A new small device made of glass for separating microplastics from marine and freshwater sediments

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Abstract

18

Separating microplastics from marine and freshwater sediments is challenging, but necessary to determine their distribution, mass and ecological impacts in benthic environments. Density separation is commonly used to extract microplastics from sediments by using heavy salt solutions, such as zinc chloride and sodium iodide. However, current devices/apparatus used for density separation, including glass beakers, funnels, upside-down funnel-shaped separators with a shut-off valve, etc., possess various shortcomings in terms of recovery rate, time consumption and/or usability. In evaluating existing microplastic extraction methods using density separation, we identified the need for a device that allows rapid, simple and efficient extraction of microplastics from a range of sediment types. We have developed a small glass separator, without a valve, taking a hint from an Utermöhl chamber. This new device is easy to clean and portable, yet enables rapid separation of microplastics from sediments. With this simple device, we recovered 94-98% of <1,000 μm microplastics (polyethylene, polypropylene, polyvinyl chloride, polyethylene terephthalate, and polystyrene). Overall, the device is efficient for various sizes, polymer types, and sediment types. Also, microplastics collected with this glass-made device remain chemically uncontaminated, and can therefore be used for further analysis of adsorbing contaminants and additives on/to microplastics.

35

Introduction

37

Microplastics, small pieces of plastic ranging from 5 mm in size down to microscopic, are ubiquitously distributed particulate contaminants that have to-date been detected in various

40 environmental samples, including freshwater lakes and rivers (Eriksen et al., 2013; Free et al.,
41 2014; Gasperi et al., 2014; Biginagwa et al., 2016), seas and oceans, including the deep-sea and
42 the polar regions (Lusher et al., 2014; Shim & Thompson, 2015; Bergmann et al., 2017;
43 Matsuguma et al., 2017; Wang et al., 2019). Once they end up in aquatic environments,
44 microplastics eventually accumulate in/on the surface water, shores/beaches and in the sediments
45 of benthic environments (Hidalgo-Ruz & Thiel, 2013; Van Cauwenberghe et al., 2013; Law &
46 Thompson, 2014; Van Sebille et al., 2015). Marine and freshwater sediments are considered to
47 possibly be the biggest sink of plastics in aquatic environments since significant numbers of
48 microplastic fragments, films, spheres and fibers have been found in multiple sediment samples
49 from various locations (Woodall et al., 2014; Bergmann et al., 2017; Matsuguma et al., 2017).
50 However, we have not yet reached a consensus about the amounts of microplastics accumulating
51 in oceanic and freshwater sediments, how these microplastics were transported into the
52 sediments, and what their impact is on benthic animals (Farrell & Nelson, 2013; Ugolini et al.,
53 2013; Law & Thompson, 2014; Green et al., 2016). Increased amounts of microplastics in/on the
54 sediment enhance the risk of microplastic ingestion by benthic animals (Van Cauwenberghe et
55 al., 2015a). Developing an efficient method for detecting microplastics from sediments is an
56 important issue for further understanding the distribution, mass and ecological impacts of
57 microplastics in both marine and freshwater environments.
58
59 One of the major concerns in microplastic studies of sediment samples is methodological – what
60 is the best way to extract microplastics from a large amount of sediments? Several methods have
61 been proposed to extract microplastics from sediment samples, including visual sorting,
62 filtration, sieving, density separation, elutriation, flotation, chemical digestion, electrostatic
63 behavior, and magnetic extraction (Hidalgo-Ruz et al., 2012; Anderson et al., 2015; Masura et
64 al., 2015; Duis & Coors, 2016; Felsing et al., 2018; Grbic et al., 2019). Density separation is a
65 commonly used method to extract microplastics from sediments or sand using the principle of
66 the difference in specific density of sediments (e.g. 2.6 g cm^{-3}) and plastics (0.1-1.7 g cm^{-3})
67 (Chubarenko et al., 2016). In density separation, dense solutions that have been proposed include
68 sodium polytungstate (density: ~3.2 g cm^{-3}), zinc chloride (ZnCl$_2$, density: ~1.8 g cm^{-3}), zinc
69 bromide (ZnBr$_2$, density: 1.7 g cm^{-3}) and sodium iodide (NaI, density: ~1.8 g cm^{-3}) (Corcoran,
70 Biesinger & Grifi, 2009; Imhof et al., 2012; Liebezeit & Dubaish, 2012; Dekiff et al., 2014; Van
71 Cauwenberghe et al., 2015b; Quinn, Murphy & Ewins, 2017; Prata et al., 2018). Due to the
72 higher densities of these salt solutions compared to most microplastics (0.9-1.5 g cm^{-3}), less
73 dense microplastics float and separate out from the more dense, sinking, sediment materials
74 (Quinn, Murphy & Ewins, 2017).
75
76 During density separation a glass beaker is often used, but adhesion of microplastics onto the
77 glass wall is a problem when pouring or sucking supernatant containing microplastics, thus
78 requiring repeating the procedure. Avoiding resuspension of decanted sediments is also
79 challenging when pouring supernatants (Masura et al., 2015). Use of a glass funnel is thus

80   employed to avoid resuspension of sediments, but only a very small amount of sediment can be
81   processed at any one time, requiring repeating the procedure (Masura et al., 2015). There is an
82   upside-down funnel-shaped microplastic separator with a shut off valve (MPSS), but it is a large
83   and heavy metal device and is thus not portable (Imhof et al., 2012). It also requires dismantling
84   of the unit, including the shut off valve, for cleaning purposes between samples (Imhof et al.,
85   2012). There is also a small and portable device based on the above large metal device (SMI),
86   but it still requires cleaning of the shut off valve between samples (Coppock et al., 2017). Also,
87   separators made of plastics (such as SMI) may not be appropriate for further analysis of
88   adsorbing contaminants and additives on/to microplastics. In evaluating existing microplastic
89   extraction devices using density separation, we identified the need for a device that allows rapid,
90   simple and efficient extraction of microplastics from a range of sediment types. Here, we report a
91   simple and small device made of glass, without a valve, for easier separation of microplastics
92   from sediments.
93
94   **Materials & Methods**
95
96   We developed a small separation unit made of glass without a valve, based loosely around a
97   Utermöhl chamber (Utermöhl, 1931), that can isolate microplastics from sediments in a single
98   step. The JAMSTEC microplastic-sediment separator (JAMSS) unit is constructed of two
99   components – one upper and one lower modified glass plate (Fig. 1a). JAMSS can handle a
100   variety of different sediment amounts by changing the volume of the tubes/containers attached to
101   the lower and upper plates. Fig. 1b shows small, middle and large models of JAMSS, consisting
102   of a cylindrical container of 30 ml, 60 ml, and 100 ml volume, respectively. The upper plate
103   incorporates an open glass tube, while the lower plate incorporates a cylindrical glass container
104   (Fig. 1a). Each glass plate is frosted (ground) on the opposite side to that from which the
105   respective tube or container is attached. The two plates are set against each other during use,
106   initially so that the lower container and upper tube become a single, cylindrical container into
107   which the sample can be poured (Fig. 1c). The plates can then be slid against each other to
108   separate the lower and upper halves of the sample, respectively, by introducing a lid and bottom
109   (the glass plate) to each half (Fig. 1d). The two plates stick together through the addition of a
110   liquid to the frosted glass surface. Silicone grease can also be applied to the longitudinal edges of
111   the glass plates to help them stick together. Metal clips can be attached to the plates for secure
112   attachment when the plates are heavy/large (e.g. large plate in Fig. 1b).
113
114   *Microplastic separation from sediments*
115   With the JAMSS unit in its initial position, the internal walls of cylinders were thoroughly rinsed
116   with distilled water prior to use. A sediment sample was placed in the lower cylindrical container
117   using a spatula. Sodium iodide (NaI) solution was prepared by dissolving the salt in distilled
118   water to achieve a final density of 1.6 g cm^{-3}. We used NaI solution in this study because it is
119   less environmentally dangerous compared to other commonly used salt solutions such as $ZnBr_2$

120   (Quinn, Murphy & Ewins, 2017). The solution was filtered through glass fiber filters (Whatman
121   GF/F) prior to use in order to remove any contaminants. Then, the filtered NaI solution was
122   poured into the JAMSS unit so that the solution filled the cylinder to at least halfway up the
123   upper tube. A glass-covered magnetic stirring bar (VWR) was used to gently mix the sediment
124   for 20 min (Fig. 1e), and then the unit was left to settle until the supernatant was clear of
125   sediment. Next, the two plates were carefully slid so that the lower tubular container was
126   completely sealed by the upper plate, ensuring there was no resuspension of sediments into the
127   upper tube. The supernatant in the upper tube was then poured and filtered through a filter (such
128   as a PTFE filter) by rinsing the internal walls of the tube with a squirt bottle filled with Milli-Q
129   water (Millipore), in order to transfer all residual microplastics onto the filter (Fig. 1f). Filters
130   were then transferred to a clean glass petri dish with a glass cover for further counting of
131   microplastics. All the above procedures took place in a laminar flow hood, with the JAMSS unit
132   being covered with aluminum foil when settling the sediment to minimize contamination.
133
134   *Validation of JAMSS*
135   To evaluate the extraction efficiencies of the JAMSS unit, first we tested the unit using finer
136   sediments with known concentrations of microplastics of different polymer types and particle
137   sizes. Secondly, we tested the unit using different sizes of sediment particles.
138
139   For the first experiment, we prepared five polymer types: polyethylene (PE, 0.92-0.97 g cm^{-3}),
140   polypropylene (PP, 0.90-0.91 g cm^{-3}), polystyrene (PS, 0.04-1.09 g cm^{-3}), polyvinyl chloride
141   (PVC, 1.35-1.45 g cm^{-3}) and polyethylene terephthalate (PET, 1.34-1.39 g cm^{-3}). These polymers
142   were prepared in three size categories: 100-500 μm, 500-1,000 μm and 1,000-2,000 μm by
143   milling plastic plates with a plastic-grinder (PLC-2M, Osaka Chemical) and then size-
144   fractionated using stainless steel mesh screens of 100, 500, 1000 and 2000 μm mesh size. We
145   chose these three size categories as they are major size classes of microplastics found in
146   sediments from various locations (Maes et al., 2017; Matsuguma et al., 2017; Peng et al., 2018;
147   Mu et al., 2019; Zhang et al., 2019). We used fine-grained aquarium sand (ADA, Amazonica) as
148   sediment, and this was ground beforehand using a mortar and pestle after adding Milli-Q water
149   to moisten the sand to ease the grinding process. Prepared microplastics (40 particles per
150   replicate) were mixed with 30 g sand (wet weight) in the unit and filtered NaI solution was then
151   added. These added microplastics were distinguishable from the contaminants in the sand
152   (mostly fibers), ensuring that only the added plastics were counted during the final enumeration.
153   In total, five replicates of microplastic-sediment samples were examined for each plastic type
154   and size. Microplastic-sediment samples were stirred in the JAMSS unit with a glass-covered
155   magnetic stirrer as described above. Following filtering onto a membrane filter, the microplastics
156   were counted under a stereomicroscope.
157
158   For the second experiment, we prepared reef carbonate sediments (Platinum reef sand, JUN
159   company limited, Japan) in three size categories: fine (31.9 ± 93.8 μm), medium (533.8 ± 119.1

160   μm), and coarse (1,165 ± 212 μm). All the sediments were soaked in the NaI solution prior to use
161   to remove possible contamination. PVC microplastics with a specific gravity of 1.35 (40
162   particles, size 500-1,000 μm) were mixed with each sediment sample in the unit (10 g dry
163   weight) and filtered NaI solution was then added. In total, five replicates of PVC microplastic-
164   sediment samples were examined for each sediment type, as described above.
165
166   The differences in the extraction efficiencies of microplastics between different polymer types or
167   different particle sizes were determined using one-way ANOVA. The normality of the data and
168   homogeneity of variance were examined and verified before ANOVA analysis using a chi-square
169   test and a Bartlett test, respectively. When the one-way ANOVA was significant, differences
170   among means were analyzed using Tukey-Kramer multiple comparison tests. A difference at
171    $P<0.05$ was considered significant.
172

173   **Results and Discussion**
174

175   Microplastics artificially incorporated into fine sand were extracted using the JAMSTEC
176   microplastic-sedimentation separator (JAMSS) unit with NaI at a density of 1.6 g cm^{-3}. No
177   significant differences were found between the mean recovery rates of each different polymer
178   type in any of the size categories ($P=0.57$ for 100-500 μm particles; $P=0.25$ for 500-1000 μm
179   particles; $P=0.06$ for 1,000-2,000 μm particles, one-way ANOVA) (Fig. 2). When comparing the
180   recovery rates of microplastics between the three size categories (i.e., 100-500 μm, 500-1000
181   μm, 1,000-2,000 μm), overall mean recovery rates slightly decreased with decreasing size of the
182   microplastic particles ($P<0.0005$, one-way ANOVA) with significant differences between 100-
183   500 μm and 500-1,000 μm particles and 100-500 μm and 1,000-2,000 μm particles ($P<0.05$
184   Turkey-Kramer). These differences were probably because of the increased chance to lose
185   smaller particles during processing of the samples (Nakajima et al., 2019). The recovery rates
186   ranged from 92.5 ± 5.6% to 96.0 ± 4.2% for 100-500 μm microplastics (overall mean 94.0 ±
187   1.5%), 96.5 ± 2.9% to 100 ± 0 % for 500-1000 μm microplastics (overall mean 97.8 ± 1.3%),
188   and 97.8 ± 1.6% to 100 ± 0% for 1000-2000 μm microplastics (overall mean 99.1 ± 1.0%). The
189   recovery rates for <1,000 μm microplastics (94.0-97.8%) were comparable with those of
190   previously developed separators, 95.5% for MPSS (Imhof et al., 2012) and 95.8% for SMI
191   (Coppock et al., 2017).
192

193   Microplastics incorporated into different granularities of reef sediment (fine, 32 μm; medium,
194   534 μm; coarse, 1,165 μm) revealed the JAMSS unit extracted microplastics efficiently (96.0 ±
195   3.4% for fine sand, 98.5 ± 1.4% for medium sand, 97.0 ± 1.1% for coarse sand) (Fig. 3). No
196   significant differences ($p=0.23$, one-way ANOVA) were found between the three grain sizes,
197   showing efficient microplastic separation out from sediments using heavy salt solution (NaI),
198   regardless of grain size.
199

200   Among the previous methods for density separation of microplastics, the classical decanting
201   method, for example the use of a beaker, is simple in design but adhesion of microplastics to the
202   inside of the container is a problem when the media is transferred, thus resulting in a relatively
203   low recovery rate (40%) (Imhof et al., 2012). The technique is often repeated 3-5 times to raise
204   the extraction efficiency, but this extends the processing time for each sample and increases the
205   chance to lose microplastic samples (Nakajima et al., 2019). Avoiding resuspension of decanted
206   sediments is also challenging when pouring supernatant (Masura et al., 2015). Conversely,
207   JAMSS completely seals the sediment in a closed space (i.e. the lower container) therefore
208   stopping the introduction of resuspended sediments into the supernatant. The microplastic-
209   containing supernatant can then be easily transferred by rinsing the inside walls of the unit,
210   allowing high extraction efficiency of microplastics in a single step.
211
212   To combat the weak points of the classical decanting method, use of a glass funnel can be
213   employed to avoid resuspension of sediments, but only a very small amount of sediment can be
214   processed at any one time, requiring repeating the procedure (Masura et al., 2015). The device is
215   also easily clogged irrevocably when processing relatively large, coarse sediments. In contrast,
216   JAMSS can handle a variety of different sediment amounts by changing the volume of the
217   tubes/containers attached to the lower and upper plates (Fig. 1b). Currently the large model of
218   JAMSS consists of a cylindrical container of 100 ml volume, allowing direct placement of the
219   entire sediment content of a core sample slice (e.g. 10 mm thickness, 80 mm diameter).
220
221   Previously, an upside-down funnel-shaped microplastic separator with a shut off valve (MPSS)
222   had been proposed as the best method for separating microplastics from sediment samples, but
223   this is a large and heavy metal device and is thus not portable (Imhof et al., 2012). It also
224   requires dismantling of the unit, including the shut off valve, for cleaning purposes between
225   samples (Imhof et al., 2012). There is also a small, portable device based on the above large
226   metal device (SMI), but it still requires cleaning of the shut off valve between processing
227   samples (Coppock et al., 2017). From this point of view, JAMSS has no valve and has a simple
228   structure, allowing rapid cleaning and reductions in sample processing times.
229
230   Though SMI is a portable unit (Coppock et al., 2017), it is made of plastic (PVC), thus it may not
231   be appropriate for further analysis of adsorbing persistent organic pollutants (POPs) and other
232   chemicals such as additives on/to microplastics. One of the advantages of the JAMSS is that it is
233   made of glass, so microplastics remain uncontaminated for use in further chemical analyses.
234
235   Overall, the JAMSS unit is a small, portable device that enables the extraction of microplastics
236   from sediments in a single step. It is simple, without a shut-off valve, and is thus easy to use. The
237   JAMSS has also proven compatible with both finer sediments and coarse sand. Because of the
238   size of this small device, all the above procedures can be done in a laminar flow hood. It should
239   be noted, however, that when the sediment is clay-like or otherwise interferes with the rotation of

240 the magnetic stirrer bar, dilution with water may be necessary. Microplastics can also get caught
241 in the silicone grease used for lubrication between the two plates, so the grease should only be
242 applied to the longitudinal edges of the plates, which do not come into contact with the sample.
243

Conclusions

245

246 We have developed a small, glass, microplastic-sedimentation separator (JAMSS), without a shut
247 off valve, taking a hint from an Utermöhl chamber. This new device is easy to clean and
248 portable, yet enables rapid separation of microplastics from sediments in a single step. In using
249 the JAMSS unit, the user has the advantage of being able to rinse the entire headspace multiple
250 times without resuspending the settled sediment, therefore reducing the need for repetitive
251 processing and limiting the possibilities of external contamination and loss of microplastics. The
252 lack of a shut off valve means there is no chance of it becoming clogged. Unlike devices made of
253 plastic, microplastics separated from sediments with this glass-made JAMSS unit remain
254 chemically uncontaminated so they can be used for further analysis of adsorbing contaminants
255 and additives on/to microplastics.

256

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259

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367

Figure 1

JAMSTEC microplastic-sedimentation separator (JAMSS) unit

(a) the upper plate (left) incorporates an open glass tube, while the lower plate (right) incorporates a cylindrical glass container. (b) small, middle and large models of assembled JAMSS, consisting of a cylindrical container of 30 ml, 60 ml, and 100 ml volume, respectively. (c) JAMSS during density floatation with sediment in the lower container. (d) separation of sediment and supernatant by sliding the two plates against each other. (e) JAMSS can be placed on a magnetic stirrer to ensure the sediments are well mixed during microplastic floatation. (f) microplastics in the supernatant in the upper tube are poured by rinsing the internal walls of the tube.
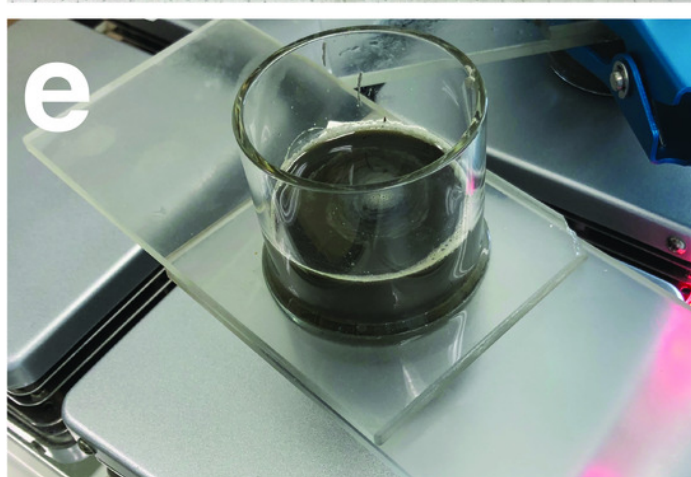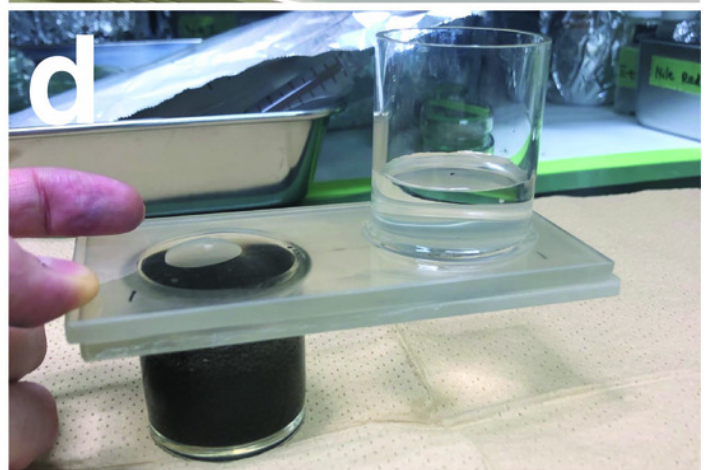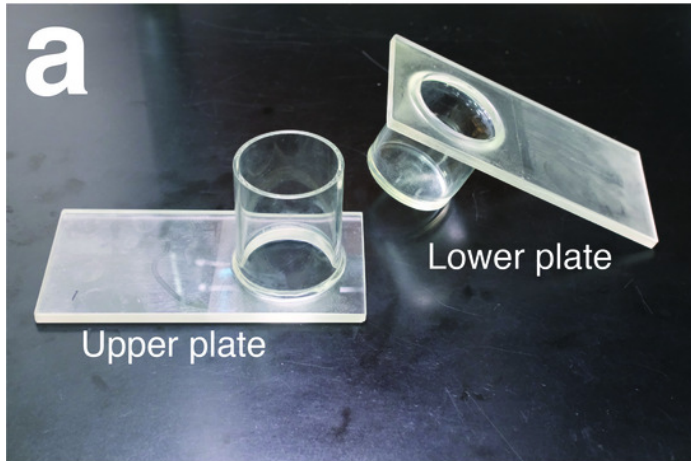
Figure 2

Recovery rate (%) of microplastics of different polymer types and particle sizes by JAMSS unit

Each bar indicates the average recovery rates of microplastics of different size particles: (a) 100-500 μm. (b) 500-1,000 μm. (c) 1,000-2,000 μm. PP, polypropylene; PE, polyethylene; PET, polyethylene terephthalate; PS, polystyrene; PVC, polyvinyl chloride. Error bars indicate standard deviation.
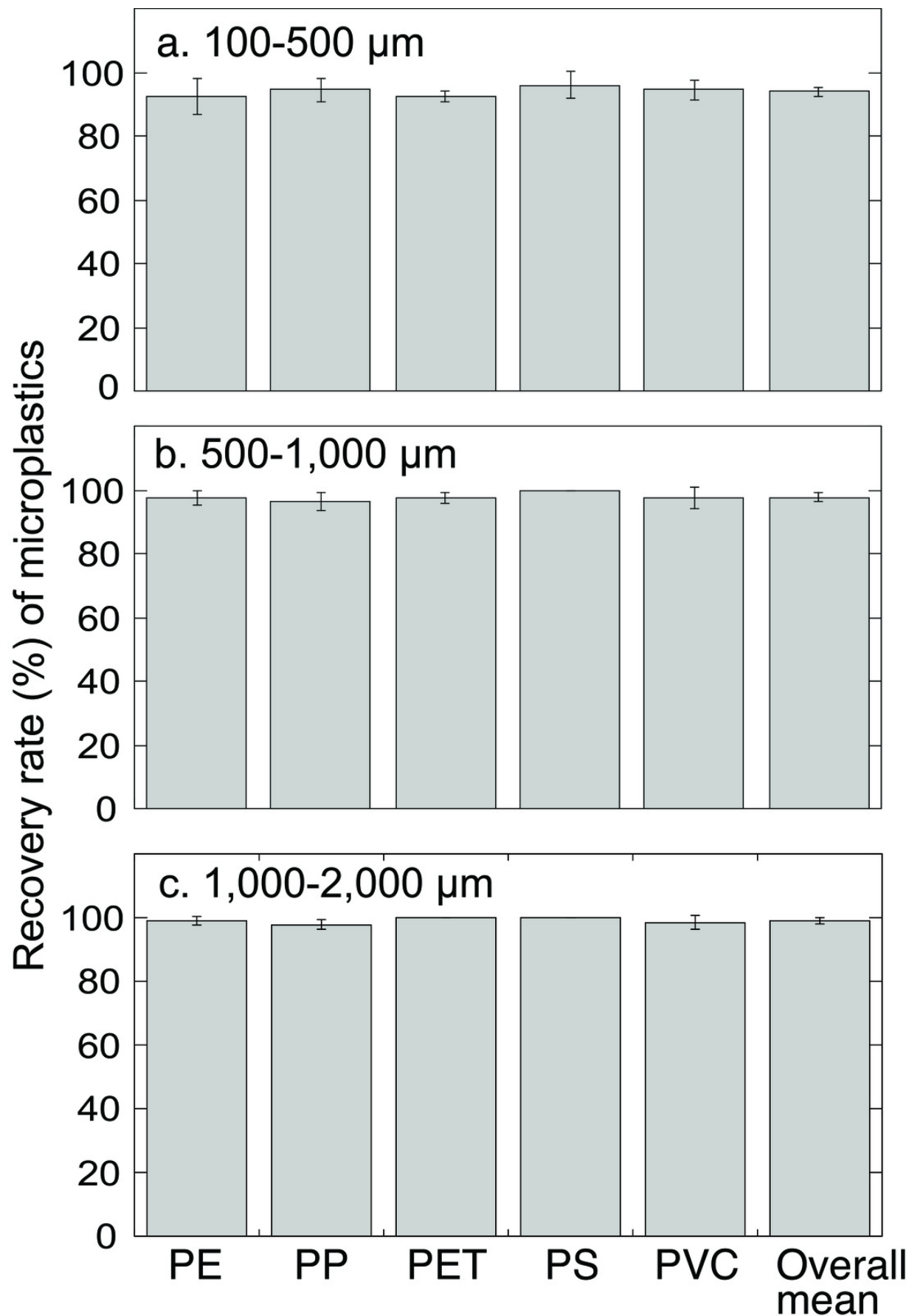
Figure 3

Recovery rate (%) of microplastics by JAMSS unit using different sizes of sediment particles.

Each bar indicates percentage recovery rate of microplastics in different granularities of reef sediment (fine, medium, coarse). Error bars indicate standard deviation.