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Separation and analysis of microplastics and nanoplastics in complex environmental samples

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Conspectus

The vast amount of plastic waste emitted into the environment and the increasing concern of potential harm to wildlife has made microplastic and nanoplastic pollution a growing environmental concern. Plastic pollution has the potential to cause both physical and chemical harm to wildlife directly, or via sorption, concentration, and transfer of other environmental contaminants to the wildlife that ingest plastic. Small particles of plastic pollution, referred to as microplastics (>100 nm and <5 mm) or nanoplastics (<100 nm), can form due to the fragmentation of larger pieces of plastics. These small particles are especially concerning due to their high specific surface area for sorption of contaminants as well as their potential to translocate in the bodies of organisms. These same small particles are challenging to separate and identify in environmental samples as their size makes handling and observation difficult. As a result, our understanding of the environmental prevalence of nanoplastics and microplastics is limited.

Generally, the smaller the size of the plastic particle, the more difficult it is to separate from environmental samples. Currently employed passive density and size separation techniques to isolate plastics from environmental samples are not well suited to separate microplastics and nanoplastics. Passive flotation is hindered by the low buoyancy of small particles as well as the difficulty of handling small particles on the surface of flotation media. Here, we suggest exploring alternative techniques borrowed from other fields of research to improve separation of the smallest plastic particles. These techniques include adapting active density separation (centrifugation) from cell biology and taking advantage of surface interaction-based separations from analytical chemistry.

Furthermore, plastic pollution is often challenging to quantify in complex matrices such as biological tissues and wastewater. Biological and wastewater samples are important matrices that represent key points in the fate and sources of plastic pollution, respectively. In both kinds of samples, protocols need to be optimized to increase throughput, reduce contamination potential, and avoid destruction of plastics during sample processing. To this end, we recommend adapting digestion protocols to match the expected composition of the non-plastic material as well as taking measures to reduce and account for contamination.

Once separated, plastics in an environmental sample should ideally be characterized either visually or chemically. With existing techniques, microplastics and nanoplastics are difficult to characterize or even detect. Their low mass and size provide limited signal for visual, vibrational spectroscopic, and mass spectrometric analyses. Each of these techniques involve tradeoffs in throughput, spatial resolution and sensitivity. To accurately identify and completely quantify microplastics and nanoplastics in environmental samples, multiple analytical techniques applied in tandem are likely required.
Plastic size - small particles are challenging to recover and detect
Matrix complexity - high biomass and heterogeneity complicates plastic separation
1. INTRODUCTION

Nearly 80% of the 8 billion tonnes of plastic produced to date is in landfills or the environment\textsuperscript{1}. Small pieces of plastic arise from fragmentation of larger pieces, synthetic fibres in clothing, and microbeads used in consumer products\textsuperscript{1,2}. These tiny pieces dominate plastic particle counts in the environment\textsuperscript{3} and are typically referred to as microplastics (>100 nm and <5 mm) or nanoplastics\textsuperscript{4}. We define 100 nm as the upper size limit for nanoplastics, rather than 1000 nm, as this threshold is generally used in environmental nanotechnology\textsuperscript{5}. Because of their small size, microplastics and nanoplastics can be ingested by biota with unknown consequences for wildlife health. Nanoplastics pose a particular concern as they can disrupt cell membranes\textsuperscript{6}.

The prevalence of and potential harm from plastic pollution necessitates sampling and analyzing the environment and wildlife to fully understand the implications and risks. However, few data exist on the smallest size fraction of plastic particles in complex matrices, including field-collected organisms and wastewater, due to practical, and in some cases fundamental, challenges for isolation and analysis. Consequently, knowledge gaps exist for plastics smaller than 100 µm.

This account specifically addresses analysis and separation of the smallest size fractions of plastics in the most challenging matrices. Here, we outline these challenges to assist researchers in pursuing new directions in developing methods to separate and analyze micro- and nanoplastics in environmental samples.

2. GENERAL APPROACHES FOR PLASTIC SEPARATION

As with any separation process, isolating plastics from complex matrices (Fig. 1) requires leveraging plastic properties that differ from their surroundings. Specifically, compared to (wet) environmental matrices, plastics tend to be (1) less dense and (2) more hydrophobic. Furthermore, when matrices are low in solids content, plastics can be separated by size-based filtration.

2.1 Density-based Approaches

Flotation is used for plastic separation from denser sediment. Typical density separations for plastics employ salt solutions to render plastics buoyant. The salt selection balances particle
recovery, processing cost, and environmental impact. Plastics float to the surface passively or assisted by elutriation. Alternatively, ethanol can be used as a flotation medium. However, solvents such as ethanol can dissolve or damage some types of plastics – especially in the case of small particles.

Flotation can achieve near complete separation in the millimeter size range. Nevertheless, flotation is seldom used for plastic particles that are too small to be manually handled due to the difficulty of recovery from the air-liquid interface. Furthermore, flotation is incompatible with the smallest size fractions of plastic since the buoyant force is low and surface fouling can significantly change the particle density. Another concern is that the attachment of bubbles to non-plastic particles can carry denser particles to the air-liquid interface.

In our view, density separation techniques common in other fields are under-utilized in plastic pollution research. Specifically, in biology, density separations are routinely conducted via centrifugation. A common approach for density gradient centrifugation is to use Percoll (suspended colloidal silica nanoparticles) as a medium.

2.2 Hydrophobicity-based Approaches

Hydrophobic separation of small microplastics from environmental matrices has not been effectively applied. Froth flotation is used to separate minerals by the strength of hydrophobic interactions, whereby hydrophobic particles adhere to the surface of the bubbles which subsequently carry the particles to the air-liquid interface. However, froth flotation may not be suitable for analytical plastic separation due to the unpredictability of bubbles resulting in high particle losses. Imhof et al. only recovered 55% of plastics from sediments using froth flotation. Crichton et al. achieved high recovery of relatively large (~1 mm) microplastics by using oil to capture plastics via oleophilic interaction. However, this technique requires a potentially plastic-damaging ethanol rinse to remove oil residue. Nevertheless, in other fields, hydrophobicity-based separation has successfully been applied down to the molecular scale where hydrophobic interaction chromatography is used to separate biomolecules. Recently, proof-of-concept magnetic separation of 15 µm plastics was achieved by hydrophobizing iron nanoparticles via silanization to cause them to bind to plastic.

2.3 Size-based Approaches
With filtration, we must balance between the ability to capture small particles and filter clogging. Sequential filtration using increasingly smaller pore sizes can minimize filter clogging. However, this approach only confirms the presence of nanometre-scale plastics and not their total numbers as a significant fraction of the nanometre-scale particles are likely lost via attachment to filters. In contrast to “dead-end” filtration, crossflow filtration followed by asymmetric flow field-flow fractionation passes samples through porous sleeves to concentrate small particles and fractionate particles by size, respectively.

**Fig. 1.** Selected density-based and sized-based separation methods. (a) density separation, (b) dead-end filtration; Reproduced with permission from ref. 14. Copyright 2017 American Chemical Society; note that 0.1 µm filtration is done twice, (c) froth filtration; Reproduced with permission from ref. 10. Copyright 2012 American Society of Limnology and Oceanography, (d) asymmetric flow field-flow fractionation reproduced with permission from ref. 17. Copyright 2015 Frontiers Media.
2.4 Adapting approaches from nanoparticle separation

While few techniques have been reported for nanoplastic separation, adapting methods developed for engineered nanoparticles could prove effective. These techniques include: magnetic field flow fractionation (MFFF), gel electrophoresis, and size exclusion chromatography (SEC).

With MFFF, nanoparticles are separated as they flow through a channel by a magnetic field pulling magnetic particles tangentially to the direction of flow. Adapting MFFF to nanoplastic separation will require a method to magnetize them, which may be more challenging than with microplastics as magnetic nanoparticles may be too large to effectively bind to nanoplastics.

Gel electrophoresis separates nanoparticles by pulling particles through a gel with an electric field. Since gels are nanoporous structures, the speed of a particle is dependent on its size and charge. Using gel electrophoresis for nanoplastic separation will likely present challenges in analysis since the nanoplastics would be embedded into the gel and electrophoresis is not plastic specific.

SEC is applied to separate particles in a sample by size. The sample is passed through a column of porous beads. Smaller particles are slowed since they have to pass through the porous matrix of the beads while larger particles pass around the beads – meaning that smaller particles exit the column last. Further, SEC is not plastic specific.

Finally, all these techniques are typically used for nanoparticles at much higher concentrations than anticipated environmentally-relevant concentrations of nanoplastics. As such, advances in the separation efficiency of these techniques may be required before reliable application to nanoplastic separation.

3. SEPARATION IN BIOLOGICAL SAMPLES

Efficient and accurate quantification of plastics in field-collected wildlife is key to advancing the quality of environmental toxicological studies in a risk assessment and management context. Of 45 peer-reviewed research papers published between 2012 and 2018, a large portion (90%)
studied aquatic organisms, with 35% on fish species, 19% on mussels, 13% on plankton, and less than 5% on other organisms (Fig. 2). In contrast, studies on terrestrial organisms were relatively limited (10% of studies; Fig. 2). Laboratory studies that relied on labeled or dyed plastics at very high concentrations were excluded from our analysis as tagging plastics in the environment prior to exposure is impractical.

Acid or base digestion is frequently used successfully to isolate microplastics from biological tissues (Fig. 1). Nitric acid (HNO₃) (18% of studies), potassium hydroxide (KOH) (16% of studies), and hydrogen peroxide (H₂O₂) (12% of studies) are effective and widely applied to destroy or digest tissue and reduce greasy tissue fractions, leaving behind recalcitrant materials. Other strong acids (e.g. hydrochloric acid) and bases (e.g. sodium hydroxide) can also be used as digestants. However, some plastics can be degraded or damaged by these acid/base treatments, particularly at higher temperatures (> 80°C), while other plastics such as polyethylene (PE) and polyvinyl chloride (PVC) are resistant to these treatments, resulting in inconsistent recoveries. Enzymes (e.g. proteinase K, chitinase, cellulase) have also been successfully applied for more specific digestion of tissue, either alone or in combination with chemical digestants.

Dissection (13% of studies) is a traditional method for assessing plastic uptake in gastrointestinal tracts of larger organisms (e.g. whale, shark, fish) or whole bodies of smaller organisms (e.g. mussels and barnacles). Dissection is inexpensive and accurate when visually identifying microplastics > 500 μm in gastrointestinal tracts. However, smaller microplastics can translocate to other tissues and organs. Fixation and cryosection methods can be useful to investigate the translocation of microplastics to other tissues (e.g. liver) prior to examination and quantification. The physical separation techniques discussed above are not typically applied to biological samples (18% of studies) due to the large biomass content.

Overall, methods exist for processing organisms prior to plastic quantification, but most are either time-consuming or high-cost, limiting their applicability to large-scale field investigations. Lengthy separation procedures result in a significant risk of contamination. The lack of studies on terrestrial organisms means our understanding of the distribution and impact of microplastics in terrestrial systems lags behind aquatic systems.
Fig. 2. Overview of the separation methods for microplastics in a variety of field-collected organisms. Chemical: hydrogen peroxide, acids, bases, and enzymes; Manual: dissection, fixation and cryosection; Physical: filtration, ultrasonic and gravity separation (45 peer-reviewed articles, full reference list in the Supporting Information Table S1).
4. SEPARATION IN WASTEWATER

Plastic transport in wastewater treatment plants (WWTPs) is poorly understood, largely due to the complexity of wastewater itself (Fig. 3). Most microplastics (95-99%) entering a WWTP are estimated to be removed with biosolids or through grease skimming. Despite overall high removal percentages, the vast quantities of water being treated lead to estimates of 10^5–10^8 plastic particles per day entering the environment with treated effluent from one plant. Along the treatment process, microplastics may become brittle and break down, melt or transform, or become biofouled, further complicating identification and quantification.

Separation methods used in recent studies on microplastics in wastewater are summarized in Table 1. Method complexity varies greatly: some scientists report a single sieving step before microplastic identification while others present two to six steps including sieving, homogenization, concentration, digestion, density separation or staining.

There is no standardized procedure for digestion of wastewater samples. In the recent literature, researchers used Fenton’s reagent (peroxide with iron), peroxide alone, enzymatic digestion followed by Fenton’s reagent, 3% NaOCl or no digestion. Digestion protocols were generally optimized case-by-case for specific samples (raw influent, secondary effluent, sludge, etc.). Methodological studies focused on microplastic extraction (e.g., effects of Fenton’s reagent dosage on plastic integrity or efficiency of several digestion reactants on organic matter loss) are currently lacking.

Digestion protocols should be adapted to a quantitative parameter that describes sample organic matter content rather than a qualitative categorization of samples. Such protocols should include fixed parameters (e.g., peroxide volume and concentration, temperature and duration) and a range of organic matter mass from the sample, similar to other standard digestion protocols used in wastewater analysis such as Chemical Oxygen Demand (COD) and Kjeldahl nitrogen. COD of samples, seldomly included in wastewater microplastic studies, should be systematically reported to define future digestion guidelines. Moreover, parameters such as pH, presence/absence of Fenton’s reagent or temperature remain unoptimized.
Table 1. Digestion protocols reported in wastewater microplastic studies

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Processing Steps</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent</td>
<td>S</td>
<td>39</td>
</tr>
<tr>
<td>Pre-treatment effluent</td>
<td>S</td>
<td>39</td>
</tr>
<tr>
<td>Secondary effluent</td>
<td>S</td>
<td>39</td>
</tr>
<tr>
<td>Tertiary effluent</td>
<td>S</td>
<td>39</td>
</tr>
<tr>
<td>Anaerobic digestor sludge</td>
<td>S</td>
<td>39</td>
</tr>
<tr>
<td>Dewatering reject water</td>
<td>S</td>
<td>39</td>
</tr>
<tr>
<td>Dried sludge</td>
<td>S</td>
<td>39</td>
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<tr>
<td>Tertiary effluent</td>
<td>S</td>
<td>38</td>
</tr>
<tr>
<td>Secondary effluent</td>
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<td>38</td>
</tr>
<tr>
<td>Pre-treatment effluent</td>
<td>S</td>
<td>36</td>
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<tr>
<td>Grit and Grease</td>
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<td>36</td>
</tr>
<tr>
<td>Primary effluent</td>
<td>S</td>
<td>36</td>
</tr>
<tr>
<td>Secondary effluent</td>
<td>S</td>
<td>36</td>
</tr>
<tr>
<td>Anaerobic biological process</td>
<td>D H</td>
<td>41</td>
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<tr>
<td>Secondary and tertiary effluents</td>
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<td>34</td>
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<tr>
<td>Biosolids</td>
<td>D O</td>
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<td>Primary effluent</td>
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<td></td>
<td>H retentate</td>
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<tr>
<td>Secondary effluent</td>
<td>S D filtrate</td>
<td>35</td>
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<tr>
<td></td>
<td>H retentate</td>
<td>35</td>
</tr>
<tr>
<td>Primary sludge</td>
<td>D H settled sludge</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>D supernatant</td>
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<tr>
<td>Secondary sludge</td>
<td>D H settled sludge</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>D supernatant</td>
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<tr>
<td>Secondary effluent</td>
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<td>Raw influent</td>
<td>S E H S</td>
<td>43</td>
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<tr>
<td>Secondary effluent</td>
<td>S E H S</td>
<td>43</td>
</tr>
<tr>
<td>Dewatered sludge</td>
<td>D S H S</td>
<td>44</td>
</tr>
<tr>
<td>Sludge, lime stabilization</td>
<td>M S S</td>
<td>37</td>
</tr>
<tr>
<td>Sludge, thermal drying</td>
<td>M S D S</td>
<td>37</td>
</tr>
<tr>
<td>Sludge, anaerobic digestion</td>
<td>M S D S</td>
<td>37</td>
</tr>
<tr>
<td>Sludge, thermal drying + anaerobic digestion</td>
<td>M S D S</td>
<td>37</td>
</tr>
<tr>
<td>Primary effluent</td>
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<td>40</td>
</tr>
<tr>
<td>Secondary effluent</td>
<td>S C H D S T</td>
<td>40</td>
</tr>
<tr>
<td>Tertiary effluent</td>
<td>S C H D S T</td>
<td>40</td>
</tr>
<tr>
<td>Tertiary effluent</td>
<td>S C H D S T</td>
<td>40</td>
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5. IDENTIFICATION AND QUANTIFICATION OF MICROPLASTICS AND NANOPLASTICS

5.1 Visual Characterization: Size and Morphology of Micro- and Nanoplastics

Visual characterization techniques are inexpensive and convenient alternatives to reduce the number of particles that are later chemically characterized. 79% of reviewed studies employed visual characterization as the first step to screen for microplastics in environmental samples\textsuperscript{45}. These techniques involve identifying physical characteristics of particles associated with plastic (i.e., morphology, color, etc.). For particles larger than \( \sim 500 \ \mu m \textsuperscript{46} \), the naked eye or light microscopy are feasible.

However, even with ideal optics, the fundamental 200 nm diffraction-limit constrains observation with conventional light microscopy\textsuperscript{47}. Scanning electron microscopy (SEM) can observe smaller particles and their surfaces\textsuperscript{48}, although the cost and complicated sample preparation is unappealing\textsuperscript{48,49}, and the technique does not confirm the presence of plastic. Transmission electron microscopy (TEM) is not effective to visualize nanoplastics due to their...
amorphous structure; heavy metal stains are required\textsuperscript{50}. Aggregated 12 nm Eu-luminescence-labeled nanoplastics inside \textit{M. exotica} were observed non-invasively with two-photon excitation and time-resolved detection\textsuperscript{51}. This technique has advantages in simplifying sample preparation compared to electron microscopy. However, it is still diffraction limited. In degradation studies, nanoparticle tracking analysis has been used to measure concentration and size distribution of suspended nanoplastics, combining properties of Brownian motion and light scattering to identify particles down to 30 nm\textsuperscript{52}.

Staining with Nile Red for fluorescence microscopy has been used to identify microplastics\textsuperscript{53}. While Nile Red can be inconsistent and is not plastic-specific\textsuperscript{54,55}, it is effective in clean samples, and a better understanding of plastic-dye interactions may lead to the use of more plastic-specific and plastic-sensitive dyes and dye combinations.

5.2 Confirmation of Micro- and Nanoplastics' Composition

5.2.1 Vibration Spectroscopy

Vibration spectroscopy coupled with optical microscopy can provide both visual information and composition of plastic particles. However, the signal obtained is dependent on the size of the particles analyzed and typically a well-separated sample is required.

5.2.1.1 Fourier-transform Infrared Spectroscopy (FTIR)

FTIR is a powerful and commonly used technique for microplastic identification. The signal depends on the change in the permanent dipole moment of a chemical bond, making it sensitive to polar functional groups (e.g., in polymers). FTIR microscopes have spatial resolutions down to 5 µm\textsuperscript{56}. However, FTIR requires a minimum sample thickness (\textasciitilde150 nm\textsuperscript{57}) and sample deposition onto an IR transparent substrate\textsuperscript{58}. Due to these limitations, FTIR is best suited for individual particles larger than \textasciitilde20 µm. However, agglomerates or films of smaller particles may be analyzed\textsuperscript{14}.

5.2.1.2 Raman Microscopy

Conversely, Raman microscopy is more suited to the microplastic fraction below \textasciitilde20 µm\textsuperscript{59}. In Raman active materials, the molecular vibrations cause the scattering of polarized light\textsuperscript{60}. The main advantage of Raman is that the complete wavelength region is used, and amorphous carbon
can be detected. As a result, the Raman spectra of microplastics exposed to UV degradation is not significantly altered\textsuperscript{60}. The spatial resolution of Raman is approximately 1 µm\textsuperscript{56}, and particle shape and thickness do not influence the measurement. These advantages make Raman a potentially more sensitive tool to identify microplastics compared to FTIR\textsuperscript{61}. However, some materials exhibit fluorescence, masking vibrational information. Raman signal is heavily influenced by dyes\textsuperscript{60}, microbiological\textsuperscript{62}, organic\textsuperscript{56} and inorganic substances\textsuperscript{56}.

5.2.2 Mass Spectroscopy Methods

In mass spectroscopy-based methods, the sample is analyzed in bulk. This trades the spatial resolution of vibration spectroscopy for potentially increased sensitivity – which can allow detection of nanoplastics. The detection signal depends on the total mass analyzed. These methods are suitable to qualitatively identify a mixture of plastic particles and certain configurations will also allow for quantification, albeit in relative quantities rather than discrete particle counts.

5.2.2.1 Thermal Desorption Coupled with Gas Chromatography-Mass Spectrometry (TDS-GC-MS)

TDS-GC-MS involves placing a sample onto a thermogravimetric balance and heating to temperatures up to 1000 °C\textsuperscript{63}. Degradation products are adsorbed onto a solid-phase which is then transferred to a thermal desorption unit. These are then desorbed by increasing the temperature, separated with a chromatography column, and analyzed by mass spectrometry. TDS-GC-MS is a technique suited for relatively high mass (up to 100 mg) samples but is limited to qualitative analysis\textsuperscript{64–66}.

5.2.2.2 Pyrolysis Coupled with Gas Chromatography-Mass Spectrometry (py-GC-MS)

Py-GC-MS involves decomposing a sample at high temperatures, separating the products via gas chromatography and analyzing by mass spectrometry. Inter-lab reproducibility is challenging with Py-GC-MS, as results are highly dependent on sample preparation, pyrolysis type and pyrolizate transfer\textsuperscript{67}. In electrical heated filament pyrolysis, the sample is inserted into a quartz tube which is then placed inside a resistively heated coil\textsuperscript{68}. In furnace pyrolysis, the solid sample is inserted into a pre-heated furnace and a carrier gas transports the pyrolyzate into the gas chromatography column\textsuperscript{68}. In Curie-point pyrolysis, the solid sample is placed inside a
ferromagnetic foil which is later placed in the induction pyrolyzer. In all cases, the
temperature increase is performed under a controlled atmosphere. In filament and Curie-point
pyrolysis, the disposable sample substrate is the main advantage, as suspensions with
microplastics and nanoplastics can be dried and further pyrolyzed. This is impossible in furnace
pyrolysis as fused quartz sample containers must be reused. The main advantage of the Curie-
point pyrolysis is that the composition of the pyrolizate does not change in the range of 480-
980°C, whereas it is temperature dependent in furnace and coil pyrolysis. Additionally, Curie-
point pyrolysis is faster, more precise, and quantification is possible, given that the temperature
is high enough to avoid unpyrolyzed residue. With filament pyrolysis, some of the pyrolizate will
condense to form a residue in the quartz tube.

In comparison to TDS-GC-MS, py-GC-MS offers greater sensitivity, making it more appealing
when trying to identify small masses of nanoplastics (down to ~50 µg). This can be relevant in
simple matrices such as drinking water, where separation is straightforward. However, in
environmental samples, where the nanoplastics are likely to be embedded or attached to other
materials, sensitivity may still be a barrier. Furthermore, the amount of sample that can be
injected is restricted by the size of the pyrolyzer, leaving room for improvement.

5.3 Signal Processing

With all these spectroscopic/spectrometric techniques, the spectra are interpreted to obtain useful
information. While developing new libraries and applying existing libraries is important to
optimize identification, a thorough understanding of the fundamental chemical implications of
the spectra is invaluable to decoding the signal.

6. BLANK AND SPIKE CONTROLS

Quality controls including blanks, spike recovery and contamination assessment are critical
aspects of microplastic and nanoplastic analysis. Blanks undergoing some or all extraction steps
are often analyzed. Background levels can represent a significant portion of the signal in
low concentration samples. Positive controls (spiked samples) were used for both solid and
liquid samples with recoveries between 70 and 95% in most studies, though
comparison between biota studies is still hampered by variation in recovery rate across size
fractions and processing methods. Spikes are generally limited to only a few types of particles,
with polystyrene most commonly employed. Spike particles are typically 100-500 μm\textsuperscript{37,40,44}. While satisfactory recovery was sometimes reported using spikes as small as 10-45 μm\textsuperscript{34}, studies using nanometer-sized spike controls are lacking. Few\textsuperscript{35} studies spike samples with fibers. Frequently, recoveries for positive controls are solely analyzed visually, but ideally, chemical analysis should be conducted to identify transformations due to extraction. Airborne contamination controls using clean petri dishes or filters have been employed, leading researchers to count fibres depending on colour\textsuperscript{35,71}. Using laminar flow hoods, clean environments and/or natural fiber clothing can limit microfiber contamination\textsuperscript{36}. Contamination is especially problematic in nanoplastic detection with mass spectroscopy methods due to the bulk nature of the analysis.

**CONCLUSIONS**

In this account, we review recent developments in microplastic and nanoplastic separation and analysis focusing on particular challenges with small plastic particles and processing complex samples including field-sampled organisms and wastewater (Fig. 3 and 4). A common theme in our account is that new techniques are required to answer key questions in the field of microplastic and nanoplastic research. To this end, we recommend combining different techniques to leverage the unique advantages of each technique. For example, the chemical specificity of spectroscopic techniques could be combined with high resolution of electron microscopy using correlative microscopy techniques. When developing methods employing combined techniques, looking to other fields could prove helpful. For example, micro- and nano-scale separations and analysis are routinely employed in fields such as cellular and molecular biology. Borrowing methodological principles from these fields could help develop new techniques to answer outstanding questions in microplastic and nanoplastic research.
Fig. 4. Overview of microplastics and nanoplastics separation and analysis methods in simple and complex matrixes.

**BIOPHAGICAL INFORMATION**

Brian Nguyen completed his Ph.D. at the University of Toronto in 2018. He developed methods for studying the prevalence and ecotoxicology of microplastics. His current research interests are developing techniques to study the smallest size fractions of plastic pollution.

Dominique Claveau-Mallet completed her Ph.D. at Polytechnique Montreal in 2017. She developed technology for domestic wastewater treatment in small-scale applications. She is now a postdoctoral researcher working on co-transport of contaminants with microplastics.

Laura M. Hernandez is a Ph.D. student working on sources and fate of nanoplastics and developing new methods to separate and characterize nanoplastics. She completed her Master’s degree at the Universidad de los Andes in 2015. She worked with anisotropic particles and surfaces for manipulation of transport phenomena properties.

Elvis Genbo Xu obtained his Ph.D. from the University of Hong Kong in 2015. He worked on the integrated risk assessment of endocrine-disrupting chemicals in marine protected areas. He completed a postdoc at the University of California. His recent research focuses on ecotoxicity of micro- and nanoplastics and developing detection methods in biological samples.

Jeffrey M. Farner received his Ph.D. from Duke University in 2016. He studied the role of aggregation and surface interactions on nanoparticle photoreactivity. Jeff is currently a
postdoctoral researcher and focuses on the environmental fate and transport of nanoparticles and nanoplastics originating from consumer products.

Nathalie Tufenkji is a Professor at McGill University. She holds a Tier 1 Canada Research Chair in Biocolloids and Surfaces. She has extensive expertise in nanoscience, colloid chemistry, microbiology, molecular biology and material engineering. She earned her Ph.D. from Yale University.

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