Analytical Methods

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Cite this: *Anal. Methods*, 2021, **13**, 5299

Microplastic extraction from sediments established? – A critical evaluation from a trace recovery experiment with a custom-made density separator†

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By now, microplastics are present in every environmental compartment of which sediments are considered one major sink. As a result, several approaches for their enrichment from sediments have been established in microplastic analysis. At the same time, the smaller microplastics gained increasing attention regarding their ecotoxicological relevance. A customized sediment separator was evaluated with trace amounts of small microplastic particles (150–300 μm) of the nine most common polymers. Separation was performed with sodium bromide ($\rho = 1.5$ g cm^{-3}). The experimental recovery comprises pristine as well as incubated polymers to include early biofouling effects. Polymer quantification was achieved exclusively using pyrolysis-gas chromatography-mass spectrometry. The results reflected an overall mean recovery of 65%. Interestingly, the observed behaviour seems to be density related. While polymers of higher densities revealed higher average extraction efficiencies (74–97%), those of less dense polymers are reduced and span between 34 and 65%. These observations hypothesize possible polarity related surface interactions as a relevant factor for microplastic particle extraction. In contrast, the density of the separation fluid seemed to be of subordinate relevance, if small microplastic particles were extracted in trace amounts. Early biofouling enhanced recoveries of some polar polymers, whereas the effect on apolar polymers was even negative in some cases. In a comparative synopsis with other published density separation approaches, a limited number of comparable experimental setups concerning particle size, polymer density range and polymer concentration were revealed. Nonetheless, some related experiments point to similar density/polarity driven extraction behaviour. In conclusion, the presented study suggests a re-evaluation of current separation approaches for extraction of low number/mass concentrations of small microplastics from sediments to enable a more comprehensive insight into factors that influence surface properties for microplastics extraction. Concurrently, it raises the question of how an ideal environment relevant recovery experiment can be designed.

Received 8th June 2021
Accepted 27th September 2021

DOI: 10.1039/d1ay00983d

rsc.li/methods

1. Introduction

Plastics have become indispensable in modern society. Their low price, durability, and versatility lead to an ever-growing demand of plastic products since the 1950s.[1] Since then, plastics enter the environment through various pathways and parts of them end up in the ocean.[2–4] In particular the smaller 5 mm so called microplastic (MP) fraction is proven to be present in every environmental compartment by now.[5]

Sediments are considered a major sink for MPs in the environment.[6,7] Nonetheless, a defined picture of MP transport processes from and into sediments and their resulting distribution is still lacking. A high pollution of sediments implies a potential threat in particular to fertile riverine and coastal habitats.

MPs smaller than 1 mm are thereby of special concern since their particle counts increase with declining particle size[8] that is related to an elevated toxic potential.[9,10] Thus, small MP particles are an essential target for microplastic analysis in sediments. State-of-the-art established polymer-specific quantification techniques (*e.g.*, FTIR-, Raman-spectroscopy, and pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS)) allow a comprehensive analysis even for very small MP particles. However, regarding environmental samples a successful application of these techniques is inevitable adherent to a preceding sample purification if complex sediment samples are in the analytical focus.[11]

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/d1ay00983d

Sediments are one of the most challenging compartments for MP analysis.[12–14] The challenge to maintain polymer integrity while reducing complex organic and inorganic matrices resulted in diverse purification approaches.[12–14] Inorganic components are commonly removed by elutriation or density separation.[12–14] These techniques take advantage of the density differences between microplastics and inorganic components like quartz and other minerals.

In particular, the focused small MP particles (≪1 mm) show less clear analytical behavior in density-dependent extraction procedures. Here, the overall specific polymer density becomes less important.[15–17] Density separation of small particles needs longer equilibration times to reach floatage[18] and is more affected by adhesion and buoyancy changes. Observed buoyancy changes are related to aggregation[19,20] and biofouling.[21–25]

Only few evaluation studies on density separation techniques accounted for small particle sizes,[26,27] while none addresses aggregation or biofouling in their recovery experiment. Furthermore, recoveries were often performed with visual controls[26–33] but were rarely associated with reliable polymer-specific quantification techniques (*e.g.*, FTIR, Raman, and Py-GC/MS).[34]

In this study we put forward a density separator that has been developed within the framework of a former project simultaneously with several other separators following the increasing demand of MP data from environmental sediments.[6,35] Ever since these times the small MP particles have become more and more relevant in the ecotoxicological context and gained increased attention regarding their analytical behaviour such as potential influence of aggregation and biofouling.

The aim of this study is to re-evaluate our separator by performing recovery tests for a broad range of relevant MP types, with low, environmentally relevant concentrations and small particle sizes. We thereby want to complement our two previous successful tests on the reproducibility of environmental sample analysis[36] and on the extraction of 1 mm particles from different sediment types (Kögel *et al.*, in prep.).

Our recovery experiments compare the extraction of pristine and incubated polymers from an artificial sediment. The incubation experiment should thereby reflect the effect of biofouling on the recovery efficiency. All recoveries are quantified exclusively by Py-GC/MS and accordingly reflect real analytical conditions.

The results are critically discussed in contrast to the current literature, and with a comparative look on currently used separation procedures. Here, we intend to review all separation procedures and related recovery experiments with special focus on the shifted and future needs in small MP analysis of sediments.

2. Materials and methods

2.1 Technical setup

The separator assembly consisted of three units. (1) A modified 3 l glass beaker (Fig. 1) fitted with a discharge funnel and a skimmer. (2) A displacement cone (Fig. S1†) that is placed in the centre of the beaker and connected to an electric stirring device (Hei-TORQUE Value 200, Heidolph Instruments). (3) A second 800 ml glass beaker to collect the skimmed solution. The 3 l beaker was modified by a professional glassblower (specifications in Fig. S1†). All parts are easy to handle, easy to cover with alumina foil during the separation process and easy to clean afterwards. During the density separation procedure buoyant particles rise to the solution surface where they are displaced by the cylinder to the outer ring of the solution surface. A constant rotation of the cylinder pushes the particles together into the skimmer which channels them down into the discharge funnel. Finally, the particles are retrieved in the collection beaker.

Fig. 1 Separation beaker with a stirring device and displacement cylinder.

2.2 Experimental setup

Sample preparation. To test the efficiency of the density separation nine relevant microplastic standards (Tables 1 and S4†) were added to fine quartz sand (grain size distribution *cf.* Table S1†). These widely demanded and utilized polymers represented a wide density range (Table 1). Spiking sets were prepared by manually preselecting particles of the respective pristine polymers with similar sizes (150–300 μm). They were weighed into aluminium weigh boats with a Cubis Ultramicro balance MSE2.7S-000-DM (Sartorius, Germany). The respective polymer weights (3–45 μg, equivalent to particle numbers between 5 and 20 *cf.* Table 1) were adjusted to enable an optimal quantification within the individual Py-GC/MS calibration curves and outside any detection limits (Table S2†). The particle size range was chosen to ensure the particle transfer into the sediment by optical control *via* a binocular.

For every replicate, one separation beaker was filled with 800 g of quartz sand (pre-treated in a muffle furnace at 550 °C for 12 h) and spiked with the prepared sets. The polymer particles were rinsed from the aluminium boats into the separation beaker with 200 ml filtered water, representing a 1 kg wet weight sediment.

To induce biofilm production on the particles in the incubation experiment the spiked sediment in the beaker was additionally spiked with a 50 ml homogenized mixture of artificial seawater and marine benthos microalgae (*Thalassiosira eccentrica*, *Amphora coffeaeformis* and *Diploneis* sp.) representative of a typical marine benthic community (North Sea, Germany). The water level was then further adjusted to create

Table 1 Overview on properties of polymers and their characteristics in the experimental setup

	Polymer properties			Experimental setup	
Abbreviation	Polymer	Density (g cm^{-3})	Particles (n)	Weighed portion (μg)	Size (μm)
PP	Polypropylene	0.9	7–9	43.1	∼300
PE	Polyethylene	0.96	7–15	42.9	∼250
PS	Polystyrene	1.04	5–10	20.3	∼210
PA6	Polyamide-6	1.13	6–10	45.2	∼300
PMMA	Polymethyl-methacrylate	1.19	5–7	20.6	∼250
PC	Polycarbonate	1.2	5–7	5.0	∼150
MDI-PUR	MDI-polyurethane	1.23	7–10	44.8	∼280
PET	Polyethylene-terephthalate	1.38	5–9	15.0	∼190
PVC	Polyvinyl chloride	1.43	15–20	42.8	∼200

a water layer on top of the sediment to allow microalgae growth. The microalgae have been pre-cultivated for ten days and autoclaved with nutrient and vitamin enriched artificial seawater. The replicates were further incubated for another three days at room temperature to allow biofouling.

Density separation procedure. To the spiked sediment replicates 1375 ml of a sodium bromide (NaBr, Grüssing GmbH) solution (>1.5 g cm^{-3}) was added. With respect to the given water content of the sample 340 g of washed NaBr was added to adjust the final density to >1.5 g cm^{-3}. The suspension was manually stirred for a few seconds with a stainless-steel spoon. This was repeated 8 times over a period of two hours. Subsequently, the solution was left to settle overnight. Now, the stirring unit was positioned into the beaker in a way that its displacement cylinder was at the level of the skimmer and overflow. The level of the NaBr solution was elevated right up to the overflow. The collection beaker (600 ml) was placed beneath the overflow and the stirring device was adjusted to a speed up to 30 rotations per minute. Over a two-hour time span the sides of the beaker, the displacement cylinder and the skimmer were rinsed successively with saturated NaBr solution from a PFTE-bottle every 15 minutes (Fig. S2†). Subsequently the skimmed solution was transferred thoroughly from the collection beaker into a separation funnel (500 ml). This was shaken vigorously to disintegrate potential aggregates and adhered particles then rinsed from the glass walls with NaBr solution from a PFTE-bottle. The solution was left to settle overnight. Then, the heavy fraction at the bottom (approximately 2/3 of the solution) was discarded. Meanwhile, the sediment extraction procedure was repeated, and the resulting solutions were combined in the respective funnel. Density separation in the funnel was continued until no particles were left to settle. The residual solution was vigorously rinsed onto a glass fibre filter (1 μm pore size, Ø 15 mm) with filtered water and 10% ethanol and stored in glass Petri dishes (5 cm).

The modular setup between the separation beaker and stirring unit allowed the parallel extraction of four samples.

Contamination measures. To keep any secondary contamination as small as possible glassware and laboratory equipment were rinsed with filtered water and 10% EtOH from Teflon bottles. All solutions were filtered through glass fibre filters (1 μm pore size). Glass fibre filters were muffled at 400 °C (4 h) before use. All experiments were performed in a frequently cleaned, but not activated fume hood. Plastic lab ware was avoided to the most possible extent. All washing bottles used were made of PTFE, not included in the polymer types analysed. In particular plastic lids were substituted *via* alumina foil. All processing steps were performed under alumina foil protection and uncovered for short intervals only if necessary. Only cotton clothes were allowed to be worn in the laboratory along with a cotton lab coat.

To exclude secondary contamination from the commercial NaBr it was prewashed and recrystallized. Solid NaBr was filled into the separation beaker of the separator, covered with pre-filtered, saturated NaBr solution and stirred with a constant addition of solution generating an overflow of the upper solution layer, which removes potential MP particles. Subsequently the saturated NaBr solution was decanted and the wet but crystalline NaBr was transferred into cup-sized aluminium bowls that allows their adjacent portioning.

Although the sand matrix used for the experiment was treated at 550 °C to remove any organics, the recovery experiments were complemented by a procedural blank. This procedural blank was processed in parallel and analogous to the incubation recovery experiments to assess any possible secondary contaminations throughout the whole density separation procedure. It is supposed to indicate any potential contamination issue, and carried out here, it takes the extended handling process adhered to the incubation step into account as well. Even though the detected polymer contents are very low or even absent (*cf.* Table S9†), they were subtracted from the respective incubation recovery raw data on an area basis. The subtraction from the pristine experiment was discarded due to high frequent intensity variances between the two Py-GC/MS measuring sequences. Procedural blank data from the same sequence for a meaningful subtraction would be needed. Nonetheless, the quantification of the procedural blank with respect to the polymers (*cf.* Table S9†) underlines its minor and almost negligible impact on the overall results. The quantified blank functions as a contamination estimate for the pristine recovery (Table S9†).

Quantification – Py GC/MS. The extracted samples were prepared for Py GC/MS measurement according to Fischer *et al.*[36] The glass fibre filters were folded into pyrolysis cups and spiked with 4 different pyrolysis injection standards (Table S5†) and tetramethylammonium hydroxide (TMAH, 25% in methanol (MeOH), Fluka, Germany) for on-line derivatisation.

The prepared sample cups were analyzed using a micro-furnace pyrolyzer (EGA/Py-3030D, Frontier Lab) connected to a gas chromatograph (Agilent 7890B) and a mass spectrometer (Agilent MSD 5977A). All measurement conditions rely on Fischer *et al.*[36,37] and given in detail in the ESI (Table S6†). Polymers were identified *via* polymer specific indicator ions (Table S8†) and then quantified by external calibrations from peak areas and peak area ratios. Basic calibration curve parameters are given in the ESI (Table S7†).

Potential contaminations identified in the procedural blanks (Table S9†) were compensated by peak area subtraction from the sample peak area before any quantification. Potential outliers were identified by the Grubbs test implemented in Origin (OriginLab Corporation, Version 2020).

3. Results & discussion

3.1 Recovery experiments

Polymer-specific extraction. All 9 polymers were successfully extracted by the sediment separator regarding all recovery experiments (4 pristine and 4 incubation experiments; see the following subsection). The overall quantified MP mass recovery regarding the sum of spiked polymers was 68 ± 35%. The observed polymer specific recovery efficiency varied highly between the different types. The highest average recoveries were achieved with PVC (97%) whilst the lowest average recovery was achieved with PE (34%) (Fig. 2).

Interestingly, arranging the polymers in accordance with their respective density, a so far inconspicuous trend becomes obvious. The differences amongst polymer recoveries seemed to be density driven and polymer recovery consistently increased with polymer density (Fig. 2). Opposite to any expected scenario that would favour a better polymer recovery with decreasing density and increasing separation fluid density, the reverse effect is observed. High-density polymers represented by PET and PVC but also by PMMA, MDI-PUR and PC as well (Table 1) are extracted and quantified with mean efficiencies above 70%. This emphasizes that a density of 1.5 g cm^{-3} represented by the utilized NaBr solution is sufficient to extract the high-density polymers included in the experiment.

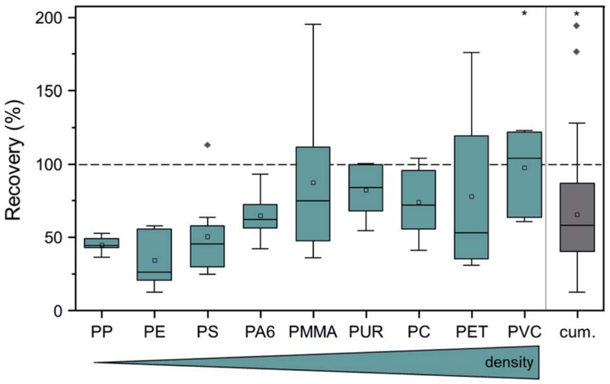


Fig. 2 Combined polymer-specific recoveries from the pristine and incubation experiment, including 8 replicates. cum. = all recoveries from every experiment and polymer. Outliers are marked as diamonds; * one outlier out of bounds at 280%.

In contrast, the lower density polymers like PE, PP and PS show surprisingly low recovery rates (around 50%). A possible reason for this behaviour might be a combined effect of low density on one hand and low polarity on the other. Low density causes buoyancy of particles and results in their accumulation at the solution surface as well as its air–glass boundary. Additionally, the apolar character of the polymers might induce a repulsion from the polar density agent to the glass surface and result in adhesion.[38,39] These interaction processes could occur at different steps of the separation procedure, in the separator itself, the collection beaker, and finally the separation funnel. In the last step, the reduction of density solution level in the tilted glass walls of the separation funnel further promotes adhesive surface interactions for polymer particles which might result in MP removal, despite intensive rinsing.

This suboptimal density and potentially polarity dependent extraction behaviour has not been described in the microplastic literature to our knowledge before. The inverted and almost expected trend would be easily traced back to an insufficient density of the selected density fluid. The usage of very low concentrated, rather small particles of the respective polymer might have revealed so far not recognized but crucial gaps of knowledge in the field of density driven microplastic extraction. In contrast to larger particles small particles are more affected by surface properties or *e.g.*, drag within the solution, while large particles are mainly affected by density differences.[18,40] It therefore might appear logical that small particles are less effectively separated from sediments than large particles. Further, high amounts and percentages of MP can directly influence the extraction behaviour.[41] The often-used high amounts might result in a kind of saturation effect, which shields MP particles from adhesion or aggregation with other material and therefore allows better extraction.

We take the high reproducibility of the density/polarity dependent results as an incentive to pay closer attention to MP extraction validation studies with low concentrations of small microplastics in sediments (Section 3.3).

The same separator and separation fluid had been used in an independent approach of a joint project. In this experiment the recovery was performed with 1 mm spherical, pristine particles. The respective results are part of a much broader study and will be published in detail in another context (Kögel *et al.* in prep). With respect to the applicated particles, three polymer types (PE, PS and PET) were overlapping with the presented study. The determined recoveries of these 1 mm spheres from three different sediments and nine extractions in total were 92.2% ± 13.9 for PE, 93.3% ± 10.0 for PE and 94.4% ± 7.2 for PET. These feasible satisfactory recoveries did not show any indication of a potential polymer discrimination. Accordingly, the supposed impact of surface properties on small particle behaviour implies a potentially different extraction behaviour compared to big particles.

Pristine *versus* incubation experiment. For most polymers, the achieved extraction efficiencies and observed variances were similar, disregarding whether they were pristine or incubated before. While PE, PP, PS, PA6, PUR and PVC were extracted in the same order of magnitude, the extraction efficiency for PMMA and PET clearly increased in the incubation experiment (Fig. 3).

Within polymer types, minor differences between pristine and incubated were observed. The variance between experiments decreased for PE and PS if particles were incubated, but was almost unchanged for PVC, PS, PA6, PUR, PC and PP. An increased variance between recoveries appeared in the case of PET and PMMA after incubation.

Early alteration processes like biofouling are known to change particle behaviour.[24,42] A biofouling layer on the particles might reduce adhesion at glass surfaces as well as inducing or reducing aggregation-affinity with other particles (organic and inorganic) and directly influence its density.[20,25,43] Accordingly, surface adhesion and polymer particle buoyancy are supposed to have a direct effect on polymer recoveries and their respective variation coefficients.

The incubation experiment of PET and PMMA indicates that early biofouling effects resulting from very basic conditions represented by the performed experiment already have a visible positive effect on extraction recovery. Since both polymers are polyesters this polymer-specific effect might be even accompanied by polymer-specific biofouling.[44,45]

A deduction of any general extraction behaviour of polymers from such incubation experiments needs large reservation regarding environmental samples. Variations in natural organic and inorganic matter might affect microplastic particles differently concerning aggregation.[21,44] Further, the specific polymer composition, shape and alteration degree affect biofouling, surface properties, aggregation behaviour, and sinking speeds.[18,40,43]

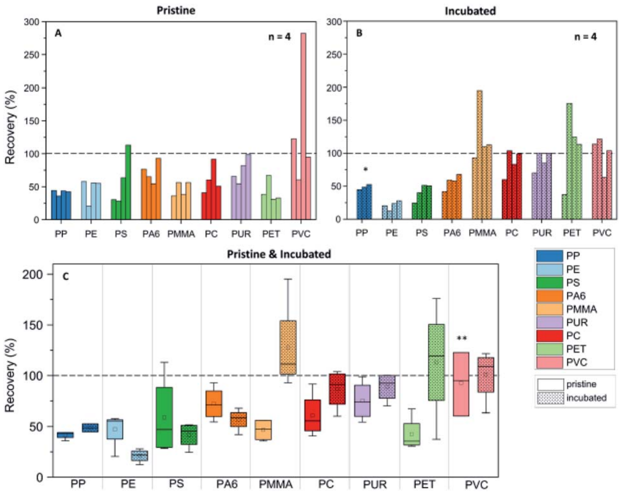


Fig. 3 Polymer-specific recoveries with pristine polymers (a), incubated polymers (b) and in comparison (c); * 4th replicate did not include PP; ** one outlier out of bounds at 280%.

Critical evaluation of the experimental setup. Disregarding the observed and discussed effects, some aspects regarding the experimental setup are supposed to be of general relevance for the reported surprising recoveries and partial variances of studied polymer types. Here, in particular the comparable low number of particles (Table 1) applied in the experiments is a weak point and must be stressed. In the experiment, they represent a compromise to get a low absolute polymer mass (<50 μg) on one hand, representative of real environmental conditions, but have still a size that allows an optical control (binocular) for an accurate spiking process on the other. Subsequently no further optical counting was possible since the MP particles were not distinguishable from any sand particles. Accordingly, the loss of just one single particle would already result in a 5% loss *e.g.* for PVC (20 particle approach) and up to 20% loss relevant for most of the polymers (5–7 particle approach) in overall recovery, determined by Py-GC/MS exclusively (Table S3†). Thus, the applied low number of spiked particles, and the loss of one or a few particles have an immediate effect on both recovery and variance as well.

Further on, for the Py-GC/MS quantification of the given process, the standard deviation of the respective polymer calibration varied between ±3% and ±13% if projected on the respective polymer recovery (Fig. S3;† calculation example). Consequently, the quantification process comprised an inherent methodical variation. Both aspects needed critical consideration regarding the observed variances in recovery data of the polymer types as well.

3.2 Environmental samples

Embedded in the successful improvement and application of the Py-GC/MS method for the MP quantification in different complex environmental samples the same separator and procedure were already applied for MP pre-concentration from muddy sediments. This application on real sediments underlines the feasibility of the separation procedure in general.[36] However, it reveals potential difficulties with respect to environmental samples as well. Such are size, shape and density dependent inhomogeneity of polymer particles in the natural environment and accordingly a challenge of environmental MP analysis.[36,46,47]

Overall, the study reflected a successful quantification of 7 high- and low-density polymer types in a muddy, intertidal sediment (Fig. 4). It gave a meaningful order of magnitude of the expected resulting polymer types, highly relevant for realistic recovery experiments. The summarized MP concentrations of the four replicates varied between 48.4 μg (C) and 166 μg (D) per kg wet weight (Fig. 4). The MP concentrations of individual polymers were almost constantly less than 50 μg per kg sediment which was implemented in the presented spiking experiments. Sizes of suspected MP particles in these environmental sample filters hardly exceeded 200 μm and are reflected by particle sizes in the presented recovery experiment.

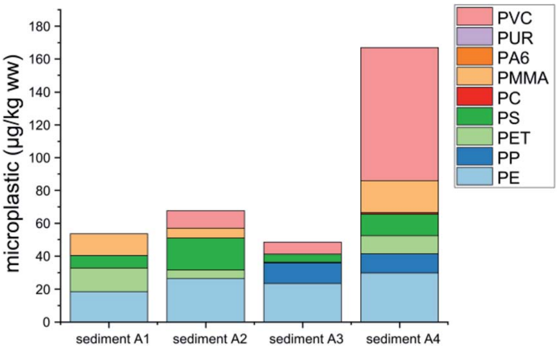


Fig. 4 Microplastic content of 4 subsamples from a muddy intertidal sediment in µg g^{-1} wet weight (adapted from Fischer *et al.*, 2019).

The overall complexity of natural samples and the associated alterations of particle properties in natural environments implicate problems if an objective transfer of extraction efficiencies is deviated from rather artificial, much less complex extraction experiments on natural sediment samples. Besides the already addressed aspects, different degrees of physicochemical as well as biological alterations have to be considered as well.

However, the results of a previous study[36] were considered as guidance for the presented recovery experiment and its capabilities to extract high- and low-density polymers at lower ppb levels.

3.3 Critical reflection with respect to literature data

As suspected sinks for MPs their investigation in sediments, sludge and soil is of increasing importance. Several isolation approaches have been developed almost simultaneously over the last years. Highly surprised about our own unexpected but reproducible results, we reviewed the existing literature for any indicators of similar density or polarity dependent extraction behaviours in former studies, in evidence with minor recoveries particularly for small MP particles at low concentrations.

This review focused on extraction methods that included recovery experiments. To fulfill comparability of the broad spectrum, it was restricted to elutriation and density separation approaches. The results are provided in Table 2. It is subdivided into three major sections: (1) the technical setup; (2) the experimental setup, and (3) parameters regarding the overall process evaluation. Here, the most meaningful and characteristic sub-criteria were emphasized. These assigned criteria describe either determined aspects mostly associated with the technical setup, or flexible/adaptable aspects, mainly related to the experimental setup or the evaluation process. All of them can easily be changed and adapted in a future re-evaluation process.

The criteria close to or matching our presented study were marked blue, additionally. In the following discussion, we focused almost only on the comparison with our results but use this line-up to emphasize crucial aspects with respect to MP analysis.

General experimental criteria. The technical setup reflects variable materials and applicable sample volumes. Often the tailored separator volume is focussed on a certain sample type and its expected MP load. Here, the sample size (Table 2, A2) has a direct impact on data quality and the achievable limit of detection, although the latter is finally defined by the subsequent analytical detection principle for the MP (Table 2, C1, C2). With respect to an inhomogeneous distribution of particulate MP already discussed,[46,47] the analysed volume should be as representative as possible for the respective sample type. Reflecting on realistic environmental sediment sample concentrations (300 particles per kg (ref. 48–50)) a volume above 500 ml should be used for feasible extractions. Only 5 of 13 studies presented separators with a respective volume.[27,28,32–34]

In principle, setups including relevant plastic parts bear an inherent risk of contamination with the corresponding polymer.[26,30] Accordingly, stainless steel or glass setups are preferred. Here, glass setups have the advantage of enabling an overall visual control.

All extraction procedures reported in Table 2 employed sufficiently dense separation fluids (Table 2, A4). However, non-toxic and less expensive density agents with sufficient density capabilities (*e.g.* ≥ 1.5 g cm^{-3}) are favourable, *e.g.* NaBr over $ZnCl_2$.[51] NaBr is commonly used with a density between 1.37 and 1.4 g cm^{-3}.[52] Its extension to densities ≥ 1.5 g cm^{-3}, as shown in the presented approach, assures the recovery of even denser polymers (*e.g.* PVC/PET).

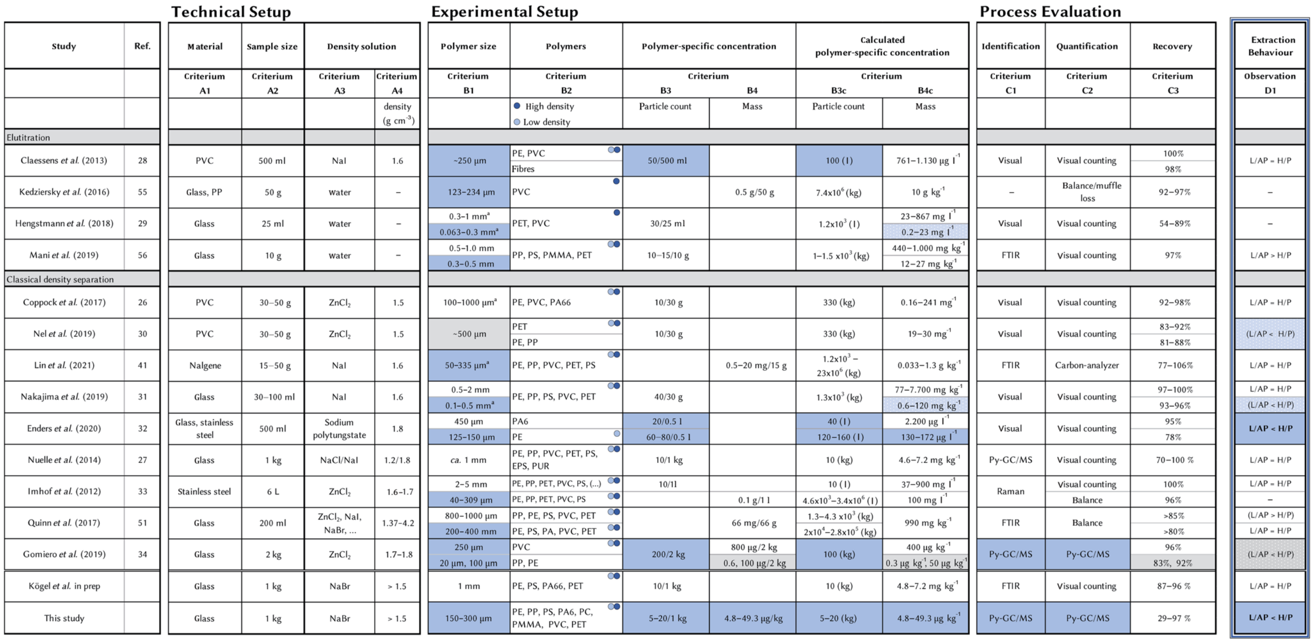
The experimental setup needs thorough examination to identify comparable conditions and thus potentially similar extraction behaviours to our study (Table 2; blue cells). Here, the most crucial factors effecting extraction behaviour are particles size (Table 2, B1), polymer density (Table 2, B2) and polymer concentration (mass; particle count) (Table 2, B3–B4).[18,40,41] An ideal experimental setup needs to include polymers of different densities/polarity to attribute respective effects.

Our study applied particle sizes between 150 and 300 µm. This makes it one of the studies that consider particle sizes at the lower end of the range studied in the contemplated approaches (Table 2, B1). Although, several studies include this size range (Table 2, B1), only five studies reported recoveries for high- and low-density polymers within this size class as well (Table 2, B2).

If very high particle counts (10^3–10^6 per kg) and high or very high masses (0.5–900 mg per kg) are applied, they are suspected to mask any of the described effects. However, since almost all of the referred studies used visual counting or balancing to determine their recoveries such high concentrations are warrantable. Focussing on realistic environmental conditions, studies reported that environmental sediment samples rarely exceeded 1000 particles per kg in the MP size range between 100 and 300 µm. More often particle counts were even below 300 particles per kg.[48–50] Environmental MP mass concentrations commonly range below <200 µg kg^{-1}.[34,36]

With respect to particle sizes and concentrations only three studies are comparable to our study.[28,32,34] Although, no study perfectly matches the conditions of small MP particles and low

Table 2 Condensed literature overview on microplastic density separation approaches



		Technical Setup				Experimental Setup						Process Evaluation			
Study	Ref.	Material	Sample size	Density solution		Polymer size	Polymers	Polymer-specific concentration		Calculated polymer-specific concentration		Identification	Quantification	Recovery	Extraction Behaviour
		Criterium A1	Criterium A2	Criterium A3	Criterium A4	Criterium B1	Criterium B2	Criterium B3	B4	Criterium B3c	B4c	Criterium C1	Criterium C2	Criterium C3	Observation D1
					density (g cm^{-3})		● High density ○ Low density	Particle count	Mass	Particle count	Mass				
Elutitration															
Claessens *et al.* (2013)	28	PVC	500 ml	NaI	1.6	~250 μm	PE, PVC ○● Fibres	50/500 ml		100 (l)	761–1.130 μg l^{-1}	Visual	Visual counting	100% 98%	L/AP = H/P
Kedziersky *et al.* (2016)	55	Glass, PP	50 g	Water	–	123–234 μm	PVC ●		0.5 g/50 g	7.4x10^6 (kg)	10 g kg^{-1}	–	Balance/muffle loss	92–97%	–
Hengstmann *et al.* (2018)	29	Glass	25 ml	Water	–	0.3–1 mm^a 0.063–0.3 mm^a	PET, PVC ●	30/25 ml		1.2x10^3 (l)	23–867 mg l^{-1} 0.2–23 mg l^{-1}	Visual	Visual counting	54–89%	–
Mani *et al.* (2019)	56	Glass	10 g	Water	–	0.5–1.0 mm 0.3–0.5 mm	PP, PS, PMMA, PET ○●	10–15/10 g		1–1.5 x10^3 (kg)	440–1.000 mg kg^{-1} 12–27 mg kg^{-1}	FTIR	Visual counting	97%	L/AP > H/P
Classical density separation															
Coppock *et al.* (2017)	26	PVC	30–50 g	ZnCl$_2$	1.5	100–1000 μm^a	PE, PVC, PA66 ○●	10/30 g		330 (kg)	0.16–241 mg^{-1}	Visual	Visual counting	92–98%	L/AP = H/P
Nel *et al.* (2019)	30	PVC	30–50 g	ZnCl$_2$	1.5	~500 μm	PET ○● PE, PP	10/30 g		330 (kg)	19–30 mg^{-1}	Visual	Visual counting	83–92% 81–88%	(L/AP < H/P)
Lin *et al.* (2021)	41	Nalgene	15–50 g	NaI	1.6	50–335 μm^a	PE, PP, PVC, PET, PS ○●		0.5–20 mg/15 g	1.2x10^3 – 23x10^6 (kg)	0.033–1.3 g kg^{-1}	FTIR	Carbon-analyzer	77–106%	L/AP = H/P
Nakajima *et al.* (2019)	31	Glass	30–100 ml	NaI	1.6	0.5–2 mm 0.1–0.5 mm^a	PE, PP, PS, PVC, PET ○●	40/30 g		1.3x10^3 (kg)	77–7.700 mg kg^{-1} 0.6–120 mg kg^{-1}	Visual	Visual counting	97–100% 93–96%	L/AP = H/P (L/AP < H/P)
Enders *et al.* (2020)	32	Glass, stainless steel	500 ml	Sodium polytungstate	1.8	450 μm 125–150 μm	PA6 PE ○	20/0.5 l 60–80/0.5 l		40 (l) 120–160 (l)	2.200 μg l^{-1} 130–172 μg l^{-1}	Visual	Visual counting	95% 78%	**L/AP < H/P**
Nuelle *et al.* (2014)	27	Glass	1 kg	NaCl/NaI	1.2/1.8	*ca.* 1 mm	PE, PP, PVC, PET, PS, EPS, PUR ○●	10/1 kg		10 (kg)	4.6–7.2 mg kg^{-1}	Py-GC/MS	Visual counting	70–100 %	L/AP = H/P
Imhof *et al.* (2012)	33	Stainless steel	6 L	ZnCl$_2$	1.6–1.7	2–5 mm 40–309 μm	PE, PP, PET, PVC, PS, (...) ○● PE, PP, PET, PVC, PS ○●	10/1l	 0.1 g/1 l	10 (l) 4.6x10^3–3.4x10^6 (l)	37–900 mg l^{-1} 100 mg l^{-1}	Raman	Visual counting Balance	100% 96%	L/AP = H/P –
Quinn *et al.* (2017)	51	Glass	200 ml	ZnCl$_2$, NaI, NaBr, ...	1.37–4.2	800–1000 μm 200–400 mm	PP, PE, PS, PVC, PET ○● PE, PS, PA, PVC, PET ○●		66 mg/66 g	1.3–4.3 x10^3 (kg) 2x10^4–2.8x10^5 (kg)	990 mg kg^{-1}	FTIR	Balance	>85% >80%	(L/AP > H/P) L/AP = H/P
Gomiero *et al.* (2019)	34	Glass	2 kg	ZnCl$_2$	1.7–1.8	250 μm 20 μm, 100 μm	PVC ○● PP, PE	200/2 kg	800 μg/2 kg 0.6, 100 μg/2 kg	100 (kg)	400 μg kg^{-1} 0.3 μg kg^{-1}, 50 μg kg^{-1}	Py-GC/MS	Py-GC/MS	96% 83%, 92%	(L/AP < H/P)
Kögel *et al.* in prep		Glass	1 kg	NaBr	> 1.5	1 mm	PE, PS, PA66, PET ○●	10/1 kg		10 (kg)	4.8–7.2 mg kg^{-1}	FTIR	Visual counting	87–96 %	L/AP = H/P
This study		Glass	1 kg	NaBr	> 1.5	150–300 μm	PE, PP, PS, PA6, PC, PMMA, PVC, PET ○●	5–20/1 kg	4.8–49.3 μg/kg	5–20 (kg)	4.8–49.3 μg kg^{-1}	Py-GC/MS	Py-GC/MS	29–97 %	**L/AP < H/P**

[a] Merged range of particle sizes (more details in respective study); (L) & (kg) = number of particles per litre or kilogram; L/AP = low-density/apolar; H/P = high-density/polar; blue colouring = similar criteria characteristic compared to our study; grey colouring = insufficient information.

concentration in both particle count and mass for high- and low-density polymers, three studies can be classified as comparable.

In most approaches, process evaluation is performed by visual counting or weighing. Here, in particular, the optical preselection can be humanly biased.[30] However, some of the reviewed studies ensured their evaluation by additional or solely instrumental identification techniques (Table 2, C1), but almost none conducted the quantification by a common polymer-specific quantification technique (Table 2, C2). The resulting polymer recoveries averaged around 90%, but reported efficiencies span broad ranges (54–100%).

Implications on extraction behaviour. The potential impact of the reviewed studies on MP extraction behaviour is given in the column "extraction behaviour" with respect to the available information (Table 2). Those studies not using polymers of different densities[29,53] or did not specify polymer specific recoveries[33] were excluded from final discussion (Table 2, D1).

The derived extraction behaviours either showed no density/polarity dependent recoveries,[26–28,31,33,41,52] lower recoveries for high-density/polar polymers[52,54] or the most interesting indication of lower recoveries for low-density/apolar polymers.[30–32,34]

Lower recoveries for high-density polymers were observed for extraction procedures with a weak flotation mechanism *e.g.*, light density solution (NaCl) or weak elutriation.[54–56] Here, apparently the buoyancy induced by the weak flotation mechanism is insufficient for high-density particles and causes minor recoveries. This is most likely causing respective effects in the reviewed studies by Quinn *et al.* and Mani *et al.*[52,54]

The majority of the studies indicate no density/polarity dependent recoveries (8 studies) (Table 2, D1). It is expected and found in our previous experiments, that large particles are more affected by density differences then small particles. Small particles are more affected by surface properties and thus less effectively separated from sediments than large particles.[18,40] This could explain no observed effects of four approaches.[26,27,31,33] Two studies used high polymer particle/mass concentrations spiked into only small matrix aliquots.[41,52] This divergent proportion of high polymer loads to low matrix volume might result in a more sufficient extraction due to the saturation effect which shields MP particles from adhesion or aggregation to other materials. Additionally, any loss of a few particles is neglectable.[41] Both arguments might have prevented any observations with respect to density/polarity driven behaviour. The remaining one study by Claessens *et al.*[28] used a combination of flotation and elutriation mechanisms for MP separation and not gravity only. Here, a combined effect of dense NaI solution and the elutriation might favour higher recovery of small light (here PE) particles (250 μm) even at low particle numbers (50 per polymer) and resulted in no density/polarity dependent recoveries.

At least an initial suspicion that less dense, apolar polymers might be more affected by density separation is indicated in the data presented by Nakajima *et al.*[31] They reported a higher variance in PE regarding the small particle fraction. This might be implied by two other studies as well, since they observed slightly less effective recovery rates of PE.[30,34] Unfortunately, both studies give insufficient details on either particle size or process evaluation, which hinders further discussion.

The most distinct evidence for a different behaviour of apolar and low density polymers is indicated in the work of Enders *et al.*[32] Their experimental setup resulted in a 17% less effective recovery of PE compared to PA6. The study stands out by using a closely related setup to our study with almost comparable polymer concentrations (particle; mass) while utilizing small polymer particle sizes. Unfortunately, it was only restricted to these two polymer types.

4. Conclusion

Recovery experiments from artificial sediments spiked with pristine and biofouled MPs were performed with a custom-made density separator, leading to unexpected results. While the precision was absolutely convincing with respect to the complex procedure a variable, polymer specific accuracy was obvious. Low density, non or less polar polymers revealed lower recoveries compared to those of higher density and polarity. The experimental setup focussed on low concentrations (5–50 μg), low particle numbers ($N = 5$–20), and small particle sizes (150–300 μm) and a broad polarity polymer range was adapted to mimic realistic environmental concentrations. This so far undescribed, counterintuitive and unanticipated extraction behaviour was suspected to indicate a density/polarity related and concentration dependent particle size/surface phenomenon inside the separation system. The fact that high-density polymers were more effectively recovered changed the determining focus from density effects of the separation fluid, towards other relevant factors in particular regarding small microplastic particles.

Published studies of MP density extraction validation studies covered almost no comparable recovery experiments concerning particle size, polymer density range and polymer concentration. However, a few studies focused on smaller particle sizes (<300 μm, low concentration ppb range/low counts), and different density polymer concentrations at least indicate evidence of this density/polarity driven extraction behaviour as well.

The presented results emphasize the complexity of microplastic analysis in particular, when complex pre-concentration steps are involved. Since authentic environmental sediments will introduce additional factors that further affect particle extraction behaviour the perfect microplastic extraction technique at trace levels and respective recovery experiment are still under construction.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

We would like to thank Joanne Yong (ICBM Planktology working group) for providing the benthic algae community for our incubation experiment as well as Anke Müllenmeister and

Sebastian Neun for their support in the laboratory. The study was kindly supported by the German Federal Ministry of Education and Research (Bundesministerium für Bildung und Forschung, BMBF) in the joint research projects BASEMAN (grant ID 03F0734D; JPI-Oceans) and PLAWES (grant ID 03F0789E; FONA III, MH).

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