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1 **Validation of density separation for the rapid recovery of microplastics from sediment.**

2
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12
13 **Abstract:**

14 Several density separation techniques using numerous brine solutions have been developed for the
15 separation of microplastics from sediment. The aim of this study was to validate the use of various
16 brine solutions in a relatively rapid, reproducible, low cost single stage method that can deliver
17 consistently high recoveries for different microplastic polymers <1 mm appropriate for monitoring
18 programmes. The recovery of environmentally relevant microplastics (200 – 400 µm and 800 – 1000
19 µm) from post-consumer products was tested against tap water and brine solutions of varying
20 density including sodium chloride (NaCl), sodium bromide (NaBr), sodium iodide (NaI) and zinc
21 bromide (ZnBr₂). As expected general trend of increasing microplastic recovery with increasing
22 solution density was observed, with NaI and ZnBr₂ having significantly ($p < 0.001$) higher rates of
23 microplastic recovery. Microplastic size was found to influence recovery rates and needs to be taken
24 into consideration when choosing a brine solution. From this work it is evident that density
25 separation recovery tests are needed to validate the use of brine solutions for microplastic recovery
26 and that ZnBr₂ is a novel and appropriate brine solution for microplastic extraction. This study
27 represents the most in depth validation of brine solutions for the density separation of microplastic
28 from sediments undertaken to date.

29
30
31 **Keywords:**

32 Density separation; brine solution; microplastic; sediment; validation

1 **1. Introduction**

2

3 Microplastics are commonly found throughout the environment and have been isolated from water,
4 sediment and biological samples throughout the world (for review see¹). Much of this research has
5 concentrated on marine sediments which are seen as the ultimate sink for all microplastic² and
6 where microplastics can represent 3.3% of the sediment by weight on heavily polluted beaches.³

7 Following sediment sample collection for microplastic analysis the potential plastic fragments need
8 to be separated from other organic and inorganic material for identification and quantification. In
9 some cases the sample may be reduced in the field by filtration or density separation,⁴ but in most
10 cases a bulk sample will be taken back to the laboratory for microplastic analysis. There are several
11 laboratory techniques commonly used for the separation of microplastics from sediment including
12 visual sorting, filtration, sieving, density separation, elutriation, flotation and chemical digestion.⁵⁻⁷

13 As with most aspects of microplastic research, the rapid development of the field and the lack of
14 standardised techniques have led to an inconsistency in the extraction methods used to quantify
15 microplastics, particularly from sediments.^{1,8} However, guidelines and protocols have been
16 produced by regulatory bodies in an attempt to standardise the separation of microplastics from
17 environmental samples^{6,9,10}. In this study we compare and validate the use of several brine solutions
18 for the density separation of microplastics from sediment.

19

20 The method used for the separation of microplastics from sediment is influenced by the physical
21 characteristics (size, density, shape) of both the sediment and the microplastics. The separation of
22 larger microplastics from fine sediments (mud or silt) by visual sorting, sieving or filtration is
23 relatively simple and commonly practiced^{5,11,12}. However the separation of small microplastics can
24 be more difficult, particularly from finer sediments with microplastic shape influencing the
25 separation ability. Several techniques have been developed to overcome these issues including
26 elutriation (separated based on size, shape and density by a stream of gas or liquid flowing in an
27 opposite direction to sedimentation) and flotation (separation based on relative buoyancy and
28 hydrophobic nature) that have been successfully used for the separation of microplastics from
29 sediments.¹³⁻¹⁷ However, density separation is the most reliable and common method for the
30 separation of microplastics from sediment or sand.

31

32 In density separation, materials of different densities are placed in a liquid of intermediate density,
33 where the less dense material floats and separates out from the more dense sinking material.

34 Changing the density of the liquid, commonly a brine solution (a solution of a salt in water) allows

1 for the floating of particles of different density, relative to the density of the solution. This technique
2 has been applied in 65% of the studies where microplastics were separated from sediments.⁵ The
3 most common brine solution is saturated sodium chloride (NaCl) with a specific gravity of 1.2 g/cm³,
4 separating only items lighter than this from the more dense sinking sediment, with a typical density
5 of 2.65 g/cm³. Density separation is normally achieved by agitating the sediment in saturated NaCl
6 solution as described by Thompson et al.¹⁸. NaCl has the advantage of being cheap, widely available
7 and environmentally benign. However although many microplastics have a density <1.2 g/cm³
8 including polypropylene (PP), polyethylene (PE), polystyrene (PS) and polyamide more commonly
9 known as nylon (see table 1) and may be floated by this solution, several others will not include the
10 more dense polyvinyl chloride (PVC) and polyethylene terephthalate (PET) which make up >17% of
11 global plastic demand¹⁹. This is of particular importance as these more dense plastics will be the first
12 to settle and be incorporated into marine sediments¹ and will therefore be under represented in
13 sediment analysis. Despite this the Marine Strategy Framework Directive (MSFD) technical sub-group
14 on marine litter recommend NaCl solution for the separation of microplastics from sediments by
15 density separation⁹.

16
17 To address this issue brine solutions with a higher density have been used for microplastic sediment
18 extraction including sodium polytungstate (1.4 g/cm³)^{20,21}, zinc chloride (1.5-1.7 g/cm³)^{15,22},
19 Calcium chloride (1.30 – 1.35 g/cm³)¹⁷ and sodium iodine (1.8 g/cm³)^{13,14,23}. Recently, NOAA
20 recommended the use of 5.4 M lithium metatungstate (1.62 g/cm³) for density separation¹⁰. To
21 ensure the separation of all plastic polymers from sediment brine solutions of a density of >1.45
22 g/cm³ have been recommended^{1,15}. However these higher density solutions are often very
23 expensive and toxic to the environment, reducing their practical use in larger scale studies and in
24 many cases their ability to recover microplastics from sediments has not been investigated.

25
26 Despite the use of several different brine solutions in the density separation of microplastics from
27 sediments, only limited validation has been undertaken on their effectiveness. Several publications
28 use larger (>1 mm) spherical microplastics in relatively coarse sediments that although provide good
29 microplastics recoveries, may not be environmentally realistic²⁴⁻²⁶. Using density separation
30 microplastic recoveries of 91-99 % have been recorded for microplastics ~1 mm,¹⁴ but with recovery
31 rates reducing to 40% for smaller (40-309 µm) particles¹⁵. The current work focuses on validating
32 the efficiency of four brine solutions and water for the separation of environmentally relevant post-
33 consumer microplastics from two different size ranges (200 – 400 µm and 800 – 1000 µm) based on
34 the original density separation method by Thompson et al.¹⁸ and further developed by Claessens et

1 al.²⁷. The aim of this work was to validate a relatively rapid, robust technique that would include
2 separation of high and low density plastics in a one stage process for use in monitoring by regulatory
3 agencies.

4
5

6 **2. Materials and Methods**

7 **2.1 Brine solution preparation:**

8 The density separation of tap water and four brine solutions (sodium chloride (NaCl), sodium
9 bromide (NaBr), sodium iodide (NaI) and zinc bromide (ZnBr₂)) with various densities (Table 1) was
10 tested. Fully saturated solutions of NaCl, NaBr and NaI were made by dissolving the relevant salt into
11 a volume of distilled water (1L) on a magnetic stirring plate until a small excess formed at the
12 bottom of the beaker and no more salt would dissolve into solution. At this stage the density of the
13 solution was tested to ensure saturation using the equation:

$$14 \quad \text{Density} = \frac{(\text{Weight of flask and brine (g)} - \text{Weight of flask (g)})}{\text{Volume of flask (cm}^3\text{)}}$$

15 As saturated ZnBr₂ has a density of 4.2 g/cm³, a 25% saturated solution was made to obtain the
16 desired density of 1.7 g/cm³. For this the appropriate amount of salt (1125 g L⁻¹) was slowly added to
17 distilled water using a stirring plate with the density checked at regular intervals using the equation
18 above.

19 The same brine solutions were reused throughout the series of density separation tests and were
20 filtered twice through Whatman Grade 540 ashless filters and a thin layer of Celite® 512 medium
21 (Sigma) between tests. The density of the solution was checked after each use as described above
22 and adjusted where necessary. The microplastic separation efficiency of these reused solutions was
23 checked regularly using a reference plastic (polyethylene (180 μm) Sigma) and was found to be
24 consistently within 2% of the reference results presented in Figure 1.

25 **2.2 Microplastic preparation**

26 Eight different types of plastic polymer were taken from 11 different post-consumer products (listed
27 in Table 2) that were broken down to secondary microplastics by various physical methods including
28 a coffee grinder, food processor and in some cases with the use of liquid nitrogen. The colour and
29 reference density was noted for each plastic sample and Fourier Transform Infrared Spectroscopy
30 (FTIR) analysis was undertaken to confirm the correct sample identification as per the Resin

1 Identification Code (RIC). Following mechanical breakdown the plastics were passed through a series
2 of sieves on a mechanical shaker and divided into size classes with the 200 - 400 μm and 800 - 1000
3 μm fraction being investigated. Although of varying shape, these secondary microplastics could be
4 considered as fragments. Commercially obtained polyethylene spheres (180 μm) were obtained
5 from Sigma. The nylon sample (thread) was cut into small pieces using a scissors with the average
6 size (340 μm , ± 174 Std Dev) calculated by observation using a graticule under a light microscope.

7 **2.3 Marine sediment preparation**

8 A sample of marine sediment was collected from a beach along the Firth of Clyde in the west of
9 Scotland. This beach and its sediment were representative of the area and of beaches commonly
10 found throughout the UK. A subsample was passed through a series of sieves on a mechanical shaker
11 with the 200 - 400 μm particle size fraction collected and cleaned by repeated (x 3) washing with
12 25% ZnBr_2 brine solution using the technique described below to remove all floating debris and any
13 traces of environmental microplastic contamination, verified by microscopic examination. This
14 sediment was stored in a glass container and kept clean following the strict contamination
15 prevention outlined below. The sediment was cleaned between each density separation using 25%
16 ZnBr_2 brine solution to remove all floating debris and ensure no contamination between each test.
17

18 **2.4 Density separation**

19 The percentage recovery of tap water and the four brine solutions was investigated using a series of
20 spiking experiments, where 66.66 g of clean sediment and 0.066g of microplastic was added to a 400
21 ml glass beaker. A volume of 200 ml of the solution to be investigated was added following the ratio
22 of 3:1 described by Claessens et al.²⁸ and the mixture was stirred using an overhead stirrer at 300
23 rpm for 3 minutes. The stirrer was lifted from the solution and the paddle rinsed with the relevant
24 solution. The solution was left to settle for 10 minutes, allowing the lighter plastic particles to float
25 or stay in suspension as the heavier sediment particles sank. The particles that accumulated on the
26 surface of the solution were recovered by vacuum suction. A glass tube connected to a vacuum
27 system by a rubber tube was moved around the surface of the solution and particles would be
28 collected into a three necked flask which acted as a trap, the rubber and glass tubing was thoroughly
29 rinsed with the relevant solution to ensure no microplastics were lost. A small amount of soap was
30 occasionally touched to the liquid surface causing the plastics to accumulate on one side of the
31 beaker and making it easier for them to be collected by suction. This top layer of solution containing
32 the plastic that was collected in the trap was filtered through a clean filter paper (Whatman No 1,

1 cellulose) using a Buchner funnel. Once filtration was complete the flask was thoroughly washed to
2 ensure no loss of sample and the filter paper transferred to a watch glass and placed in an oven (70
3 °C) for 10 min. Once dry the microplastics were transferred to a pre-weighted filter paper, weighed
4 using an analytical scale and the percentage recovery calculated following the equation:

$$5 \quad \% \text{ Recovery} = (\text{final weight of plastic} / \text{initial weight of plastic}) \times 100$$

6

7 The lower density solutions (water, NaCl and NaBr) required 3 washes for microplastics extraction as
8 per Thompson et al. ¹⁸ with the more dense NaI and ZnBr₂ brine solutions only requiring a single
9 wash. This protocol was used to test the separation efficiency of water and four brine solutions on
10 selected microplastics from two size ranges (200 – 400 μm and 800 – 1000 μm). The microplastics
11 were tested for each solution individually and as a mixture made from an equal mass of each
12 polymer (200 – 400 μm only).

13

14 **2.5 Contamination mitigation**

15 To prevent microplastic contamination a strict contamination mitigation protocol was adhered at
16 every step of the procedure. This protocol was outlined in detail in Murphy et al. ²⁹ and is
17 summarised below. Clean cotton white lab coats were worn at all times with no synthetic fibres
18 underneath. The protocol involves three steps: 1) all surfaces and equipment were cleaned three
19 times with 70% ethanol and distilled H₂O and equipment examined under a dissection microscope
20 for microplastic contamination before use. 2) All laboratory benches were examined for particle
21 contamination using the taping technique used in forensic science. This was undertaken before and
22 after all procedures with the tapings being examined microscopically and by FTIR where necessary.
23 3) Atmospheric microplastic contamination was investigated using the taping technique and by
24 leaving clean filter papers in petri dishes for the duration of the lab work that were later checked for
25 contamination.

26 Care was taken to limit the amount of time a sample was exposed to air both during the density
27 separation protocol and after the microplastics had been recovered. Filter papers containing
28 microplastics were kept in sealed petri dishes to prevent contamination. Blank runs were
29 undertaken to ensure glassware was properly clean, using the density separation protocol with no
30 sediment sample, allowing the determination of potential microplastic particles remaining from
31 previous runs.

32 **2.6 FTIR polymer identification**

1 Before being broken down into secondary microplastics each item was positively identified by
2 Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR) using a Perkin Elmer
3 Spectrum One FTIR. This was also randomly undertaken on microplastic samples to ensure no
4 contamination. Infrared radiation from 600–4000 cm^{-1} was used with a spectral resolution of 4 cm^{-1}
5 with 4 scans taken to produce the specific spectra, which was compared to reference spectra
6 present on the Thermo Scientific™ OMNIC™ software to identify the plastic polymers. FTIR allows
7 for the identification of chemical bonds present in the samples and gives a characteristic signal in the
8 “fingerprint” region.

9

10 **2.7 Statistical analysis**

11

12 Microplastic recovery data (%) was expressed as the mean \pm the standard deviation. Sample
13 numbers of $n=9$ and $n=3$ were used for the 200 – 400 μm and 800-1000 μm fractions respectively
14 giving a total number of 450 and 105 density separations for each size fraction respectively. Data
15 normality and distribution (homogeneity of variances) were tested using the Shapiro-Wilks and
16 Levene`s tests respectively. Where normality and equal variance was demonstrated analysis of
17 variance was performed and critical differences between groups appraised using the Bonferroni t-
18 test. Following transformation if data were found to fail one or other of these tests nonparametric
19 analysis of variance was performed using Kruskal-Wallis one way ANOVA on ranks. All statistics were
20 undertaken using Statistica (Version 6).

21

22

23 **3. Results and Discussion**

24

25 Although various degrees of validation of the different methods for separating microplastics from
26 sediment (flotation, elutriation and density separation) have been undertaken,^{13-15,28} it has been
27 mentioned that validation tests on recovery rates for microplastic extraction from sediments are
28 rarely reported.¹⁴ The results from the series of 200 – 400 μm microplastic experiments clearly show
29 a general trend of increasing microplastic recovery with increasing solution density, with NaI and
30 ZnBr_2 having significantly ($p<0.001$) higher rates of recovery for all polymers (except PVC (P) with
31 ZnBr_2) (Figure 1). Tap water consistently had the lowest microplastic recovery rates, as would be
32 expected being the least dense solution. This was particularly true for the denser PET samples and a
33 low recovery rate (~70%) was found for the microplastics mixture. Despite this recovery rates of

1 >95% were found for nylon fibres. These results are in accordance with data recently published by
2 Alomar et al.¹² who most commonly found microplastic fibres when using distilled water for density
3 separation of marine sediments. It would appear that density separation with water would actively
4 select fibres as they have a large surface to volume ratio and given their shape they are easily
5 trapped in the surface tension film.

6

7 Of the four brine solutions investigated, NaCl showed the lowest recovery rates (85-95% and <90%
8 for mixed microplastics) and largest error bars (indicating greater variability) for the 200 – 400 µm
9 microplastics (Figure 1). Interestingly only four of the tested twelve samples (nylon, PVC (UP) and
10 both HDPEs) showed significantly higher recover rates than water. This is particularly relevant as
11 although they have similar densities (Table 1), NaCl is the most popular brine solution for
12 microplastic density separation and is recommended for use by the Marine Strategy Framework
13 Directive (MSFD) Technical Sub- group on Marine Litter⁹ since it is low cost, abundant and
14 environmentally benign. NaCl was the original brine solution used for microplastic density
15 separation by Thompson et al.¹⁸ and is still commonly used throughout the world.³⁰⁻³³ These
16 recovery rates are similar to the 68.8-97.5% recoveries found by Claessens et al.²⁸ using the
17 Thompson protocol, but in this work no details were provided on the recovery rates for specific
18 polymers. Interestingly, low recovery rates were found for PET 1 using both NaCl and water,
19 particularly compared against the PET 2 sample. Although having the same resin identification code
20 (RIC) these samples were taken from a carbonated soft drink bottle (PET 1) and a water bottle (PET
21 2) and are indicative of how the same polymer can contain different additives or wetting agents that
22 can impact on their density and subsequent separation. This has been observed by Wang et al.¹⁶
23 who found the addition of various wetting agents to a polymer can significantly decrease its
24 floatability.

25

26 To the authors knowledge this is the first time NaBr has been used as a brine solution for the density
27 separation of microplastics. Having a medium density (1.37 g/cm³) it consistently showed better
28 microplastic recoveries than both NaCl and water, with significantly higher recoveries than water for
29 nine of the twelve tested microplastics, with the lighter PE samples and PET 2 being the exception
30 (Figure 1). However it showed no significant differences in recovery rates compared to NaCl, with
31 the exception of nylon fibres. Therefore for microplastic of the 200 – 400 µm size range, NaBr does
32 not hold any significant advantage for the replacement of the commonly used NaCl solution. A brine
33 solution of sodium polytungstate with a similar density (1.4 g/cm³) has been used to separate out
34 microplastics from marine sediments,^{20,34} but to our knowledge no recovery test for this solution

1 have been undertaken. Another brine solution of calcium chloride, with a similar density (1.30 – 1.35
2 g/cm³) showed recovery rates of ~55% for PE (100 – 1000 μm) enriched sediment using an air
3 venting separation technique.¹⁷ From these results it would appear that a brine solution of >1.4
4 g/cm³ is needed to ensure effective microplastic separation, as previously recommended by several
5 authors.^{1, 15}

6
7 The NaI brine solution was particularly effective for the separation of the 200 – 400 μm
8 microplastics, showing significantly higher recovery rates than water and for nine and three of the
9 twelve microplastics compared to NaCl and NaBr respectively (Figure 1). NaI also showed higher
10 recovery rates than ZnBr₂ for four of the tested microplastics, being significantly higher for PVC (P).
11 NaI has previously been used for the density separation of microplastics from sediments.^{13, 14, 23} In
12 recovery experiments using 1mm microplastics (PE, PP, PVC, PET, PS and polyurethane (PUR))
13 recovery rates of between 91-99% were obtained.¹⁴ This paper represents one of the few in depth
14 studies to investigate microplastic recovery rates. However comparison is difficult as they used a 2
15 step method to extract microplastics from sediments involving air-induced overflow (AIO) based on
16 fluidisation in a NaCl solution to reduce the material volume, followed by flotation using NaI
17 solution. As saturated NaI solution has sufficient density (1.6 g/cm³) to separate the heavier additive
18 containing polymers it has been recommended as a suitable brine solution above NaCl for
19 microplastic separation.³⁵

20
21 This study is the first to report the use of ZnBr₂ for the density separation of microplastics from
22 sediments. A 25% saturated solution (density of 1.71 g/cm³) was used, as when fully saturated ZnBr₂
23 has a density of 4.5 g/cm³, dense enough to cause sediment floatation. ZnBr₂ gave the highest
24 recovery rates for eight of the twelve microplastics in the 200 – 400 μm size range, being
25 significantly higher than both water and NaCl for all but PVC (P) and HDPE (gum) (NaCl only). Despite
26 having the highest recovery rates, these were not significantly higher than NaI. ZnBr₂ showed
27 particularly good recoveries (99%) for the mixed microplastic sample that most closely represents
28 environmental samples. This brine solution also provided relatively tight error bars indicating a good
29 level of reproducibility for all polymers investigated. ZnBr₂ has a similar density to zinc chloride
30 (ZnCl₂, 1.7 g/cm³) which has been previously used for microplastic separation.^{15, 22} In recovery
31 experiments ZnCl₂ brine solution was found to have a 100% and 95% recovery rate for large (1-5
32 mm) and small (<1 mm) microplastics (respectively) from seven environmentally relevant plastic
33 types (PA, PE, PVC, PC, HDPE, PET, PP).¹⁵ However comparison with the present data is difficult as a
34 Munich Plastic Sediment Separator (MPSS) device was used for density separation on mostly virgin

1 plastic pellets. Using the classic Thompson¹⁸ technique, recovery rates of ~40% were found for the
2 small microplastics. This is considerably lower than the current study, but was thought to be due to
3 the significant loss of microplastics during handling as they stuck to the wall of the apparatus. Due to
4 their similar densities, both ZnBr₂ and ZnCl₂ brine solutions are good candidates for microplastic
5 density separation.

6
7 The density separation of the larger microplastic size class (800 - 1000 μm) showed a similar but
8 more pronounced trend of increasing recovery rates with increasing brine solution density.
9 Interestingly the recovery rates for both water and NaCl are lower for the larger microplastic size
10 compared to the smaller particles of the same polymer, being below 80% and 85% respectively
11 (Figure 2). These recovery rates fell to ~60% for the heavier PVC and PET samples indicating that
12 neither NaCl nor water are suitable for the extraction of these heavier plastic fragments. Although
13 this decrease in recovery rates is substantial (Figure 2), due to the non-parametric statistical
14 methods used, it was not significant. The results for the remaining brine solutions show a clear
15 stepwise increase in recovery with increasing density from NaBr, NaI to ZnBr₂. This increase is
16 significant for the lighter microplastics (PP, HDPE and both PS samples), but not for the heavier PVC
17 and PET, again most likely due to the non-parametric statistical methods used. For these polymers
18 only ZnBr₂ shows significantly higher recoveries than water. It is evident from these results that for
19 larger microplastics class ZnBr₂ shows the best recoveries with rates consistently >95%, higher than
20 those obtained for the smaller (200 - 400 μm) particles. In contrast, although not significant NaBr
21 and NaI solutions show higher recovery levels for the smaller rather than larger microplastics.

22
23 Although not always reported in the literature, the size of the microplastics used in recovery tests
24 can impact on their recovery rates. As microplastics include particle up to 5 mm and separating them
25 from sediments can often become more difficult at smaller sizes, most authors only include plastics
26 >1 mm for both environmental sampling^{16, 24-26, 34, 36} and recovery experiments^{14, 34, 37}. More attention
27 needs to be paid to influence of brine solution on particle size as small microplastics (<1 mm)
28 represent 35-90% of all microplastics in the marine environment^{26, 38-41} and therefore need to be
29 included in environmental sampling to avoid a serious underestimation of microplastic
30 contamination in sediments.

31
32 In this work we focused on making the validation method as realistic as possible to field sampling
33 conditions by using post-consumer plastics commonly found in the environment in non-uniform
34 shapes and sizes and sediment (cleaned and graded to 200-400 μm to help standardisation) taken

1 from the environment. The technique used closely resembled that by Thompson et al.¹⁸ and further
2 developed by Claessens et al.²⁸ and was kept as simple as possible to help reduce contamination and
3 allow a relatively rapid process time that is needed by regulatory agencies for monitoring
4 programmes involving large numbers of samples. Although there have been several published
5 techniques that produce high microplastic recovery rates such as elutriation,¹³ MPSS¹⁵ and a two-
6 step air-induced overflow (AIO) followed by NaI density separation,¹⁴ our aim was to validate a
7 relatively rapid, reproducible, low tech, low cost approach that can deliver consistently high
8 recoveries for different polymers <1 mm appropriate for monitoring programmes. Both NaI and
9 ZnBr₂ also have the advantage of only needing a single wash of the sediment for microplastic
10 removal, as opposed to the three washes needed for NaCl solution, making the process more
11 efficient. NaI showed similar recovery rates to ZnBr₂ and is cheaper and less environmentally
12 dangerous, but when used on sediment samples from the environment it turned the filter paper
13 black, making it very difficult to isolate the microplastics. This was thought to result from the excess
14 sodium iodide creating free iodine which reacts with starch in the paper. Although these tests
15 indicate ZnBr₂ is the most suitable brine solution for the extraction of microplastics from sediment it
16 is expensive and severely hazardous to the environment.¹⁴ However these issues are overcome by
17 the successful reuse of this brine solution following careful filtration and clean up. Although the
18 solution can change to a light brown colour and needs the density checked and adjusted where
19 necessary, this is simple process and will allow the solution to be used indefinitely offering a
20 relatively cheap and environmentally responsible method for density separation of microplastics
21 from sediment.

22
23

24 **4. Conclusion**

25

26 There are numerous brine solutions that are potentially suitable for the density separation of
27 microplastics from sediment samples. However, before they can be used for monitoring purposes
28 their ability to separate out a wide range of environmentally relevant polymers with the appropriate
29 shape and sizes that are commonly found in the environment needs to be investigated. Although the
30 recovery tests outlined in the present work are relatively simple and indeed are designed to be so,
31 this validation step is essential to ensure that a proper representative microplastic sample is
32 obtained to prevent an underrepresentation of microplastics (particularly <1 mm) in the
33 environment. Given the wide range of techniques and brine solutions currently used to separate
34 microplastics from sediment it is increasingly difficult to compare the results generated from various

1 studies undertaken around the world to get a true representative picture of the scale of microplastic
2 pollution. Only through the use of standardised, validated protocols can we ascertain the most
3 suitable methods and get a true picture of the scale of microplastic contamination.

4
5

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9
10

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1 **Figures & Tables**

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4 **Table 1.** The density of the different brine solutions and water tested for microplastic recovery by
5 density separation in the study.
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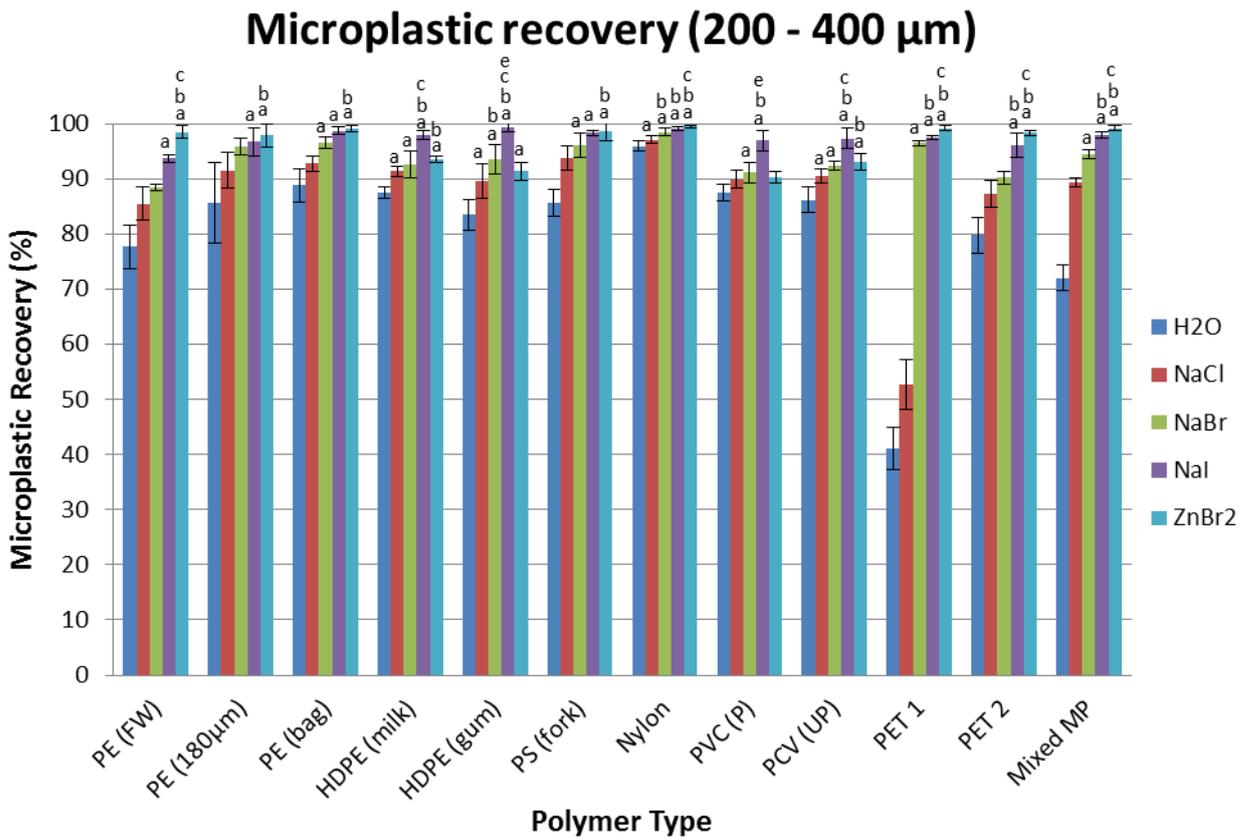
Solution	Density (g/cm ³)
Water (H ₂ O)	1.0032
Sodium Chloride (NaCl)	1.1708
Sodium Bromide (NaBr)	1.37
Sodium iodide (NaI)	1.566
Zinc bromide (ZnBr ₂) 25%	1.71

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10 **Table 2.** The polymer type, source, resin identification code (RIC) used for recycling, colour and
11 density of the microplastics used in the density separation validation studies.
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Plastic	Source	RIC	Colour	Density
Polypropylene (PP)	Plastic Container*	5	Clear	0.855 – 0.946 g/cm ³
Low density Polyethylene (LDPE)	Face wash	4	Blue	0.915 – 0.925 g/cm ³
Polyethylene (PE 180µm)	Sigma	4	White	0.926 – 0.940 g/cm ³
Polyethylene (PE)	Supermarket Bag	4	Clear	0.926 – 0.940 g/cm ³
High density Polyethylene (HDPE)	Milk carton Chewing gum box*	2	White black	0.94 to 0.97 g/cm ³
Polystyrene (PS)	Plastic Fork* Coffee cup lid*	6	White	0.96 – 1.04 g/cm ³
Polyamide (nylon)	Thread	7	Green	1.13-1.15
Plasticised Polyvinyl Chloride (PVC P)	Electrical cable*	3	Black	1.1 – 1.35 g/cm ³
Un-plasticised Polyvinyl Chloride (PVC UP)	Window frame*	3	White	1.35 – 1.45 g/cm ³
Polyethylene terephthalate (PET)	Soft drink bottle 1 Water bottle 2*	1	Clear	1.38 g/cm ³

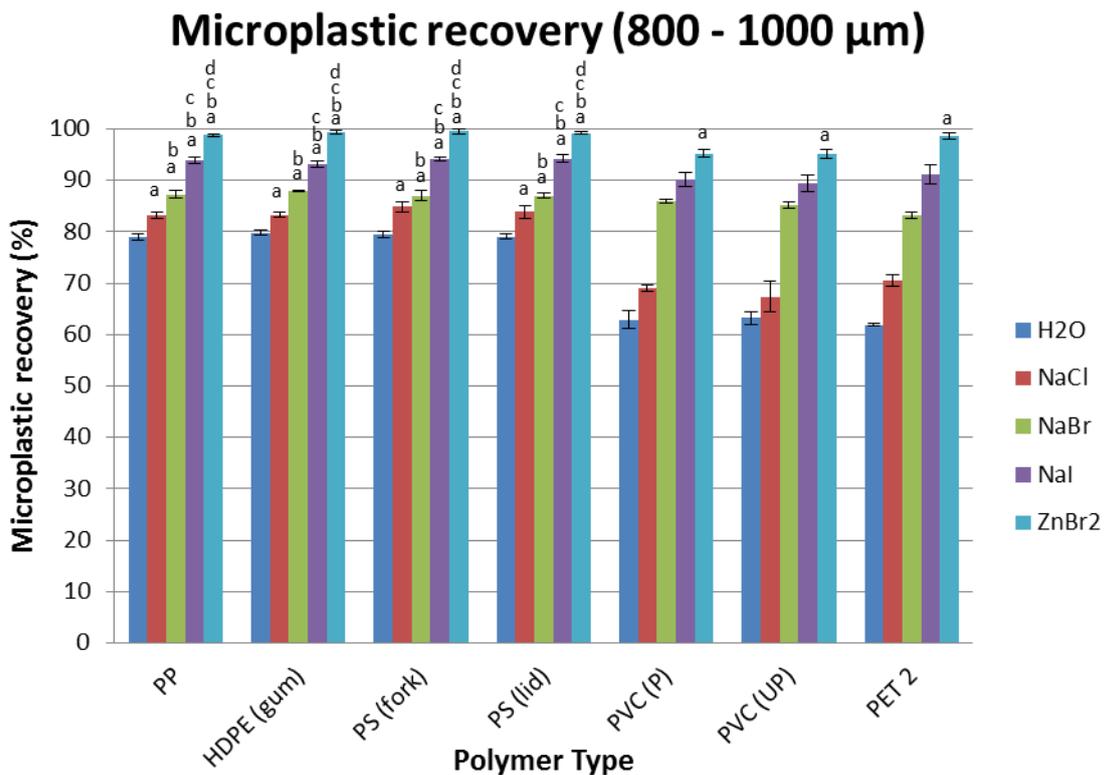
13 *Also used for the larger microplastics (800 – 1000 µm) recovery tests.
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1 **Figure 1.**
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5 **Figure 1.** Microplastic recovery (% mean (n=9) ±Std Dev) for various polymers sized 200 – 400 μm
6 tested individually using water and sodium chloride (NaCl), sodium bromide (NaBr), sodium iodide
7 (NaI) and zinc bromide (ZnBr₂) brine solutions. Polymer type abbreviations are provided in Table 1.
8 Letters indicate significantly higher recovery compared to H₂O (a), NaCl (b), NaBr (c), NaI (d) and
9 ZnBr₂ (e).
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1 **Figure 2.**
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6 **Figure 2.** Microplastic recovery (% mean (n=3) ±Std Dev) for various polymers sized 800 – 1000 μm
7 tested individually using water and sodium chloride (NaCl), sodium bromide (NaBr), sodium iodide
8 (NaI) and zinc bromide (ZnBr₂) brine solutions. Polymer type abbreviations are provided in Table 1.
9 Letters indicate significantly higher recovery compared to H₂O (a), NaCl (b), NaBr (c) and NaI (d).

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