Isolation and Extraction of Microplastics from Environmental Samples: An Evaluation of Practical Approaches and Recommendations for Further Harmonisation

Amy L. Lusher[1], Keenan Munno[2], Ludovic Hermabessiere[2,3], Steve Carr[4]

[1]Norwegian Institute for Water Research, Gaustadalleen 21, Oslo, Norway

[2]Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Ontario, Canada

[3] Department of Civil and Mineral Engineering, University of Toronto, Toronto, Ontario, Canada

[4] Sanitation District of Los Angeles County, Whittier, CA 90607-4998, United States

Corresponding author: amy.lusher@niva.no

Abstract

Researchers have been identifying microplastics in environmental samples dating back to the 1970s. Today, microplastics are a recognized environmental pollutant attracting a large amount of public and government attention, and in the last few years the number of scientific publications has grown exponentially. An underlying theme within this research field is to achieve a consensus for adopting a set of appropriate procedures to accurately identify and quantify microplastics within diverse matrices. These methods should then be harmonized to produce quantifiable data that is reproducible and comparable around the world. In addition, clear and concise guidelines for standard analytical protocols should be made available to researchers. In keeping with the theme of this special issue the goals of this focal point review are to provide researchers with an overview of approaches to isolate and extract microplastics

from different matrices, highlight associated methodological constraints and the necessary steps for conducting procedural controls and quality assurance. Simple samples, including water and sediments with low organic content, can be filtered and sieved. Stepwise procedures require density separation or digestion before filtration. Finally, complex matrices require more extensive steps with both digestion and density adjustments to assist plastic isolation. Implementing appropriate methods with a harmonised approach from sample collection to data analysis will allow comparisons across the research community.

Keywords: organic matter removal; density separation; analytical methods; digestion; biota; sediments; water

1. Introduction

Identifying appropriate methods is a compelling theme within the field of microplastic pollution research. Comparative methods are essential as data generated underpin our ability to examine studies from different locations and research groups[1]. Calls for standardisation and harmonisation have emerged[2-4] including calls from local level monitoring programs to global level implementation studies, such as NOAA marine debris program (US), GESAMP-WG40 (UN) and CleanSeas (EU). As valid as the requirement is, the ability of many research groups and laboratories to achieve full standardisation is heavily reliant on access to funding and facilities to make this possible. Not every method is suitable for every laboratory, nor is every laboratory able to implement high-level and high-cost procedures. Similarly, as the research field continues to expand, new and novel approaches emerge in the scientific literature, as does the ability of researchers and instruments to identify smaller and smaller particles[3]. This renders comparisons between methods an almost impossible task. Furthermore, identifying appropriate methods for specific matrices can complicate the matter. As an example, complex

matrices such as wastewater influent cannot be processed with a single processing step. They require a complex protocol which increases costs and experience required for efficient and effective particle isolation.

When designing and implementing an appropriate study of microplastics in a particular matrix, researchers must start by addressing all steps required from sample collection to results analysis and interpretation[1,5]. Along the way, some of the steps are heavily reliant on the former being appropriate and accurate. Following sample collection, microplastics which vary in polymer, size, colour and morphology[6] must be removed and isolated from what can be a complicated matrix. Isolating microplastics in an appropriate manner is paramount to achieving high extraction efficiencies, preservation of particles and accurate data generation. This is made more difficult because the type of extraction required is media specific and can vary within sample types.

Particle separation and isolation from different matrices can be a problem if methods are not chosen properly or tested before processing commences. Choosing appropriate methods for microplastic isolation must consider sample complexity as well as the complexity of required methods. Thus, researchers must assess how a matrix performs before processing it. For example, the wastewater matrix possesses what can be considered an extreme level of matrix-associated interferences. The overwhelming presence of fats, oils and grease coupled with the extreme quantities of toilet paper residues present obvious challenges to cleanup methods[7]. The exploitation of density and other physical properties that are suitable for facilitating microplastic isolation in most matrices are found to be highly challenging or totally ineffective for primary influent[7].

Compared to natural biological and other inorganic fragments, plastics typically possess several distinctive characteristics that are readily noticeable to an experienced analyst[8]. Particles in sieved residues, for example, typically have distinctive colors, irregular physical profiles, or geometries that differentiates them from surrounding biological residues. Plastic fragments are also resistant to crushing or deformation when pressed or probed with a micro spatula or other appropriate tools. In addition, solid plastic fragments will typically survive hot acid or highly oxidative digestion. In general, post-digested non-polymeric solids residues also have physical properties like density, friability and crystallinity that differs from common polymers. Once cursory qualitative screenings are conducted it is recommended that the analyst perform confirmatory analyses using Fourier transform infrared spectroscopy (FTIR), Raman spectroscopy, thermal analyses (e.g. Pyrolysis-GC/MS, Thermal Extraction Desorption-GC/MS) or other accepted instrumental methods for polymer confirmation[9].

Some methods may be reliant on mechanical processes such as sieving and mixing. These steps, although effective for particle isolation from samples, can increase procedural error if particles are brittle and fragment, this will affect particle count data. For some matrices, research groups have begun to naturally gravitate towards a common method, but for others, there are many emerging approaches that are still being examined in detail through extraction efficiencies and interlaboratory comparisons. As already mentioned, wastewater influent and sludge cannot be prepared with a single processing step and require a complex protocol. Similarly, some biota tissues cannot be digested with simple alkaline digestion because of high proportions of fats and oils[10]. A common example here are the differences observed between pelagic fish. Salmon and herring are very oily and have lipid-rich tissues which hinder the ability of potassium hydroxide (KOH) digestion, whereas whole myctophid stomachs can be digested using KOH[11]. On the other hand, KOH extraction protocols for the processing of bivalves are almost

commonplace with minor modifications between research groups[12-14]. Furthermore, where an organism feeds will impact the type of inorganic material that ends up in the organism's digestive tract, sometimes complicating extraction. For example, benthic-feeding fish may have a larger volume of sediment or sand in their gastrointestinal (GI) tracts. A density separation step can be added to enzymatic and chemically digested benthic-feeding fish stomachs with high sand and sediment content[15].

Such an array of methods can be overwhelming for researchers when designing a study plan with appropriate methods. Many researchers therefore turn to reviews and guidelines to offer direction. Over the past few years, a number of reviews have addressed the methods for biota[2-3,16-17], sediment[18-19], water[4, 20-21], wastewater treatment plants[22-23], terrestrial[24], freshwater [25-28], and marine matrixes[29]. Many reviews have highlighted the need for researchers to efficiently separate microplastics from sample material through reduction of sample mass and the removal of biological material, whilst maintaining particle properties. However, what many reviews lack is a thorough comparison between matrix and environment. Consequently, the goal of this focal point review is to critically present a comparison of extraction methods from simple procedures to stepwise and more complex processes. We aim to identify the most suitable extraction approach for each sample type, highlight associated methodological constraints, discuss necessary steps for conducting procedural controls and quality assurance based on the methodology applied.

2. Approach

Microplastic research is saturated[30] with novel methodological approaches and publications utlising different processing and isolation steps. In order to assess the state of the science we have chosen to focus on reviews published in the past five years (Suppl. Material, Table S1) as

well as utilising a brief primary literature review focusing on data published between January - July 2019. Publications were acquired using the following search terms on Google Scholar: *microplastics* AND *review* OR *sediment/ biota/ fish/ bivalve/ water/ seawater/ drinking water/ wastewater.* Reviews were used to identify publications prior to 2018 which could be included in the literature assessment. Data obtained from the publications specifically focused on isolation techniques and was organised into a database. This database was then used to compile a summary and critique of the available methods for microplastic isolation from different matrix types, as well as identify recommended methodological approaches. Three common themes were identified between methods. As such methods have been divided into three groups: (1) simple (single processing steps), (2) stepwise (two or three steps required to achieve samples for analysis), and (3) complex (multiple processing steps and extended treatment duration).

3. Isolation methods for simple matrices

Samples which are relatively easy to process are those from simple matrices, by this, we refer to samples that can undergo very little pretreatment before filtering, sorting and analysis. These methods tend to be cheaper and less labour intensive and can be carried out with limited resources. However, these methods can yield "quick and dirty" results related to methodological constraints. Simple isolation steps include filtering clean water samples, mechanical separation of beach sediment and visually sorting vertebrate digestive tracts.

3.1. Filtering relatively clean water samples

Clean water samples, irrespective of sample collection, can simply be filtered onto filter papers or meshes for visual analysis and chemical validation. Sample types which fall into this category primarily include drinking water samples[4] and other beverages, and on rare occasions

offshore water samples collected in areas with few biological particles[31-32]. Some effluent samples may also be included within this category including tertiary treated wastewater or recycled water for direct nor indirect potable use[33]. These simple extractions require no pre-processing and the resulting filters can be manually inspected or automatically scanned for microplastics. There are a number of different filtering systems used, although vacuum filters including Buchner set-ups are by far the most common. Filter or mesh pore sizes used between research groups vary greatly and will have a significant effect on the overall number of particles collected as they determine the lower size of microplastics detected. A review conducted in 2017, glass fibre filters were identified as most commonly used (incl. Whatman® GF/A, GF/C or GF/F), along with nitrocellulose filters and isopore filters[18]. Anodisc filters (Alumina oxide) are now being introduced for automated scanning μFTIR[9]. Unfortunately pore size of filters is an analytical inconsistency between studies and filters can range from 0.2 μm (Alumina oxide), 1.2 μm (GF/C), 5 μm (Silicon, silver) and nylon mesh 250 μm[34-36]. Smaller pore sizes can result in the obstruction of samples by organic material and samples may require further processing (see **Section 4**). With varying lower limits of particles captured during filtering, direct comparisons cannot be made unless such information is accessible in published research[1]. This further highlights that researchers should use several size categories, or bins, when reporting data to allow the assessment of comparable data ranges[37].

Recommendations: Clean water samples including beverages, field samples with low biological content and some wastewater effluent can be processed using filtration alone. When working with clean water samples, researchers are reminded to consider appropriate sample volume before commencing research[4]. It is recommended that such samples are filtered onto appropriate filters depending on individual study aims and analytical isolation capabilities.

Sample volume, filter type and pore size should be recorded. Procedural controls must be included.

3.2. Mechanical separation

Sieving is used most frequently for the separation of microplastics from sediment. Sediment samples which are dry and mostly free of fine organic matter can be sieved to remove large stones and debris (inc. plastics and organic material). Many visual observation studies carried out on beaches use this technique and separate large plastic items from smaller plastic items. The resulting items are counted and categorized. This method is normally implemented in studies focusing on plastics which can be separated out by eye with sieves of 1mm, 2mm and 5mm commonly used to define the lower size limits[38]. Many beach studies are performed *in situ*, thus limited contamination control is carried out in the field. In such studies, plastics are simply removed and retained for visual processing at a later stage. This approach is not adequate for small microplastics (<1 mm) and isolation steps must be performed under laboratory conditions. As with water samples, if smaller mesh sizes are used, organic and mineral matter may obstruct the identification of plastic particles, thus further processing may be required using organic matter removal or density separation (see **Section 4**).

Samples which are collected in the field but returned to the laboratory for processing under controlled conditions can facilitate the inclusion of smaller particles along with procedural controls to monitor contamination. Samples can be homogenised and split using standard sediment protocols before microplastic isolation. Microplastics can be separated via size-based fractionation when solid content is low[39]. Both wet and dry sieving can be used, however, wet sieving may be less accurate at separating particles because the water can make them stick to

194 one another. In wet sieving, a long duration of rinsing is required to adequately separate the
195 particles. Fractioning samples using sieve stacks with or without the aid of water will divide
196 the sample into smaller subfractions based on size bins created by the sieves. The volume in
197 each subfraction will be less than the total, thus increasing the likelihood that some subfractions
198 will contain few solids. The subfractions that contain few to no solids may not require any
199 further steps to isolate microplastics (see **Section 4 and 5**).
200
201 Although effective for separating samples, sieving can cause brittle particles to fragment. This
202 may affect final particle counts and an over-estimation of smaller sized particles. When using
203 sieves to separate samples, the cleaning of the sieves is of utmost importance. One of the best
204 approaches for cleaning sieves is to perform reverse flow flushes using a strong water or air
205 jet. Mechanical scrubbing with detergent and scouring with fine steel wool or brushes can also
206 be effective. A sonicator can also be used where available.
207
208 In an attempt to simplify the preparation and isolation of microplastics from environmental
209 samples, Felsing and colleagues[40] utilised the electrostatic properties of plastics to facilitate
210 their separation. The method used a modified electrostatic separator, Korona-Walzen-Scheider
211 electrostatic bell separator, to reduce sample mass and concentrate plastics based on their
212 physical properties: sediments have conductive properties, which can be separated from non-
213 conductive microplastics. Dried and unconsolidated samples are introduced to the separator via
214 a vibrating conveyor where samples are electrostatically charged with up to 35 kV. Four
215 different materials were separated into size fractions with nearly 100% recovery of spiked
216 samples and a reduction of the original sample volume by almost 99%. The advantages of this
217 approach includes a shorter processing time and the almost complete removal of biological
218 material. Another alternative approach for separating microplastics from sample matrices is the

magnetic removal of plastics which takes advantage of plastic's hydrophobic surface to magnetize plastic particles[41]. Grbic and colleagues proposed that this method could be used stand alone for cleaner samples, such as drinking water, but also as part of a stepwise process following density extraction. This method is not without its limitations. There was variation in recovery rates which could be related to lower surface area to volume ratios of medium sized microplastics (200 µm to 1 mm) and lower recovery rates from sediments as soil particles can impede extraction. Magnets were also seen to cause more brittle microplastics to fragment. Finally, the presence of lipophilic substances, or biota, in sediment samples along with the non-specific binding of nanoparticles may reduce the effectiveness of isolation.

Recommendations: All three approaches are suitable for the mechanical separation of microplastics from sediments containing little organic matter. Sieves must be thoroughly cleaned between samples and procedural controls must be included. Procedural controls include processing of blank samples to ensure no contamination is introduced through the separation process, and to ensure that the equipment is properly cleaned. Samples can be wet or dried sieved, but care should be taken to avoid further fragmentation of brittle particles. All procedural steps should be recorded, including original sample volume, processed sample volume, mesh size and sample condition (wet/dry).

3.3. Visual sorting of biota digestive tracts or sieved water and sediment samples

In the early years of microplastics research, visual sorting was the primary method for separating microplastics from water, sediment and biota samples. In regards to biota, dissecting out and visually sorting the contents of digestive tracts, including stomachs and intestines of larger animals including fish, birds and sea turtle was the most common approach (e.g., [2, 16-17,

[20]. Tissues are visually sorted under a microscope and potential plastics isolated and counted. In a review of 120 studies, 26% studies used visual sorting of the digestive tract[16]. Dissection alone was used in 13% of studies for assessing the uptake of plastics in the gastrointestinal (GI) tracts of larger organisms or whole bodies of smaller organisms[39]. Furthermore, 53% of 55 studies investigating seafood products relied solely on visual identification[2].

Visually sorting through GI tracts of biota under a microscope has been adopted by the Marine Strategy Framework Directive Technical Subgroup of Marine Litter (MSFD-TSML) who recommend that the entire digestive tract is assessed under a dissecting microscope. This form of investigation is relevant for microplastics >500 µm in size as isolation is limited to the visual acuity of the researcher carrying out the task [42-43]. Dissection and subsequent visual identification of microplastics >500 µm is inexpensive and relatively accurate for GI tracts and whole bodies of some organisms[39]. Smaller biota are harder to process by hand and require additional processing (see **Section 4.2**).

Similarly, sieved sediment and water samples can be sorted visually if the subfractions contain few to no solid, such as sandy beach sediments or surface water samples[44-45]. Samples can be sorted under a microscope and plastics can be isolated. Hanvey and colleagues[18] reviewed sediment sample processing and found that sorting was used for 20/42 reviewed studies, 14 (33%) used sieving as a stand-alone process, whereas six used sieving in a stepwise process (see **Section 4**).

Visual sorting has its advantages that there are no chemical hazards, it can be applied to many sample types and has low cost, however it is unreliable due to human error. Visual sorting of samples is reliant on confirmation of isolated particles using further analytical techniques.

Unfortunately, in earlier studies, visual isolation was often carried out without considering procedural or airborne contamination or QA/QC related to polymer identity[3,18]. Furthermore, there is still ongoing discussion on the appropriate sample size required for representative results from biota. For example, some studies use the recommended number of individuals to compare to long term monitoring data of other contaminants (e.g., 20 individuals per site)[12] whereas OSPAR and MSFD-TSML recommended researchers to use 50 individuals per site and is supported by recent reviews [2,3]. That said, when Markic and colleagues reviewed biota studies dating back to 1972 they found that visual sorting, even with large sample sizes (N>1000) yielded a very low frequency of microplastic occurrence[17]. The number of individuals must be suitable for the study plan and if fewer than 50 individuals are used, the reasoning must be justified. Long-term spatial and temporal monitoring may require a reduced sample size per sampling event due to the intensity of laboratory processing required for monitoring programs[12]. What is clear is that sample sizes with few individuals are not sufficient to provide a realistic estimate of microplastic abundance in biota.

Recommendations: Visual sorting should only be used for particles >500 µm. Smaller size ranges may be considered (>100 µm) providing it is supported by chemical validation of polymers. Visual sorting of biota digestive tracts must be carried out in controlled conditions and procedural controls must be included. An appropriate number of individuals is required, but further investigations into sample sizes should be conducted. Samples should be washed externally prior to opening to remove potential contamination following dissection. All instruments must be cleaned between individuals and visually inspected using a microscope before use. A wet filter can be used next to the dissected organism to estimate airborne contamination if no other method for blanks is feasible. Also, samples of all materials used

during dissection can be collected to provide references for visual identification and polymer confirmation (e.g., fibers from lab coats, kim wipes, fragments from gloves etc.).

4. Stepwise methods

As mentioned above, samples often require additional steps to aid in the isolation of microplastics. Stepwise methods include the use of density to separate out particles from environmental material and digestive agents to remove biological material. Density separation, gravity separation and elutriation can aid in separating microplastics from environmental material whereas digestion procedures can be applied to samples to remove organic and other non-target particles. These methods can be slightly more labour intensive than simple methods, but they have the ability for a better yield of target particles.

4.1 Separation utilising density incl. gravity separation and elutriation

Microplastics have inherent properties which can be utilised to aid their separation from environmental samples. Plastics have different densities which are dependent on polymer type, additive concentration, as well as adsorbed substances and associated organisms. These densities can be used to facilitate their differentiation from organic matter (Table I) . Processes can be as simple as letting a sample stand and allow gravity to enable separation or involve liquids of known density or air to facilitate separation.

Gravity sorting has been utilised in some studies to separate plastics from samples containing large amounts of organic material, although it is probably the least used direct method for separation of microplastics from field collected organisms (4% of 45 studies[39]). This method sees a sample placed into a large cylinder, such as a volumetric cylinder, and allows samples

315 to naturally separate over a known period of time. It is a common method applied by plankton
316 biologists to determine plankton biomass but can be applied to separate less dense plastic
317 particles[34, 46-47]. Buoyant particles, either collected in freshwater or saltwater matrices, can
318 then be syphoned off leaving the biological material for further analysis (**see Section 4 and 5**).
319
320 Liquids of different densities can be used to isolate plastics from samples and has been applied
321 to different sample types to varying degrees[43]. In simple terms, a saturated salt solution with
322 a known density can be carefully mixed with a sample and left to settle. The overlying material
323 is then collected and filtered off for further investigation. Density extraction of plastics from
324 environmental samples can be extremely effective as common environmental samples, soil and
325 sand typically have a much higher density than most polymers making separation efficient. For
326 most marine sediments, solutions with a specific density >1.2 g cm^{-3} are commonly used to
327 extract particles which will have settled to sediment as they are more dense than seawater.
328 Density extractions using seawater are able to recover particles including polyethylene (PE)
329 and polypropylene (PP). By increasing the density of the solvent, it is possible to create a
330 solution where higher density polymers can be collected (Table I). It must be noted that
331 microbial communities may colonize microplastics in certain environments where nutrient
332 levels are high. The biofilms subsequently formed on microplastic surfaces processes can
333 impact the density of these plastic particles[48], complicating isolation and separation.
334
335 Sodium chloride (NaCl) is one of the most commonly used solutions[18] as it is cheap, easily
336 available and eco-friendly. Reagent grade NaCl is recommended as it can achieve slightly
337 higher densities and extract slightly heavier polymers including high density polyethylene,
338 HDPE[49]. Solutions with higher densities, such as sodium bromide (NaBr), sodium iodide
339 (NaI) and zinc chloride ($ZnCl_2$), are able to extract a wider array of particles however these

solutions start to have some considerable environment, health and safety concerns[50]. NaCl is recommended by many researchers due to low costs and low toxicity, including the MSFD-TSML, NOAA and the BASEMAN consortium[50]. However, an assessment of several salt solutions determined NaCl to have the lowest recovery of microplastics of those tested, and it only had significantly higher recovery than tap water alone for four types of plastic[51]. Sodium polytungstate (SPT) and its derivatives have been used by some researchers[52-53]. However, SPT is extremely expensive (although recyclable), can be hazardous, and therefore not a first choice suitable for most routine monitoring[50,54].

NaI should also be considered as appropriate, even though it is expensive, it can be recycled, and the volume used can be reduced when used with aeration in an elutriation column[55-56]. Similarly, $ZnCl_2$ can be used in connection to sediment separators with very high recovery rate and less expensive cost [57-59], but it is extremely hazardous and corrosive. Calcium chloride ($CaCl_2$) can achieve a density (1.4 g/cm³) above NaCl (1.25 g/cm³) but not as high as the other salts, is inexpensive, and is a food additive so it is not hazardous. A less explored salt solution is saturated potassium formate (HCO_2K). The solution has a density of 1.6 g/cm³, is stable and has a low viscosity, relatively cost-effective as it can be filtered and reused[60,61]. Oils have hydrophobic properties which can be utilized to separate plastics from environmental samples and help improve recovery rates[21, 62]. They can reduce the surface tension and helped remove plastics from sediment samples, although recovery rates have varied between studies, 55- 96% [58,63].

Irrespective of the density solution applied, samples must be thoroughly mixed to ensure that polymers detach from the sample matrix. Mixing can be carried out through vigorous manual shaking[64], mechanical shaking[65], or with a centrifuge[66,67]. Stirring can be performed

365 manually or with a magnetic stirrer, or by the process of aeration and inversion[68,69]. As to the
366 length of mixing and stirring required, there is no clear indication of tested and validated
367 durations. Many studies do not provided length of mixing and those which do range from 30
368 seconds to two hours[70]. This should be quantified and assessed in detail. Settling time of
369 samples in density solutions varies within published literature. A range of times have been
370 reported from as short as two minutes[71] and can be up to 24 hours[64]. The duration of settling
371 is heavily dependent on the sample type. Coarse sediments settle out relatively quickly but
372 samples with fine particulate matter require a longer duration. Again, this is a subjective
373 element which should be quantified and assessed in further detail.
374
375 Density separation may require more than one extraction, or using multiple salt solutions (e.g.,
376 [64, 70, 72, 73]. For example, on average only 30.2% (12.5 - 45%) of microplastics were recovered
377 after the first extraction which reached between 88.7% and 100% following four extractions[70].
378 Many separation procedures utilise falcon tubes, volumetric flasks or separating funnels.
379 Although, some laboratory devices have been developed to aid with density separation (Table
380 II).
381
382 Elutriation devices have been developed for use with complex samples including wastewater
383 effluent[74] and sediment[56]. They can be used with or without salt solutions. Most elutriation
384 devices use a liquid which is injected into the bottom of a column allowing the separation of
385 buoyant particles from organic matter and sediments which settle[56]. This method is cheap and
386 efficient for large volumes of sediments and reducing the need for a reduction of sample
387 volume when density extraction is carried out. However, samples can be labour intensive and
388 require pre-separation into to the required size range. Similarly, pressurized fluid extraction
389 using methanol, hexane and dichloromethane can extract microplastics from soils under high

temperature and pressure[75], although limitations include specialised equipment and solvents, high costs, potential environmental pollution and the pyrolysis of particles under high temperature and pressure leading to inaccurate recovery related to the mass of particles.

Density separation is not free of limitations. An understanding of study design and sample types can inform whether density separations should be applied, and which type of separation is most suitable. The environmental matrix may provide indication for potential loss of microplastics during density separation. For example, fouling of microplastics by organic and inorganic material can alter a particle's density and cause microplastics to remain in non-buoyant fractions of density-separated material, thus requiring subsequent manual sorting of microplastics from the non-buoyant material[76]. As mentioned above, performing multiple rounds of density separations reduces the likelihood of loss in the non-buoyant material[70]. Thus, matrices containing high organic content should be processed accordingly. Floatation is also insufficient for small microplastics as the buoyant force is low and bubbles in the solution may cause floatation of non-buoyant particles[39]. The time required to achieve separation will vary with sample type and matrix composition. Differences in suspended solid densities could be exploited to improve partitioning and enhance microplastic aggregation. The application of centrifugation can assist in the isolation of microplastic residues.

Some polymers may be missed in separation more frequently than others, and this will differ depending on the density separation solution applied. For example, polyvinyl chloride (PVC) and polyethylene terephthalate (PET) were observed to have relatively low recovery compared to other plastic polymers tested using NaCl as they are more dense than other polymers tested[51]. The likelihood of missing some other polymers is even higher. Teflon (Polytetrafluoroethylene: 2.1-2.3 g cm^{-3}) is more dense than many solutions used in density

separation, so it is much more likely to be missed than PE (0.91-0.97 g cm^{-3}), a less dense polymer. The density of microplastics will also vary slightly depending on the inclusion of additives[77]. If density separations are used to isolate microplastics, it is important to report the density of the solution used, as this impacts which polymers are likely to be underrepresented in the resulting data. Furthermore, some considerations are needed when working with different salt solutions, for example, NaI can react with cellulose turning them black which complicates visual identification[51]. Density separation should be employed with the understanding that it can be challenging and time-consuming to perform multiple extractions, and that each round of extraction introduces additional routes for potential contamination[78]. Even with additional rounds of extractions, it is difficult to obtain high precision for high density polymers[78].

Recommendations: As with all processing methods, researchers must carry out procedural controls. All salt solutions must be prepared and filtered to remove impurities and prevent the introduction of contamination into samples. More than one extraction is recommended, and samples should be thoroughly mixed following the addition of salt solutions. For studies intending to collect and analyze small particles, size fractionation is recommended before density separation. Floatation should not be performed on small size fractions where bubbles may interfere with the floatation process; however, floatation may be suitable for large size fractions[39]. Taking all the available data into consideration, including operator safety and price of materials, into account, NaI is recommended as the most suitable approach in terms of cost, hazards, extraction efficiency and recyclability. Further augmentation studies to assess the differences between salts are encouraged. As with clean water samples, it is recommended that such samples are filtered on the appropriate filter depending on the aim of the individual study. Sample volume, filter type and pore size should be recorded.

439 **4.2 Digestion of samples containing biological and organic material**

440 Many researchers use digestion to facilitate the isolation of microplastics from biological
441 matrices. This can include soft tissues of biota, or biofilms formed on microplastics which can
442 hamper polymer identification. Digestion has become the most commonly used method in
443 recent years for microplastic isolation from biota tissues[16-17]. Additionally, digestion can also
444 be applied to sediments and water samples containing organic matter[18,79]. Digestion
445 approaches can be used in combination with density separation to further optimise sample
446 extraction, as this process can become more complicated they are included under Complex
447 Methods (**Section 5**)

448

449 Digestion methods may involve some form of pre-treatment to increase efficiency of digestion.
450 For example, mussel soft tissue is often extracted from the shell[80-82], thereby reducing the
451 complexity of the matrix for digestion. Once removed from the shells, mussels can be treated
452 similarly to other soft tissue biota (e.g. fish fillet). Extraction of mussels from shells should be
453 carried out with caution to ensure microplastics are not lost in the shell (i.e. rinse the inside of
454 the shell or examine visually for larger microplastics). Also, extraction of mussels from the
455 shells includes an additional stage of preparation thereby increasing the risk of airborne
456 contamination as the tissues are exposed for a longer period. Railo and colleagues[83] digested
457 both shelled and unshelled mussels and observed consistently higher fiber concentrations in
458 unshelled mussels. Therefore, removing tissue may reduce matrix complexity but additional
459 measures should be taken to assess and reduce airborne contamination from the tissue
460 extraction process. For example, wet filters can be placed in the vicinity of the dissection to
461 assess the rate of airborne contamination coming into contact with the tissue.

462

Many digestion approaches have been developed including bases such as sodium hydroxide, NaOH [83-85] or KOH[14, 83, 86, 87]; acids such as nitric, hydrochloric acid and perchloric acid, HNO_3, HCl, $HClO_4$[84-85,88]; oxidants such as hydrogen peroxide (H_2O_2), peracids, sulfuric acid[56,85]. Enzymatic digestion requires a more complicated procedure[84] and is included as a complex method (**Section 5**). In the following section, advantages and limitations to some of the chemicals used for digestion are presented, including the degree to which chemicals are destructive to various polymer types. Not one method is perfect and outcomes depend on concentrations and molarities of digestive agents, the ratio of solution used per g of tissue, temperature and duration of the digestive process.

Acid digestion: Several approaches using acids to dissolve organic material have been introduced to microplastic research[84,85,88]. However, there are many limitations for acid digestion. Acids can have a high level of destruction of biogenic compounds, between 94-98%, however they can also dissolve polymers. Some polymers have a low resistance to acids and can be degraded at high concentrations and temperatures[89]. Nitric acid and perchloric acid (69% HNO_3 + 70% $HClO_4$) was recommended by ICES[90] but has been seen to have detrimental effects on common plastic polymers, polyamide (PA), polyurethane (PU) and to a lesser extent acrylonitrile butadiene styrene, polymethyl methacrylate and polyvinyl chloride[88]. Heating nitric acid allows samples to be digested 26 times faster[91], unfortunately, these temperatures are high enough to damage weaker polymers[92]. Temperatures exceeding 60°C were observed to melt PE-based microbeads in boiling tests of several microplastics isolated from personal care products[92]. Also, HCl is not recommended since it does not destroy all organic matter, and when used at concentrations with high digestion efficiency, 37% at 25°C, it causes PET to melt[88]. Similarly, the ICES[90] mixture (69% HNO3 + 70% $HClO_4$) led to complete destruction of PA, PU and black tire rubber elastomer; and affected the

structure of other polymers (incl. polymethyl methacrylate, PVC[88]. Subsequent heating to 80°C increased destructive effects of ICES mixture[88].

While some acid digestion methods have proven effective, the simultaneous removal or destruction of some microplastics is cause for great concern. It may lead to the underestimation of microplastics in environmental samples as a result of the destructive nature of acids. As several polymers are impacted by acidic digestion, it should be avoided and used with great caution when alternative methods do not suffice.

Alkaline digestion: Bases provide another method of digestion. NaOH at 1 M has an efficiency of 90%[84] and an increase in molarity and temperature provides a more effective digestion. Potassium hydroxide, KOH, in a 10 M solution can completely remove organic matter[93]. Many different versions of this procedure have been carried out, including standing at room temperature for 2-3 weeks and, speeding up the reaction at 40°C or 60°C in an incubator with continuous rotation[12, 94, 95]. KOH is efficient in digesting fish tissue. A 10% KOH solution was found to have an efficiency ranging from 97.1-98.9% for ground fish tissue at temperatures from 25-50°C[85]. On the other hand, digestion of fish stomachs with saturated KOH solution (1120 g/L H_2O) resulted in a layer of floating black/brown slime[88]. Also, the use of 4 M KOH at room temperature was not sufficient in completely removing plant-based cellulosic material[92]. Alterations to the method such as a 1:1 combination of KOH and NaClO was found to be more efficient in digesting fish tissue than KOH alone[88]. A solution of 10% KOH incubated at 40°C for up to 72h completely digested a whole fish when combined with NaI density separation to separate out the bones[87].

512 However, as with acids, increased temperatures and molarity can discolour and degrade some
513 plastic polymers including polycarbonate, cellulose acetate, PET and PVC[64, 95]. KOH may
514 discolour some plastics when used at excessive concentrations and for prolonged durations
515 [85,92]. Incubated KOH (>50°C) also resulted in reduced recovery of PET particles[85]. It is also
516 not able to completely digest hard materials and fats[88]. More complex protocols have been
517 suggested for better digestion and recovery rates[96].
518
519 Alkaline digestion has been frequently recommended for the digestion of biota; but it's
520 limitations must not be overlooked. Incubating KOH at temperatures >50°C may result in the
521 destruction of some PET particles and recovered PET particles may display altered surface
522 texture[85]. A saturated KOH solution (1120 g/L H_2O) can cause spectral deviations and lower
523 quality Raman spectra relative to undigested polymers[85,88]. Most recently, it was demonstrated
524 reduced temperatures are preferable for KOH (40°C) as at 60°C KOH can destroy rayon[10].
525 The use of KOH to process biota presents an example of how the ratio of KOH to gram of
526 tissue can influence effectiveness. For example, 10 ml of 1M KOH added to samples ranged
527 from 0-10 g was not sufficient to process bivalve tissue[81], whereas between 100 and 300 ml
528 of 10% KOH can be required for samples with a mass <6g[13]. While KOH is effective for
529 digestion of biota, it is recommended in combination with other extraction methods for more
530 complex matrices.
531
532 **Oxidative digestion:** Hydrogen peroxide, H_2O_2, is an efficient oxidizer for use when removing
533 organic material. Although there have been polymeric changes identified such as transparency
534 and shrinking in size when a 30% solution is applied[85, 97]. H_2O_2 has been observed to degrade
535 PA[85], and in some instances its use has lead to the formation of a foam and a reduced extraction
536 efficiency[56,85]. Temperature and incubation period will influence the efficiency of peroxide

digestion[98]. Incubation of H_2O_2 at 50°C increased digestion efficiency but created additional white particles in the solution[85]. Furthermore, H_2O_2 can become unstable over time, and stability can vary from batch to batch[99], although there has been some discussion over this [100,101]. A reduced strength, 10%, solution is recommended[50] and this method can be optimised using an iron catalyst (see **Section 5.2**).

Recommendations: When working with digestion methods, researchers must carry out procedural controls. All digestive agents must be prepared and filtered to remove impurities and prevent the introduction of contamination into samples. All methods are recommended to be tested for extraction efficiencies in laboratories before and during use as efficiencies can vary between personnel. Alkaline digestion is recommended for biota samples, but temperatures and molarity should be kept low. KOH in a 1-2 M or 10% is recommended; although some method alteration will be needed to digest complex samples (**Section 5.2**). Regardless of the digestion treatment, incubation should be used with caution. It is not recommended to apply temperatures above a threshold of 40°C. This is the threshold for samples that may contain weaker polymers, including rayon. H_2O_2 as a stand-alone oxidative digestion method requires low temperatures and a reduced strength. As the procedure is less straightforward, it is recommended that H_2O_2 methods are adapted to use an iron catalyst to work in reduced temperatures (see **Section 5.2**). All of these procedures can be applied before or after density separation. Acid digestion has several limitations and many polymers can be affected therefore it is recommended that they are avoided, and only used when alternative methods are not available. As with all other previously discussed samples, it is recommended that samples are filtered on the appropriate filter depending on the aim of the individual study. Sample volume, filter type and pore size should be recorded.

5. Complex methods

Samples from wastewater treatment plants are probably the best example of complicated matrices. They often require a number of treatment steps, can be labour intensive and costly. Enzymatic digestion often requires multiple treatments with different enzymes and can take days to complete[84,102]. Similarly, wet peroxide oxidation (WPO) can be controlled at a lower temperature with an iron catalyst (Fe^{2+}) but is labour intensive. In the following section, the advantages and limitations of methods which require multiple steps to work with complicated matrices are presented. As with previous section, not all methods are appropriate for every matrix and the complexity of methods will heavily depend on the organic content of the sample.

5.1 Enzymatic digestion

Enzymes were introduced to microplastic processing in 2014 as an alternative to more aggressive digestion methods as they are less hazardous, can be selected to target particular biological materials for breakdown and do not impact microplastics contained within the sample[80,81,84]. Enzymatic digestion protocols may be preferential due to the biological specificity of enzymes. However, using enzymatic digestion to target specific types of organic matter for digestion will either require some knowledge of the type of organic matter present in the matrix, or a combination of several enzymatic digestions to prove effective[102]. Enzymatic digestion with Proteinase-K was found to have an efficacy of 88.9 ± 1.5% in digesting biota-rich seawater samples[84]. The resulting filter contained a thin film of glutinous material post-digestion, though microplastics were deemed visible through the film of biological material[84]. Some biological materials are not broken down by Proteinase-K, including shell, carapace, wood and other types of anthropogenic litter[84]. The method was adapted using $CaCl_2$ and H_2O_2 to digest fish tissue with a 97% recovery rate[62]. However, calcium deposits were observed which can complicate characterization and this method

586 requires grinding with a mortar and pestle[84] which may cause fragmentation of MPs. Further
587 fragmentation of microplastics will affect estimates of the quantity of MPs. There have been
588 further attempts to assess digestive efficiencies of additional enzymes as Proteinase-K is
589 relatively costly.
590
591 Other enzymes include trypsin, collagenase, papain[80] and commercially isolated pancreatic
592 enzymes (PEz)[81]. No difference in efficiency was observed among trypsin, collagenase and
593 papain, and the efficiency in digesting mussel soft tissue was determined to be approximately
594 86%[80]. PEz was slightly more efficient in digesting mussel soft tissue[81]. More complex
595 sample matrices may include a wide variety of organic matter and tissue types, such as bone,
596 chitin and plant matter. Additional enzymes have been assessed for efficiency in the breakdown
597 of more complex sample matrices. Protease, cellulase and chitinase have been assessed in
598 combination with optional additional enzymes (lipase and amylase), H_2O_2, SDS and a $ZnCl_2$
599 density separation[62,102]. While this protocol was effective (sample mass reduced by 98.3%),
600 the protocol requires multiple phases of digestion, several materials and up to 16 days to
601 complete. Even though there is no requirement for multiple sample preparation steps[84],
602 samples which are processed with enzymes used in a combination require longer processing
603 times. Furthermore, each additional step has the potential to introduce procedural
604 contamination.
605
606 *Recommendations:* Enzymatic digestions are complex and time-consuming procedures which
607 are a viable option for digestion depending on the complexity of the matrix, time allotted for
608 digestion, access to financial resources and materials. Researchers must assess the suitability
609 for enzymatic procedures when designing their studies as enzymatic digestion may require
610 some prior knowledge of the types of organic materials to be digested. Even though enzymatic

procedures can eliminate the requirement of preprocessing steps, they can be a lengthy procedure. Enzymes reduce the need for pretreatment but can also be applied after density separation. Enzymatic digestion is not recommended for high sample throughput, monitoring studies, and is more suited to analytical investigations using fewer samples or projects supported with adequate finances. As with all other methods, researchers must carry out procedural controls. This is especially important when there are multiple steps carried out over several days. All enzymes must be prepared and filtered to remove impurities and prevent procedural contamination. Extraction efficiencies should be investigated before and during use. Incubation should be used with caution to ensure weaker polymers are not affected, an upper threshold of 40°C is recommended. It is recommended that samples are filtered on the appropriate filter depending on the aim of the individual study. Sample volume at all treatment steps, filter type and pore size should be recorded.

5.2. Fenton's reagent (H202 with Fe^{2+})

Wet peroxide oxidation (WPO) is an oxidative digestion method which can be carried out on its own, using solely H_2O_2[103]. However, the reaction requires elevated temperatures which can damage plastic particles[64, 104]. An alternative approach is to carry out WPO in the presence of an iron catalyst (Fe^{2+}) to lower the reactive temperature. Fenton's reagent utilises Fe^{2+} to initiate and catalyze H_2O_2 decomposition, leading to the *in-situ* generation of hydroxyl and hydroperoxyl radicals. Working at lower temperatures preserves weaker polymers ensuring more accurate data acquisition. This method, although complex to carry out, has been shown to be effective when working with complex and organic rich samples. It can be carried out at low costs and has shown reduced sample preparation times when compared to other methods[105] and it is an effective processing tool when large samples cannot be processed with more simple processing procedures. Fenton's can be used to isolate microplastics from organic

635 rich samples, including wastewater[105], sediments[106], sludge[64], and biota[107] can be used
636 effectively as a pre-treatment for FPA-μFTIR[35]. The reagent has little to no impact on MPs,
637 including surface chemistry and particle size [64, 105]. Fenton's can also be used in combination
638 with density separation [64,104].
639
640 Fenton's reagent and WPO is not without its limitations. Some microbeads tested in an
641 assessment of chemical digestion methods were significantly impacted by Fenton's reagent[92].
642 Boiling tests suggest that the application of heat <60°C (or heat generated by the chemical
643 reaction) leads to loss of some types of microbeads, thus requiring the use of an ice bath to
644 maintain a temperature below this critical threshold throughout the procedure[92]. The use of an
645 ice bath to maintain temperature below a critical threshold requires additional labour and time
646 spent observing the reaction to prevent the loss of some MPs. Fenton's has also resulted in the
647 discoloration of PE and PA[26]. Discoloration of microplastics may affect visual identification
648 of the microplastics if color is of interest.
649
650 *Recommendations:* Fenton's reagent is effective in digesting samples rich in organic matter
651 that may be challenging to digest using alkaline or oxidative digestion alone. Suitable samples
652 include complex matrices, such as samples from wastewater treatment plants, where organic
653 content is high and sample volumes are large as alternative methods may be too costly or time-
654 consuming. Methods requiring many processing steps have many opportunities for the
655 introduction of contamination. As with all other methods, researchers must carry out procedural
656 controls and all reagents must be prepared and filtered to remove impurities. Extraction
657 efficiencies should be investigated before and during use due to the variety of organic matter
658 that may be present in complex samples. The reaction generates heat, even with the addition of
659 Fenton's reagent, so the temperature should be monitored throughout the reaction and an upper

660 threshold of 40°C is recommended to reduce destructive effects on weaker polymers. It is
661 strongly recommended that the reaction be performed in an ice bath as the temperature may
662 increase rapidly and become volatile. Due to the potentially volatile reaction, samples must be
663 monitored closely requiring more labour than some alternative digestion procedures. This
664 procedure should be performed with the understanding that sample loss may occur should the
665 reaction become volatile, and discoloration of microplastics may occur[26,92]. Again, it is
666 recommended that samples are filtered on the appropriate filter depending on the aim of the
667 individual study. Sample volume at all treatment steps, filter type and pore size should be
668 recorded.

669 **5.3 Combination methods**

670 All of the previously mentioned methods can be used in combination. For example, WPO can
671 be carried out before or after density separation. This has been successfully applied for samples
672 collected from a wastewater treatment plants and soils where digestion was performed using
673 H_2O_2 and NaClO followed by density separation with $ZnCl_2$[108,109], or NaCl density separation
674 followed by H_2O_2[110]. An alternative approach was to use NaI before and after Fenton's reagent
675 on soils and sludge samples[64]. Extraction efficiencies varied between 80 - 95.6%, 67 - 100%
676 and 79 - 98% for H_2O_2 and NaClO followed by $ZnCl_2$, NaCl followed by H_2O_2 and for both
677 combinations of NaI, respectively.
678

679 **6. Recommendations and future work**

680 It is evident that there is no one-size-fits-all method for the isolation of microplastics from
681 environmental samples. Different matrices require variations in which methods are applied but
682 they can be divided into three categories: simple methods, stepwise methods and complex

683 methods. Researchers are encouraged to rigorously assess the suitability of methods based on
684 the complexity, cost and processing time. Figure 1 presents a summary of methods by sample
685 type. Researchers are reminded that throughout sample processing and data analysis quality
686 control and quality assurance steps must be followed and reported[1,5]. All methods are
687 recommended to be tested in laboratories for extraction efficiencies before and during use as
688 efficiencies can vary between personnel. Researchers should have a clear protocol and be
689 prepared for differences between sample types.

690 **6.1 Liquid samples:**

691 Samples collected for the assessment of microplastics in liquid matrices can range from bottled
692 beverages to sewage influent at wastewater treatment plants. Therefore a range of approaches
693 are required:

694 - *Simple:* Samples with little organic content can be filtered directly onto chosen filters
695 for visual and chemical analysis. These include tap water and other beverages. Effluent
696 and some offshore waters may be processed with filtering only, but an assessment of
697 organic content must be made prior to filtration to ensure filters do not clog and organic
698 particles obscure microplastic quantification.
699 - *Stepwise:* Samples with some biological material will require some processing to isolate
700 microplastics. Such samples should be digested and the use of KOH is recommended
701 at 40°C. Samples may instead be separated by density using a salt solution where NaI
702 is recommended. Alternative salts may be more suitable for specific research teams
703 therefore limitations of the chosen salt should be clearly stated when reporting findings.
704 - *Complex:* Influent should first be disinfected then processed using WPO with Fenton's
705 reagent. Samples can be filtered after digestion or further processed with density

extractions if required. Researchers are encouraged to use suitable sample sizes and replicates.

6.2 Sediment samples:

Sediment matrices can range in organic matter content and therefore a number of different approaches are required to isolate MPs:

- *Simple:* Samples can be separated mechanically using either sieving, magnetism and or electrostatics, and then visually sorted. Beach sediments with large sample sizes can be sieved but a lower size limit must be established if samples are processed in the field. Researchers are reminded that rigorous sieving may further fragment brittle particles and caution is advised.
- *Stepwise:* Sediment with low organic matter content such as benthic sediments can be separated with density separation. This also facilitates the extraction of smaller microplastics from beach sediments. NaI is recommended for all sediment types as it can isolate a wider range of particles. If researchers choose to use alternative they are encouraged to list the limitations and report extraction efficiencies.
- *Complex:* Samples with high organic matter, including some freshwater sediment, biosolids and sludge from wastewater treatment processes will need more than one procedure to isolate MPs. Organic matter removal with Fenton's reagent and density separation should be used in combination. Researchers are encouraged to use suitable sample sizes and replicates.

6.3 Biota samples:

- *Simple:* Large organisms, such as marine vertebrates, can be dissected and their whole digestive tracts visually sorted for microplastics >500 µm. This lower size limit should

be observed as below this limit there is huge variation between researchers, if lower size categories are extracted they must be confirmed with further analytical methods.

- *Stepwise:* Biota tissues, such as fish fillets or whole soft bodied organisms, can be digested with KOH at 40°C. This is a widely recommended method and is encouraged. If modifications (e.g. extraction of soft tissue from shelled organisms) or other methods are used the limitations must be understood and extraction efficiencies should be reported.
- *Complex:* Enzymes are not cost efficient for most monitoring programs but if affordable they are encouraged providing researchers assess all steps of procedural contamination. Fenton's reagent can be used on samples that cannot be digested using KOH and density separation can be introduced if digestion results in incomplete isolation.

6.4. Other matrices of interest:

Wastewater treatment plants: Many samples from wastewater treatment plants have been mentioned above. It is important to note that within a single WWTP there may be many different sample types which will all require different sample processing. Initial screenings can employ a combination of visual, tactile and physical properties to assess samples. So microscopic examination coupled with simple tactile technique can be a very effective and reliable way to assist with screening plastic residues in complex matrices. It is imperative that personal protective equipment, biohazard protocols and disinfectants are carried out on these types of sample

Road run-off: Research has begun to look at road derived microplastics[111,112], however few methods have shown their efficiency. Particles are expected to be generated from road paint, tire wear, plastics recycled into asphalt and salt applied to roads in winter[113-115]. Microplastics

753 in road samples tend to have high densities which will complicate density procedures. Samples
754 should be free of organic matter before filtration, making working with this matrix a stepwise
755 process. Samples containing a large proportion of sediment may make the differentiation
756 between microplastics and sediment tricky therefore increasing pressure on visual analysis. All
757 particles should be analysed with further analytical techniques, but problems with FTIR exist[9].
758
759 **Air.** Monitoring the atmosphere for microplastics, namely microfibres, is interesting for
760 researchers looking to understand the potential source for intake of microplastics by
761 humans[116] or the role of the atmosphere in transporting particles[117]. Currently data
762 surrounding atmospheric microplastics is sparse but attempts to quantify microplastics in the
763 atmosphere have emerged[91,118,119]. Microplastics and passive samplers allow large air volumes
764 to be filtered and analysed, although samples may contain high levels of organic matter and
765 may require complex digestion processes.

766 **6.5 Contamination monitoring**

767 Use of appropriate filters or greased surfaces can be used to trap and collect airborne
768 microparticulates and microfibers in dust from air. The use of fibrous media for filtration media
769 that are prone to developing electrostatic charges may not be suitable for microplastic or
770 microfiber collection. In some cases, microfilters have been observed to have a repulsive effect
771 on airborne fibers[120]. Some of these static dynamics might be controlled by adequate
772 grounding of filtration assemblies. All methods should use appropriate monitoring of
773 procedural and airborne contamination and we encourage readers to refer to the parallel focal
774 point review[5].

6.6 Future research

There is still room for improvement for optimising isolation and separation techniques within this research field. Further method development to work with smaller sized particles is welcomed. Currently, working with smaller sized particles can be tricky. Density separations are ineffective as particles between 1 nm and 1 µm are not generally subject to gravity or density partitioning and can remain perpetually suspended in the liquid phase through Brownian action in solution. Methods which facilitate automatic separation and analysis through a single process, eradicating human error and contamination introduction are urgently required.

7. Conclusion

One of the biggest shortcomings of the extensive microplastic data generation in recent years are the varied methodological approaches for separation and isolation of particles from different matrices. Each type and method possess their own limitations and advantages. Applied methods can affect density, size, morphology and polymeric composition of microplastics which can impact final results. A clear understanding of methodological constraints is vital when selecting an isolation protocol, as this will provide an insight on how results may be affected. Potential constraints must be reported alongside results to ensure any impacts can be taken into consideration when interpreting and comparing across studies. It is likely that harmonised methods will differ based on the sample matrix and complexity as no single method fits all matrices.

In developing these recommendations, we wanted to allow for the development of new or improved techniques to reduce potential impacts on microplastics. Further research is required to improve upon existing methods or develop new methods that also take into consideration

the time and effort required to extract samples, the cost of each procedure, the simplicity of the method (allowing for method harmonisation) and the potential for the introduction of contamination. As shown here, isolation of microplastic particles presents a significant challenge for many researchers in the field of microplastics. New or improved methods will significantly advance research efforts will allow for long term monitoring, extraction of challenging sample matrices and facilitate comparison among studies.

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Figures and Tables:

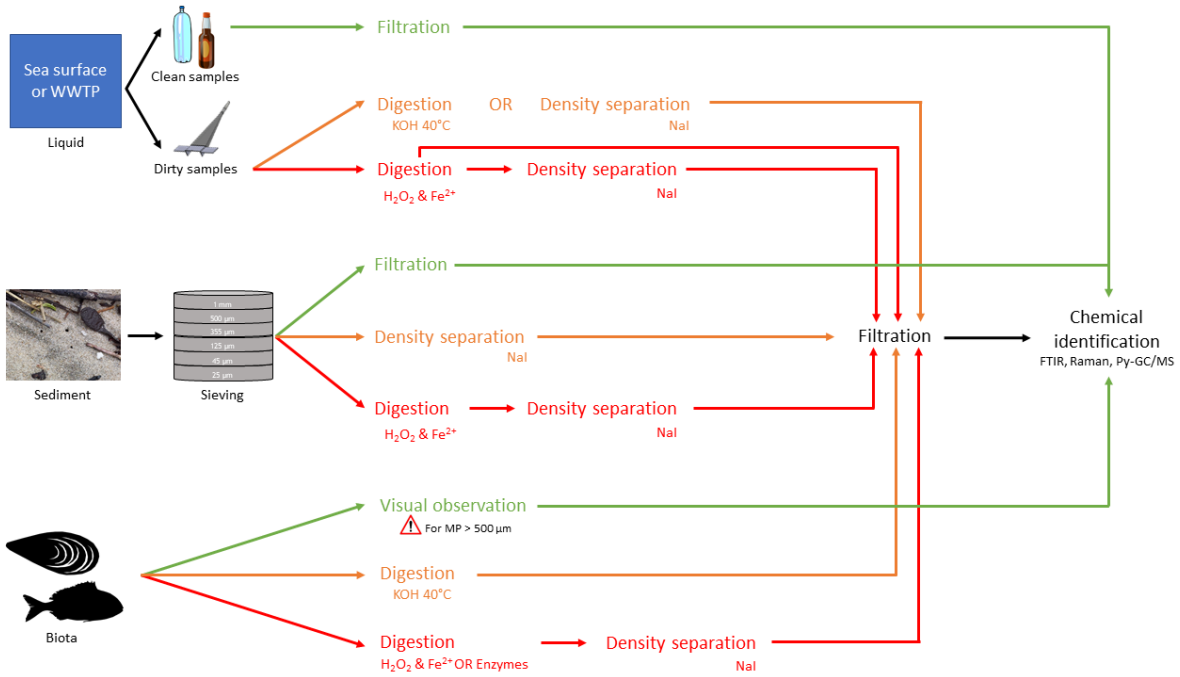
Figure 1. Recommended processing steps for the isolation of microplastics from different matrices. Coloure lines represent Simple (Green), Stepwise (Orange) and Complex methods (Red).

Table I. Isolation abilities of different density solutions compared to some of the common polymers. Note that polymer density can be affected by additives (Crawford and Quinn 2017, Prata et al. 2018, Enders et al., 2015).

Table II. Efficiencies of different sediment separators and novel methods beyond density separation.

Table SI 1. Summary of the reviews included in assessment of isolation methods for microplastics

Table SI 2. Summary of sample matrices divided into broad categories of Liquid, Sediment, Biota, Air and other. *depending on the organic matter content may require further processing.



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1363 Figure 1. Recommended processing steps for the isolation of microplastics from different

1364 matrices. Colour lines represent Simple (Green), Stepwise (Orange) and Complex methods

1365 (Red).

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1367 Table I. Isolation abilities of different density solutions compared to some of the common polymers.
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			Buoyancy in freshwater (FW)	Density solutions (xx/g cm^{-3})							
Abbr.	Polymer	Density		FW (1.0)	NaCl (1.2)	$CaCl_2$ (1.30-1.35)	$KHCO_2$ (1.5)	NaI (1.6)	$ZnCl_2$ (1.6-1.7)	$ZnBr_2$ (1.7)	SPT (2.94-3.10)
PP	Polypropylene	0.85-0.92	Positive	+	+	+	+	+	+	+	+
LDPE	Low-density polyethylene	0-89-0.93	Positive	+	+	+	+	+	+	+	+
EVA	Ethylene Vinyl Acetate	0.94-0.95	Positive	+	+	+	+	+	+	+	+
HDPE	High-density polyethylene	0.94-0.98	Positive	+	+	+	+	+	+	+	+
(E)PS	(expanded) Polystyrene	0.01-1.06 (1.04-1.1)	Negative	-	+	+	+	+	+	+	+
Acrylic	Acrylic	1.09-1.20	Negative	-	+	+	+	+	+	+	+
PA	Polyamide	1.12-1.15 (1.02-1.05)	Negative	-	+	+	+	+	+	+	+
PA 66	Nylon 6,6	1.13-1.15	Negative	-	+	+	+	+	+	+	+
PM(M)A	Polymethyl (meth)acrylate	1.16-1.20	Negative	-	+	+	+	+	+	+	+
PC	Polycarbonate	1.20-1.22	Negative	-	+-	+	+	+	+	+	+
PU	Polyurethane	1.20-1.26	Negative	-	+-	+	+	+	+	+	+

PVA	Polyvinyl alcohol	1.19-1.31	Negative	-	+-	+-	+	+	+	+	+
PET	Polyethene terephthalate	1.38-1.41	Negative	-	-	-	+	+	+	+	+
PVC	Polyvinyl chloride	1.38-1.41	Negative	-	-	-	+	+	+	+	+
POM	Polyoxymethylene	1.41-1.61	Negative	-	-	-	+-	+-	+	+	+
PTFE	Polytetrafluoroethylene	2.10-2.30	Negative	-	-	-	-	-	-	-	+

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1371 Table II. Efficiencies of different sediment separators and novel methods beyond density separation.
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Device	Principle	Sample type (volume)	Size of particles extracted	Density solution	Polymers	Reported efficiency	Reference
Sediment-Microplastics Isolation Unit (SMI)	Density flotation	Intertidal (50g)	100-2000 µm	$ZnCl_2$	PE, Nylon, PVC, LDPE	92 - 98%	Coppack et al., 2017
Elutriation column	Elutriation, aeration and centriguation	Coarse (500ml)	<1mm	H2O2 NaCl NaI	PVC, PE	97-98%	Claessens et al., 2013
Elutriation column	Elutriation with aeration followed by density separation	Sediment not described (40g)	1.0 (L) × 4.0 (W) × 2.0 mm	$ZnCl_2$	HDPE, PVC	80-94%	Mahon et al., 2016
Munich sediment separator (MPSS)	Aeration with a ball valve	Fine (6 kg)	1-5 mm <1 mm	$ZnCl_2$	PVC, PA, PS, PET, PC, PP, HDPE	95.5 - 100%	Imhof et al.,2012
Munich sediment separator (MPSS)	Aeration with a ball valve	Marine and organic rich sediments	460 µm	$ZnCl_2$	PET	13-39%	Zobkov and Esiukova 2017
Electrostatic separator	Utilizes electrostatic nature of particles	Freshwater, Beach (150g)	63-5000 µm	n.a.	HDPE, LDPE, PET, PP, PS, PVC, PMMA, PA, PE, tire wear	<100%	Felsing et al., 2018
Pressurised fluid extraction	Pressurised fluid extraction	Municiple waste and soil	50µm, 1 mm	n.a.	HDPE, PVC, PS, PET, PP	84-111%	Fuller and Gautam 2016
Magnetic extraction	Hydrophobic Fe nanoparticle bind to plastic allowing magnetic recovery	Sediments	200µm-1mm	n.a.	PE, PS, PU, PVC,PP	78-84%	Grbic et al., 2019

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1375 Table SI 1. Summary of the reviews included in assessment of isolation methods for microplastics

Reference	Review focus	Date range	Critical review
Dehaut et al., 2019	Seafood	n.r.	No
Hu et al., 2019	Wastewater systems	n.r	Yes
Koelmans et al., 2019	Freshwaters and drinking water	1972- August 2018	Yes
Markic et al., 2019	Ingestion by marine fish	1972- January 2019	Yes
Nguyen et al., 2019	Complex environmental samples	2012-2018	No
Prata et al., 2019a	Water and sediments	1972- May 2018	Yes
Stock et al., 2019	Methods	n.r.	No
Sun et al., 2019	Waterwater treatment plants	1972-2018	No
Zhang et al., 2019	Methods	n.r..	No
Hermsen et al., 2018	Biota	1972- June 2017	Yes
Rezamia et al., 2018	Aqautic environmetnst and biota	n..r	no
Silva et al., 2018	Not extrensive	2015-2018	no
Hanvey et al., 2017	Mps in sediments	2003-2016	Yes
Lusher et al., 2017	Biota	1972-2017	Yes
Miller et al., 2017	Recovery of MPs from marine samples	1972- April 2017	No
Renner et al., 2017	Opinion and overview of methods for MP analysis	2015-2017	No

Qiu et al., 2016	Methods: all matrices	n.r.	No
Rocha-Santos and Durate et al., 2015	Methods: all matrices	n.r.	No

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1377 Table SI 2. Summary of sample matrices divided into broad categories of Liquid, Sediment, Biota, Air and other. *depending on the organic matter
1378 content may require further processing.

		Simple	Stepwise	Complex
Liquid	Clean water	x		
	Beverages	x		
	Offshore waters	x	x*	
	Freshwater	x	x*	
	Effluent	x	x*	
	Influent			x
Sediments	Beach	x	x*	
	Intertidal/Benthic		x	
	Freshwater		x*	x

	Soil			x
	Sludge			
Biota	Digestive tracts	x		
	Soft tissue		x	
	Fish fillets		x	x
Air		x	x	

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