confirm the accumulation of SMPs in organisms in the field, mainly due to sampling and methodological limitations. Considerable progress has been made for identification and quantification of SMPs, and the new knowledge gained can now be transferred to the analysis of SMPs in organisms, as discussed in more detail below. Controlled laboratory research has demonstrated that SMPs can be taken up by (micro)organisms [5] and accumulate in their tissues e.g., in crop plants and mussels [6]. Given the co-occurrence of microplastics and SMPs, and the growing body of evidence of uptake of microplastics by biota, it is reasonable to assume the uptake of SMPs alongside the larger microplastics.

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https://doi.org/10.1016/j.nantod.2021.101296
1748-0132/

The physicochemical properties of SMPs, such as size, shape, and polymer composition, can influence their uptake and biological fate (e.g., biodistribution, localization, bioaccumulation, and clearance) in an organism. To date it remains largely unexplored whether and to what extent, exposed organisms translocate SMPs after uptake from the environment. It is unknown how the physicochemical properties of SMPs determine their biological fate in organisms, as well as their excretion from organisms.

Gaining insight into the biological fate of SMPs requires analysis of the SMPs and their fragments in biological matrices. Techniques for identification (mass and chemical composition), characterization (e.g., size distribution, shape etc.) and quantification (particle number) of SMPs are available [7,8]. However, when SMPs are present in an organism, the similarity of the chemical composition of some SMP to the surrounding biological matrices and the intensity of signals generated by the background matrices make their identification analytically challenging. Moreover, the small size of SMPs, their heterogeneous size distribution, expected trace levels in the environment and lack of quality assurance and quality control (QA/QC) tools all complicate the analyses. Any selected analytical technique will have to contend with the low concentration of SMPs and the high background concentrations of other biogenic polymers such as proteins, lipids, polysaccharides, celluloses, etc. [9] as these materials have a similar chemical composition to some SMPs, which complicates the identification e.g. due to generating similar signals. Currently, appropriate analytical techniques to address these challenges for SMPs are in their early stages of development [10,11], and large efforts are currently being undertaken to overcome these limitations [12].

Each physicochemical property of SMPs requires the researcher to make informed decisions on how to combine appropriate sample preparation approaches and a fit-for-purpose set of analytical techniques to obtain data for each property of interest. So far, there are no established standardized protocols available to facilitate decision making by researchers in planning and conducting investigations of SMPs in organisms. Some methods have been developed for analysis of microplastics in environmental samples, mostly through identification of their chemical fingerprint. Some progress has also been made in tracking carbon-based nanomaterials (engineered carbon nanotubes, graphene materials, etc.) in complex matrices by using proxies, e.g., utilizing stable isotope or radio-labeling techniques. Recently, labeled or tagged SMPs were also investigated in various model environmental systems using metal-doped plastics as proxies in laboratory experiments using bench-top or pilot-scale systems [13]. Such approaches allow a novel perspective for tracking SMPs in organisms [14] and can advance understanding of how SMPs might behave as a function of their physicochemical properties in physiological media and how this correlates with their biological fate.

Herein, we propose a structured and transparent workflow that enables analysis of SMPs in biological matrices to determine the possible biological fate of SMPs in organisms. We describe approaches for identification and quantification of the SMPs load. The workflow contains three steps: sample preparation (separation, enrichment, stabilization), identification and quantification (Fig. 1) which is tailored to the experimental design or approach for SMPs in organisms. For each proposed approach, we briefly highlight the challenges that researchers are faced with when analyzing SMPs in biological matrices. Note that this perspective does not cover approaches to assessing toxicity of SMPs to organisms.

The state of the art of analyzing SMPs in biological matrices

There are currently no non-destructive approaches available tailored to in-situ analysis of SMPs in biological matrices. The identification of microplastics in deionized water is well established and a multitude of different techniques can be used, including Fourier-transform infrared spectroscopy (FTIR) and Raman spectroscopy [9,15,16]. These techniques are routinely used but have a low spatial resolution (~1–20 µm for FTIR and ~1 µm for Raman spectroscopy), which makes them unsuitable for identifying SMPs. In the last years, however, there has been considerable improvements in the identification of SMPs in aqueous samples, which we believe can be used for identifying SMPs in organisms, following suitable sample preparation, as discussed in the next section.

A number of review papers emerged recently discussing the methodologies required for analysis of microplastics and SMPs in complex environmental matrices [17–19]. These papers present a wide variety of techniques for measuring mostly microplastic in environmental samples [20,21], though they make it clear that no single technique on its own is sufficient to address all questions on the biological fate of SMPs in organisms. It was concluded that none of the methodologies reviewed in these papers could be directly applied to determine the biological fate of SMPs in organisms.

In the next sections, we offer guidance on method selection and solutions to these recognized challenges. For this we make use of knowledge from the previous development of methods in the field of nanomaterials and microplastics in biological matrices. Our focus on the submicron size fraction in this paper allows us to highlight the considerable differences regarding separation, identification, characterization, and quantification between SMPs and their microplastics counterpart. For example, SMPs have a smaller size compared to microplastics and require analytical techniques that feature high sensitivity and lower size detection limits. Moreover, the smaller size of SMPs implies a larger volume-specific surface area and a high surface energy, which can influence the colloidal stability of the SMPs during handling and measurement. This makes them more prone to agglomeration and other transformations (e.g., degradation, surface oxidation etc.), thus challenging the application of existing sample preparation protocols for SMP identification and quantification, particularly measuring their loads on a particle number basis.

Sample preparation methods for SMPs in biological matrices

The primary goal of an effective SMP preparation method is to extract one or several target SMPs intact from a biological matrix. Note that it is not currently feasible to develop a generic sample preparation method that can be applied to different environmental samples e.g., water, soil, sediment or biological tissues. The background matrix in which SMPs reside can drastically influence the way sample preparation must be developed to selectively extract the SMPs. For example, the sample preparation method used for a freshwater matrix might not work for a biological matrix because the type of particulate (organic and inorganic) materials in each matrix are different. This necessitates applying different digestion agents to remove each matrix. The method should be tailored to removing the biological matrices and any matrix-related interferences and should be capable of extracting the particles as well as concentrating the SMPs to above the detection limit of the selected analytical techniques. Since correlation of the physicochemical properties of SMPs to their biodistribution, localization, bioaccumulation, and clearance, is critical to the understanding of their biological fate, the sample preparation method must have no or minimal impacts on the SMPs properties. It is therefore important to use a stepwise sample preparation method specifically developed for different biological matrices and to optimize the parameters, e.g., digestive agents, time, temperature, pH etc., in each step to minimize particle losses (e.g., due to degradation, agglomeration and attachment to tubing) and interferences while obtaining high mass and number recoveries of SMPs.

Commonly used chemical methods based on acid digestion can break the matrix in which the SMPs reside, however, they are likely

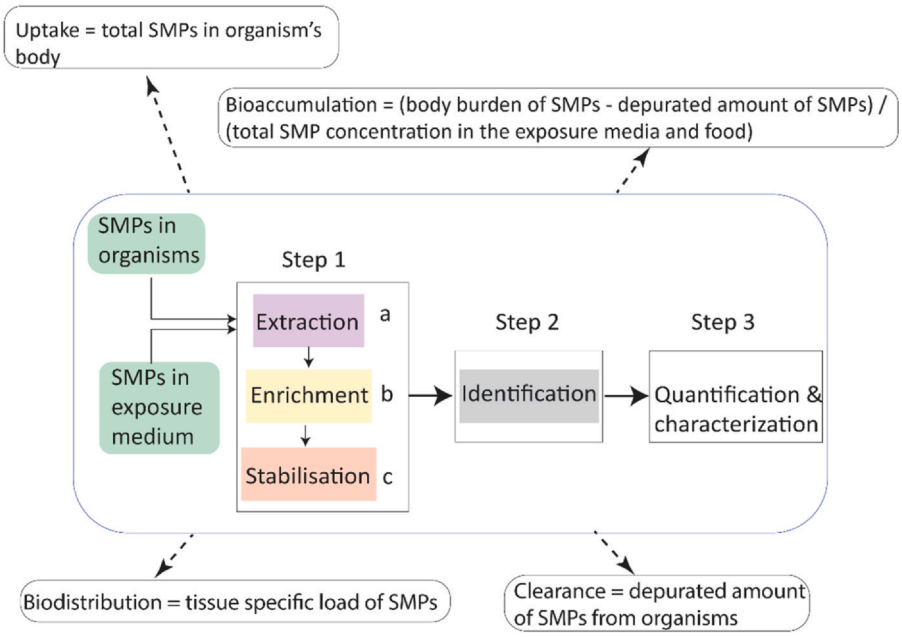

Fig. 1. Workflow for identification, quantification and characterization of SMP in biological tissue. The workflow contains 3 main steps. The sample preparation step which involves SMP extraction (step 1a): the SMPs are extracted from the organisms' bodies; SMP enrichment (step 1b): increasing the concentration of the SMPs in the samples; and SMP stabilization (step 1c): stabilizing the SMPs against particle agglomeration. After sample preparation, the extracted particles are identified (Step 2): determining the chemical identity of the SMPs, and quantified/characterized (step 3): measuring the mass concentration, number, size distribution and shape of the particles. The dashed arrows show some of the expected output data that can be used to quantify SMP uptake, bioaccumulation, biodistribution and clearance.

to damage some types of polymers such as PS, PE and PP, resulting in inconsistent recoveries and artifacts [22]. Fig. 2 illustrates examples of sample preparation options for animal and plant tissue. SMPs in animal tissues and cells may be extracted by digesting the biological matrix using an enzymatic digestion method (for instance: Proteinase-K) or an alkaline digestion method, e.g., tetramethylammonium hydroxide (TMAH) [26,33], at different temperatures (Fig. 2a). Enzymatic and alkaline digestion methods were reported to minimize particle loss due to polymer degradation [25]. Note that using potassium hydroxide for alkaline digestion might partially degrade PS particles [26]. We recommend that the type of digestive agents used for alkaline digestion and the parameters (e.g., temperature, the quantity of digestive agents, time of digestion, and the pH) are optimized for the specific experimental conditions, and it is critical to validate the method(s) for each type of physiological environment such as blood, animal tissue, cells, plant tissues etc. and for each SMP composition. Attention must be paid to the fact that some tissues of organisms, such as carapaces which are typically present in daphnids and shrimp, are not digested using enzymatic or alkaline methods and can degrade to small biological particles interfering with the SMP analysis. Moreover, digestion of some plant materials, such as cellulose (in cases where a plant is the organism of interest) and some pigments, cannot be achieved using alkaline digestion (Fig. 2b). To overcome this problem, additional steps of wet oxidation using hydrogen peroxide (H_2O_2) and enhanced temperature (approximately 60 °C) could be an option [27]. Some polymers such as PVC and PE are resistant to diluted H_2O_2 [37,38], and thus do not degrade under wet oxidation conditions. One must also be careful as a high concentration of H_2O_2 may, on the other hand, damage PE and PP [30]. If PE and PP are the SMPs of interest, the concentration of H_2O_2 must be optimized to minimize the degradation damage while ensuring sufficient recoveries and minimal changes in size of these polymers (Fig. 3).

The concentrations of extracted SMPs in suspension are expected to be low in biological samples. They are likely to be in low concentrations in some tissues of an organism exposed to a high concentration of SMPs in laboratory experiments because they might be unable to pass physiological barriers. Unlike for dissolved chemicals, steady-state assumptions, which consider the equilibrium between accumulation of a compound and its excretion from organisms, do not hold for SMPs. An enrichment step using ultracentrifugation (UC) for SMPs that are denser than water or crossflow ultrafiltration for low density SMP is suggested to increase the concentration of the SMPs in the extract (Fig. 2c). Note that unlike their large microplastic counterparts, SMPs experience Brownian motion in a fluid. Thus, buoyancy does not influence SMPs and their dispersion into the fluid. This makes density differentiation challenging for SMPs. Thus for SMPs that have a density lower than water, liquid-liquid particle extraction, where the SMPs diffuse from the water phase to a hydrophobic phase, is potentially an option, as previously applied for ultrafine particles [31] and surface-modified metallic nanomaterials [32].

After the enrichment step, the dispersed SMPs could be stabilized using a suitable stabilizing agent e.g., cetyltrimethylammonium bromide, tween 80, sodium dodecyl sulfate (SDS) or dextran 40 (Fig. 2d) followed by sonication [33]. The effect of sonication on the SMPs, particularly fiber-like SMPs, should be evaluated by transmission electron microscopy (TEM) as it has been documented that tip sonication may fragment polymers [34] as well as fiber-like SMPs.

A dispersion of extracted SMPs may be heterogeneous in particle size, shape and composition. Asymmetric flow field-flow fractionation (AF4) has a robust size-based separation capacity and is capable of separating a mixture of nanomaterials based on their hydrodynamic sizes. Recently, the technique was successfully applied to separate SMPs [45,47]. Off-line coupling of AF4 with the subsequent polymer-type characterization such as pyrolysis gas chromatography mass spectrometry (Py-GC/MS) could provide insight as to whether different size fragments are enriched in certain polymer types. This may allow one to infer differences in rates of ingestion of different polymer types and sizes by an organism. Note that SMPs with the same hydrodynamic size but with different morphologies and chemical composition cannot be separated from each other via AF4 alone.

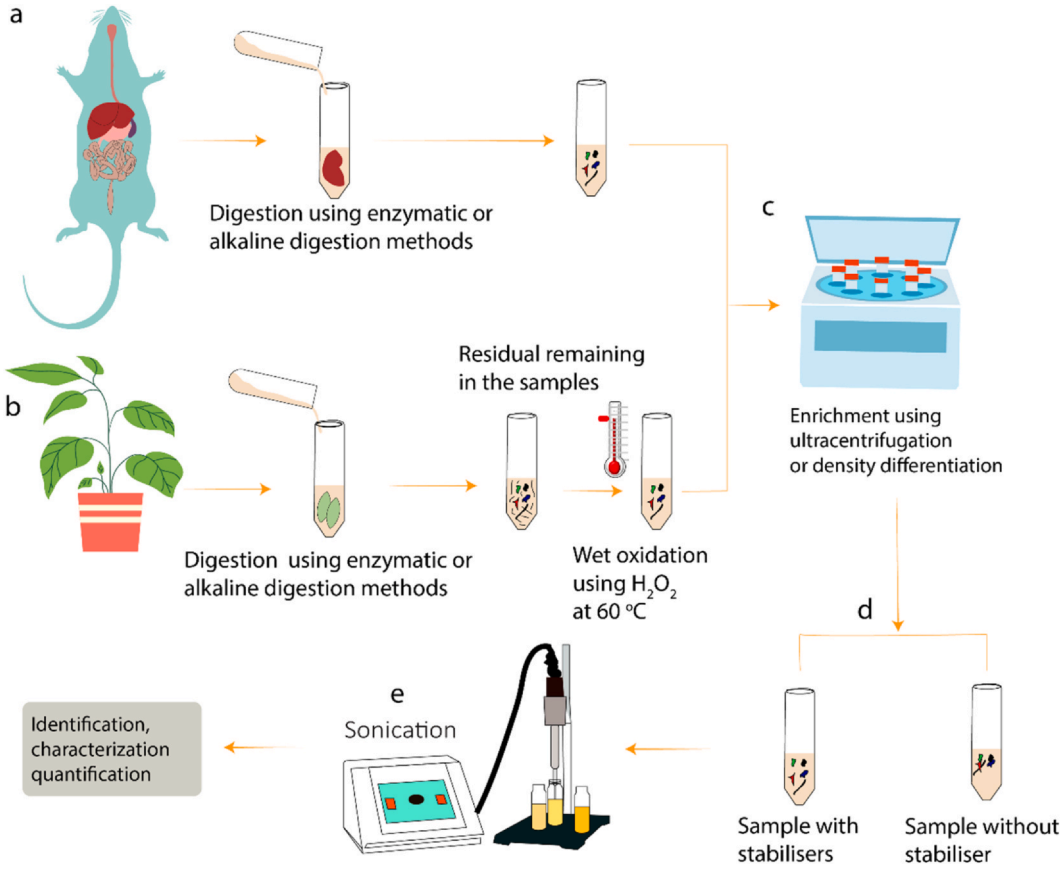

Fig. 2. Example sample preparation methods for extraction of SMPs from animal and plant tissues: a) Dissection and digestion of individual animal tissues (organs) to remove the biological matrices and bring the SMPs into a dispersed state. With this step, it is essential to assess the impact of the digestion protocol on the SMPs, and to perform proper method validation to ensure that the digestion process itself is not forming new SMPs in the sample. b) Separation and digestion of plant tissues (e.g., leaves, roots) to extract SMPs. After digestion of a plant sample, some residues such as cellulose might remain in the sample and requires additional treatment. Wet oxidation using H_2O_2 and heat is subsequently used to remove these residuals. c) Enrichment step, using e.g., ultracentrifugation, to increase the concentration of SMPs in the sample. d) Use of a stabilizer (such as sodium dodecyl sulfate) to avoid SMP agglomeration and maintain a stable dispersion where characterization of particle size and/or quantification of particle number is required. Note that the presence of stabilizers on the surface of particles may change the hydrodynamic size of the SMPs. Sonication facilitates the detachment of agglomerated SMPs. However, the power and time of sonication should be adjusted because it may impact some SMPs causing degradation. e) The stabilized SMPs can be identified using e.g., Py-GC-MS and characterized in terms of different physicochemical properties, and the abundance of particles can be quantified using established methods such as microscopy, NTA etc. This sample preparation procedure is applicable to organisms collected in the field and to laboratory-based experiments. Control samples and procedures are described in Fig. 3.

It is critical to control contamination and minimize the influence of the background particles to avoid false positives (e.g., measuring particulate materials of the background matrices and SMPs shed from containers) in order to generate reliable and reproducible data. It is expected that SMPs present in the environment, even in indoor air, makes the samples prone to secondary contamination during collection, transport, processing, handling and analysis [36]. Thus, one must maintain good field and laboratory practices to minimize secondary contamination resulting from e.g., deposition of SMPs onto the samples, release of SMPs from plastic equipment and tools, and shedding of SMPs from synthetic textiles [37]. In some cases, even the laboratory materials applied such as solidum chloride were reported to contain plastic particles [38]. Laboratory materials need to be analyzed to ensure they contain no plastic particles before using them in experiments and, if possible, chemicals should be of analytical grade. Procedural blanks are important to measure in order to characterize and identify any background contamination.

Additional experiments to assess particle losses and /or degradation due to the extraction steps are essential to assess the error (s) and bias(es) introduced by these processes (Fig. 3). This can be achieved by dispersing SMP standards (of the same polymer and size as the target analyte) in pure water to evaluate how the extraction processes may influence the apparent physicochemical properties of the particles and/or lead to particle losses. Attention must be paid to the fact that commercially available SMPs are often synthesized with a surfactant coating such as Tween 20 and SDS to minimize agglomeration of the particles during storage or to facilitate dispersion of the particles in water. The digestion process or temperature may remove the surface stabilizers, possibly leading to particle agglomeration and, consequently, lower SMP number recoveries.

To ensure that the digestion procedures successfully digest all of the background matrix (organic and inorganic materials) of biological samples, additional control experiments must be performed. Accordingly, a biological matrix of a clean sample (preferably the same tissue of an organism kept in the laboratory) can be used. Note that it does not matter whether this step of method development is intended to be used for sample preparation for SMPs in laboratory or field organisms. This is because the purpose of this step is to ensure that the background non-polymeric particles are totally removed after digestion to minimize interferences with SMPs identification and characterization. It is also possible that careful control experiments are performed and correlated with the polymer fragmentation and molecular weight. Spiking fabricated SMPs (of the polymer of interest) into clean biological matrices (representing the sample of interest) followed by performing particle extraction can assist in optimizing the extraction steps to completely remove the organic residue and evaluate the influence of the biological matrices on particle extraction and particle characteristics.

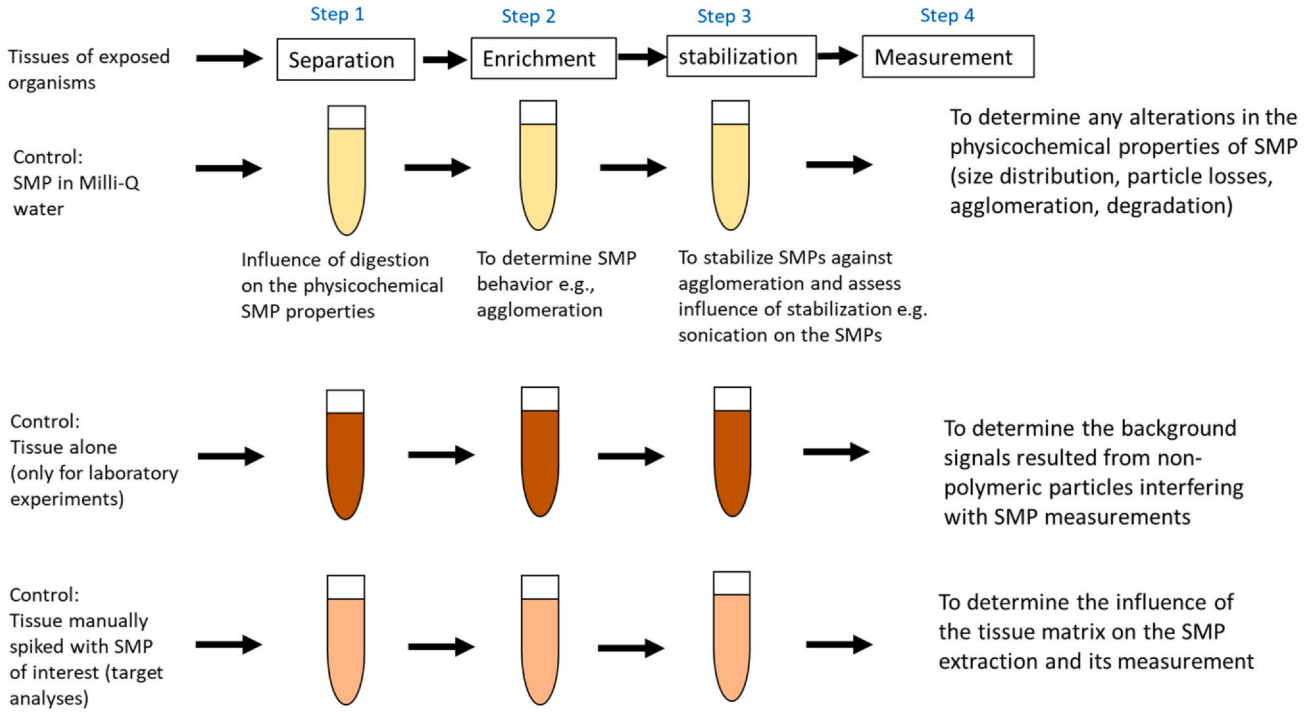

Fig. 3. The controls required to assess the possible alteration of the SMP properties upon sample preparation (dispersion and extraction steps).

Chemical identification

Under controlled conditions in a laboratory setting, where organisms are usually exposed to SMPs of known compositions, targeted analysis can be applied to track and measure the SMP fate and accumulation in organisms' bodies. Note that the size distribution of SMPs in organisms may change after uptake due to biotransformation, regardless of the initial SMPs size distribution. This will be even more complicated in the field, where organisms might be exposed to SMPs of different chemical compositions and size distribution, which may accumulate in their bodies. Thus, one may apply the developed methods to search for a specific SMP type in organisms or must perform a complicated non-target screening analysis to identify the SMP present in an organism. The latter approach is more applicable for environmental risk assessment of SMPs. Note that there is no difference in the applied SMP identification techniques between a laboratory and a field organism as long as the existing techniques can identify SMPs extracted from the organisms.

There have been improvements in identification of microplastics smaller than 1 µm. For example, coherent Raman scattering (CRS) microscopy, such as coherent anti-stokes Raman scattering (CARS) or stimulated Raman scattering (SRS), can be used for analysis of plastic items as small as ~130–300 nm [39]. Progress has been made in improving the spatial resolution of Raman spectroscopy [7] to measure SMPs, down to 100 nm, in water. Py-GC/MS [40] and the combination of thermogravimetric analysis (TGA) and solid-phase extraction with thermal desorption (TED)-GC/MS [8], in principle, can be used for in-situ analysis of SMPs to provide chemical structural information about polymers in complex matrices by analyzing their thermal degradation products. It was reported that TGA-FTIR-GC-MS can also be used successfully for in-situ identification of the polymer types of plastics collected from the environment [41]. The sensitivity and low size detection limits of the emerging techniques make them potentially suitable for analyzing a wide range of SMPs, in practice, it is still challenging to use these techniques for in-situ SMPs analysis in biological matrices. The problem is that the presence of other biological molecules and particles (organic and inorganic), such as proteins and nucleic acids that occur in animal tissues, cellulose, natural rubber, and lignin in plants, could interfere with the identification of the SMPs. Moreover, the concentration of SMPs is expected to be low in biota and their size distribution and chemical composition are likely to be heterogeneous. A promising solution is to develop a sample preparation method to extract SMP from biological matrices and purify the particles, followed by a suitable enrichment method to bring the concentration of the SMPs to a level measurable by the existing techniques, i.e., to above their limit of detection. For instance, using Py-GC-MS, different types of SMPs were identified after extraction from spiked fish tissues in the $\mu g \cdot g^{-1}$ wet weight range [42] (roughly equal to 1.8×10^{15} spherical PS particles of 100 nm size and 1.06 g cm^{-3} density) and in water samples in the $\mu g\ L^{-1}$ ($> 20\ \mu g\ L^{-1}$) range [23]. The detection limit depends on the chemical composition of the polymers [23]. Py-GC/MS and TED-GC/MS have not yet been exploited for detection of SMPs in biological matrices to their full extent, but these techniques have the potential to deliver comprehensive quantitative information on the identity of SMPs in biota if coupled with a sample preparation method tailored to SMPs in organisms. Similarly, TGA-FTIR-GC-MS can be applied to mesoscale plastic samples for definitive identification of polymer types including the influence of some additives. Method development for extraction of SMPs from biological matrices is in the early stages, although the development of methods for detecting and quantifying SMPs in water [43] and even in more complex systems such as algae [44] and soil [10] have been reported. Note that sample preparation methods are intended to prepare (extract, purify, enrich, and stabilize) SMPs for identification using fit-for-purpose identification techniques regardless of whether the SMPs are from laboratory or field organisms.

Number-based quantification and qualitative characterization of SMPs

The application of chemical identification techniques only provides information about the mass and chemical composition of SMPs. Determination of the SMPs' shape and provision of

quantitative data about SMP size and number is the minimum information needed when reporting on SMPs internalized by organisms and for correlation of the biological fate to the properties of the SMPs. Although there are many other physicochemical properties of interest that may influence the biological interactions of SMPs such as their glassy or rubbery properties (reflected in their glass and melting transition temperatures), hydration state and surface chemistry (oxygen content, functional groups and biomolecule corona coating), we only focus on particle size, shape, and number here.

In some cases, existing protocols developed for characterization of engineered nanomaterials in terms of particle size, shape and number might be applicable to SMPs in pure water. For example, TEM and scanning electron microscopy (SEM) can be used for measurement of morphology, size distribution and number concentration of SMPs. However, one must be aware that, unlike metal-based nanomaterials, the low contrast between SMPs and biological matrices may challenge the direct application of electron microscopic techniques for quantification and characterization of SMPs in-situ in biological matrices. Electron microscopy analysis requires dehydration of the samples, although application of correlative approaches [45] such as correlated confocal and electron microscopy have been successfully used for quantification of metallic nanoparticles where the TEM images were mathematically reconstructed to their hydrated size. The electron beam of TEM can damage some SMPs in the sample, leading to artifacts such as polymer degradation [46]. The minimal electron dose needed to obtain sufficient contrast depends on the type of polymer. Scanning-TEM with a radiation dose as low as 0.3 $e^-/Å^2$ per image, which was performed for imaging of live bacteria [47], could be used to decrease the amounts of destructive electrons needed for SMPs imaging. By decreasing the time of beam focusing on the sample one can minimize the damage, e.g., degradation, resulting from the electron beam.

Ensemble techniques are well established for measuring particles with sizes smaller than 1 µm in demineralized water. Nanoparticle tracking analysis (NTA), multi-angle light scattering (MALS) and dynamic light scattering (DLS), which are routinely used to measure nanoparticle size and (in the case of NTA) to quantify the amounts of particles, can be used for SMPs characterization when they are alone in suspension. These techniques can thus only be used when the SMPs are extracted from the biological matrices because cells, biomolecules and micelles of the biological matrices will interfere with the SMP characterization.

Application of tracers or doped SMPs

Labeled or doped SMPs have been introduced as an approach to trace and characterize SMPs in complex media [48]. While this approach cannot be used in field samples, in laboratory settings it can be a potential methodology to assess the biological fate of SMPs in organisms. For instance, doping SMPs with rare elements, isotopes or molecules that are not typically present in the environment and using these materials as tracers can circumvent some of the analytical challenges associated with the SMPs analysis workflow. A number of different doping techniques have been put forth in recent years, including the addition of fluorescent dyes [49], inorganic metal-doping [50], radio-labeling or stable-isotope labeling of the carbon polymer backbone [51]. The commercial availability and ease of use of fluorescently labeled SMPs have made them a popular choice amongst researchers assessing the ingestion and biological fate of particulate plastic in the past, although there are challenges with elution of dyes under biological conditions. Staining with fluorescent labels followed by quantification of bioaccumulated SMPs via confocal microscopy is a possible choice for SMPs, including the option of assessing their co-localization within targeted organelles, such as early endosomes or lysosomes [52]. However, fluorescent labels have begun to fall out of favor as it has been shown that the presence of organic matter can quench the tracer in complex media and the fluorescent dye often leaches from the particles under biological conditions [51]. This problem can be partially solved by dialysis prior to use [53] and control experiments under simulated biological conditions, e.g., in gut and lysosomal fluids, cell lysates.

Using metal-doped SMPs has been shown to have several key advantages, since existing standard methods for trace metals analysis exist and can be exploited for measuring metal-doped plastic materials [54]. Metal-doped SMPs in tissues can, for example, be completely decomposed through microwave-induced acid digestion and the metal content analyzed by inductively coupled plasma-mass spectrometry (ICP-MS) to back-calculate plastic concentrations. Moreover, techniques such as single-cell ICP-MS may be used to quantify metal-doped SMPs on a cell-by-cell basis [6], owing to the sensitivity of the technique, at levels as low as attograms of metal per cell.

In our opinion, metal doping is therefore the current technique of choice when it is anticipated that: (1) the SMPs consist of a polymer which is unlikely to degrade over the experimental timespan, (2) many samples need to be taken, and thus a high throughput technique is needed, and (3) when a large amount of SMPs is needed, as fabrication of these materials is comparatively inexpensive. However, some important aspects should be taken into further consideration, such as the need to select a metal that does not itself have a high natural abundance in the test organism or environment. Ultimately, the density of doped particles depends on the amount of metal incorporated. Metal-doped SMPs may therefore behave differently than their un-doped counterparts when a high metal content is added. Considering that much of the transport of nanomaterials is controlled by Brownian motion, and not by density alone, in many instances the impact of this density difference may be negligible, although this needs to be confirmed for doped SMPs.

Conversely, when the research question hinges on the biodegradability of plastic and/or incorporation of plastic into an organism, labeling the carbon backbone of the polymer could be a more appropriate choice [55]. The use of ^{13}C- and ^{14}C-labeled SMPs can provide an effective way to establish whether the plastic is mineralized or whether it resists biodegradation. ^{14}C labeled SMPs have been used to investigate the uptake, biodistribution and depuration of SMPs in mollusks exposed to environmentally relevant concentrations ($< 15\ \mu g\ L^{-1}$) of SMPs [56]. This allowed for the application of radio-tracing tools, that are routinely used for chemicals, to ^{14}C labeled SMPs. For instance, visualization of uptake and localization of ^{14}C labeled SMPs in biological matrices can be carried out using single-photon emission computed tomography, autoradiography, and positron emission tomography. Detection and quantification of ^{14}C labeled SMPs can be achieved using direct measurement of radio-carbon labeled SMPs using scintillation counters or measurement of the radio-carbon labeled CO_2 efflux upon SMP degradation, by means of wavelength-scanned cavity ring-down spectroscopy. Radio labeling can also be used to measure SMPs in a non-destructive manner in living organisms (in vivo) over time by means of e.g., in vivo gamma counting [57] and positron emission tomography. This facilitates the provision of images that reveal information about the biological fate of SMPs, even in humans. The generation of fragments from ^{14}C labeled plastic pellets or materials might be a first step in obtaining non-spherical SMPs which may be a more relevant proxy for field occurring SMPs arising from environmental degradation of plastic waste. The workflow shown in Figs. 1 and 2 are applicable to all the labeled variants of SMPs, although the required control steps (and safety considerations) will differ, as shown in Fig. 3.

Perspective and future directions

Given the existing knowledge gaps and the existing analytical limitations surrounding SMPs, more research into method development and optimization for SMPs in biological matrices is needed to measure trace levels of particles in tissues. Method development and validation should continue in order to optimize such workflows for different polymer compositions and to facilitate direct analysis of SMPs in organisms collected from the field. Although the availability of standard reference materials for evaluating the steps of the sample preparation method is required, no standard reference materials for all type of SMPs and even for microplastics are available. Developing some standard reference materials at reasonable cost (that can be affordable by many laboratories) could facilitate studies focusing on method development for SMPs.

Repurposing of methodological workflows from the fields of environmental nanoscience and nanomedicine is possible and could be fostered by researchers focusing on SMPs in biological matrices. Studies that attempt to apply fit-for-purpose methodologies to understand the fate of SMPs in organisms can be informed from assessment of the environmental health and safety of engineered nanomaterials, and potentially circumvent challenges which are applicable to both fields of research. Application of metal-doped polymers or isotopic carbon labeled polymers as models of SMP, which allow the employment of well-established techniques to measure metals or isotope carbon as proxies of the SMPs, offer a reliable way to understand the biological fate of SMPs.

As the need to develop specific techniques is shared across fields such as environmental science, toxicology and analytical chemistry, it is critical that this process be guided by the collective effort of these research communities e.g., collaborative studies, sharing knowledge, performing intra-laboratory experiments etc. The quality assurance and quality control procedures developed for most environmental contaminants cannot be directly used for SMPs because of the distinct characteristics of SMPs, such as their variation in chemical composition, shapes and sizes. The proposed strategies in this Perspective can facilitate a major step toward developing harmonized methods and generation of more reliable and reproducible data and will allow better comparisons across laboratories. After optimization the approach may also be transferrable to analysis of SMPs occurring in e.g., consumer products such as food and cosmetics.

CRediT authorship contribution statement

F.A.M. conceptualized, wrote, and reviewed the paper and designed the figures. **M.G.V.**, **D.M.M.**, **I.L.**, and **H.A.L**. contributed to conceptualizing and editing the paper and the design of the figures. **Z.G.**, and **P.Z.**, contributed to editing the paper. **W.J.G.M.P.**, and **E.V.J.** contributed to conceptualizing, editing, and reviewing the paper.

Declaration of Competing Interest

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

Acknowledgements

This study was supported by the University of Eastern Finland, Finland water research program (the Saastamoinen Foundation), H2020 project RiskGONE, UK (814425), H2020 project ACEnano, UK (720952), the Swiss National Science Foundation, Switzerland, Ambizione grant number PZP002_168105 and Eccellenza grant number PCEFP2_186856, and the H2020 supported project PLASTICFATE, Netherlands (grant agreement number 965367).

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