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Comparision protocols for extraction of microplastics in water samples

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ABSTRACT

Microplastics (MPs) are increasing recognized as emerging pollutants in various environmental components. However, protocols for sampling, analyses and standardization of measurements in MPs research are under development. The extraction method is a cruciak factor that affects the accuracy and comparability of microplastic data. In this study, we evaluated and compared the effectiveness of four different protocols (D, MJ, MA, and S) for separating MPs from water of different types (brackish, marine, and river). Known combinations of MP particles (polyethylene (PE), polyethylene terephthalate (PET), polystyrene (PS) and polyvinyl chloride (PVC) with size ranging between 150 µm and 700 µm were spiked into water samples. Our results showed that the average recovery effectiveness of microplastics using four studied methods ranged from 53% to 86%. Notably, the recovery efficiency of light-density MPs was higher than that of heavy-density MPs. For purified water samples (PW) obtained from a filtration system, using only H_2O_2 was effective in recovering MPs with an efficiency of 80 ± 6.61%. The S method for MP extraction, which combines SDS, Bioenzyme, H_2O_2 30%, and a saturated salt solution using NaCl gave the highest average MP recovery of 78.13 ± 2.39% in PW and 69.72 ± 4.81% in surface water. This method had several advantages over the other three methods, such as low cost, environmental friendliness, and compatibility with various water samples, making it suitable for analyzing large amount of MPs. Our study highlights the importance of carefully selecting the appropriate extraction protocol for accurte and reliable microplastic analysis in different water samples.

Keywords: Extraction method, microplastics, recovery, water sample.

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INTRODUCTION

Plastic materials are widely used in human life. Every year, hundreds of millions of plastic tons are produced. Total annual global plastic production has increased by approximately 337 million tons, from 30 million tons in 1970 to 367 million tons in 2020, over the past 50 years [1]. However, only 9% of plastic waste was recycled, 12% was incinerated, and the rest was buried or discharged into the environment [1]. Most plastic waste has a slow decomposition rate and can be broken down into small plastic particles/fragments and then into microplastics (particles < 5 mm in size) due to the impact of physical, chemical, and biological processes [2]. Since 1972, scientists have discovered microplastics in the marine environment by scientists [3, 4]. Microplastics in the marine environment are derived from the mainland or aquaculture and fishing activities [5, 6], and there are over 5 billion microplastic particles floating in the ocean. Microplastics have been identified in several remote regions, such as Antarctica [7] and six continents [8]. Human consumption products also contain microplastics, such as seafood, commercial sea salt [9], and drinking water [10]. Due to their small size, organisms quickly ingest microplastics and accumulate in their bodies. Microplastics are dangerous and negatively affect the environment, organisms and human health [11, 12].

Hitherto, several methods have been developed to extract microplastics from water and sediment samples, including filtration, sieving, density separation, flotation, chemical decomposition, electrostatic separation, optimization of elution column, and magnetic extraction using coated Fe nanoparticles to magnetize the resin [13]. However, density separation is a commonly applied method to separate microplastics based on the difference in specific gravity between the plastic and water/sediment. Saturated salt solutions with different densities, such as NaCl (1.2 g.cm-3) [14, 15], $ZnCl_2$ (1.6–1.7 g.cm-3) [16–18], NaI (1.8 g.cm-3) [19], $CaCl_2$ (1.4 g.cm-3) [20] was used and gave mixed results in plastic recovery efficiency. In addition, acids, alkalis, fentones, and hydrogen peroxide (H_2O_2) solutions are used simultaneously to remove organic matter from the sample matrix. The methods leading to different microplastic recovery rates present many challenges associated with microplastic research. Choosing a proper purification procedure to remove organic materials from environmental samples is critical for accurate microplastic particle identification by chemical identification using various vibrational spectroscopic and mass spectrometric methods. Both sodium hydroxide (NaOH) and potassium hydroxide (KOH) have been proven to effectively decompose environmental samples, particularly animal tissue, in a short period [21]. However, one disadvantage of alkaline treatment is the breakdown of specific plastic particles. If microplastics are exposed to a 12 M NaOH solution for seven days at room temperature, the PC film will dissolve entirely, and PET will lose significantly weight. Nitric acid, hypochloric acid, and peroxymonosulfuric acid solutions have proven efficient in degrading organic compounds but are exceptionally destructive to polymers [22].

One of the top concerns for MP quantification in the natural environment is to choose a cheap, simple, and cost-effective method. Therefore, this study aimed to evaluate the MP recovery efficiency of four methods for the extraction of microplastics from water samples. The standard separation methods were selected for testing, including oxidation by H_2O_2 30% (D), oxidation by Fenton and H_2O_2 30% combined with density separation by NaCl (MJ), oxidation by H_2O_2 30% combined with density separation of $ZnCl_2$ 30% (MA) and oxidation by SDS, Bioenzym, H_2O_2 combined with density separation by NaCl (S). Spiked MP polymers (PS, PE, PVC, and PET) were performed with four types of water (pure filtered water (PW), river water (RW), estuarine brackish water (BW), and marine water (MW) samples). The advantages and disadvantages of each method were discussed to recommend the most effective and reasonable method for the microplastic extraction of from the water environment.

MATERIALS AND METHODS

Materials

Preparation of microplastic spiked samples

Four types of plastic that are widely used in daily life, including plastic bags (PE), plastic pipes (PVC), plastic bottles (PET), and standard plastic PS (CRT 332.00, V2020-0064), were selected in the present study (Table 1). Plastic products were washed with alcohol and dried naturally in laboratory conditions. PE, PET, PS, and PVC were cut, crushed by the Retsch Model ZM 200 centrifugal sample mill, sieved through a metal mesh with a size of less than 500 µm, collected, and stored in glass bottles. The size of each piece of plastic was measured using a stereo microscope (Leica S9i Microscope) with image analysis software (Leica Application Suite X). PE, PET, and PVC microplastics with sizes from 300–700 µm and PS with sizes from 150 µm to 250 µm were selected in the microplastic recovery test. Different plastic colors are selected for identification.

Table 1. Properties of the studied microplastics (polymer types, sources, colors)

Polymers	Size (µm)	Density (g.cm^{-3})	Shape	Color	Souce
Polystyrene (PS)	150–250	1.04–1.08	Fragment	Black	CRT 332.00 (V2020-0064)
Polyetylen (PE)	300–700	0.91–0.93	Fragment	Blue	Supermarket bag
Polyvinyl clorua (PVC)	300–700	1.3–1.58	Fragment	Yellow	Plastic tube
Polyetylen terephthalate (PET)	300–700	1.29–1.4	Fragment	Green	Soft drink bottle

Chemicals and preparation of solution for density separation

Hydrogen peroxide (H_2O_2 30%, Merck), Sodium Dodecyl Sulfate (SDS, Merck), Bioenzym SE (protease and amylase, Spinnrad), Bioenzym F (lipase, Spinnrad), Fenton Fe (II) (Xilong) were used in the present study. Two density separation solutions were investigated: sodium chloride (NaCl, 1.2 g.cm^{-3}); and zinc chloride ($ZnCl_2$, 1.7 g.cm^{-3}). The saturated NaCl, and $ZnCl_2$ were prepared under a fumehood by dissolving the salt powders in distilled water using a magnetic stirrer plate. Each solution was filtered using 1.2 µm glass fiber (GF/A, Whatman) to remove any microplastic and remaining salt particles and stored in a pre-cleaned glass bottle at laboratory temperature (25^{o}C).

Microplastic separation method

Sampling

Purified water from the filtration system (UV/UF-TOC (Thermo Scientific - USA), river water (To Lich river), estuary brackish water (Lach Tray estuary), and marine water (Hon Dau - Do Son) were used in triplicate. For samples from rivers, estuarine and oceans, 20 L were filtered through a 20 µm planktonic mesh in January 2022. The filtered samples were transferred to a glass bottle and refrigerated at -4^{o}C before analysis. The number of samples was repeated three times at each sampling site.

Table 2. Locations of sampling water

No.	Water source	Sign	Sampling location information	Longitude	Latitude
1	Brackish water	BW	Lach Tray river estuary	20.7741	106.7494
2	Marine water	MW	Hon Dau - Do Son	20.6677	106.8122
3	River water	RW	To Lich river, the section flowing through Hoang Quoc Viet road	20.9632	105.8180

Figure 1. Map of three sampling locations in this study

Microplastic separation

Four selected protocols we tested were performed by choosing procedures with a wide range of literature-documented procedures and performed easily and inexpensive for MPs' separation from water. The differences between the four methods are presented in Table 3. Four types of microplastics were spiked to the water samples (n = 10 particles/1 type of microplastic, 40 particles/sample) to determine the microplastic recovery efficiency of methods. Samples were processed using the methods described in Table 3.

Table 3. Methods for separating microplastic in water

No.	Method	Sample screening (µm)	Sample treatment	Density separation	Microplastic size (µm)
1	Mingxiao Di (D) [23]	48	H_2O_2 30%	-	48–5,000
2	Julie Masura (MJ) [24]	300–5,000	Fenton (Fe (II) 0.05 M, H_2O_2 30%)	6 g NaCl (~5 M)	300–5,000
3	Áron Mári (MA) [25]	8	H_2O_2 30%	$ZnCl_2$ (1.7 $g.cm^{-3}$)	< 5,000
4	Emilie Strady (S) [26]	250–1,000	SDS Bioenzyme SE Bioenzyme F H_2O_2 30%	NaCl (1.18 $g.cm^{-3}$)	300–5,000

Protocol D [26]: This protocol is a digestion method with hydrogen peroxide 30% H_2O_2. The water sample was filtered through 48 µm stainless steel sieves, then 30% H_2O_2 was added to process the sample for 12 h. The solution was filtered (GF/A 1.6 um, Ø = 47 mm, Whatman) using a glass filter, and dried at 50°C. The filter was stored in a glass Petri dish for further the examination.

Protocol MJ [27]: The water sample was through a 0.3 mm stainless steel sieve, then Fenton solution (20 mL Fe(II) 0.05 M and 20 mL H_2O_2 30%) was added and heated at 75°C for 30 minutes. NaCl was added and heated at 75°C. After 24 h, the solution was filtered (GF/A 1.6 um, Ø = 47 mm, Whatman) using a glass filter and kept in a glass Petri dish.

Protocol MA [28]: This protocol is based on density separation using a dense solution of $ZnCl_2$. The water sample was through an 8.0 µm filtered membrane, then placed in a beaker containing $ZnCl_2$ solution and sonicated for 5 min. The solution was extracted for 60 min using a glass apparatus, and the supernatant was further treated with 30% H_2O_2 at 70°C and stirred at 400 rpm for 60 min. Finally, the solution was filtered 8.0 µm a glass filter (MCE, Ø = 47 mm) and kept in a glass Petri dish.

Protocol S [29]: This is a digestion and density separation method. The water sample was first filtered through a 1 mm stainless steel sieve. The prepared samples were treated using SDS, Bioenzyme, and H_2O_2 30% combined with a saturated salt solution using NaCl. The supernatant was filtered (Whatman glass microfiber filters, Grade GF/C, 0.45 µm) three times through a 1.6 um Whatman GF/A filter and observed under a stereo microscope. All filters were examined under a microscope (Leica MZ12 stereomicroscope at a 16-160-fold magnification). The number of microplastic items determined after density separation in each method was used to calculate the recovery efficiency.

Microplastic recovery efficiency (H) = [microplastics collected and counted on the filter/spiked microplastics] × 100 (n = 3).

Quality control

All steps were performed inside a fume hood to avoid microplastic contamination from the surrounding environment during the experiment. Laboratory experiments were always in clean conditions. Experimental instruments were washed with distilled water that was filtered through a GF/A filter (1.6 µm) to remove every possible contamination and encased in aluminum foil.

RESULTS

Evaluation of the efficiency of microplastic recovery in filtered water samples (blank sample)

The recovery percentages of PET, PE, PVC, and PS microplastics in PW (blank sample) of the four protocols are shown in Figure 2. The microplastic recovery (n = 3) exhibits medium values ranging from 58% to 80% in all four methods. The microplastic recovery efficiency was arranged as follows: D method (80 ± 6.61%) > MA method (79.17 ± 2.89%) > S method (78.13 ± 2.39%) > MJ method (58.33 ± 7.64%). Four tested plastics are recovered at high efficiency. However, the MJ method did not recover PVC microplastics. There is a significant difference between PE, PS, and PET recovery efficiency compared PVC ($p < 0.01$).

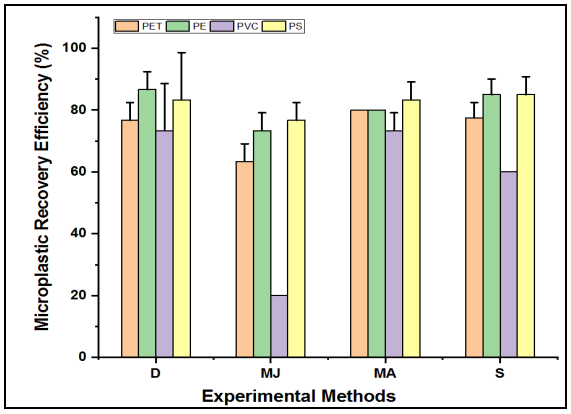

Figure 2. Average recovery of microplastics in blank sample

Overall, the microplastic recovery of the tested methods for PS microplastic was high, with an average of 82.71 ± 4.48%; followed by PE 81.46 ± 6.25%, PET 74.38 ± 7.50% and PVC 56.67 ± 25.24%. For PS microplastics, the recovery efficiency of the S (87.5%) > D, MA (83.3%) > MJ (76.7%). For PET and PE microplastics, the order of microplastic recovery efficiency is as follows: S (77.5%; 87.5%) > D (76.67%; 85.00%) > MA (80%; 80%) > MJ (63.3% and 73.3%). For PVC microplastics, the microplastic recovery efficiency of methods D and MA resulted in 73%, while S and MJ methods gave lower recovery efficiency of 60 and 20%, respectively. The current investigation found that the D procedure achieved the highest microplastic recovery effectiveness for PW

samples that did not include organic compounds or suspended solids. However, the water samples contain many other substances, such as organic matter and suspended solids. Therefore, the water samples from rivers, estuaries, and coastal areas were used to evaluate the recovery efficiency of the four test methods.

Evaluation of the efficiency of microplastic recovery in real samples with added standards

There was a similarity in the microplastic recovery efficiency in the studied water samples (river water, brackish water, marine water) compared with the blank sample. The D method resulted in the highest average microplastic recovery (79.44 ± 5.67%), with microplastics recovered in RW at 75%, in BW at 86%, and in MW at 78%; the S method yielded the second average microplastic recovery (69.72 ± 4.81%), with microplastics recovered in BW and MW were 73% and 72.5%, RW at 62.5%; the MA method led to the third average microplastic recovery (66.39 ± 6.31%), with microplastics recovered in RW and MW samples were 71% and 69% while BW was 59%. The MJ technique had an average microplastic recovery (48.89 ± 12.73%), microplastics recovered in RW at 52%, BW at 35%, and MW at 60%. The highest microplastic recovery is PET (73.89 ± 3.85%), the second is PE (73.61 ± 4.74%), the third is PS (71.39 ± 8.22%), and the fourth is PVC (45.56 ± 3.37%).

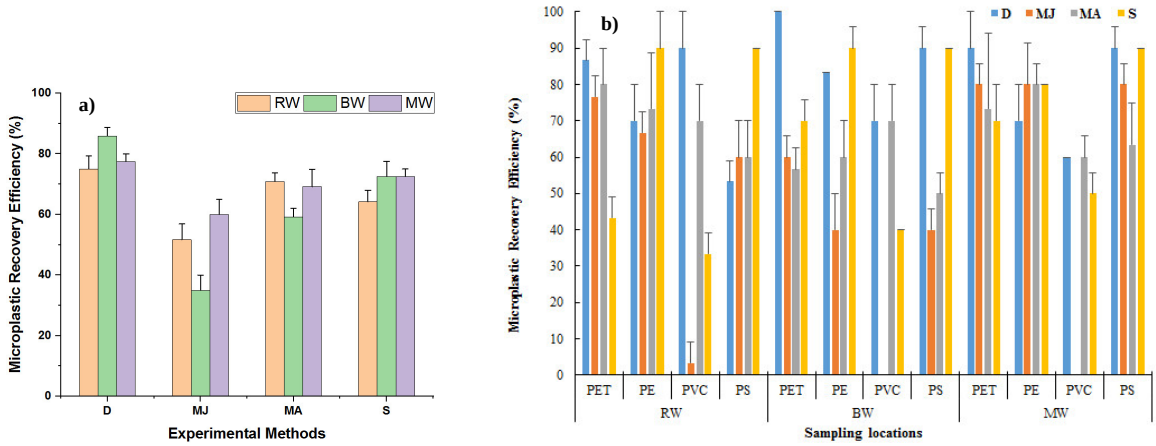

Figure 3. Average recovery of microplastics by method (a) and by type of plastic (b)

In general, three evaluated techniques may recover four different types of microplastics, each with its benefits and drawbacks. Oxidizing agents were often used to remove organic matter present in the matrix [27]. Organic removal with hydrogen peroxide (H_2O_2, 15–35%) was found to be more effective than with alkaline (NaOH) or acid (HCl) solutions [22]. The D method was simple: using H_2O_2 30% solution to remove organic matter. The solution after treatment was filtered through filter paper and microplastics were recorded with the highest recovery efficiency of about 79.44 ± 5.67%. Mak et al., (2020) also recorded similar results when using H_2O_2 30% for water samples and obtained the recovery efficiency of PE, PS, PVC, and PET microplastics at 90.8 ± 3.0%; 85.0 ± 12.7%; 87.5 ± 12.3%; and 46.5 ± 6.5%, respectively [28]. However, using metal sieves with a size of 48 µm retains microplastics but at the same time keeps suspended and organic substances that make for confusing, complicated, and time consuming microplastic identification. In addition, water quality should be considered, especially with organic-rich water samples that require oxidation at a longer time and higher temperatures. According to Karami et al., (2016), using H_2O_2 30% solution at 50°C for a long time can lead to partial dissolution and

discoloration of bioplastic particles, changing the color of PET, and structural degradation of polymers, especially PVC and PS [22]. Sample treatment with Fenton solution combined with H_2O_2 and NaCl salt solution (MJ) resulted in low efficiency of organic matter treatment and microplastic recovery, with a range of 48.89 ± 12.73%, significantly since this method did not recover PVC microplastics. It was likely that, the oxidation Fenton, which caused precipitation and discoloration of the filter paper, made it difficult to observe and identify microplastics. Weisser et al., (2021) reported a similar phenomenon occurring in some cases because the Fenton reaction can produce oxidized iron, resulting in an orange precipitate [29]. Another limitation of the Fenton reaction was that the high amount of heat released from the reaction could affect the properties of the microplastics [30]. Using H_2O_2 in combination with $ZnCl_2$ saturated salt solution (1.7 $g.cm^{-3}$) (MA method) resulted in microplastic recovery efficiency in the range of 66.39 ± 6.31%. However, for samples with many suspended solids, difficulties observation and identification of microplastics were noted. This phenomenon can be explained by using $ZnCl_2$ as a flotation agent, whichincreases the environment viscosity when flotation can lead to small organic substances drifting along the microplastics, thus affecting microplastics recovery from the water samples that are rich in organic matter [31]. Using $ZnCl_2$ saturated salt solution resulted in high microplastic recovery efficiency; however the following issues need to be considered: (i) cost of $ZnCl_2$ salt, (ii) toxicity of $ZnCl_2$ to aquatic animals and plants, and the requirement of proper treatment before being discharged into the environment, (iii) $ZnCl_2$ solution can also be harmful to technicians in cases of inhalation and skin contact who may have to requiree treatment and specific care [13]. Using H_2O_2 and saturated NaCl solution (method S) to separate microplastics with good results, the microplastic recovery efficiency reached 69.72 ± 4.81%. Using NaCl salt was cheaper than $ZnCl_2$ salt. It was also not harmful to users and the environment. Remarkably, treatment of organic compounds with SDS, Bioenzyme, and H_2O_2 30% at a low temperature of 40^oC still removed organic matter and did not affect the properties of microplastics. Filtering the sample through a metal sieve before sample processing reduces in the number of chemicals used in microplastic separation and flotation and reduces suspended solids present in alluvium-rich samples. The disadvantage of this method appeared to be that the recovery of high-density microplastics was low, such as PVC. However, the microplastic recovery efficiency can be improved by repeating the flotation step several times on the same sample [32].

According to the findings, many aspects influencing the choice of microplastic measurement technique in water need to be considered, including water quality and microplastic density. For example, water samples rich in organic matter and high in suspended matter (high total suspended solids) would face many difficulties in the flotation process, and microplastic observation and recognition should be preferred method S; in contrast, clean water and low suspended matter samples, method D can be applied. Separation of microplastics uses a salt solution with high density for flotation, such as $ZnCl_2$ (1.6–1.7 $g.cm^{-3}$), NaI (1.3–1.8 $g.cm^{-3}$), $CaCl_2$ (1.35 $g.cm^{-3}$) would achieve a higher microplastic recovery efficiency than using NaCl (1.18–1.2 $g.cm^{-3}$) but were not recommended because these chemicals are toxic to users and harmful to environment [13]. The obtained results showed that the S method using SDS, Bioenzym, and H_2O_2 30% combined with density separation by NaCl led to the average microplastic recovery efficiency with an average of 78.13 ± 2.39% in PW and 68.33 ± 5.20% in surface water samples, which can be considered as a suitable method in research and monitoring of microplastics for surface water samples.

CONCLUSION

Many methods have been developed to assess microplastic pollution in environments. As a result, evaluating the microplastic recovery from various methods and selecting

the best approach is critical. Microplastic recovery efficiency in PW and surface water samples (BW, MW, and RW) using four methods as the D, MA, S, and MJ methods were 80 ± 6.61% and 79.44 ± 5.67%; 79.17 ± 2.89% and 66.39 ± 6.31%; 78.13 ± 2.39% and 69.72 ± 4.81%; 58.33 ± 7.64% and 48.89 ± 12.73%, respectively. However, the D method was only suitable for clean water samples with little organic matter. The MA method was highly efficient in recovering microplastics (in surface water), but it was expensive and caused environmental pollution. The S method provided many microplastic recovery efficiency, low cost, environmentally friendly, and was found to be suitable for different surface water samples.

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