Evidence of underestimation in microplastic research: a meta-analysis of recovery rate studies.

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1. Abstract

Research on microplastics in the environment is of high interest to many scientists and industries globally. A key to the success of this research falls with the method used to isolate the microplastic from environmental media. However, with microplastics now being found in new complex media, many multifaceted methods are developed to research the quantities of these pollutants. To validate new methods, recovery studies can be undertaken by spiking the test medium with known quantities of plastics. The method is typically run as normal, and the recovered plastics counted to give a recovery rate. A current issue in this field is that methods are rarely or poorly validated in this way. Here, we conducted a meta-analysis on 71 recovery rate studies. We found sediment was the most studied medium and saline solutions were the most used reagents. Polyethylene and polystyrene were the most used spiking polymers, which is relevant to the most common polymers in the environment. We found that recovery rates were highest from plant material, whole organisms and excrement (>88%), and lowest from fishmeal, water and soil (58-71%). Moreover, all reagents but water were able to recover more than 80% of the spiked plastics. We believe we are the first (to our knowledge) to provide an overarching indication for the underestimation of microplastics in the environment of approximately 14%, varying with the methods used. Furthermore, we recommend that the quality, use and reporting of recovery rate studies should be improved to aid the standardisation and replication of microplastic research.

1.1. Keywords

Microplastics, Recovery Rate, Method, Validation, Standardisation, Underestimation

2. Introduction

Currently, global microplastic research has a high public profile, is of high importance and includes many research avenues within one field. Crucially, it is primarily focused on the amount of these pollutants in different environmental matrices. For example, microplastics have now been found in wastewater and sludge from China (Li *et al.*, 2018), Finland (Railo *et al.*, 2018) and Australia (Ziajahromi *et al.*, 2017), in soil samples from Chile (Corradini *et al.*, 2019) and Switzerland (Scheurer and Bigalke, 2018), and in aquatic sediments from Belgium (Claessens *et al.*, 2011), England (Horton *et al.*, 2017) and the Arctic (Kanhai *et al.*, 2019). Research has also focused on the sources of this pollutant. For instance, it has been estimated that a single washing machine load of clothing could release approximately 700,000 microplastic fibres into waste water systems (Napper and Thompson, 2016), and similarly one use of a face wash could release up to 94,000 microbeads (Napper *et al.*, 2015). Some of this research has resulted in policy change, like the banning of facial cleansers containing microbeads (Guerranti *et al.*, 2019).

However, a key to successful microplastic research lies within the method used to extract these small pollutants. Researchers in this discipline face criticism for their lack of standardisation and comparative approaches (Underwood *et al.*, 2017). Methods can vary significantly; Density separation methods use many different saline solutions such as sodium chloride (NaCl) (Nuelle *et al.*, 2014, Pagter *et al.*, 2018, Quinn *et al.*, 2017), zinc chloride ($ZnCl_2$) (Imhof *et al.*, 2012, Wang *et al.*, 2018) and sodium iodide (NaI) (Nuelle *et al.*, 2014, Roch and Brinker, 2017); various acids, bases and oxidising agents have been used (Bianchi *et al.*, 2020, Schirinzi *et al.*, 2020, Yu *et al.*, 2019). Enzymes (Catarino *et al.*, 2017, Loder *et al.*, 2017), and oils (Radford *et al.*, 2021) are also being utilised, with or without the use of additional reagents such as dispersants. Many of the methods are used in combination – for example combining in sequence oxidising agents with density separation methods. Also, new equipment and devices are being developed to assist in the extraction of microplastics (Coppock *et al.*, 2017, Imhof *et al.*, 2012, Nakajima *et al.*, 2019). However, with some methods inaccessible due to cost or limited access to equipment, this is not always achievable. For example, spectroscopic equipment such as Fourier transform infrared (FTIR) and Raman spectroscopy, used to identify polymers often come at very high cost, with some systems priced between US$200,000 - 300,000 (Primpke *et al.*, 2020). Similarly, saline solutions used in density separations can be expensive when needed in large quantities. For instance, NaI may cost C$90 for just 100ml and $ZnCl_2$ can cost C$922 for just 30 litres (Crichton *et al.*, 2017). More complex matrices such as fishmeal (Gündoğdu *et al.*, 2021, Thiele *et al.*, 2021) and terrestrial soils (Corradini *et al.*, 2019) are being found to contain microplastics, thus many multifaceted methods are being developed and published to cater for this, or current methods are being developed further to combat current limitations.

For this suite of methods to be replicated and used by others, they should be verified and validated[1] in some way. However, method verification/validation is not as common as it may need to be in this developing field of research. For example, Underwood *et al.* (2017, p.1337) stated *"Methods used have not been analysed experimentally to determine the relative importance of the different thermal, physical and chemical techniques on rates of recovery and dissolution of different sizes and polymers of microplastic"*. To verify and validate new methods, so called "recovery rate" studies are sometimes undertaken alongside the main microplastic extraction. This entails 'spiking' the studied matrix with known types and configurations of spiking polymers, running a method considered for use in further study of that matrix, and then establishing the amount of spiking plastics recovered. This provides an indication of how effective the method is at extracting plastics from a specific matrix, typically as a percentage recovery rate. When implemented effectively, this could provide an insight in to how well a method could perform compared to others. Further to this, a recovery below 100% could suggest how using a certain method may underestimate the amount of microplastics in a matrix, and a recovery over 100% could show a potential for overestimation.

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[1] *Method verification* is an assessment that focuses on how a specified analytical test procedure is suitable for its intended use under authentic experimental conditions. *Method validation* is an evaluation process on the performance characteristics of a recognised analytical procedure via laboratory studies with all performance characteristics meeting the anticipated analytical applications. An analytical method should be scrutinised from a range of positions to prove that the arising test result is reliable, replicable authoritative and can be appropriately applied to its intended purpose.

This meta-analysis aims to identify the recovery rates from multiple studies, and critically review how they vary when using different methods to extract microplastics from a wide range of matrices. The analysis is the first (to our knowledge) to provide an estimate of how much microplastic research may be under or over-estimating current levels of microplastics based on the methods utilised and the recovery rates found. Finally, recommended reporting criteria are provided for future recovery rate studies to allow for improved validation and simpler replication.

3. Method

3.1. Methodology for literature search – Identification

The methodological approach of this meta-analysis was carried out by following the guidance of the PRISMA 2020 flow diagram (Page *et al.*, 2021) (Figure 1). Sections of the PRISMA 2020 checklist (Page *et al.*, 2021) were also complied and followed, with the inclusion of the eligibility criteria, information sources, a full search strategy, study selection, the data collections process and the data extracted.

During January 2021, a database search was undertaken using Web of Science, Scopus, GreenFILE and PubMed search engines. The search was conducted using the following search terms:

“recovery rate” OR “recovery efficiency”

AND

microplastic OR plastic OR nanoplastics

AND

extraction OR identification OR validation

The search was filtered further to only include peer-reviewed articles; however, no limit was put on date of publication. Following from the database searches, 890 records were found and a reference manager (Endnote) was used to organise the articles. Duplicates were removed, leaving 791 papers to be screened for suitable titles and abstracts (Figure 1).

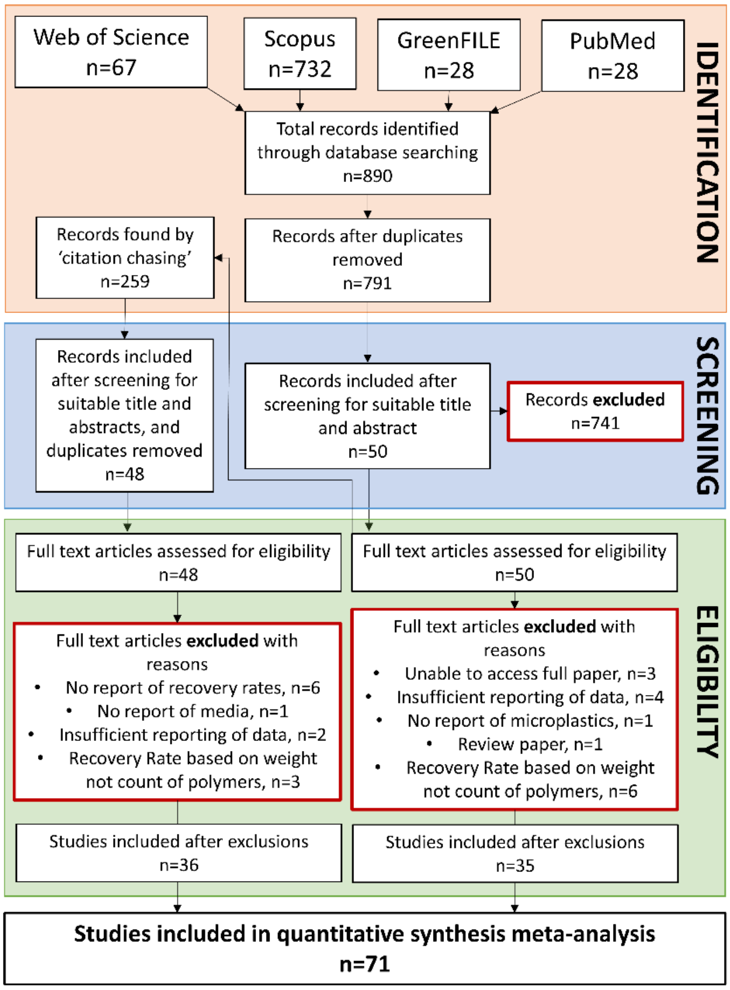

Figure 1. ***Literature search flowchart.*** *Including literature identification, screening and eligibility process. Including number of articles found and/or excluded at each stage.*

3.2. Criteria for inclusion – Screening and Eligibility

During the screening for suitable titles and abstracts, certain inclusion criteria were applied. Articles incorporated must include plastics that are either below 5 mm (large microplastics) or plastics between 1 µm-1 mm (microplastics) (International Organization for Standardization, 2020). The titles and/or abstract must also indicate that the method used was validated in some way, either by including a recovery rate or using another term such as efficiency. The media tested in the studies were not limited.

Following on from screening for suitable title and abstracts, 50 full text articles were assessed for eligibility. Articles were excluded for the following reasons: No access to the full paper and data, insufficient reporting of data, no report of using microplastics in the study or spiking trial, no report of recovery rates, any review papers and recovery rates calculated by weight difference, not count.

Due to recovery rate studies being often undertaken as a side project alongside microplastic extraction/identification studies, many recovery rate studies may have not been identified during screening titles and abstracts. Therefore, “citation chasing” (Barrett, 2005) was carried out to counterbalance this. When reading the full text articles, suitable references were identified and pooled. 259 potentially suitable articles were identified and managed within the reference manager. After duplicates were removed and abstracts and titles were screened for the same inclusions mentioned previously, 48 articles were selected to be checked for full paper eligibility.

After all articles were assessed for eligibility, including those found by citation chasing, 71 papers were included for the meta-analysis.

3.3. Data Extracted

Data extracted from the articles included basic information such as the authors’ names, the journal name and date of publication. Other material extracted included a short detail on the method used, the test media, the types of reagent used, the spiking microplastic polymer types, the spiking microplastic shapes, the spiking microplastic sizes and the recovery rates found.

The quantitative analysis was further conducted in Microsoft Excel and RStudio (version 3.6.1). The microplastic size category was further subdivided into MP (microplastic) (any microplastics between 1µm and 1mm) and LMP (large microplastic) (any microplastics between 1mm and 5mm) (International Organization for Standardization, 2020). Similarly, the test reagents were categorised into oils, alcohols, dyes, acids, oxidising agents, bases, saline solutions, water, enzymes and solvents. The test media were categorised into plant material (vegetal plant material), air, fishmeal, biological material (biofilm), excrement, whole organisms, tissues of organisms, soil (horticultural/agricultural soil, farmland soil, compost), wastewater effluent/sludge, water and sediment (marine and freshwater sediment/beach and river sediment). All information on studies included is provided in the supplementary material (Table S1).

Due to the lack of control samples used in recovery rate studies, and lack of reported sample sizes for the recovery rate part of a study, a sample effect size was not able to be calculated. However, this limitation will be examined in the discussion.

3.4. Quality of selected studies

The quality of the selected studies in this analysis are assessed by ranking each study subjectively from 1 to 5 (1 being low quality, 5 being high quality). The criteria (Table S3) are adapted from Porter *et al.* (2014) and Fidai *et al.* (2020), and is based on the quality of the recovery rate method, comprising of the inclusion of the test media, the reagent used and information on the spiking plastics used. Furthermore the criteria included whether the studies have potential for replication and the clarity and presentation of results.

4. Results

4.1. Summaries of studies included in meta-analysis

4.1.1. Quality of selected studies

The purpose of reviewing the quality of included studies is to highlight the areas of recovery rate studies which need improvement. The mode score for the 71 studies included in this meta-analysis is 4. With only 14 studies achieving the rank of 5, it shows there are many limitations of recovery rate studies to be discussed.

4.1.2. Media and reagent used

A total of 12 different types of media were studied, including fishmeal, plant material, air, biological material, excrement, whole organisms, tissues of organisms, soil, wastewater/sludge, gastrointestinal tracts, water and sediment (for a breakdown of these media categories see section 3.3 Data Extracted). One study did not report the medium used (N/A in Figure 2). The most tested medium is sediment (n=26), followed by water (n=14) and gastrointestinal tracts (n=12) (Figure 2).

Several different reagents were used in the studies when performing recovery rate trials. These include solvents, enzymes, dyes, bases, acids, oxidising agents, water, alcohol, oil and saline solutions. The most frequently used reagents were saline solutions (n=39), followed by oxidising agents (n=31), oxidising agents combined with saline solutions (n=17) and bases (n=14) (Figure 2). The most commonly used saline solutions include sodium chloride (n=15), sodium iodide (n=10) and zinc chloride (n=10) (Table S2). Moreover, five studies did not state what reagent was used in the recovery trial (N/A in Figure 2).

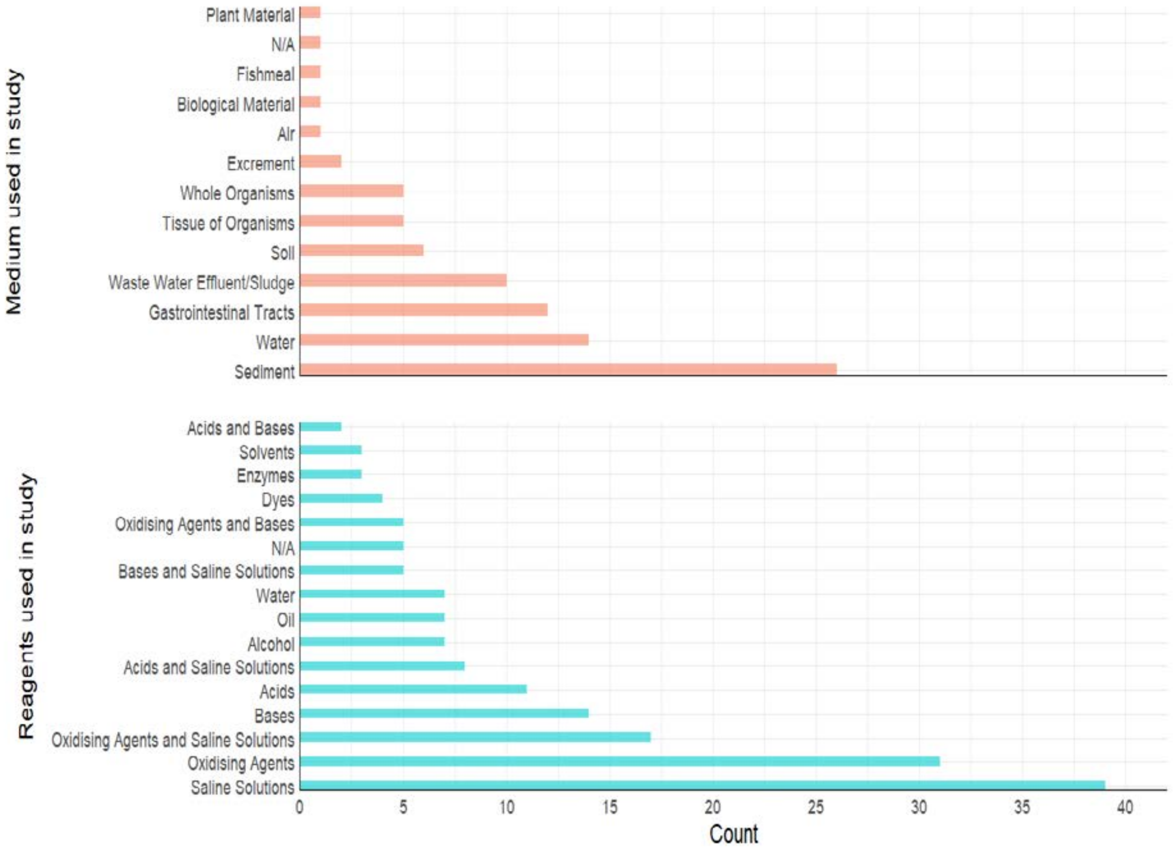

Figure 2. ***Count of studies included in meta-analysis using different media and reagents.*** *The count of studies included in this meta-analysis which used each medium and each reagent during a recovery rate experiment. N/A represents number of studies which did not report the medium or reagent used.*

4.1.3. Type of spiking polymer used

A total of 27 different spiking polymers were used in the microplastic recovery experiments reviewed. The most commonly used polymer was polyethylene (PE) (n=44), followed by polystyrene (PS) (n=36) and polyethylene terephthalate (PET) (n=35) (Figure 3). One study did not report the type of spiking polymer used. From here forward, the eight most used polymers (used in more than eight studies), were further analysed. These eight polymers have been further categorised into high- density (PET, PVC and PA) and low-density (PE, PS, PP, LDPE and HDPE) polymers (Figure 3). At least one or more of these polymers are used in 98.5% of the studies selected for this meta-analysis (70 out of 71 studies).

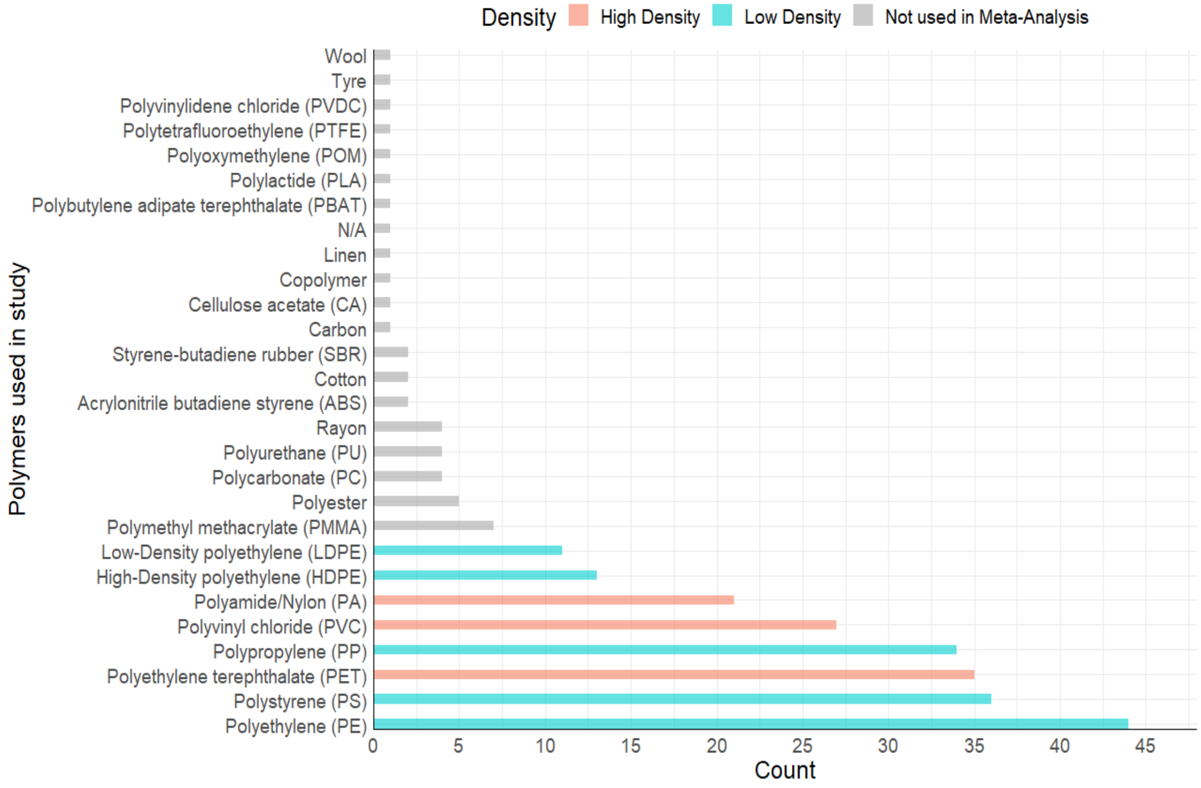

Figure 3. ***Count of the types of spiking polymers used in the studies examined in this meta-analysis.*** *Those polymers used in more than 8 studies are further split into high and low-density polymers for further investigation*

4.1.4. Shape and size of spiking polymers used

The most common shape spiking polymer used was fragments (n=27), followed by fibres (n=22) (Figure 4). A large number of studies did not report the shape of the spiking polymer (n=10). Furthermore, 11 studies used the word “particle” to describe the spiking polymer used. This is an ambiguous term which could be interpreted and described as many shapes, so this term was given its own category. With regard to the size of spiking polymers used, the majority of the studies (n=60) used microplastics (1µm-1mm) as their spiking polymers. However, four studies did not report the size of the spiking polymer used (Figure 4).

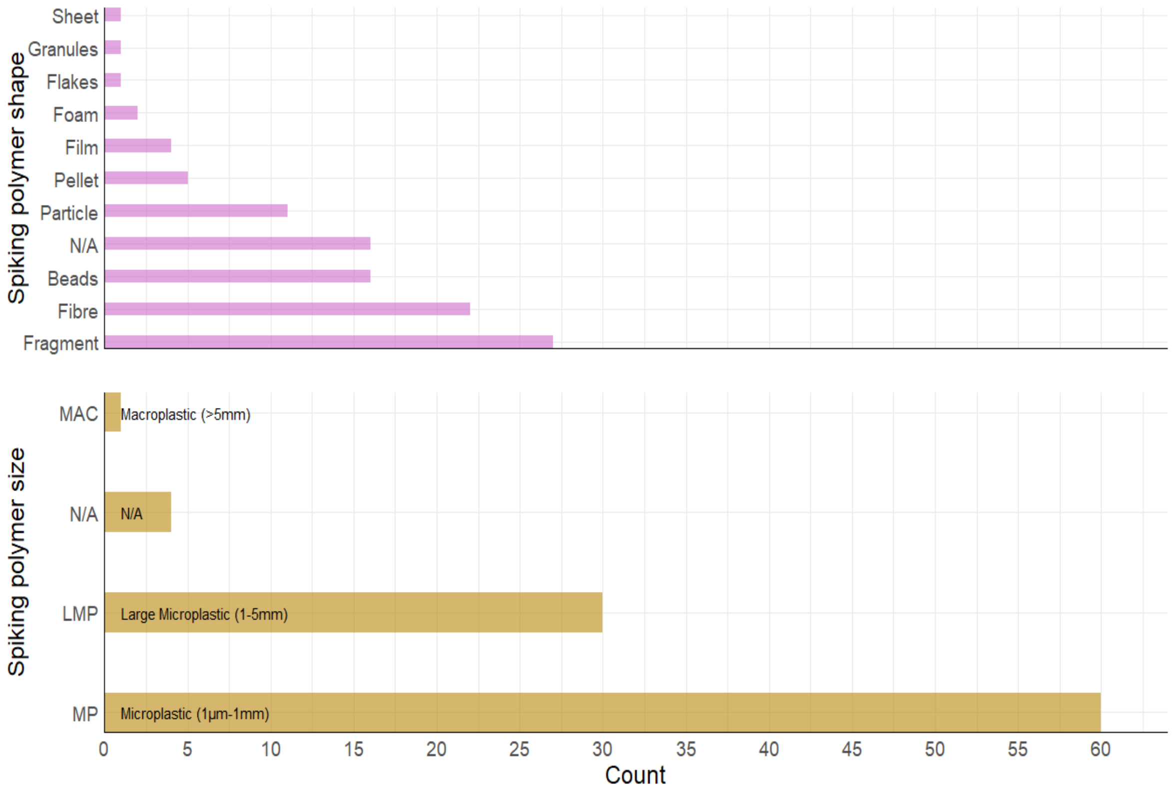

*Figure 4. **The count of different shape and size of spiking plastics used in the studies selected for this meta-analysis.** Large microplastics are those classed between 1 mm-5 mm, microplastics are those classed between 1 μm-1 mm (International Organization for Standardization, 2020). N/A represents the number of studies not reporting the spiking polymer shape or size*

4.2. Meta-analysis of recovery rates across studies

4.2.1. Recovery rates of polymers from different media

The majority of the lower recovery rates in each media type came from the high-density polymers (PVC, PET and PA). This is the case for fishmeal, water, wastewater/sludge, tissues of organisms and whole organisms (Figure 5). However, in the studies which have used gastrointestinal tracts and excrement as the study medium, the opposite is found, with lower recovery rates of low-density polymers (PS, PP, PE, LDPE, HDPE). Overall, polymers were recovered more effectively from plant material (all 100%), biological material (96%), whole organisms (91-95%) and excrement (88-95%); and recovered least from fishmeal (58-70%), water (67-82%) and wastewater effluent/sludge (76-89%) (Figure 5). The difference in recovery rates between high and low-density polymers is much larger in some media compared to others. For example, 22% more low-density polymer were recovered from soil than high-density polymers. However, from tissues of organisms only 3% more low-density polymers were recovered than high-density polymers (Figure 5).

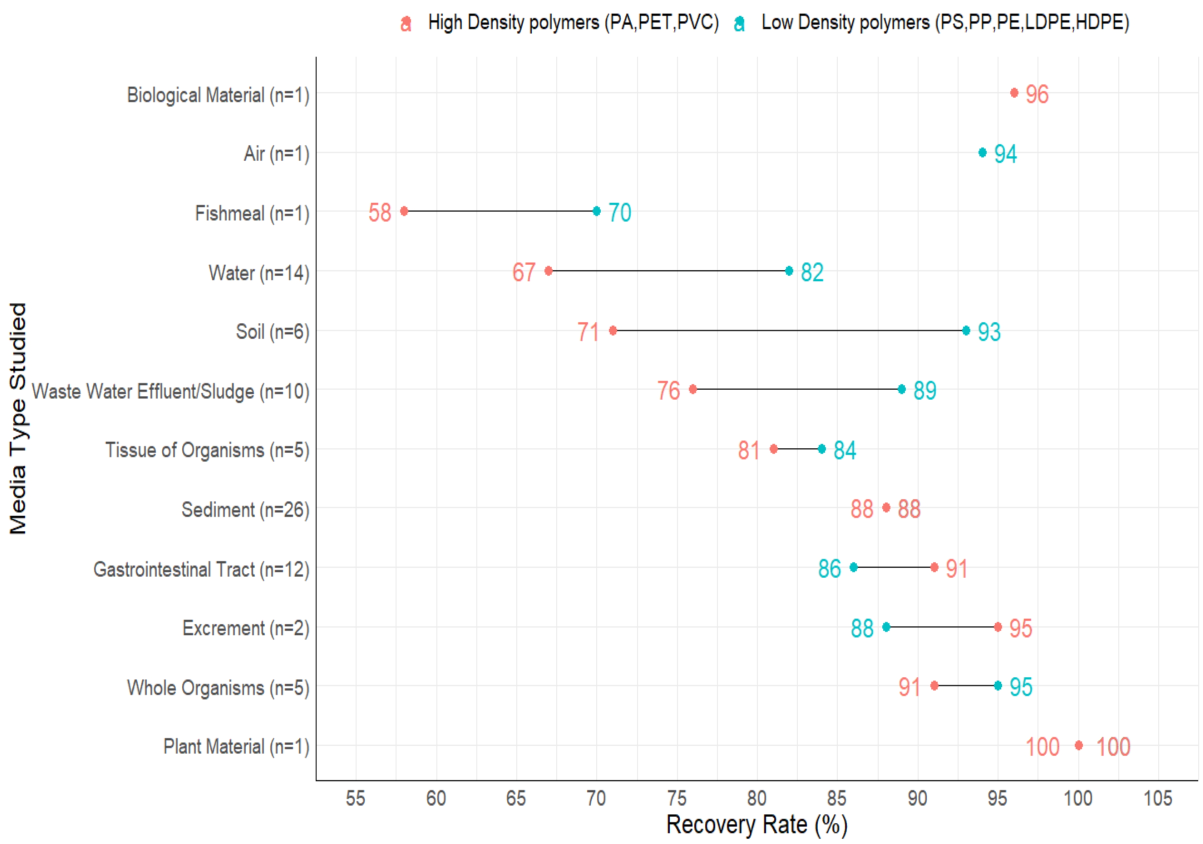

Figure 5. ***Average recovery rates across studies of high and low-density polymers when extracted from different media.*** *Numbers against the media represent number of studies in this meta-analysis using each medium.*

4.2.2. Recovery rates of polymers using different reagents

Similarly to the trend found in the recovery of polymers in different media, most reagents recovered more low-density polymers than high density polymers, which is the case for water, saline solutions, oxidising agents, bases and dyes. However, the opposite is found when studies used solvents, alcohols, acids and oils, which removed more high-density polymers. Moreover, all but one reagent (water) recovered more than 80% of spiking polymers on average. However, the studies which used water as a reagent to recover the polymers showed the lowest recovery rates (averages 53% for high-density polymers, 65% for low-density polymers) (Figure 6).

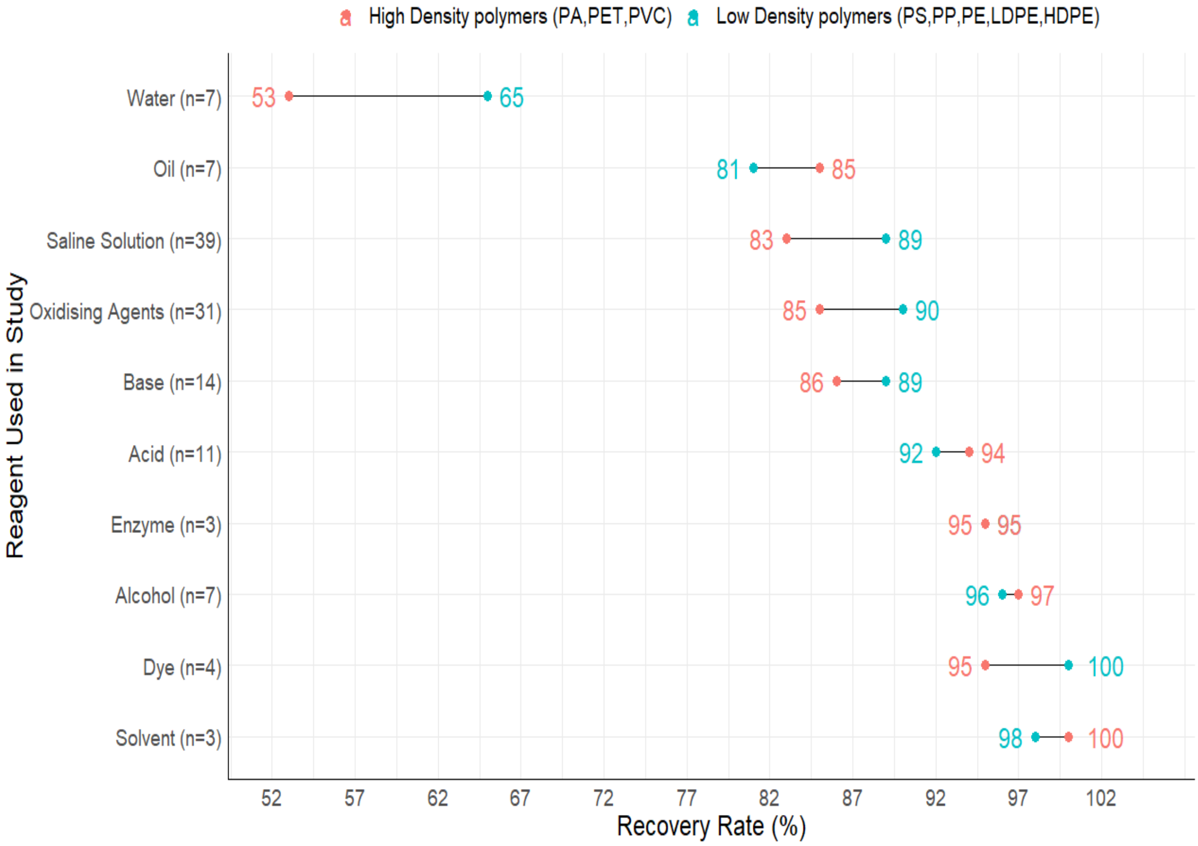

*Figure 6. **Average recovery rates across studies of high and low polymers when extracted using different reagents**. Numbers against the reagents represent number of studies in this meta-analysis using each reagent.*

4.2.3. Combination of different reagents and media on the recovery rates of polymers

Individually, reagents and type of media have an effect on recovery of microplastic polymers (Figure 5 & 6), however they can also have an effect on recovery when combined (Figure 7). For example, the use of an acid as a reagent results in higher recovery than other reagents when used in the same media. This is the case for excrement, sediment and whole organisms. However, when an acid is used to recover polymers from wastewater/sludge and water, lower recovery rates are found (Figure 7). The use of oxidising reagents recovered the most polymers from air, excrement, gastrointestinal tracts and plant material, however, these reagents resulted in very low recoveries of high-density polymers from soil (Figure 7).

Similarly, saline solutions recover high amounts of polymers from air and whole organisms, but lower amounts from media such as excrement, fishmeal, soil, tissues of organisms and wastewater/sludge (Figure 7).

Moreover, the use of an oil as reagent to recover plastics produced high recovery rates in soil. However, much lower recovery rates were found when using the same reagent to extract polymers from gastrointestinal tracts and tissues of organisms.

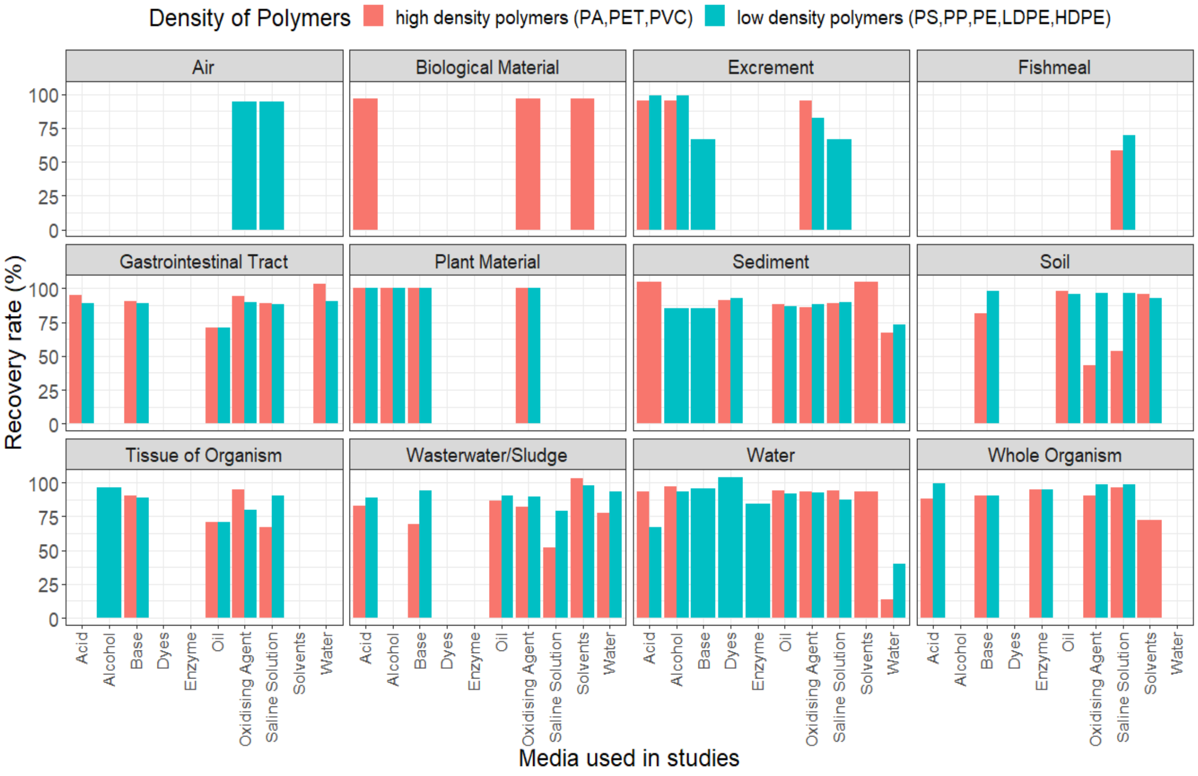

Figure 7. ***Recovery rates of media and reagents combined.*** *Average recovery rates across studies of high and low-density spiking polymers when using different reagents and test on different media.*

4.3. Assessment of underestimation

As seen in Figures 5-7, very few combinations of reagents and media tested result in 100% recovery of spiking microplastics, meaning there is a level of underestimation when using these methods to extract polymers. Due to the lack of consistent information reported and the low importance given to recovery experiments in much microplastic research, an effect size could not be calculated for this meta-analysis. Therefore, we have counterbalanced this by calculating a weighted mean based on equations provided by Gurnsey (2017). Here we estimate that microplastic research could be underestimating how many microplastics are found by approximately 14% (calculation in supplementary material (Equation S1)), based on the type of reagents and medium used. We recommend taking this approximate underestimation in to account when concluding how many microplastics are found in environmental samples. However, this underestimation may well be higher or lower depending on the method used, including the medium and reagents used.

5. Discussion

This meta-analysis has gathered recovery rates from studies that have used a wide array of media (Figure 2), including plant material, fishmeal, biological material, air, excrement, whole and tissues of organisms, soil, waste water treatment plant products, gastrointestinal tracts, water and sediments. There are benefits of studying such different types of media as it has been increasingly evident that microplastic contamination of the environment is enormously widespread. For example Ross *et al.* (2021) have found polyester fibres in remote environments such as the Arctic. However, with regards to the method used with these new media types, problems can arise, specifically with the ability to standardise. Microplastic research has been calling for standardisation when it comes to methods for extraction (Skalska *et al.*, 2020). However, a “one-size-fits-all” kind of method is extremely difficult to achieve when properties of the study medium varies so drastically. Lusher *et al.* (2020) explained how methods could be divided depending on their complexity and the number of steps needed.

Similarly, with new methods being developed to extract microplastics from complex media, often new reagents are used. This meta-analysis found a range of reagents including solvents, enzymes, dyes, bases, acids, oxidising agents, water, alcohols, oils and saline solutions (Figure 2). These were either used individually or sometimes combined. With the aim of microplastic research to identify harmful microplastics in the environment to eventually find solutions for their removal, it could be argued that harmful/toxic reagents should not be used in methods. For example zinc chloride ($ZnCl_2$) and sodium hypochlorite (NaOCl) are commonly used to extract microplastics (Collard *et al.*, 2015, Coppock *et al.*, 2017), however both of these reagents can be toxic to the environment and marine life and have multiple hazard statements in safety data sheets. For example, zinc chloride can alter bone development of zebrafish (Salvaggio *et al.*, 2016), and similarly sodium hypochlorite can cause acute toxicity on the same species (Emmanuel *et al.*, 2004). However, high recovery rates (>80%) of microplastics have been found when using less harmful alternatives such as sodium chloride (Quinn *et al.*, 2017). Moreover, it could be the case that certain regents are more suited at extracting microplastics from certain media. For example oil works as a better reagent to recover microplastics from soil than gastrointestinal tracts and tissues of organisms (Figure 7). Reasons for this could be due to the majority of soils having less than 30% of organic matter, allowing oil to work well as a density separation (Radford *et al.*, 2021). Whereas oil may not work as well at separating microplastics from biological material such as gastrointestinal tracts or tissues, which often need to be digested beforehand with use of a strong oxidising agent such as hydrogen peroxide (H_2O_2) (Avio *et al.*, 2015).

As a part of a recovery rate study, spiking polymers/microplastics are used. This meta-analysis identified that a wide range of type, shape and size polymers were used (Figures 3 and 4), with little reasoning why in each of the studies. The most commonly used spiking polymers were PE, PS, PET and PP. It would be most reflective of real environmental conditions if the spiking polymers used would be the same as those commonly found in the environment. Phuong *et al.* (2016) found that most studies use more plastics in experiments than those in the environment, but the most common microplastics found in the environment are polyethylene, polypropylene and polystyrene. Therefore, the four most widely used spiking polymers in this meta-analysis are environmentally relevant if used in the correct quantity. Similarly, it is important that the shape and size of the spiking plastics is environmentally relevant. The most common shape used in the studies in this meta-analysis is fragments (Figure 4). A review by Phuong *et al.* (2016) confirmed that this is also the most commonly found shape in sediment and water samples, however other shapes such as fibres were also predominant depending on the type of method used. The shape of the spiking polymer is an important aspect to consider as different shape microplastics may be recovered easier than others. For instance, researchers have reported some microplastics sticking to glassware (Thiele *et al.*, 2019). Also, foam-like microplastics such as polystyrene have a low density of 0.028-0.045 g/cm^3 (British Plastics Federation, 2020) which enables it to float more readily than other denser microplastics, thus enabling easier density separation. Micro-sized plastics (1 µm- 1 mm) (International Organization for

Standardization, 2020) were the most commonly sized spiking plastic identified in this meta-analysis. This is environmentally relevant. However, it is becoming apparent that smaller nano-sized (<1µm) particles may be more abundant in the environment but have yet to be studied in depth due to technological limitations. An example of this limitation is the ability to identify and quantify such small particles. Even if nanoplastics are in high abundance, their mass could be so low that it is difficult to detect with current technology and methods, or nanoplastics may be found aggregated to other particles due to their size, making them difficult to isolate (Jakubowicz *et al.*, 2021). Furthermore, these type of spiking recovery studies typically use new or 'virgin' plastic to spike the sample. However, true extractions from environmental media will usually involve isolating material that has been subjected to some ageing and weathering thus will behave differently from the virgin spiking material. Routine spiking studies with weathered microplastics would be challenging to deliver but is an area that could reward some further study.

When looking at the recovery of microplastics from different media types, microplastics were recovered at higher rates from some types over others. For example, plant material, biological material, air, whole organisms and excrement had recovery rates over 95%, whereas fishmeal, water, soil and wastewater effluent/sludge had recovery rates below 80% (Figure 5). This could be due to some of the properties of those media types. For example, there would be less organic material to breakdown in air than in fishmeal and soil. Radford *et al.* (2021) found organic material was one of the key factors in hindering the recovery of microplastics. Similarly, particle size may influence the ability to extract microplastics as some nano and micro-sized plastics may take longer to float than larger sized plastics (Wang *et al.*, 2018). Moreover, the range of recovery between low and high-density microplastics varies considerably between the different media types. For example, there is 22% percent difference between low and high-density microplastics recovered from soil (71-93%) (Figure 5), but only 3% different from those recovered from tissues of organisms (81-84%). This could be due to the complexity of the test media. For example, the soil used in the different studies may vary considerably in regards to particle size distribution and organic matter which depending on the quantity of each, may benefit the lower-density plastics, but hinder the high-density plastics.

Similarly, this meta-analysis has revealed that using different reagents can yield different recovery rates. The majority of the reagents (oil, saline solutions, bases, acids, oxidising agents, enzymes, alcohols, dyes and solvents) recovered more than 80% of the spiking plastics (Figure 6). However, in the studies which used water, recovery rates were below 65% (53-65%) (Figure 6). This is not surprising as the density of water is approximately 0.99 g/cm^3 (Tanaka *et al.*, 2001), which is lower than many plastics (PET: 1.37 g/cm^3, PVC: 1.38 g/cm^3 (British Plastics Federation, 2020)). However, what is surprising is that in some cases when using oils, alcohols and solvents, more high-density polymers were recovered than low density polymers (Figure 6). A reason for this could be due to the density of these reagents. Chloroform has a density of 1.49 g/cm^3 but is corrosive enough to attack plastics (National Center for Biotechnology Information, 2021), this could allow the higher density plastics to float, but cause the smaller plastics to corrode.

What is overwhelmingly clear from the results of this meta-analysis is that it is rare for all spiking plastics to be recovered, thus a 100% recovery rate is seldom achieved. This meta-analysis found that on average microplastics could be underestimated by approximately 14% (See Supplementary information for calculation). More so, studies rarely account for this underestimation brought about by the methods used. If this underestimation accounted for, the amounts of microplastics estimated to be in the environment could be a lot larger than originally anticipated.

Overall, this meta-analysis has highlighted many issues within recovery rate studies and microplastic research. Firstly, recovery rate studies are rarely used to validate methods in published studies. For example, the 71 studies found and used in this analysis is a minute size compared to the large number of microplastic research papers and methods that have been published over time (Provencher *et al.*, 2020). For example, in 2004 when the first microplastics related research was published by Thompson *et al.* (2004), a Google Scholar search for “microplastics” only yielded 89 results, whereas the same search for 2020 yielded 11,200 results. Furthermore, those papers that are published with a recovery rate study, they are often poorly executed with key information missing, such as sample size and the type, shape and size of the spiking plastic used. With this missing information, it is difficult to make further inferences regarding the effect size and publication bias, also this makes it problematic for others to replicate the method used. Often recovery rate results are poorly displayed and are seen as unimportant compared to the main results of a study. Also a standardisation needs to be agreed on in several aspects of these studies. Firstly, it should be agreed on whether recoveries are calculated by weight difference or difference by count; and secondly, the terms used to describe the shapes of the spiking polymers, often the term ‘particle’ is used, which can be interpreted in many ways. Due to the aforementioned limitations we have assembled recommended reporting criteria specifically for recovery rate studies, with the intention of making validation of microplastic extraction methods clearer to others.

6. Conclusions and Recommendations

The varying range of recovery rates found in the studies included in this meta-analysis were dependent on the media types and reagents used. However, very rarely were 100% of the spiking plastics recovered, and overall an underestimation of 14% was discovered, meaning the amount of microplastics in the environment could be higher than estimated from research studies to date. From this meta-analysis it is clear that recovery studies are not utilised enough and, on the occasion, when they are, they are often poorly executed. It could be argued, that with a more holistic approach to validating methods, by studying the properties of the test medium, and clearly and concisely reporting the recoveries, it could help with the ever-growing issue of standardisation in microplastics research. This meta-analysis flagged several limitations within recovery rate studies, which we recommend improvements:

Report all raw or average recovery rates with variance in both tabulate AND graphical form. Include this in supplementary material if needed. Many studies either reported a single percentage in the text or displayed recovery rates in graphical form, often making it difficult to extract an exact percentage, thus making it difficult for others to accurately assess the effectiveness of the method.

Calculate the recovery rate by count of recovered plastics. Few studies calculated the recovery rate by change in weight, these studies were removed from this meta-analysis as they were not comparable to the majority which use counts. If this is adopted by all, it allows for standardisation.

Use triangulation: have multiple researchers count recovered plastics in a study. If counted by eye, counts of recovered microplastics could be different depending on the observer's experience carrying out this task.

Report the number of samples used in the recovery rate study. Many studies did not report the sample size, making it difficult for further analysis.

Report the shape, size, type and size of spiking plastics used. The reporting style of the spiking plastics across the studies varied considerably. For example, one study did not state the type of polymer used, ten studies did not state the shape of the polymer used, eleven studies used the word 'particle' to describe the shape, which could be interpreted differently by others, and four studies did not report the size of the polymer used. We recommend reporting these properties clear enough for replication and to use environmentally relevant quantities which are reported in the literature for each test medium.

Do the recovery rate study on the same media which is to be tested for the main experiment. Methods will work differently on media with different properties, thus different recovery rates will be found.

The aim of this meta-analysis is to highlight the importance to researchers of using a recovery rate study/trial to validate their methods, with the proposal that in the future this becomes a "new normal" during method development, and the quality of these types of studies are up to a standard that can be replicated. Furthermore, if the amount of underestimation, brought about by the methods used is accounted for in each study, the amounts of microplastics reported will probably be higher but more realistic, which can offer more robust evidence for policy makers.

7. Credit author statement

Chloe Way: Conceptualisation, methodology, validation, formal analysis, investigation, data curation, writing-original draft, visualisation. **Malcolm Hudson:** Supervision, project administration, funding acquisition, writing-review and editing. **Ian Williams:** Supervision, writing-review and editing. **John Langley:** Supervision, writing-review and editing.

8. Data availability

Data supporting this study are openly available from the University of Southampton repository at: [insert link]

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