

Article

Comparison of Different Procedures for Separating Microplastics from Sediments

Veronica Nava  and Barbara Leoni 

Department of Earth and Environmental Sciences, University of Milano-Bicocca, Piazza della Scienza 1, 20126 Milano, Italy; barbara.leoni@unimib.it

* Correspondence: v.nava15@campus.unimib.it; Tel.: +39-02-448-2705

Abstract: The separation of microplastics from environmental matrices is still challenging, especially for sediments where microplastics can accumulate affecting benthic organisms. Many authors have adopted different procedures, but their effectiveness has been rarely compared. The present study aims to compare the recovery rate of three different methodologies for the separation of dense microplastics from fine sediments and provide insights about contamination processes occurring in microplastic separation techniques. The protocols tested are a density separation method with NaCl and NaI, a density separation with NaI followed by a centrifugation step, and a digestion method with 10% KOH (*m/v*). The recovery yields of two high-density polymers of three different dimensional classes were tested. The highest recovery rate was reported for the first protocol. However, this method proved to be expensive, and unsatisfactory results were found when using merely NaCl. The digestion method was the one that was proven to be simple, reproducible, and affordable. The contamination tests highlighted as multiple filtration steps can increase the number of fibers deriving from airborne contamination. Since a unified approach for microplastic separation from sediments is still not selected, this study is of paramount importance as it provides data about the reliability of different methods widely adopted.

Keywords: density separation; digestion method; recovery rate; method validation; plastic methodology



Citation: Nava, V.; Leoni, B. Comparison of Different Procedures for Separating Microplastics from Sediments. *Water* **2021**, *13*, 2854. <https://doi.org/10.3390/w13202854>

Academic Editor: Sarper Sarp

Received: 26 September 2021

Accepted: 7 October 2021

Published: 13 October 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Plastics are the most versatile materials invented by man. These same substances that have allowed technological advancements may ultimately lead to a significant environmental problem. Indeed, a huge quantity of plastic debris is accumulating in the aquatic ecosystems, where it breaks down to form microscopic fragments, called 'microplastics' (MPs). Besides these degradation products (secondary MPs), MPs can also be produced as such (primary MPs) [1]. Microplastics can be ingested by organisms, accumulate in specific tissues, and be transported along food chains [1,2]. Moreover, these particles, due to their small size (large surface/volume ratio), may act as a medium to concentrate and transfer chemicals and persistent, bioaccumulative, and toxic substances to organisms [2]. In addition to this, plastic particles can pose further toxicological risks, as residual monomers or incorporated additives such as fillers, stabilizers, flame retardants or plasticizers may leach [3]. As these polymers are highly resistant to degradation, quantities of microplastics in aquatic environments will most likely continue to increase over time and, consequently, microplastics will represent a long-lasting problem that future generations will have to face [4].

Densities of plastic materials range mainly from 0.85 to 1.41 g cm⁻³ [2]. Since this range includes material of lower, equal, or higher density than water, microplastics can accumulate in both pelagic and benthic compartments: low-density plastics occupy a surface environment, while high-density plastics are found at the bottom [2,5]. Degradation and biofilm formation on the surface of floating plastic particles may facilitate the attachment of

organic coatings, inorganic material, or other plastic debris. Due to this additional weight, microplastics tend to settle in sediments [1,6]. Moreover, the ingestion of microplastics by aquatic organisms can lead to further accumulation in sediments. Indeed, plastic fragments can be ingested by zooplankton and small mesopelagic fishes and then can be transferred to larger predators, sink with the bodies of dead organisms, or be defecated and thus accumulate in sea and lake bottom [7,8]. Therefore, sediments can represent a long-term sink for microplastics [5,9]. This suggests an underestimation of the plastic contamination in aquatic systems for all studies measuring only the abundance of floating debris [1,2,5]. Besides this, microplastics can affect the marine and freshwater benthos, which includes ecologically and commercially important species [10]. Moreover, sediment may become a sink of different organic and inorganic pollutants and, thus, the bioaccumulation of MPs in sediment can enhance contaminant biomagnification [11].

Despite the occurrence of microplastics in sediments and the importance of reliable data about their abundance in the benthic zone of aquatic environments, various methodological issues remain to be addressed to design an effective and unified method [12]. As reported in the review of Hanvey et al. [13], the most common approach to extracting microplastics from sediments is density separation combined with filtration. The separating solution is frequently a concentrated salt solution such as NaCl of varying densities, usually 1.2 g mL^{-1} , as it is cheap and inert [14]. Due to the low density achievable using this salt, other studies have proposed different solutions, for instance, zinc chloride (ZnCl_2), sodium iodide (NaI), and sodium polytungstate [15–17]. Various authors have adopted different procedures, combining different steps and/or different brine solutions, but the effectiveness of the several methods adopted has been rarely compared as many authors have tried to develop their own methods, failing to implement those already devised.

In this work, we evaluated the efficiency of different protocols for separating microplastics from fine sediments, testing three procedures that are more frequently adopted and/or suitable for the separation of microplastics from fine sediments simulating as far as possible real conditions. In particular, we collected deep sediments from Lake Idro (Northern Italy), that we spiked with a known number of plastic particles of two different polymers of different sizes: polyethylene terephthalate (PET) and polyamide (nylon). On these samples, we performed the three selected protocols, and we counted the number of particles that we were able to extract following each methodology. We also assessed the possible contamination linked to both air and water and, for this last, we compared filtered and not filtered ultrapure water. The data collected allowed identifying the method with a high recovery rate and that combine, at the same time, simplicity, reproducibility and affordability. Moreover, the advantages and drawbacks of each protocol were pointed out.

2. Materials and Methods

2.1. Preparation of “Spiked Samples”

To simulate real conditions, we used deep lake sediment collected using a gravity corer (UWITEC, Austria) in 2015 from Lake Idro (Northern Italy, $45^\circ 46' \text{ N } 10^\circ 32' \text{ E}$) [18–20]. This sediment was selected as it is very rich in organic substances and colloidal, and therefore the separation of microplastics from this kind of matrix can be particularly challenging.

The sample was dried (48 h, 60° C), and inspected with a dissecting microscope ($40\times$) to remove any particles that could potentially be plastics. Then, 10 g were weighed and put in a glass container. Plastic fragments, that we generated in the laboratory, of two different polymer types were added: white pellet of polyethylene terephthalate (PET) and green filament of polyamide (Nylon). We selected these plastic polymers as they are characterized by two different density values (PET: 1.38 g cm^{-3} ; Nylon: 1.15 g cm^{-3}), but that are denser than water. Indeed, considering this feature, it is likely that polymers of similar density can easily sink and accumulate in bottom layers and thus they may be more abundant in sediment matrices [1,2]. Raman spectra of the two polymers were acquired to allow a characterization (Figure S3) [21].

The fragments of both the polymers were divided into three different dimensional classes based on their largest dimension, i.e., small (250–500 μm), medium (500–2000 μm), large (2–5 mm) size. In each sample, we added five different fragments for each dimensional class for both Nylon and PET; then, the samples have been carefully mixed in order to allow the incorporation of the spiked plastics. Three replicates were performed for each protocol. Pictures of each fragment were acquired (see an example of the particles used in Figure S1) using the high-resolution Leica ICC50 camera and the dimensions of each fragment were measured using the Leica AirLab app (Version 3.4). The average values of the microplastic dimension with the standard deviation are reported in Table 1.

Table 1. Mean dimension of the particles added to the standard protocols: protocol 1, P1; protocol 2, P2; protocol 3, P3. The first dimensional class (D1) includes particles in the range 2–5 mm, the second (D2) in 500–2000 μm , and the third (D3) in 250–500 μm .

Protocol Number	Dimensional Class	Mean Particles Dimension (\pm SD) (mm)
P1	D1	3.12 \pm 0.33
	D2	1.44 \pm 0.17
	D3	0.42 \pm 0.05
P2	D1	3.04 \pm 0.29
	D2	1.38 \pm 0.28
	D3	0.42 \pm 0.03
P3	D1	3.07 \pm 0.27
	D2	1.21 \pm 0.26
	D3	0.41 \pm 0.06

2.2. Protocols for Plastic Separation

The selection of the three protocols that we tested was performed choosing procedures that are more frequently adopted and/or suitable for the separation of microplastics from fine sediments. After the application of each protocol on the “spiked samples” [22], the recovery rate was evaluated by counting under a dissecting microscope (40 \times) the number of microplastics and comparing the shape and dimension of the particle recovered using the pictures taken for each particle in each sample. In this way, we were confident that the identified and counted particles were the ones that we added to the samples. Microplastic recovery data (%) was expressed as the mean \pm the standard deviation. A description of the different protocols is reported below.

2.2.1. Protocol 1

The first protocol (P1) selected was a density separation method [23]. To test the performance of different dense solutions a step procedure was followed. In particular, a solution of NaCl was prepared by dissolving the salt in ultrapure water until reaching a density of 1.2 g cm⁻³. The density was measured by the weighing method. The same procedure was followed for the preparation of the NaI (Sigma-Aldrich, 99%) solution and the density reached was equal to 1.8 g cm⁻³. The solutions were filtered (Whatman glass microfiber filters, Grade GF/C, 0.45 μm) three times to remove any contaminants. The prepared samples (sediment and plastic particles) were put into a cylinder and a volume of NaCl solution equal to double the volume of the sediment was added. The solution was energetically shaken for 1 min and the sediment was left to settle for 2 h. Then the particles floated to the surface of the solution were collected with a glass pipette (used in the inverted way to have a large opening) and filtered (Whatman glass microfiber filters, Grade GF/C, 0.45 μm). We repeated the procedure two more times, and we kept separate filters every time to analyze the recovery efficiency of each serial step. The third time the sediment was left to settle for 12 h. Then, the last step was performed using the NaI solution: the NaCl solution was replaced with NaI, which was left for 12 h before collecting and filtering (Whatman glass microfiber filters, Grade GF/C, 0.45 μm) the supernatant.

In total, we performed four steps and, for each one, a filter was analyzed to estimate the recovery of the different steps and the overall procedure.

2.2.2. Protocol 2

The second protocol (P2) is based on a density separation with the use of a dense solution (NaI) and a centrifuge system [24]. The dried sample was disaggregated with a previously filtered deionized water; then the sample was washed through 250 μm stainless steel sieves. The retained material was collected and placed into a 50 mL centrifuge tube. We prepared a sodium iodide (NaI) solution, with a density equal to 1.8 g cm^{-3} . The solution was filtered (Whatman glass microfiber filters, Grade GF/C, 0.45 μm) three times to remove any contaminants. Twenty-five milliliters of the solution were added to the centrifuge tube previously filled with the samples. The 50 mL tubes were capped and centrifuged at 1500 rpm for 5 min. The lower-density fraction was pipetted off and vacuum-filtered through a 0.45 μm micropore glass microfiber filter. The filters were then inspected using a dissecting microscope (40 \times).

2.2.3. Protocol 3

The third protocol (P3) is a digestion method with 10% KOH (*m/v*) combined with a sieving procedure. We optimized this method, usually used for the separation of subfossil Cladocera remains from sediment cores, for microplastic extraction [25]. In a 1000 mL beaker placed on a hotplate, we added 10 g of the prepared sample and 200 mL of a 10% KOH (*m/v*) solution. The sample was left heating to 60 $^{\circ}\text{C}$ for 30 min with constant gentle stirring. Mixing was performed with a magnetic stirrer to ensure proper homogenization. After the time necessary, 600 mL of water was added and the KOH-sediment mixture was poured onto a 250 μm sieve and washed using ultrapure water, previously filtered (Whatman glass microfiber filters, Grade GF/C, 0.45 μm), until the rinsing water comes through clear. To remove possible carbonates residuals, a few drops of 10% HCl were added and the sample, then, was thoroughly rinsed to remove acid remains. It is important to remove the acid as detrimental effects on some plastic polymers are reported, e.g., on nylon fibers which are known to be sensitive to acids and alkalis [26].

2.3. Evaluation of Possible Sources of Contamination

Additional analyses were performed to assess the possible contamination deriving from different steps involved in the microplastic separation methods discussed before and, in general, commonly used in several protocols concerning microplastic analysis. All the particles that appear to be similar to plastics were considered, as the visual inspection step is still necessary for microplastics analysis and adding more confounding and potentially contaminating particles can lead to an increase in sample processing time and unreliable results. In particular, we evaluated the contamination in terms of fibers and fragments deriving from air during the vacuum filtration step, and from the use of ultrapure water (both filtered and not filtered). For the investigation of air contamination linked to filtration procedures (thereafter 'AIR'), a glass microfiber filter (Whatman, Grade GF/C, 0.45 μm) was inspected under a dissecting microscope (40 \times) to assess the possible presence of particles. If particles were visually detected, the filter was discarded. The selected clean filter was then placed on the vacuum filtering system and left for 10 min with the pump switched on. After that, the filter was collected, put in a glass Petri dish, and inspected under a dissecting microscope (40 \times). For the different water analyses, glass microfiber filters (Whatman, Grade GF/C, 0.45 μm) were accurately inspected as described before. Ultrapure water (1000 mL) was collected in a previously rinsed flask, filtered on the inspected filter through the vacuum filtration system, and stored in a glass Petri dish for the examination with the stereomicroscope ('UNF'). For the evaluation of the contamination linked to the filtration step, 1000 mL of ultrapure water was collected in a flask and filtered three times through a glass microfiber filter. The last filtration was performed on an inspected and clean filter,

which was collected and evaluated under the microscope ('UF'). For all the procedures, we performed three replicates.

2.4. Cost Assessment and Statistical Analyses

We estimated the average cost for each replicate. We considered the cost derived only from the preparation of the different solutions (i.e., consumable costs); the cost of the instrumentations is not included, as neither of the protocols requires special equipment.

To evaluate how the adopted dimension and protocol influence the recovery rate, non-parametric analysis of variance was performed using Aligned ranks transformation ANOVA (ART ANOVA) implemented in the R package 'ARTool' [27,28]. Post hoc comparisons for main effects were performed using the package 'emmeans' [29]. All statistics and figures were produced using R (3.6.1.).

3. Results

3.1. Recovery Rate

The percentage recovery of Nylon and PET of the three protocols for the different sizes of microplastics is reported in Figure 1. Protocol one has several sequential steps (see Section 3.2); therefore, in the present section, we evaluate the overall recovery of the whole procedure.

Considering the Nylon fragments (Figure 1a), a complete recovery ($100 \pm 0\%$) was found for the particles belonging to the largest dimensional class (D1, 2–5 mm) for all the protocols in all the replicates. For the second dimensional class (D2, 500–2000 μm), a complete recovery was reported for protocols 1 and 3 ($100 \pm 0\%$), and a recovery of $86.7 \pm 12\%$ for protocol 2. A complete recovery was not obtained for the smallest dimensional class (D3, 250–500 μm), with values of $86.7 \pm 12\%$, $66.7 \pm 31\%$, $66.7 \pm 23\%$ for protocol 1 (P1), protocol 2 (P2), and protocol 3 (P3), respectively. The protocol that shows the lowest variability among replicates is protocol 1, followed by protocol 3, which shows some differences just for the lowest dimensional class. The ART ANOVA highlighted as only the dimension represents a significant factor influencing the recovery ($p < 0.001$), and no significant difference was found when considering the different protocols. The contrast tests pointed out as the difference are significant between D1–D3 ($p < 0.001$) and D2–D3 ($p < 0.05$).

Figure 1b reports the percentage recovery of PET fragments of the three protocols for the different dimensional classes of microplastics. As reported for Nylon particles, the microplastics belonging to the first dimensional class were completely recovered, with a percentage of $100 \pm 0\%$ for all the protocols in all the replicates. For the second dimensional class, a complete recovery was reported only for protocol 3 ($100 \pm 0\%$); instead, a recovery of $93.3 \pm 12\%$ was reported for protocol 1 and $73.3 \pm 31\%$ for protocol 2. The lower recovery was obtained for the particles with the smallest size (D3), with values of $73.3 \pm 31\%$, $46.7 \pm 12\%$, $60.0 \pm 0\%$ for protocol 1, protocol 2, and protocol 3, respectively. Protocol 3 is highly consistent among the replicates, indeed the standard deviation in all the dimension classes is zero. Significant differences were highlighted for the various dimensional classes ($p < 0.001$) and the protocols ($p < 0.01$). For the dimension factor, the contrast tests highlighted as the difference are significant among all the classes, with a p -value < 0.001 for all the comparisons (i.e., D1–D2, D1–D3, D2–D3). Concerning the protocol, significant differences were highlighted between P1–P2 ($p < 0.01$) and P2–P3 ($p < 0.05$); no differences were highlighted between P1–P3.

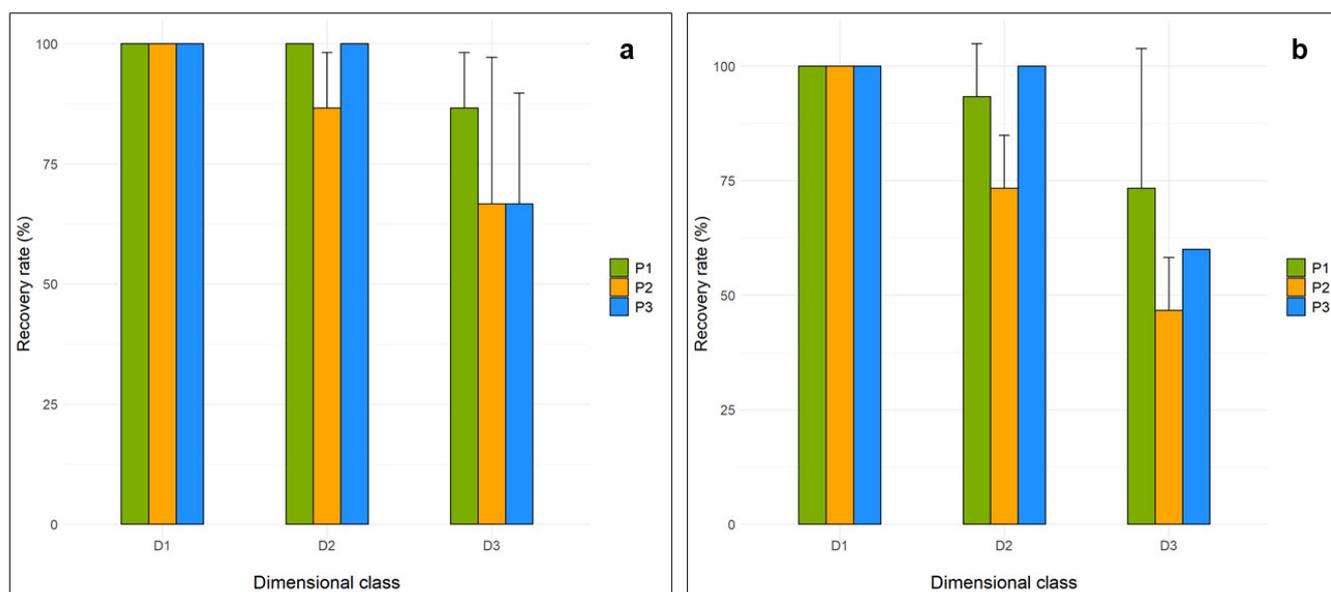


Figure 1. Percentage recovery rate of (a) Nylon and (b) PET particles for the three protocols testes (P1, P2, P3) grouped by the different dimensional classes (D1: 2–5 mm; D2: 500–2000 μm ; D3: 500–250 μm).

3.2. Sequential Recovery of Protocol 1

A more detailed description is provided for protocol 1, as several steps that provide different results were executed. The four sequential steps carried out during the application of protocol 1 yielded different percentage recovery (Figure 2).

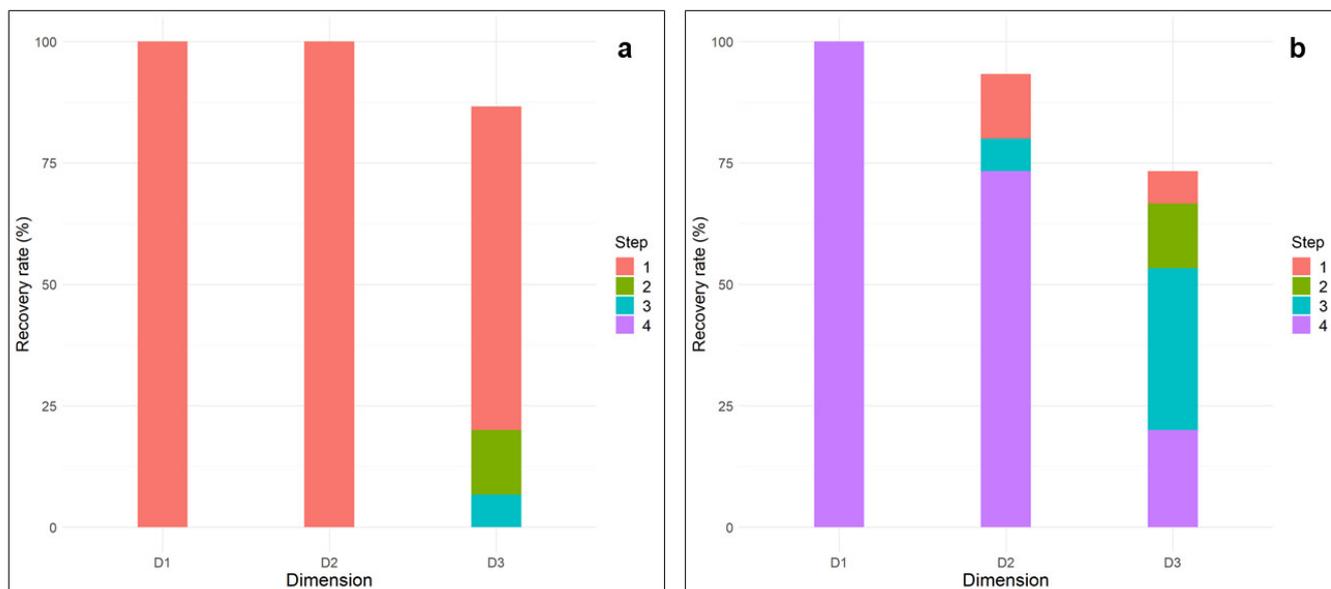


Figure 2. Average percentage recovery rate for the sequential steps (1–3: density separation with NaCl, 4: density separation with NaI) of Protocol number 1 for the different dimensional classes (D1, D2, D3) for (a) Nylon particles and (b) PET particles.

Considering the Nylon particles, the complete recovery ($100 \pm 0\%$) was reported for the particles belonging to the biggest dimensional class (D1) and the medium-size particles (D2) during the first step using the NaCl solution. For the Nylon particles of smaller dimensions (D3), the highest recovery was reported during the first step, with a value of

$66.7 \pm 12\%$. An additional recovery was obtained during the second and the third step with a percentage on the overall total of $13.3 \pm 12\%$ and $6.7 \pm 12\%$, respectively (Figure 2a).

We obtained different results for the recovery of PET particles (Figure 2b). Indeed, a complete recovery of the biggest particles (D1) was obtained for all the replicates during the last step, using the NaI solution. For the particles belonging to the second dimensional class (D2), the highest recovery was obtained with the last step ($73.3 \pm 23\%$), but also some particles were recovered during the first ($13.3 \pm 23\%$) and the third step ($6.7 \pm 12\%$). For the smallest PET particles (D3), we were able to recover a small amount from each step: $6.7 \pm 12\%$ for step 1, $13.3 \pm 12\%$ for step 2, $33.3 \pm 31\%$ for step 3, and $20.0 \pm 0\%$ for step 4 (percentage on the overall total).

3.3. Contamination Evaluation

Figure 3 reports the number of contaminating particles detected for the different tests performed, divided between fibers and fragments. Fibers were detected in all the procedures performed, with the highest value in the 'AIR' sample. High values were also detected for the filtered samples of ultrapure water ('UNF'). Instead, fragments were less abundant, and no fragments were detected in the 'AIR' samples and in the ultrapure water filtered ('UF'). In general, the water filtration step proves to allow removing the contamination with fragments but leads to major contamination with airborne fibers.

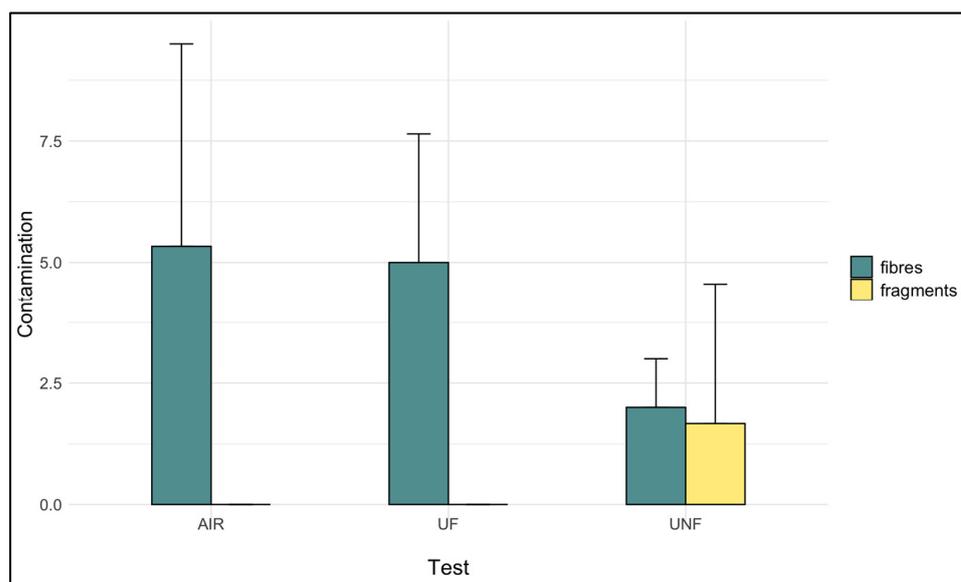


Figure 3. Average number of contamination particles (fibres and fragments) for the different tests performed (i.e., 10 min of air filtration 'AIR'; 1000 mL of ultrapure water not filtered and filtered) with standard deviation values. 'AIR' air evaluation; 'UF' Ultrapure water filtered; 'UNF' Ultrapure water Not filtered.

3.4. Cost Assessment

We evaluated the cost for the performance of each protocol on one single sample. The estimation is reported in Table 2. The highest cost is reported for protocol number 1. Indeed, sodium iodide (anhydrous, free-flowing, Redi-Dri™, ReagentPlus®, $\geq 99\%$) costs around 0.30 €/g, sodium chloride (anhydrous, free-flowing, Redi-Dri™, ReagentPlus®, $\geq 99\%$) 0.03 €/g, and sodium hydroxide (BioXtra, ≥ 85) 0.13 €/g. For the application of protocol 1 (P1) to one sample, we used 17 g of NaCl and 75 g of NaI for a total cost of EUR ~23 for one sample. For the second protocol (P2), 37.5 g of NaI were employed for a total cost of EUR ~11 for one sample. Finally, for the third protocol (P3), we used 23.6 g of KOH, to obtain a solution at 10%, for a total cost of EUR ~3. To allow a comparison between the different protocols, we also evaluated the relative cost unit.

Table 2. Cost assessment for the processing of each sample with the different experimental protocols: protocol 1, P1; protocol 2, P2; protocol 3, P3.

Protocol Number	Cost (€) ¹	Relative Cost Unit
P1	23	7.7
P2	11	3.7
P3	3	1

¹ The costs are referred to Sigma-Aldrich (accessed on 14 August 2019) considering the 1000 g pack-size.

4. Discussion

The presence of microplastics in sediments of both deep and littoral areas of marine and freshwater environments is increasingly reported in various recent studies and it is widely recognized as sediments can represent a major sink for these pollutants [9,24,30,31]. The accumulation of microplastics in these environments may affect the benthic organisms, which include ecologically and commercially important species [10]. Moreover, sediment may become a sink of different organic and inorganic pollutants and, thus, the bioaccumulation of MPs in sediment can enhance contaminant biomagnification [11]. Despite the pivotal role that sediments can play in the accumulation of microplastics, a standard and unified analytical technique for measuring microplastics in sediments is not adopted and many authors have tried to develop their own methods, failing to implement those already devised. Moreover, the effectiveness of the several methods used has been rarely compared and, thus, the data about microplastic abundance in these matrices could be hardly comparable. Our study contributes to filling this gap, as we performed a comparison of the percentage recovery of spiked microplastics carrying out three different methods that are widely used for separating microplastics from sediments. We evaluated not only the recovery but also the cost for the application of the different procedures. Moreover, we provided information about contamination that can occur in different steps involved in the separation techniques (i.e., use of water for wet sieving or solution preparation, use of vacuum filtration system), providing a more comprehensive analysis.

A complete recovery of all the spiked microplastics across all size classes was not possible with none of the procedures applied. This should be taken into account when reporting data about microplastics. Indeed, monitoring in organic-rich particle matrices such as sediments is in its infancy, and standardization of methods for microplastic separation is still being discussed [9,32]; therefore it is desirable for future studies to include analytical control when separating and quantifying microplastics from sediments and soils to provide uncertainty measurements about the data reported. Based on our results, the percentage recovery was different for the various dimensional classes of the particles. Indeed, the microplastic retrieval, regardless of the protocol and polymer considered, was inversely related to the dimensional class, as the number of particles separated from the sediment matrix is smaller for the particles belonging to the smallest dimensional classes. Conversely, for the biggest particles, i.e., with the largest dimension in the range of 2–5 mm, a complete recovery was possible. Thus, the size of the microplastics can significantly affect recovery. Previous studies have already reported a relationship between dimension and recovery rate, highlighting as the separation of smaller sizes can be more challenging compared to large microplastics [33].

Other microplastic features can also affect separation ability [33,34]. For instance, the color can represent a factor influencing the recovery, as particles with colors similar to the interfering particles are more difficult to separate [32,34]. Colorful plastics, such as the green Nylon fragments that we used, are easier to identify during the visual inspection and, thus, the highest recovery highlighted in our study for the Nylon fragments can be related to this. Moreover, during the phase of the visual identification, having a clear filter, with few sediments remains, can be important in the identification of the different particles as they are more visible, and interfering materials (both organic and inorganic) likely do not overlap them. The third protocol, based on a sieving procedure, is the one in which fewer sediment remains were observed on the final filter (Figure S2). In general, using a very

high-density solution ($1.6\text{--}1.8\text{ g cm}^{-3}$), such as carried out in protocol 2 and, especially, in protocol 1, the fine sediment remained in suspension and consequently higher amounts of sediments can be collected together with the plastic particles [14].

In addition to the color, another determining feature in the separation of the microplastics is the shape of the particles. Especially, differences were highlighted when comparing filaments (i.e., fibers and lines) to the fragments. The combination of the methodology adopted, and the particle shape has paramount importance. For instance, the use of a sieving step can lead to a potential loss of some particles as the sieve mesh is square shaped and the length of the diagonals is greater than the specified mesh value. The sieve retains particles when their two smaller dimensions are larger than the mesh diagonals [35]. For the fibers and lines-shaped particles, there is a higher probability that the sieves do not retain them, as they are fine, and the flow can drag them down. However, our results showed as the recovery of Nylon fragments of protocol three compared to protocol one is the same for the first two dimensional classes (D1, D2), highlighting as the sieves cannot affect importantly the recovery of the biggest particles.

Depending on the driving research question and the instruments available for the identification of microplastic polymers (e.g., Py-GC-MS, FTIR, Raman), the methodology can be modified, for instance, selecting different sieve meshes. Moreover, steps belonging to different methodologies can be merged to obtain a higher percentage recovery. However, it is necessary to take into account the cost, as this represents a limiting factor. Indeed, as reported by Eerkes-Medrano et al. [2], the methods employed for the separation of microplastics from environmental samples should not only be precise, accurate, and simple to ensure sufficient replication but should also keep cost low enough to enable method accessibility. Usually, microplastic studies require processing several samples and the procedure for the identification of the polymer through for instance spectroscopic analysis, which follows the separation step, is expensive. Indeed, it is commonly recognized that visual identification is still necessary but not enough, with potentially high error rates, especially for smaller particles, and several studies highlighted the discrepancy between the visual identification and spectroscopic methods [36–39]. Consequently, it is important to adopt a methodology that allows a fast, but reliable, separation of microplastics from the sediments to reduce the cost of the initial phases (i.e., the separation phase). Protocol 1 is the most expensive, even if the filtration and reuse of solutions for density separations can reduce the cost; on the contrary, the protocol that proved to be cheap for fine sediments is the third one. Moreover, considering contamination processes, we highlighted as all the procedures that require more filtration steps expose the sample to a higher risk of contamination, especially regarding fibers. Indeed, the potential contamination with fragments can be managed, but it is more difficult to preserve the sample from airborne contamination, whose presence we detected in each test performed.

The use of density separation is widely reported for the separation of microplastics from complex matrices such as sediments [1,23], but the application of different brine solutions influences the number of MPs recovered [14,33,40]. For instance, the density of saturated NaCl solution (1.2 g cm^{-3}) does not offer consistent separation of higher density polymers such as polyoxymethylene, polyvinyl chloride, and polyethylene terephthalate [23,32,40,41]. However, the NaCl solution is commonly used as it is a cheap and inert option in microplastic studies, but its use could result in an underestimation of the abundance of plastics found, particularly high-density plastics [14]. Just taking into account the first three steps carried out for protocol number one (i.e., density separation with NaCl solution), we were able to recover merely Nylon particles and some of the smaller and lighter PET fragments. If the procedure had stopped at this point, without the use of a denser salt, a low recovery value would be obtained. As we are discussing methodologies for the separation of microplastics from sediments, it is important to be able to separate denser and heavier particles that can sink and are likely to be present in sediments. The step performed with NaI allows the recovery of the denser particles, as we achieve a density of 1.6 g cm^{-3} with this solution. However, this salt is highly expensive and should be handled

with caution [14,42,43]. Even if other brine solutions have been proposed (e.g., $ZnCl_2$, sodium polytungstate), problems of toxicity and/or cost arise from all of them. Therefore, for fine sediments, the use of density separation methodology appears to be not a suitable solution, also considering that the fine sediment can float as well. The possibility to use a sieve is important to reduce the sample mass, even if there can be issues for filaments, which may be lost during this step due to their shape. Our results showed as the digestion protocol (protocol 3) is a suitable methodology, being effective and easy to implement. Moreover, this method also provided relatively tight error bars indicating a good level of reproducibility.

5. Conclusions

There is still a lack of consensus on methodological approaches for the determination of microplastic contamination in sediments. However, standard extraction techniques should be adopted by the research and regulatory community, especially for monitoring purposes. Therefore, this study has provided important information about methodologies for separating microplastics from fine sediments, comparing the efficiency of three different protocols under simulation of real conditions, taking into account both the cost of the analysis and the potential sources of contamination. Based on our comparison, we highlighted that density separation may be effective only when denser salt is used (e.g., NaI), otherwise, a low recovery rate has been recorded. The digestion method that we proposed for plastic separation may be a good compromise and we recorded a high recovery rate, even if it requires a filtration step that may lead to the potential loss of filaments and fibers.

Since a unified approach for microplastic separation from sediments is still not selected, this study is of paramount importance as it provides data about the reliability of different methods and it provides guidance for researchers aiming to further characterize microplastic contamination in sediments, which are argued to be long-term sinks for these contaminants.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/w13202854/s1>, Figure S1: Pictures of the microplastics added to the samples for the recovery test, Figure S2: Example of the filters obtained for the different protocols, Figure S3: Raman spectra of the two polymers used.

Author Contributions: Conceptualization, V.N. and B.L.; methodology, V.N. and B.L.; formal analysis, V.N.; investigation, V.N. and B.L.; writing—original draft preparation, V.N.; writing—review and editing, B.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by University of Milano-Bicocca (FA).

Data Availability Statement: The data presented in this study are available in the Section 3.

Acknowledgments: We would like to thank Monica Tolotti (Fondazione Edmund Mach) for providing us with the sediment core collected from Lake Idro.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Horton, A.A.; Walton, A.; Spurgeon, D.J.; Lahive, E.; Svendsen, C. Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. *Sci. Total Environ.* **2017**, *586*, 127–141. [[CrossRef](#)]
2. Eerkes-Medrano, D.; Thompson, R.C.; Aldridge, D. Microplastics in freshwater systems: A review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. *Water Res.* **2015**, *75*, 63–82. [[CrossRef](#)]
3. Schrank, I.; Trotter, B.; Dummert, J.; Scholz-Böttcher, B.M.; Löder, M.G.; Laforsch, C. Effects of microplastic particles and leaching additive on the life history and morphology of *Daphnia magna*. *Environ. Pollut.* **2019**, *255*, 113233. [[CrossRef](#)]
4. Galloway, T.S.; Lewis, C.N. Marine microplastics spell big problems for future generations. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 2331–2333. [[CrossRef](#)] [[PubMed](#)]

5. Morét-Ferguson, S.; Law, K.; Proskurowski, G.; Murphy, E.K.; Peacock, E.E.; Reddy, C.M. The size, mass, and composition of plastic debris in the western North Atlantic Ocean. *Mar. Pollut. Bull.* **2010**, *60*, 1873–1878. [[CrossRef](#)] [[PubMed](#)]
6. Nava, V.; Leoni, B. A critical review of interactions between microplastics, microalgae and aquatic ecosystem function. *Water Res.* **2021**, *188*, 116476. [[CrossRef](#)] [[PubMed](#)]
7. Cózar, A.; Echevarría, F.; González-Gordillo, J.I.; Irigoien, X.; Úbeda, B.; Hernández-León, S.; Palma, T.; Navarro, S.; García-De-Lomas, J.; Ruiz, A.; et al. Plastic debris in the open ocean. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 10239–10244. [[CrossRef](#)] [[PubMed](#)]
8. Lusher, A.; McHugh, M.; Thompson, R. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. *Mar. Pollut. Bull.* **2013**, *67*, 94–99. [[CrossRef](#)]
9. Van Cauwenberghe, L.; Devriese, L.; Galgani, F.; Robbins, J.; Janssen, C. Microplastics in sediments: A review of techniques, occurrence and effects. *Mar. Environ. Res.* **2015**, *111*, 5–17. [[CrossRef](#)]
10. Fang, C.; Zheng, R.; Zhang, Y.; Hong, F.; Mu, J.; Chen, M.; Song, P.; Lin, L.; Lin, H.; Le, F.; et al. Microplastic contamination in benthic organisms from the Arctic and sub-Arctic regions. *Chemosphere* **2018**, *209*, 298–306. [[CrossRef](#)]
11. Bellasi, A.; Binda, G.; Pozzi, A.; Galafassi, S.; Volta, P.; Bettinetti, R. Microplastic Contamination in Freshwater Environments: A Review, Focusing on Interactions with Sediments and Benthic Organisms. *Environments* **2020**, *7*, 30. [[CrossRef](#)]
12. Piperagkas, O.; Papageorgiou, N.; Karakassis, I. Qualitative and quantitative assessment of microplastics in three sandy Mediterranean beaches, including different methodological approaches. *Estuar. Coast. Shelf Sci.* **2019**, *219*, 169–175. [[CrossRef](#)]
13. Hanvey, J.S.; Lewis, P.; Lavers, J.; Crosbie, N.D.; Pozo, K.; Clarke, B.O. A review of analytical techniques for quantifying microplastics in sediments. *Anal. Methods* **2017**, *9*, 1369–1383. [[CrossRef](#)]
14. Coppock, R.L.; Cole, M.; Lindeque, P.K.; Queiros, A.M.; Galloway, T.S. A Small-scale, portable method for extracting microplastics from marine sediments. *Environ. Pollut.* **2017**, *230*, 829–837. [[CrossRef](#)]
15. Van Cauwenberghe, L.; Claessens, M.; Vandegehuchte, M.B.; Mees, J.; Janssen, C.R. Assessment of marine debris on the Belgian Continental Shelf. *Mar. Pollut. Bull.* **2013**, *73*, 161–169. [[CrossRef](#)]
16. Imhof, H.K.; Schmid, J.; Niessner, R.; Ivleva, N.P.; Laforsch, C. A novel, highly efficient method for the separation and quantification of plastic particles in sediments of aquatic environments. *Limnol. Oceanogr. Methods* **2012**, *10*, 524–537. [[CrossRef](#)]
17. Corcoran, P.L.; Biesinger, M.C.; Grifi, M. Plastics and beaches: A degrading relationship. *Mar. Pollut. Bull.* **2009**, *58*, 80–84. [[CrossRef](#)]
18. Viaroli, P.; Azzoni, R.; Bartoli, M.; Iacumin, P.; Longhi, D.; Mosello, R.; Rogora, M.; Rossetti, G.; Salmaso, N.; Nizzoli, D. Persistence of meromixis and its effects on redox conditions and trophic status in Lake Idro (Southern Alps, Italy). *Hydrobiologia* **2018**, *824*, 51–69. [[CrossRef](#)]
19. Milan, M.; Bigler, C.; Tolotti, M.; Szeroczyńska, K. Effects of long term nutrient and climate variability on subfossil Cladocera in a deep, subalpine lake (Lake Garda, northern Italy). *J. Paleolimnol.* **2017**, *58*, 335–351. [[CrossRef](#)]
20. Milan, M.; Bigler, C.; Salmaso, N.; Guella, G.; Tolotti, M. Multiproxy reconstruction of a large and deep subalpine lake's ecological history since the Middle Ages. *J. Great Lakes Res.* **2015**, *41*, 982–994. [[CrossRef](#)]
21. Nava, V.; Frezzotti, M.L.; Leoni, B. Raman Spectroscopy for the Analysis of Microplastics in Aquatic Systems. *Appl. Spectrosc.* **2021**, *26*, 12109–12122. [[CrossRef](#)]
22. Lares, M.; Ncibi, M.C.; Sillanpää, M.; Sillanpää, M. Intercomparison study on commonly used methods to determine microplastics in wastewater and sludge samples. *Environ. Sci. Pollut. Res.* **2019**, *26*, 12109–12122. [[CrossRef](#)]
23. Hidalgo-Ruz, V.; Gutou, L.; Thompson, R.; Thiel, M. Microplastics in the Marine Environment: A Review of the Methods Used for Identification and Quantification. *Environ. Sci. Technol.* **2012**, *46*, 3060–3075. [[CrossRef](#)]
24. Turner, S.; Horton, A.A.; Rose, N.L.; Hall, C. A temporal sediment record of microplastics in an urban lake, London, UK. *J. Paleolimnol.* **2019**, *61*, 449–462. [[CrossRef](#)]
25. Leoni, B.; Patelli, M.; Nava, V.; Tolotti, M. Cladocera paleocommunity to disentangle the impact of anthropogenic and climatic stressors on a deep subalpine lake ecosystem (Lake Iseo, Italy). *Aquat. Ecol.* **2021**, *55*, 607–621. [[CrossRef](#)]
26. Crawford, C.B.; Quinn, B. *Microplastic Pollutants*, 1st ed.; Elsevier: Amsterdam, The Netherlands, 2017; ISBN 9780128104699.
27. Wobbrock, J.O.; Findlater, L.; Gergle, D.; Higgins, J.J. The Aligned Rank Transform for nonparametric factorial analyses using only ANOVA procedures. In Proceedings of the Conference on Human Factors in Computing Systems, Vancouver, BC, Canada, 7–12 May 2011; Association for Computing Machinery: New York, NY, USA, 2011; pp. 143–146.
28. Kay, M.; Wobbrock, J.O. ARTool: Aligned Rank Transform for Nonparametric Factorial ANOVAs, R Packag, Version 0.10.0. 2019. Available online: <http://depts.washington.edu/acelab/proj/art/index.html> (accessed on 25 September 2021).
29. Lenth, R. emmeans: Estimated Marginal Means, aka Least-Squares Means. R Packag, Version 1.4.1. 2019. Available online: <https://cran.r-project.org/web/packages/emmeans/index.html> (accessed on 25 September 2021).
30. Woodall, L.C.; Sanchez-Vidal, A.; Canals, M.; Paterson, G.L.; Coppock, R.; Sleight, V.; Calafat, A.; Rogers, A.D.; Narayanaswamy, B.; Thompson, R.C. The deep sea is a major sink for microplastic debris. *R. Soc. Open Sci.* **2014**, *1*, 140317. [[CrossRef](#)]
31. Blair, R.M.; Waldron, S.; Phoenix, V.R.; Gauchotte-Lindsay, C. Microscopy and elemental analysis characterisation of microplastics in sediment of a freshwater urban river in Scotland, UK. *Environ. Sci. Pollut. Res.* **2019**, *26*, 12491–12504. [[CrossRef](#)] [[PubMed](#)]
32. Zarfl, C. Promising techniques and open challenges for microplastic identification and quantification in environmental matrices. *Anal. Bioanal. Chem.* **2019**, *411*, 3743–3756. [[CrossRef](#)]

33. Quinn, B.; Murphy, F.; Ewins, C. Validation of density separation for the rapid recovery of microplastics from sediment. *Anal. Methods* **2017**, *9*, 1491–1498. [[CrossRef](#)]
34. Shim, W.J.; Hong, S.H.; Eo, S.E. Identification methods in microplastic analysis: A review. *Anal. Methods* **2016**, *9*, 1384–1391. [[CrossRef](#)]
35. Kedzierski, M.; Le Tilly, V.; Bourseau, P.; Bellegou, H.; César, G.; Sire, O.; Bruzard, S. Microplastics elutriation from sandy sediments: A granulometric approach. *Mar. Pollut. Bull.* **2016**, *107*, 315–323. [[CrossRef](#)]
36. Ivleva, N.P.; Wiesheu, A.C.; Niessner, R. Microplastic in Aquatic Ecosystems. *Angew. Chem. Int. Ed.* **2016**, *56*, 1720–1739. [[CrossRef](#)]
37. Löder, M.G.J.; Kuczera, M.; Mintenig, S.; Lorenz, C.; Gerdt, G. Focal plane array detector-based micro-Fourier-transform infrared imaging for the analysis of microplastics in environmental samples. *Environ. Chem.* **2015**, *12*, 563–581. [[CrossRef](#)]
38. Eriksen, M.; Mason, S.; Wilson, S.; Box, C.; Zellers, A.; Edwards, W.; Farley, H.; Amato, S. Microplastic pollution in the surface waters of the Laurentian Great Lakes. *Mar. Pollut. Bull.* **2013**, *77*, 177–182. [[CrossRef](#)] [[PubMed](#)]
39. Li, J.; Yang, D.; Li, L.; Jabeen, K.; Shi, H. Microplastics in commercial bivalves from China. *Environ. Pollut.* **2015**, *207*, 190–195. [[CrossRef](#)] [[PubMed](#)]
40. Shruti, V.; Jonathan, M.; Rodriguez-Espinosa, P.; Rodríguez-González, F. Microplastics in freshwater sediments of Atoyac River basin, Puebla City, Mexico. *Sci. Total Environ.* **2018**, *654*, 154–163. [[CrossRef](#)] [[PubMed](#)]
41. Claessens, M.; Van Cauwenberghe, L.; Vandegheuchte, M.B.; Janssen, C.R. New techniques for the detection of microplastics in sediments and field collected organisms. *Mar. Pollut. Bull.* **2013**, *70*, 227–233. [[CrossRef](#)] [[PubMed](#)]
42. Li, J.; Liu, H.; Chen, J.P. Microplastics in freshwater systems: A review on occurrence, environmental effects, and methods for microplastics detection. *Water Res.* **2018**, *137*, 362–374. [[CrossRef](#)]
43. Pagter, E.; Frias, J.; Nash, R. Microplastics in Galway Bay: A comparison of sampling and separation methods. *Mar. Pollut. Bull.* **2018**, *135*, 932–940. [[CrossRef](#)]