



# Sample preparation methods for the analysis of microplastics in freshwater ecosystems: a review

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## Abstract

The vast amount of plastic waste emitted into the environment is of increasing concern because there is mounting evidence for various toxic effects of microplastics on living organisms. In particular, despite freshwater ecosystems are essential sources of water supply, they have been less investigated than marine ecosystems for microplastic pollution. Here, we review 150 freshwater studies for techniques used to separating microplastics from water and sediments. We compare major chemicals utilized in digestion and density separation steps. Sodium chloride is the most prevalent salt used in separating microplastics from freshwater environments. Hydrogen peroxide and Fenton's reagent are most frequently used in digestion of organic materials.

**Keywords** Microplastic pollution · Freshwater systems · Polymer · Extraction · Density separation

## Introduction

Plastic products are widely manufactured, many of which are applied only once. Global plastic production has continued to rise, but recycling has lagged behind. It is estimated that between 4.8 and 12.7 million tons of plastic waste end up in oceans annually, via river inputs (Jambeck et al. 2015). Different effects of various types of pollutants have been widely studied (Mirzajani et al. 2015, 2016; Padash Barmchi et al. 2015; Rezaei Kalvani et al. 2019) and recently, there has been more research conducted on emerging pollutants (Jafari Ozumchelouei et al. 2020), such as microplastics. Microplastics are synthetic particles with regular or irregular shape and with size ranging from 1 to 5 mm, which are insoluble in water and have primary (manufactured in micro-sized dimensions) or secondary (large plastics broken down via degradation forces) origin (Frias and Nash 2019; Razeghi et al. 2021a; Zhang et al. 2021). While, there are huge efforts on removal of pollutants from wastewater (Mojoudi et al. 2018, 2019), including biological methods (Alavian et al. 2018; Mansouri et al. 2013; Mirzajani et al. 2017), some evidences suggest that key sources of microplastic pollution in freshwater sources are wastewater effluent and terrestrial run (Hamidian et al. 2021; Lasee et al. 2017). The potential harm to humans' health and organisms associated with microplastics can be categorized into three forms, including

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physical harms, chemicals, and microbial pathogens of biofilms (Campanale et al. 2020a; Naqash et al., 2020; Prinz and Korez 2020). Removal of microplastics by adsorption, filtration, chemical methods to treat microplastics, biological removal, and ingestion methods has been also reported (Othman et al. 2021; Padervand et al. 2020; Tofa et al. 2019). However, this important topic needs more attention from the scientific community.

Prevention or minimization of plastic production, identifying the current state of pollution and filtration are three important strategies toward removal of small-sized plastic particles in environment. After selecting appropriate sampling methods and tools for microplastic detection in environmental samples (Razeghi et al. 2021b), microplastic isolating procedure is the next important stage in microplastic studies, before identifying physical and chemical characteristics of plastic particles. Lack of standard methods in sampling, isolating, and instrumental analysis of microplastic particles from environmental samples leads us to review the literature on microplastic contamination in freshwater environments. Several separation techniques using numerous density separation and digestion solutions have been developed to isolate microplastics from water, sediment, and biological tissues. As a result, a full report on laboratory isolation methods/materials of microplastics and their frequency of use in freshwater studies is presented in this review study. Advantages and limitations of each method are then discussed throughout the paper. As an attempt, the following questions would be answered:

- a. What are microplastic isolating procedure steps in freshwater studies?
- b. Which are the most prevalent chemicals utilized in digestion and separation steps?
- c. What are scientific advantages and disadvantages of water and sediment isolating methods and chemicals?

## Data acquisition

Relevant scientific studies were gathered through online search in the databases of ISI Web of Knowledge, Science Direct, and Google Scholar. Keywords, including “microplastic” OR AND “freshwater,” OR AND “plastic particle,” OR AND “plastic fragment,” OR AND “pellets” OR AND “river” OR AND “estuary” OR AND “lake” were considered. Then, the retrieved papers were screened with respect to types of freshwater, including rivers, estuaries, reservoirs, lakes, etc. It should be noted that microplastic research focusing only on microplastics in freshwater species was excluded. However, a combination of water or sediment studies with biota or three of them simultaneously were included. A total of 150 published studies during 2010–2020

were selected and evaluated. Initial data were extracted and recorded in an EXCEL spreadsheet for subsequent analysis.

## Isolating procedures of microplastics

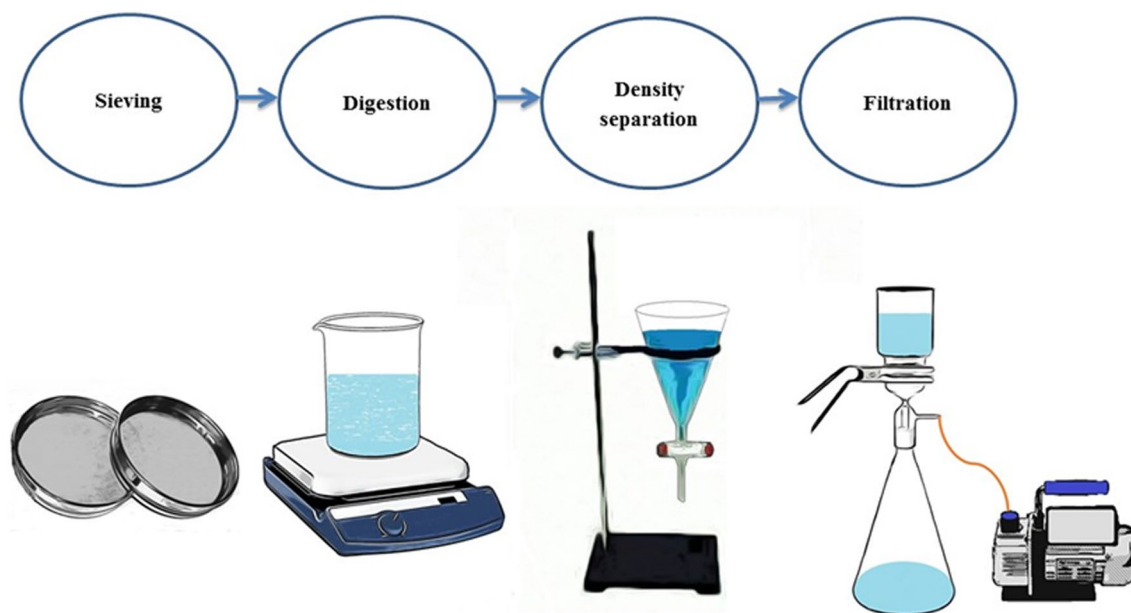
After collecting water, sediment, or biological samples from environment, plastic particles in the samples should be separated from organic and inorganic materials. Separation makes it possible to calculate quantity and quality of plastic particles. Pretreatment is done to improve accuracy of subsequent processes for plastic particles, such as isolation, material identification, and counting/weighing (Michida et al. 2019). Challenges in detection of microplastics in environment comprise of three main aspects: (1) the ability to capture plastic particles from water, sediment, and biota samples; (2) separation of plastic particles from other matter (organic and inorganic); and (3) the exact identification of plastic types (Li et al. 2020).

In general, the collected samples from environment go through all or some steps, including size selection, digestion, density separation, and filtration (Fig. 1). After that, the prepared samples are used for further examination and identification. Although, there is not a unique standard method for analysis of microplastics, currently, the main basis of studies includes the guidelines developed by the national oceanic and atmospheric administration (NOAA) (Masura et al. 2015) with slight modifications. A combination of chemical, thermal, physical, and mechanical processes can be used to prepare samples before instrumental analysis (Yonkos et al. 2014).

Order of the steps may be different in some studies or may be omitted in the others regarding complexity of the sample matrix, organic matter load, and plastic particles' size distribution (Vaughan et al. 2017). Zbyszewski and colleagues cleaned visible particles in an ultrasonic bath with deionized water in order to remove sand and other potential surface residues and plastics were carefully separated by hand (Zbyszewski and Corcoran 2011; Zbyszewski et al. 2014).

## Sieving

Sieving is an important step to separate large natural materials like sticks, leaves, grass, shells, or human-made waste material. Regarding the accepted defined large size for microplastics, which is equal to 5 mm, most of the studies have used one or a few sieves with different sizes at the beginning of detection of micro-sized plastic particles. In some papers, the collected samples have been subjected to a size selection step using sieves or filters with various pore sizes and other steps have been omitted. For example, water samples from the “Three Gorges Dam,” China, were prepared only by passing the sampled water through the



**Fig. 1** Microplastic main separation steps in freshwater studies. The collected samples from environment go through all or some steps

1.6 mm sieve. Then, liquid was transferred into a separating funnel. Materials retained on the sieve were assessed by the naked eye to pick the suspected plastic debris. Samples were allowed to settle for a week. The floating debris on surface was transferred to petri dishes for identification step (Zhang et al. 2015). Sadri and Thompson (2014) filtered water sample through a set of sieves with varying mesh sizes and then transferred particles onto petri dishes for detection (Sadri and Thompson 2014). Although sieving and visual identification are important and essential steps in microplastic identification, they may not be sufficient, especially for complex sediment samples. Sieving may result in size distribution artifacts, with different particle morphologies, but given nature of the collected material, it is important to remove larger materials as much as possible (Vaughan et al. 2017). It seems that there is a consensus on moving toward a combination of size and density separation using sieves and density separation techniques, which will be discussed in the following.

### Digestion

Digestion is a commonly used method to remove non-plastic organic materials that otherwise may negatively interfere with isolation and identification of microplastics. Oxidation, enzymatic digestions, and acid–alkaline digestion are used for organic digestion. Each digestion method is discussed further.

### Oxidation

Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) is a chemical compound with oxidizing ability. In microplastic studies, hydrogen peroxide could be used alone or in combination with a catalyst to increase speed of chemical reaction. Fenton's reagent or wet peroxide oxidation method is a common digestion procedure in the microplastic studies. In this method, a combination of hydrogen peroxide as oxidizing agent and Fe (II) as catalyst are used to digest organic matter. It has been noted that plastic is resistant to the wet peroxide oxidation method (Baldwin et al. 2016; McCormick et al. 2014). Prata et al. (2019) suggested that  $\text{H}_2\text{O}_2 + \text{Fe}$  is appropriate for removal of plant material and KOH (potassium hydroxide) for animal tissues and virgin and weathered plastics did not change in the presence of these oxidizing agents, except for cellulose acetate (Prata et al. 2019). Some studies have reported that this method may alter or potentially digest some of materials in samples (especially nylon and low-density polyethylene). Certain low-density polymers, such as nylon and low-density polyethylene are known to be reactive in exposure to 30%  $\text{H}_2\text{O}_2$  (Anderson et al. 2017). Most of the studies have used 30% or 35% peroxide to digest organic matter. However, wet digestion with 50%  $\text{H}_2\text{O}_2$  has been used in the previous research as well (Liu et al. 2019). Oxidative digestion is inexpensive but temperature needs to be controlled.

## Enzymatic digestion

Enzymatic digestion is a less damaging process that could be a suitable alternative for the wet peroxide oxidation method but it also could be very time-consuming especially for samples containing several different types of organic material (e.g., cellulose, chitin, proteins, and lipids) (Lusher et al. 2018; Michida et al. 2019). Sometimes, a combination of digestion and enzymatic methods is used. Materials of biological origin have been degraded with lipase, protease, amylase, chitinase, and cellulase, in combination with peroxide oxidation (Mani et al. 2015). In a study, protease, amylase, and lipase with hydrogen peroxide were used in microplastic assessment of Saigon River, in Vietnam (Lahens et al. 2018). Samples from urban and highway stormwater retention ponds from Denmark were wet-oxidized on the filters for 2 days by adding 50%  $H_2O_2$ . Subsequent digestion was performed by enzymatic digestion (enzymes of Cellubrix, Viscozyme, and Alcalase) (Liu et al. 2019). Depending on degree of biogenic or silicate debris, some sediment samples from Warnow Estuary, Germany, underwent enzymatic treatment (Enders et al. 2019). Extraction protocol for stormwater pond samples from Viborg City, Denmark, consisted of enzymatic digestion with cellulase followed by oxidation with Fenton's reagent (Olesen et al. 2019).

## Acid–alkaline digestion

Acid digestion is rapid but can degrade some polymers. A combination of nitric acid ( $HNO_3$ ) and hydrochloric acid (HCl) has been employed to digest biogenic matter (Noik and Tuah 2015). A mixture (1:3, v:v) of hydrogen peroxide solution (30%) and concentrated sulfuric acid ( $H_2SO_4$ ) was used to destroy natural debris in study of microplastics in river shores' sediments of the Rhine-Main area in Germany (Klein et al. 2015). Dubaish and Liebezeit treated the retained inorganic particles with hydrofluoric acid (HF). However, it was noted that polystyrene and polycarbonate may be lost due to their susceptibility toward this acid (Dubaish and Liebezeit 2013). Sediment samples from Lake Bolsena and Lake Chiusi, Italy, were treated with hydrochloric acid for 48 h at room temperature and were additionally digested under heat in order to destroy or at least leach lipid contents of organic material (Fischer et al. 2016). The sediment samples from beach of Lake Garda, Italy, were treated with 100–200 mL of peroxymonosulfuric acid ( $H_2SO_5$ ) in order to remove organic residue (Imhof et al. 2016, 2018).

Alkaline digestion causes minimal damages to most of polymers in comparison with acid digestion but it damages cellulose acetates (Michida et al. 2019). Potassium hydroxide is a commonly used chemical compound, especially for digesting biological tissues. Alkaline hydrolysis has been employed for hydrolyzing protein compounds with sodium

hydroxide (NaOH) and has been verified as a good tool for separating animals' soft tissue (Nan et al. 2020).

The use of a combination of different methods has been reported in research papers. The samples from urban and highway stormwater retention ponds underwent oxidation with Fenton's reagent and 0.1 M NaOH to further remove organic matter (Liu et al. 2019). Removing organic material via alkaline digestion using KOH/NaClO (Hitchcock and Mitrovic 2019) or two-step protocol using  $H_2O_2$  followed by sodium hypochlorite solution (NaClO) (Tamminga et al. 2019) has been done in the reviewed studies. Campbell et al. (2017) utilized Fenton's reagent, 10% NaClO solution and subsequently  $HNO_3$ /NaClO solution to digest fish's gastrointestinal tracts (Campbell et al. 2017). Some sediment samples from Warnow Estuary, Germany, were treated with enzymes, both acid and base ones, including HCl and NaOH depending on degree of biogenic or silicate debris (Enders et al. 2019). Treatment of biological tissue with  $HNO_3$ , NaOH, and  $H_2O_2$  led to loss of particle fluorescence as well as a strong agglomeration of particles. Tetramethylammonium hydroxide caused a slight decrease in particle fluorescence, resulting in an incomplete dissolution of tissues (Rist et al. 2017).

## Density separation

Density separations are conducted by subjecting environmental samples to the concentrated or saturated salt solutions, followed by filtration or other separation techniques to decrease sample mass and mineral matter. Density separation has been adopted for most of the reviewed papers. Various methods and materials have been used in different studies to separate microplastics from water, sediment, and biota samples. Some authors have reported density separation using water for polystyrene, polyethylene, and polypropylene (Vaughan et al. 2017). However, this procedure seems to be insufficient for separation of polymers with higher density. Chemicals used for density separation of microplastics in freshwater studies are listed in Table 1. These solutions represent different density separation limits, and with respect to their densities they can separate polymers smaller than this density. For example, sodium chloride (NaCl), sodium polytungstate (SPT), sodium iodide (NaI), and zinc chloride ( $ZnCl_2$ ) solutions have densities of 1.2 (Kataoka et al. 2019), 1.4 (Hidalgo-Ruz et al. 2012), 1.6 (Claessens et al. 2013), and  $1.7 \text{ g cm}^{-3}$  (Imhof et al. 2012), respectively. It has been suggested that for sediment samples, microplastic density separation can be performed with sodium chloride, sodium bromide (NaBr), sodium iodide, or zinc bromide ( $ZnBr_2$ ) (Hu et al. 2018). Sodium chloride is suitable for separation of many microplastics, such as polyethylene, some blends of polypropylene, and foamed polystyrene, which are typically the most common types of plastic found in aquatic

**Table 1** Chemicals used for microplastic density separation step in freshwater studies

Density separation chemicals	Solution density (g cm <sup>-3</sup> )	Key references
Sodium chloride (NaCl)	1.20	Briggs et al. (2019), Gallagher et al. (2016) and Yonkos et al. (2014)
Sodium iodide (NaI)	1.60–1.80	Lusher et al. (2018), Merga et al. (2020) and Willis et al. (2017)
Two-step separation using NaCl + NaI	1.20 (NaCl) + 1.60–1.80 (NaI)	Di et al. (2019), Hurley et al. (2018b) and Kapp and Yeatman (2018)
Zinc chloride (ZnCl <sub>2</sub> )	1.58–1.80	Liu et al. (2019), Shruti et al. (2019) and Zhao et al. (2014)
Potassium formate (KHCO <sub>2</sub> )	1.54	Xiong et al. (2018), Zhang et al. (2016) and Zhang et al. (2019)
Calcium chloride (CaCl <sub>2</sub> )	1.30–1.35	Grbić et al. 2020
Potassium fluoride (KF)	1.50	Fan et al. (2019)
Sodium polytungstate Na <sub>6</sub> (H <sub>2</sub> W <sub>12</sub> O <sub>40</sub> ) (SPT)	1.40	Corcoran et al. (2019), Dean et al. (2018), Enders et al. (2019) and Turner et al. (2019)
Lithium metatungstate (LMT)	1.60	Eo et al. (2019)
Oil	–	Dong et al. (2020) and Mani and Burkhardt-Holm (2020)
Water	–	Vaughan et al. 2017

Various chemicals with different density solutions have been employed to isolate microplastics from environmental matrices.

environment (Crawford and Quinn 2016). Sodium chloride is commonly used in density separation techniques for microplastic particles and has advantages over other salts, because it is inexpensive, readily available, and has less potential for negative environmental effects (Hendrickson et al. 2018). However, the use of sodium chloride solutions for density separation has been found to be inefficient for separating more dense plastic polymers like polyvinyl chloride (density = 1.30–1.70 g cm<sup>-3</sup>) and polyethylene terephthalate (density = 1.40–1.50 g cm<sup>-3</sup>) from environmental matrices (Crawford and Quinn 2016).

Other salts, such as zinc chloride, sodium iodide, and sodium polytungstate solution are less commonly used due to their high cost and substances interfering with sediments that might be extracted as well (Wang et al. 2017a). However, they have the advantage of sufficient density for polymers with higher densities, e.g., polyvinylchloride and polyethylene terephthalate and all of them have been used successfully (Crawford and Quinn 2016). Each of these salts has different densities. Most sediment grains have a density of approximately 2.6 g cm<sup>-3</sup>, which is higher than densities of salty solution; therefore, they sink to the bottom when standing still (Lusher et al. 2018).

Two-step density separation methods have been applied in some studies. The basic idea in this method is using a combination of different density saturated salts. In this method, fluidization of particles occurs in a lower density salt (NaCl) followed by flotation of microplastics in a higher density salt (NaI) (Di et al. 2019; Di and Wang 2018; Hurley et al. 2018b). However, two-step extraction is more time-consuming than flotation using only one type of salt. Air-induced overflow method is sometimes used in two-step separation to force specific lighter particles to move more quickly and frequently to the top layer of the solution (Nuelle et al. 2014).

Separation solution has been prepared in some studies by dissolving potassium formate (KHCO<sub>2</sub>) in deionized water to a density of 1.5 g cm<sup>-3</sup> (Xiong et al. 2018; Zhang et al. 2016, 2017, 2019) and calcium chloride (CaCl<sub>2</sub>) to a density of 1.4 g cm<sup>-3</sup> (Grbić et al. 2020). Lithium metatungstate solution (LMT) with the density of 1.6 g cm<sup>-3</sup> was used to separate microplastics from denser inorganic particles. It was noted that the original density of lithium metatungstate is 2.95 g cm<sup>-3</sup> but it was diluted with water to the specific density (Eo et al. 2019; Watkins et al. 2019a). This is also true for other salts.

Recently, microplastic separation by means of hydrophobic interactions and using oils (e.g., silicone oils, paraffin oils, and corn oil) has been reported as well. In this technique, lipophilic microplastics are extracted from their environmental matrix by attracting the microplastics to an oil layer and non-microplastic particles are segregated in a separation funnel. However, no generally valid recommendations can be given in this method (Dong et al. 2020; Mani and Burkhardt-Holm 2020).

In a study, for efficiently extracting the microplastics from sediment and checking performance of each salt, sodium chloride, sodium iodide, zinc chloride, and potassium formate were tested as separation solutions. Potassium formate was finally chosen due to its relatively better recovery for samples from Pearl River, in China (Fan et al. 2019).

The use of high-density solutions increases extraction efficiency, but very dense solutions cause floating of other wastes in the sample or even sediments, thus reducing efficiency of separation process. The extraction process must be repeated at least three times to achieve the maximum efficiency.

## Filtration

Filtration step is the last stage in all the research activities on microplastics and is done before visual/instrumental polymer identification. In this stage, the micro-sized plastic particles in solution or water samples are retained on the top of filter paper or sieve, with specific mesh size. Vacuum pump filtration is used to accelerate and to facilitate this process. Gridded filter paper can make it easier to search and count particles under a microscope.

## Other methods for isolating microplastics from environmental samples

In a research, elutriation step using a 1-m long tube fitted with 63  $\mu\text{m}$  mesh at the bottom was performed to reduce amount of sediment to be further treated for assessing 18 streams in and around Auckland City, New Zealand (Dikareva and Simon 2019). Froth flotation is a process that uses affinity for hydrophilicity or hydrophobicity of water to separate materials and is used for separation of plastics (Alter 2005). Centrifugation is a good method to collect supernatants from residue after flotation. Sometimes more than one extraction stage is utilized for better particle separation (Han et al. 2020; Phillips 2020). Sodium hexametaphosphate has been applied in some studies as a dispersant to disperse any microscopic aggregates in sediment samples and then, samples have been subjected to ultrasonic dispersion or sieving (Browne et al. 2010; Egessa et al. 2020; Firdaus et al. 2020; Vermaire et al. 2017). In some papers, extraction protocol and purification step have been started using sodium dodecyl sulfate (SDS) as an anionic surfactant, which is supplemented to avoid agglomeration and to ensure stability of microplastic suspensions (Enders et al. 2019; Lahens et al. 2018; Liu et al. 2019; Mintenig et al. 2020; Olesen et al. 2019). For disaggregation of sediment particles, Rodrigues et al. (2018) employed sodium polyphosphate before sieving sediment sample and stirred the sample for a specific time (Rodrigues et al. 2018). Diluted hydrochloric acid was used for washing the microplastics to remove metal attached to them (Wang et al. 2020b).

Imhof et al. (2012, 2018) extracted plastic particles using the developed semi-automated device called as Munich plastic sediment separator, which was constructed for sediment samples with recovery rates of 100% for large microplastic particles (large microplastics, in the size range of 1–5 mm) and 95.5% for small microplastics. Zinc chloride is used as a separation fluid (Imhof et al. 2012, 2018). This device has advantages like high recovery and reduced time, but it is a highly specialized piece of equipment that is not widely available. Elutriation is a process, in which particles are

separated based on their shape, size, and density using a stream of gas or liquid flowing in a direction opposite to that of sedimentation. The technique was first used to separate microplastics from sediment by directing an upward flow of water through a column (Claessens et al. 2013).

For separating microplastic particles lower than 0.5 mm, Wessel et al. (2016) designed a separation process with a series of PVC pipes and connectors that used density differences to mechanically separate sand and plastic particles (Wessel et al. 2016). Plastic debris that escaped the 1 mm sieve with the bulk of sand was separated using a low-cost fluidized density separation system, which used air pump (Noik and Tuah 2015).

Horton et al. (2017) processed sediment samples in three steps in order to determine efficiency of these steps in removing microplastics. The steps included visual inspection of the whole sample, flotation technique, and post-flotation visual inspection. The most effective method of particle removal was flotation, which extracted between 51 and 82% from the total particles. The final post-flotation visual inspection extracted less than 3% of the total particles recovered for three sites of this study (Horton et al. 2017).

Wang et al. (2020) demonstrated that hybrid biochar sand filter is able to remove 60–80% of microspheres presented and has strong potential for removal of microplastic spheres with a size of 10  $\mu\text{m}$ . Plastic particles are immobilized through stuck, trap, and entanglement in biochar porous media (Wang et al. 2020c). The techniques used for separating microplastics in freshwater studies are summarized in Tables 2, 3, 4, 5.

## Discussion

There are numerous brine solutions used for density separation of microplastics from environmental samples. Despite lower efficiency of the sodium chloride solution in separating all types of polymer, sodium chloride is the most prevalent salt used in separating microplastics from freshwater studies. The advantages of this salt over other options include ease of access, cheapness, and less potential for negative environmental effects. It has been suggested that the saturated sodium chloride solution commonly used in studies could float up the materials with a density lower than 1.2  $\text{g cm}^{-3}$ ; therefore it might underestimate microplastic concentration (Wang et al. 2017a). For ensuring about appropriate estimation of the total microplastics, especially in sediment samples, it is recommended to use alternative methods and salts. Solutions with the required density can be prepared by adding distilled water to heavy solutions (Crawford and Quinn 2016).  $\text{ZnCl}_2$  is the second option among different materials (Fig. 2). It has been revealed that  $\text{ZnCl}_2$  and  $\text{NaI}$  solutions are not commonly used due to their high

**Table 2** Microplastic separation and digestion in freshwater media

Study Compartment	Study Area	Separation/Digestion method	References
River–Estuary			
	Three Gorges Dam-China	Filtered through a 1.6 mm stainless steel sieve, picked out using stainless steel tweezers	Zhang et al. (2015)
	The North Shore Channel (NSC) in Chicago, Illinois (IL), USA	Picked out using sterilized forceps, filtered through 2 mm and 330 µm stacked sieves, NaCl, 30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II)	McCormick et al. (2014)
	29 Great Lakes tributaries, USA	Filtered through a series of Tyler sieves of 4.75, 1.00, and 0.355 mm stainless steel mesh, 30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II), filtered by a 125 µm mesh sieve	Baldwin et al. (2016)
	Inflow (Red and Assiniboine rivers) and outflow (Nelson River) of Lake Winnipeg, Canada	Filtered through a 355 µm mesh brass sieve, 30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II)	Warrack et al. (2017)
	Tamar Estuary, UK	Filtered through a set of 3 mm, 1 mm and 270 µm mesh sieves	Sadri and Thompson (2014)
	Los Angeles and San Gabriel Rivers, USA	Filtered through Tyler sieves 4.75 mm, 2.8 mm, 1.0 mm	Moore et al. (2011)
	Danube River, Austria	Water bath and a density separation	Lechner et al. (2014)
	Four Estuarine Rivers in the Chesapeake Bay (Patapsco, Magothy, Rhode, and Corsica Rivers), USA	Filtered through nested 5.0 mm and 0.3 mm stainless steel sieves, NaCl, 30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II)	Yonkos et al. (2014)
	Yangtze Estuary and East China Sea, China	Filtered through a 32 µm steel sieve, ZnCl <sub>2</sub> , 30% H <sub>2</sub> O <sub>2</sub> , filtered over gridded 1.2 µm cellulose nitrate filters	Zhao et al. (2014)
	Rhine River-Switzerland	Filtered through a stacked series of metal sieves 5 mm, 1 mm, 300 µm, NaCl, 30% H <sub>2</sub> O <sub>2</sub> and enzymatic treatment	Mani et al. (2015)
	Solent estuarine complex (Hamble, Itchen, and Test Rivers), UK	Super-saline solution (NaCl)	Gallagher et al. (2016)
	Three urban estuaries (Jiaojiang, Minjiang and Oujiang Estuaries), China	Filtered through a 333 µm steel sieve, enzymatic digestion protocol, filtered through 1.2 µm pore size	Zhao et al. (2015)
	Pearl River along Guangzhou city and Pearl River estuary, China	Filtered through a 50 µm stainless steel sieve, 30% H <sub>2</sub> O <sub>2</sub> , filtered through 0.45-µm filter paper	Yan et al. (2019)
	Saigon River, Vietnam	Sodium dodecyl Sulfate, enzymatic digestion protocol and ZnCl <sub>2</sub> , H <sub>2</sub> O <sub>2</sub> , filtered through 2.7 µm glass fiber filters	Lahens et al. (2018)
	Hudson River, USA	Vacuum filtered through a 0.45 µm gridded filter paper	Miller et al. (2017)
	Raritan River, New Jersey, USA	Filtered through 4000, 2000, 500, 250, 125, and 63 µm aperture size sieves, NaCl, 30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II)	Estahbanati and Fahrenfeld (2016)
	Elechi creek, Rivers State, Nigeria	Filtered through a stainless steel sieves of 300 µm pore size, NaCl, 30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II)	Briggs et al. (2019)
	Meuse, Rhine, Europe	NaCl, Filtered through a 0.7 µm filter paper	Brandtsma et al. (2013)
	Goose Creek, Little Kickapoo Creek, and East Branch of the DuPage River, USA	Filtered through 4.75 mm and 330 µm stacked sieves, NaCl, 30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II)	McCormick et al. (2016)

Table 2 (continued)

Study Compartment	Study Area	Separation/Digestion method	References
	Rhine, Daiälven, Danube and Po Rivers, Europe	Filtered through 5 mm and 0.16 mm sieves	van der Wal et al. (2015)
	Jade system, South North Sea, Germany	Filtered through a 40 µm steel sieve, 30% H <sub>2</sub> O <sub>2</sub> , 40% HF	Dubaish and Liebezeit (2013)
	Snake River and Palisades Reservoir, USA	Filtered through a 0.45-µm filter	McDevitt and Perez (2016)
	29 Rivers, Japan	Filtered through a 100 µm mesh size, NaCl	Kataoka et al. (2019)
	Snake River and Columbia River, USA	Filtered through a 100 µm stainless steel mesh sieve, NaCl/NaI, 30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II)	Kapp and Yeatman (2018)
	Rhine River, Germany	Filtered through 1000 and 300 µm stainless steel mesh, NaCl	Mani et al. (2019a)
	Gallatin River watershed, USA	Filtered through a gridded 0.45-µm filter	Barrows et al. (2018)
	Changjiang Estuary, China	Filtered through a 60 µm stainless pore size steel sieve, 30% H <sub>2</sub> O <sub>2</sub> , filtered through 0.45-µm filter	Zhao et al. (2019)
	Changjiang Estuary, China	Filtered through a stainless steel sieve with a mesh size of 70 µm, 30% H <sub>2</sub> O <sub>2</sub> , filtered through a 0.45 µm pore size	Xu et al. (2018)
	Pasig River, Philippines	Filtered through a stacked arrangement of 5.6 mm and 0.3 mm mesh sieves, NaCl, 30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II)	Deocaris et al. (2019)
	Danube River, Austria	Filtered through a cascade of sieve, NaCl, 30% H <sub>2</sub> O <sub>2</sub>	Liedermann et al. (2018)
	Muskegon River, Milwaukee River, and St. Joseph River, USA	30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II), filtered through a 0.45 µm pore size	McNeish et al. (2018)
	Clyde, Bega and Hunter estuaries, Australia	Filtered through a 20 µm sieve, NaCl, 30% KOH/NaClO	Hitchcock and Mitrovic (2019)
	Douro estuary, Portugal	Filtered through a 300 µm mesh size, NaCl, 30% H <sub>2</sub> O <sub>2</sub>	Rodrigues et al. (2019)
	Ofanto River, Italy	Filtered through a 300 µm and a 5 mm stainless steel sieve, 30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II), NaCl, filtered through a 1.2-µm glass microfiber filter	Campanale et al. (2020b)
	Yellow River, China	Centrifuged at 3000 r/min for 5 min, NaCl, filtered through stainless steel sieves with mesh sizes of 50, 100, 200 and 500 µm, 30% H <sub>2</sub> O <sub>2</sub> , filtered through a 0.45-µm filter membrane	Han et al. (2020)
	Swiss Rhine River catchment at Brugg and the downstream German–Dutch border at Rees (Germany and Switzerland)	Filtered through 300 µm, 1000 µm and 5000 µm mesh, Oil, filtered through a 300 µm mesh and 25-µm filter paper	Mani and Burkhardt-Holm (2020)
	Urban waters of seven cities in the Tuojiang River basin, China	Filtered through a 5000 µm stainless steel sieve, 30% H <sub>2</sub> O <sub>2</sub> , filtered through 0.45-µm filter paper	Zhou et al. (2020)
	Manas River, China	Ultrasound, NaCl, filtered by 45-µm filter membrane, 30% H <sub>2</sub> O <sub>2</sub> , HCl, ethanol	Wang et al. (2020b)
	Minjiang River watershed, Southeast China	Filtered through a 300 µm stainless steel sieve, NaCl, filtered through 0.7 µm pore size	Huang et al. (2020)



Table 2 (continued)

Study Compartment	Study Area	Separation/Digestion method	References
Lake-Reservoir	Meuse river and in Netherlands and the Dommel, Germany	Filtered through stainless steel sieves with mesh sizes of 300 $\mu\text{m}$ , 100 $\mu\text{m}$ and 20 $\mu\text{m}$ , sodium dodecyl sulfate, KOH, 32% $\text{H}_2\text{O}_2$ , $\text{ZnCl}_2$ , 0.2 $\mu\text{m}$ pore size filters	Minteniig et al. (2020)
	Cherating river and mangrove, Malaysia	Filtered through a set of sieves with mesh size 5000 $\mu\text{m}$ , 1000 $\mu\text{m}$ and 100 $\mu\text{m}$	Pariatamby et al. (2020)
	Yulin River, China	Filtered through a filter paper 0.45 $\mu\text{m}$ pore size, 30% $\text{H}_2\text{O}_2$	Mao et al. (2020b)
	Qing River, Beijing, China	Filtered through a stainless steel screen mesh with a pore size of 5000 $\mu\text{m}$ , passed through stainless steel filter membranes with a pore size of 10 $\mu\text{m}$ , 30% $\text{H}_2\text{O}_2$ + 0.05 M Fe (II), $\text{ZnCl}_2$ , passed through stainless steel filter membranes with a pore size of 10 $\mu\text{m}$	Wang et al. (2020a)
	3 connected urban lakes and drainage playawetlands, Lubbock, Texas, USA	Filtered through a series of sieves > 300 $\mu\text{m}$ , 250–299 $\mu\text{m}$ , 180–249 $\mu\text{m}$ , 106–179 $\mu\text{m}$ , and 53–105 $\mu\text{m}$ , NaCl, 30% $\text{H}_2\text{O}_2$ + 0.05 M Fe (II)	Lasee et al. (2017)
	Lake Hovsgol (mountain remote lake), Mongolia	Filtered through a series of sieves 0.355–0.999 mm, 1.00–4.749 mm, and > 4.75 mm, NaCl, 30% $\text{H}_2\text{O}_2$ + 0.05 M Fe (II)	Free et al. (2014)
	Laurentian Great Lakes (Lakes Superior, Huron and Erie), USA	Salt water, Filtered through 0.355–0.999 mm, 1.00–4.749 mm, > 4.75 mm sieves	Eriksen et al. (2013)
	Lake Winnipeg, Canada	Filtered through a 250 $\mu\text{m}$ mesh brass sieve, 30% $\text{H}_2\text{O}_2$ + 0.05 M Fe (II), filtered through a 250 mm brass sieve	Anderson et al. (2017)
	Dongting Lake and Hong Lake, China	Filtered through a stainless steel sieve with a mesh size of 50 $\mu\text{m}$ , 30% $\text{H}_2\text{O}_2$ , passed through the 0.45- $\mu\text{m}$ filter papers	Wang et al. (2018a)
	Western Lake Superior, USA	Filtered through 4.0 mm and 250 $\mu\text{m}$ metal sieves, 30% $\text{H}_2\text{O}_2$ + 0.05 M Fe (II), NaCl, filtered through 180- $\mu\text{m}$ filters	Hendrickson et al. (2018)
	Lake Michigan, USA	Filtered through 4.75 mm, 1.00 mm and 0.355 mm stainless steel sieves, 30% $\text{H}_2\text{O}_2$ + 0.05 M Fe (II)	Mason et al. (2016)
	Lake Maggiore, Iseo and Garda, Italy	Filtered through < 1 mm, 1 mm < d < 5 mm, > 5 mm sieves, 30% $\text{H}_2\text{O}_2$	Sighicelli et al. (2018)
	Urban Lakes in Changsha, China	30% $\text{H}_2\text{O}_2$ + 0.05 M Fe (II)	Yin et al. (2019)
	Feilaixia Reservoir in the Beijiang River, China	Filtered through mesh sizes of 10, 20, 240 sieves, filtered through 1 $\mu\text{m}$ filters	Tan et al. (2019)
	Lake Ulanstuhai, China	Filtered through a 48 $\mu\text{m}$ stainless steel sieve, 30% $\text{H}_2\text{O}_2$ , filtered by 0.45 $\mu\text{m}$ pore size	Wang et al. (2019)
	Mecklenburg Lake District in Mecklenburg-Western Pomerania, Germany	30% $\text{H}_2\text{O}_2$ , NaClO, filtered by filter paper 413 and 5–13 $\mu\text{m}$	Tammenga et al. (2019)

Table 2 (continued)

Study Compartment	Study Area	Separation/Digestion method	References
Stream	Wuliangshuhai Lake, Northern China	Filtered through a 75 µm sieve, 30% H <sub>2</sub> O <sub>2</sub> , filtered through 0.45-µm filter paper	Mao et al. (2020a)
Stream	Six Mile Creek and Fall Creek streams, USA	Filtered through 4.6 mm and 0.3 mm sieve, NaCl, 30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II), filtered by 0.45-µm filters	Watkins et al. (2019b)
Stream–Lake	Streams and wetlands, Victoria, Australia	Filtered through a 20 µm pore size membrane, NaOH, filtered through 0.45 µm membranes	Nan et al. (2020)
Pond	North of Jutland, Denmark	Filtered through 10 µm and 2000 µm stainless steel mesh, 50% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II), sodium dodecyl sulfate, enzymatic digestion, ZnCl <sub>2</sub> , NaOH, filtered on 10-µm steel filters	Liu et al. (2019)
River–Estuary–Lake	Urban lakes and urban reaches of the Hanjiang River and Yangtze River, Wuhan, China	Passed through a 50 µm stainless steel sieve, 30% H <sub>2</sub> O <sub>2</sub> , filtered through a filter paper with a pore size of 0.45 µm	Wang et al. (2017b)
City creeks–Rivers–Estuary and coastal waters	City creeks (Shanghai), rivers (Suzhou River and Huangpu River), an estuary (Yangtze Estuary) and coastal waters (East China Sea), Yangtze Delta area, China	Filtered through a 20 µm pore size, 10% KOH, filtered through 20 µm pore size	Luo et al. (2019)
River–water–wastewater–total atmospheric fallout	Greater Paris–Seine River, France	Filtered through a 1.6 µm mesh size filter	Dris et al. (2015)
River–atmospheric fallout–urban runoff–WWTP effluents–CSOs	River Marne, France	–	Dris et al. (2018)
Surface water, storm water runoff, agricultural runoff, and treated wastewater effluent	Lake Ontario of the Laurentian Great Lakes in Canada	Filtered through a 10-µm filter, sieved down to 25 and 125 µm pore sieve, CaCl <sub>2</sub> , filtered onto 10-µm filter	Grbić et al. (2020)
Urban prairie creek	Wascana Creek, northern outskirts of Regina, Canada	Filtered through 4750, 500, and 75 µm mesh size stacked series, 30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II), 10% NaClO solution, 30 mL HNO <sub>3</sub> /NaClO, filtered on 5.0-µm membrane filter	Campbell et al. (2017)

Microplastics in these studies were separated from water matrix. Order of the steps may be different in some studies or may be omitted in the others

**Table 3** Microplastic separation and digestion in freshwater media

Study compartment	Study area	Separation/digestion method	References
River–Estuary	Rivers and tidal flat of urban districts, Shanghai, China	NaCl, filtered on 1- $\mu$ m filter paper	Peng et al. (2018)
Littoral sediment	Changjiang Estuary, China	NaCl, 30% H <sub>2</sub> O <sub>2</sub> , filtered on filter paper	Peng et al. (2017)
Benthic sediment	Beijing River littoral zone, China	NaCl, filtered on 1- $\mu$ m filter paper	Wang et al. (2017a)
Littoral sediment	St. Lawrence River, Canada	Sieved through a 500 $\mu$ m mesh sieve	Castañeda et al. (2014)
Benthic sediment	Tamar Estuary, UK	Sieved through 32, 16, 8, 5.6, 4, 2.8 and 2 mm sieves	Browne et al. (2010)
Littoral sediment	Rivers Rhine and Main, Germany	NaCl, 30% H <sub>2</sub> O <sub>2</sub> , 0.5% sodium hexametaphosphate	Klein et al. (2015)
Littoral sediment	Thames River Basin, UK	Sieved with 63, 200, and 630 $\mu$ m mesh size sieves, NaCl, 30% H <sub>2</sub> O <sub>2</sub> + H <sub>2</sub> SO <sub>4</sub>	Horton et al. (2017)
Littoral sediment	Gulf of Mexico estuaries (Mobile Bay, AL), USA	Sieved with mesh size 1–2 mm and 2–4 mm sieves, ZnCl <sub>2</sub> , filtered through 1.2- $\mu$ m filter papers	Wessel et al. (2016)
Littoral sediment	10 rivers, Northwest UK	Sieved through a series of 5 mm, 4 mm, 2 mm, 1 mm, 0.5 mm mesh sizes sieves, mechanical density separator, 200 $\mu$ m capture sieve in the density separator device	Hurley et al. (2018b)
Benthic sediment	Two sandy beaches (Santubong and Trombol) in Kuching, Sarawak, Malaysia	NaCl/NaI, H <sub>2</sub> O <sub>2</sub> , filtered through Whatman 1 filter paper No. 1	Noik and Tuah (2015)
Littoral sediment	Atoyac River Basin, Central Mexico	Sieved through a 1000 $\mu$ m mesh size stainless steel sieve, HCl and HNO <sub>3</sub> , NaCl, filtered using Whatman filter paper No. 1	Shruti et al. (2019)
Benthic sediment	Urban river in Scotland (River Kelvin), UK	30% H <sub>2</sub> O <sub>2</sub> , ZnCl <sub>2</sub> , filtered through a 1.2- $\mu$ m filter paper	Blair et al. (2019)
Littoral sediment	Derwent Estuary, Tasmania, Australia	Sieved into the following size classes: 2.8 mm, 2.0 mm, 1.4 mm, 1.0 mm, 0.71 mm, 0.5 mm, 0.355 mm, 0.25 mm, 0.18 mm, 0.125 mm, 0.09 mm, and 0.063 mm, NaCl, filtered through 11 $\mu$ m cellulose filters	Willis et al. (2017)
Benthic sediment	Rhine River, Germany	Passed through a sieve stack, 30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II), NaI, filtered with a 1.2- $\mu$ m membrane filter	Mani et al. (2019b)
Benthic sediment	Vitória bay estuarine system (SVB), Brazil	Passed through a set of < 2 mm, 2–5 mm, > 5 mm geological sieves, ZnCl <sub>2</sub> , 30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II), filtered through 0.45 $\mu$ m pore size filter paper	Neto et al. (2019)
Benthic sediment	River Tame and four of its tributaries, Birmingham, UK	NaCl, filtered through 0.8 $\mu$ m pore size filter paper	Tibbetts et al. (2018)
Benthic sediment	Brisbane River, Australia	Passed through a set of 63 $\leq$ 250 $\mu$ m, 250 $\leq$ 1000 $\mu$ m, 1 $\leq$ 2 mm, and 2–4 mm sieves, ZnCl <sub>2</sub>	He et al. (2020)
Benthic sediment		ZnCl <sub>2</sub> , filtered through 0.45 $\mu$ m pore size filter paper	

Table 3 (continued)

Study compartment	Study area	Separation/digestion method	References
	Liaohu estuary, Daliao River and Shuangtaizi River Benthic sediment	ZnCl <sub>2</sub> , 30% H <sub>2</sub> O <sub>2</sub> , filtered through 5 µm pore size stainless steel filter	Xu et al. (2020)
	Thames River, Ontario, Canada Benthic sediment	Sieved using stainless steel mesh sizes of 5.6, 2.0, and 0.063 mm mesh sizes, sodium polytungstate, transferred into a 53 µm sieve	Corcoran et al. (2019)
	Warnow estuarine, Germany Benthic sediment	Sodium polytungstate, 37% HCl, passed through a stainless steel sieve with apertures of 500 µm (density separated using a Microplastic Sediment Separator), an array of treatments with sodium dodecyl sulfate, enzymes, NaOH, HCl or a repeated density separation	Enders et al. (2019)
	River Yongfeng, China Benthic sediment	NaCl, filtrating using 30 µm sieve, 30% H <sub>2</sub> O <sub>2</sub> and 65% HNO <sub>3</sub>	Rao et al. (2020)
	Jagir Estuary, Surabaya City, Indonesia Benthic sediment	Disaggregation by sodium hexametaphosphate, sieved using 5.0-mm and 0.3-mm filters, 30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II), NaCl, filtered through 0.3 mm pore size filter	Firdaus et al. (2020)
Stream	Seven water streams surrounding the lagoon of Bizerte, Northern Tunisia Littoral sediment	NaCl, filtered onto 7–10 µm pore size filters	Toumi et al. (2019)
Lake, Reservoir	Subalpine Lake Garda, Italy Littoral sediment	ZnCl <sub>2</sub> , filtered through 0.3 µm pore size filters, 30% H <sub>2</sub> O <sub>2</sub> and 95% H <sub>2</sub> SO <sub>4</sub> –Munich Plastic Sediment Separator	Imhof et al. (2013)
	Lake Ontario, Canada Littoral sediment and Benthic sediment	Sieved through 5.6 mm, 2.0 mm, and 0.063 mm mesh size sieves, sodium polytungstate, filtered through 0.053 µm sieve or 25-µm filter paper	Ballent et al. (2016)
	Lake Ontario, Canada Littoral sediment and Benthic sediment	Sieved through 0.5 mm, 0.5–0.71 mm, 0.71–0.85 mm, 0.85–1 mm, and > 1 mm grain size fraction, sodium polytungstate	Corcoran et al. (2015)
	Remote lakes in Tibet plateau, China Littoral sediment	Sieved with a 1 mm mesh size sieve, potassium formate, filtrated onto 1.2-mm filter	Zhang et al. (2016)
	Beaches of Lake Huron, Canada Littoral sediment	Sonicated using ultrasonic bath to remove sand	Zbyszewski and Corcoran (2011)
	Great Lakes, North America (Lake Erie and St. Clair), USA Littoral sediment	Sonicated using ultrasonic bath to remove sand	Zbyszewski et al. (2014)
	Edgbaston Pool, Birmingham, UK Littoral sediment and Benthic sediment	Sieved through 1 mm and 500 mm mesh size sieves, density separated using water	Vaughan et al. (2017)
	Setúbal Lake, Portugal Littoral sediment	Sieved through a 350 µm mesh size stainless steel sieve, NaCl, 30% H <sub>2</sub> O <sub>2</sub>	Blettler et al. (2017)
	Beaches of Lake Garda, Italy Littoral sediment	ZnCl <sub>2</sub> , 30% H <sub>2</sub> O <sub>2</sub> and H <sub>2</sub> SO <sub>4</sub> , sieved using a 750 µm stainless steel sieve, filtered through 0.2 or 2.2-µm filter paper–Munich Plastic Sediment Separator	Imhof et al. (2018)
	Lake Erie, Canada Littoral sediment and Benthic sediment	Sieved through a < 63 µm mesh size sieve, sodium polytungstate, 30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II), filtered through 25-µm filter paper	Dean et al. (2018)

Table 3 (continued)

Study compartment	Study area	Separation/digestion method	References
Subalpine Lake Garda, Italy	Littoral sediment	ZnCl <sub>2</sub> , 30% H <sub>2</sub> O <sub>2</sub> and H <sub>2</sub> SO <sub>4</sub> , sieved using a 750 µm stainless steel sieve, filtered through 0.2- or 2.2-µm filter paper–Munich Plastic Sediment Separator	Imhof et al. (2016)
		Sieved through a 5 mm mesh size sieve, 30% H <sub>2</sub> O <sub>2</sub> , potassium formate, filtered through 10 µm pore size membranes, 30% H <sub>2</sub> O <sub>2</sub> , filtered through 1 µm pore size membranes	Zhang et al. (2019)
Hampstead Pond (Lake), UK	Benthic sediment	Sieved through 1 mm and 500 µm stainless steel sieves, sodium polytungstate, passed through 0.45 µm pore filter paper	Turner et al. (2019)
		NaI, sieved through 0.36 µm and 0.75 µm pore size filter paper, passed through 2.7 µm pore size filter paper, 30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II), 10% KOH (for plankton samples)	Lusher et al. (2018)
Lake Mjøsa and Lake Femunden, Norway	Benthic sediment	Disaggregation by sodium hexametaphosphate, sieved using 5 and 0.3 mm sieve, NaCl, 30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II), passed through 0.45 µm pore filter paper	Egessa et al. (2020)
		Corn oil, passed through 5 µm pore filter paper, ethanol	Dong et al. (2020)
Lake Victoria, Uganda, Africa	Littoral sediment and Benthic sediment	NaI, passed through 0.1 µm sieve, 10% KOH (for fish samples)	Merga et al. (2020)
		Passed through a 1 mm stainless steel sieve, ZnCl <sub>2</sub> , 30% H <sub>2</sub> O <sub>2</sub> , filtered through filter paper	Yin et al. (2020)
River–Estuary–Lake	Vembanad Lake, Kerala, India	Sieved through 5 mm mesh size sieve, 30% H <sub>2</sub> O <sub>2</sub> , NaCl, filtered using filter paper	Sruthy and Ramasamy (2017)
		30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (III), ZnCl <sub>2</sub> , filtered using filter paper	Wen et al. (2018)
Coastal plain river network (Wen-Rui Tang River watershed) in Eastern China	Benthic sediment	Sieved through a 50 µm mesh sieve, 30% H <sub>2</sub> O <sub>2</sub> , passed through the 0.45-µm filter paper	Wang et al. (2018b)
		30% H <sub>2</sub> O <sub>2</sub> , filtered through a 53 µm stainless steel sieve, NaCl, passed through a 1.2 µm pore size filter	Sarijan et al. (2018)
Lagoon	Lagoon of Venice, Italy	Iodate salt, passed through the 0.45-µm filter paper	Blasković et al. (2018)
		NaCl, filtered on a 32 µm steel sieve, filtered on a 0.7-µm filter	Vianello et al. (2013)
Complex Lagoon–Channel of Bizerte, Northern Tunisia	Littoral sediment	NaCl, passed through filter with particle retention 7–10 µm	Abidli et al. (2017)

Microplastics in these studies were separated from sediment matrix. Order of the steps may be different in some studies or may be omitted in the others

**Table 4** Microplastic separation and digestion in freshwater media

Study compartment	Study area	Separation/digestion method	References
River–Estuary	Three Gorges Reservoir, China	Filtered with a 48 µm stainless steel sieve, 30% H <sub>2</sub> O <sub>2</sub> , filtered through a 0.45-µm filter paper (water samples) NaCl, poured through a 48 µm stainless steel sieve, NaI, 30% H <sub>2</sub> O <sub>2</sub> , filtered through a 0.45-µm filter paper (sediment samples)	Di and Wang (2018)
	Five urban estuaries of KwaZulu–Natal, South Africa	Filtered through 1000, 500, and 250 µm sieves (water samples), NaCl, passed through 1000-, 500-, 250-, 100- and 20-µm filters (sediment samples)	Naidoo et al. (2015)
	Pearl River along Guangzhou City, China	Filtered through a stainless steel sieve with mesh size of 20 µm, 30% H <sub>2</sub> O <sub>2</sub> , NaCl, filtered through a 5 µm filter sample (water samples), NaCl, filtered through a 20-µm filter, KOH, NaCl, filtered over a clean 5-µm membrane filter (sediment samples)	Lin et al. (2018)
	Antuá River, Portugal	Filtered through a stacked series of metal sieves 5 and 0.055 mm, 30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II), ZnCl <sub>2</sub> , passed through 0.45-µm filter (water samples), Disaggregation by sodium polyphosphate, filtered through a stacked series of metal sieves 5 and 0.055 mm ZnCl <sub>2</sub> , 30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II), passed through 0.45-µm filter (sediment samples)	Rodrigues et al. (2018)
	Ottawa River, Canada	Passed through a 30 µm mesh filter, 30% H <sub>2</sub> O <sub>2</sub> , filtered a second time through a clean 100 µm (water samples), Disaggregation by sodium hexametaphosphate, passed through a 300 µm sieve, 30% H <sub>2</sub> O <sub>2</sub> , sieved through a 100 µm sieve, NaCl, passed through a 100 µm sieve (sediment samples)	Vermaire et al. (2017)
	Charleston Harbor and Winyah Bay, two developed estuaries in US	Passed through a stainless steel sieve 2 mm mesh size, poured through a series of 500, 150, and 63 µm nested sieves, 30% H <sub>2</sub> O <sub>2</sub> , passed through a 38 µm sieve (water samples), NaCl, poured through a series of 500, 150, and 63 µm nested sieves, 30% H <sub>2</sub> O <sub>2</sub> , passed through a 38 µm sieve (sediment samples)	Gray et al. (2018)
	Slum and industrial area of Ciwalengke River, Majalaya, Indonesia	Filtered through 1.2-µm filter paper (water samples), NaCl, filtered through 1.2-µm filter paper (sediment samples)	Alam et al. (2019)
	Pearl River catchment, China	Filtered through a 5 mm stainless steel sieve, 30% H <sub>2</sub> O <sub>2</sub> , filtrated through 0.7-µm glass microfibre filters (water samples), Sieved through 1 mm, 0.45 mm and 0.1 mm mesh sieves KF, filtered through 8-µm membrane filters (sediment samples)	Fan et al. (2019)
	Wei River, China	30% H <sub>2</sub> O <sub>2</sub> , NaCl, passed through 0.45-µm filter papers (water samples), NaCl, 30% H <sub>2</sub> O <sub>2</sub> , passed through 0.45-µm filter papers (sediment samples)	Ding et al. (2019)

Table 4 (continued)

Study compartment	Study area	Separation/digestion method	References
	Tibet Plateau Rivers, China (Buqu River (the source of the Yangtze	30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II), ZnCl <sub>2</sub> , filtered through a 0.22 µm pore size (water samples), passed through a 2 mm stainless steel sieve, ZnCl <sub>2</sub> , filtered through a 0.22 µm pore size (sediment samples)	Jiang et al. (2019)
	Middle and lower reaches of the Yangtze River, China	Sieved through a set of two nested sieves 2 mm and 100 µm, NaCl, 30% H <sub>2</sub> O <sub>2</sub> , filtered onto a 1.2-µm filter (water samples), ZnCl <sub>2</sub> , filtered onto a 1.2-µm filter (sediment samples)	Xiong et al. (2019)
	Nakdong River, South Korea	Passed through a 20 µm sieve, 35% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II), passed through a 20 µm sieve, lithium metatungstate, filtered onto 5-µm filter paper (water samples), lithium metatungstate, passed through a 20 µm metal sieve, 35% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II), filtered onto 5-µm filter paper (sediment samples)	Eo et al. (2019)
	Mohawk River, USA	NaCl, 30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II)	Smith et al. (2017)
	Ebro River Delta, Northeastern Iberian Peninsula, Spain	Passed through a 0.7 µm pore size filter paper (water samples), NaCl, passed through 0.7 µm pore size filter paper (beach sediment samples), Wet sieve (> 2 mm, > 63 µm and < 63 µm), NaCl, 30% H <sub>2</sub> O <sub>2</sub> , passed through 0.7 µm pore size filter paper (benthic sediment samples)	Simon-Sánchez et al. (2019)
	Yongjiang River, Nanning City, South China	30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II), passed through a 0.45 µm pore size filter paper (water samples), NaCl, passed through a 0.45 µm pore size filter paper, NaI, 30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II), passed through a 0.45 µm pore size filter paper (sediment samples)	Zhang et al. (2020)
	Maozhou River, China	30% H <sub>2</sub> O <sub>2</sub> , passed through a 0.45 µm pore size filter paper (water samples), 30% H <sub>2</sub> O <sub>2</sub> , ZnCl <sub>2</sub> , passed through a 0.45 µm pore size filter paper (sediment samples)	Wu et al. (2020)
	Chao Phraya River, Bangkok, Thailand	NaI, sieved through stainless steel sieves with mesh sizes of 5.0, 1.0, 0.5, and 0.05 mm, 30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II) (water samples), NaI, sieved through stainless steel sieves with mesh sizes of 5.0, 1.0, 0.5, and 0.05 mm, 30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II) (sediment samples)	Ta et al. (2020)
	Ravi River in urban center (predominant drains and canals of Lahore district), Lahore, Pakistan	Passed through filtration assembly prepared by joining PVC funnels and 5 mm and 150 µm mesh, 35% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II) (water samples), Passed through 5 mm and 150 µm mesh stainless steel sieve, 35% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II), filtered using a 50 µm steel sieve, NaCl in Sediment Microplastic Isolation (SMI) unit, filtered through 50 µm steel mesh, filtered through a series of 300, 150, and 50 µm stainless steel sieves (sediment samples)	Irfan et al. (2020)

Table 4 (continued)

Study compartment	Study area	Separation/digestion method	References
Lake-Reservoir	Magdalena River, Colombia	Sieved with a 2 mm metallic mesh (sediment samples), NaCl, filtered through a 0.45 µm pore size (water and sediment samples)	Martínez Silva and Nanny (2020)
	Lake Bolsena and Lake Chiusi, Italy	Passed through sieves with 1, 0.5 and 0.3 mm mesh size, NaCl (water samples), Passed through a 5 mm mesh size sieve, NaCl, HCl + Hot digestion, membrane filtration particle retention	Fischer et al. (2016)
	Dongting Lake, China	5–13 µm (sediment samples) 30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II), ZnCl <sub>2</sub> , passed through a 0.22 µm pore size (water samples), Passed through a 2 mm stainless steel sieve, ZnCl <sub>2</sub> , 30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II), passed through a 0.22 µm pore size (sediment samples)	Jiang et al. (2018)
	Six dams near Ithaca, USA	Sieved through a #4 sieve and onto 0.335 mm mesh, 30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II), NaCl, passed through 0.45 µm pore size (water samples), Sieved through a #4 sieve and onto 0.335 mm mesh, lithium metatungstate, 0% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II), NaCl, passed through 0.45 µm pore size (sediment samples)	Watkins et al. (2019a)
	Danjiangkou Reservoir, China	30% H <sub>2</sub> O <sub>2</sub> , filtered through a 0.45-µm glass microfiber filter paper (water samples), NaCl/NaI, 30% H <sub>2</sub> O <sub>2</sub> , filtered through a 0.45-µm glass microfiber filter paper (sediment samples)	Di et al. (2019)
	Lakes along the middle and lower reaches of Yangtze River Basin, China	30% H <sub>2</sub> O <sub>2</sub> , filtered with a 0.45-mm glass microfiber filter paper (water samples), NaCl, 30% H <sub>2</sub> O <sub>2</sub> , filtered with a 0.45-mm glass microfiber filter paper (sediment samples)	Li et al. (2019)
Stream	18 streams in and around the city of Auckland, New Zealand	Filtered through a 63 µm mesh sieve, 30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II), NaCl, filtered over a 1.2-µm glass filter (water samples), Sieved through a 5 mm sieve, elutriation step with 63 µm mesh, filtered onto a 1.2-µm glass filter (sediment samples)	Dikareva and Simon (2019)
Fish ponds	Central and Eastern European region	NaCl, 30% H <sub>2</sub> O <sub>2</sub> , filtered through 0.2-µm filter paper–Munich Plastic Sediment Separator (water and sediment samples)	Bordós et al. (2019)
River–Estuary–Lake–WWTPs	River Barrow, River Nore, Lough Lurgan (Cushina, Co. Offaly) and River Liffey (Newbridge, Co. Kildare), Ireland	30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II), NaCl (water and sediment samples)	Cedro and Cleary (2015)

Microplastics in these studies were separated from water and sediment matrix. Order of the steps may be different in some studies or may be omitted in the others



**Table 5** Microplastic separation and digestion in freshwater media

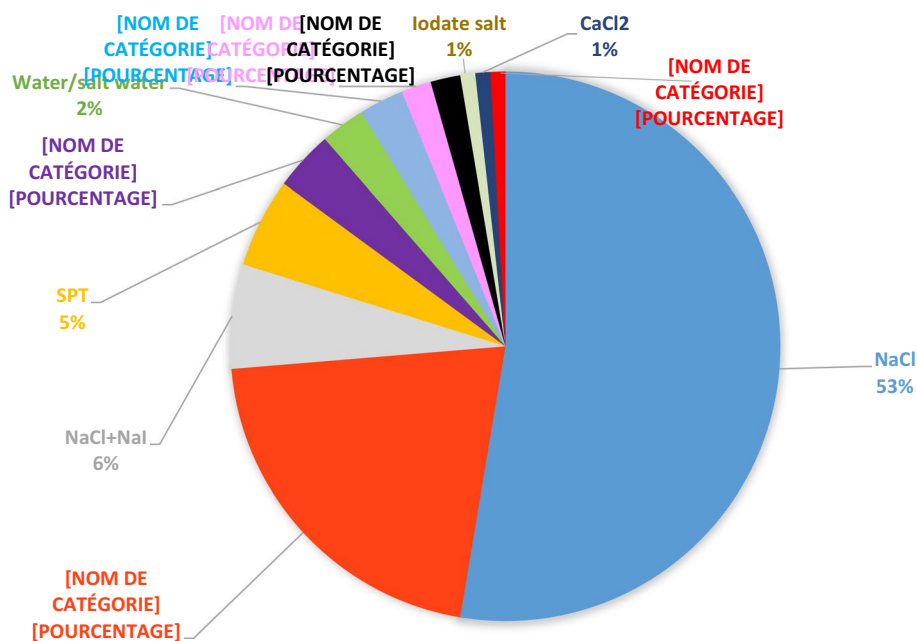
Water compartment	Study area	Separation/digestion method	References
River–Estuary	Xiangxi Bay of Three Gorges Reservoir, China	Passed through a 1 mm mesh size stainless steel sieve, potassium formate, filtered onto 1.2 µm pore size filter, (water samples), Passed through a 1 mm mesh size stainless steel sieve, potassium formate, filtered onto 1.2 µm pore size filter (sediment samples), 10% KOH (biota samples—planktonic samples)	Zhang et al. (2017)
Lake	Qinghai Lake, China	Passed through a 1 mm mesh size stainless steel sieve, potassium formate, 30% H <sub>2</sub> O <sub>2</sub> , filtered onto 1.2 µm pore size filter (water samples), Passed through a 2 mm mesh size stainless steel sieve, potassium formate, filtered onto 1.2 µm pore size filter, 30% H <sub>2</sub> O <sub>2</sub> (sediment samples), 10% KOH, filtered onto 1.2 µm pore size filter (biota samples—fish samples: <i>Gymnocypris przewalskii</i> )	Xiong et al. (2018)
	Taihu Lake, China	Filtered through 100 µm (for plankton net samples) and 5 µm (for surface water samples), 30% H <sub>2</sub> O <sub>2</sub> , filtered again (water samples), NaCl, filtered with a 5 µm pore size, 30% H <sub>2</sub> O <sub>2</sub> , filtered again (sediment samples), 30% H <sub>2</sub> O <sub>2</sub> , NaCl, filter with a 5 µm pore size (biota samples—Asian clam: <i>Corbicula fluminea</i> )	Su et al. (2016)
	Lake Geneva, Switzerland	Sieved through a 5 mm sieve (water samples), sieved with 5 mm and 2 mm sieves, floating using water (sediment samples—first method), collecting coarse plastic fragments on the beaches directly (sediment samples—second method), intestines were collected, dried and observed with a stereomicroscope (biota samples—fish and birds: northern pikes ( <i>Esox lucius</i> ), common roaches ( <i>Rutilus rutilus</i> ), common breams ( <i>Abramis brama</i> ) and black-necked Grebe ( <i>Podiceps nigricollis</i> )	Faure et al. (2012)
	Poyang Lake, China	30% H <sub>2</sub> O <sub>2</sub> , filtered using a 0.45-µm filter paper (water samples), NaCl, filtered with a 50 µm stainless steel sieve, NaI, 30% H <sub>2</sub> O <sub>2</sub> , filtered using a 0.45-µm filter paper (sediment samples), 10% KOH, 30% H <sub>2</sub> O <sub>2</sub> , filtered using a 0.45-µm gridded filter paper (biota samples—wild fish: <i>C. auratus</i> )	Yuan et al. (2019)

**Table 5** (continued)

Water compartment	Study area	Separation/digestion method	References
River–Estuary–Lake	Six of the largest Swiss lakes and some rivers-Switzerland	NaCl, ZnCl <sub>2</sub> , 30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II), passed through a 300 mm sieve (water samples), Gravity separation, NaCl, ZnCl <sub>2</sub> , 30% H <sub>2</sub> O <sub>2</sub> + 0.05 M Fe (II), passed through a 300 mm sieve a (sediment samples), Gut content rinsed, sieved and observed through microscope (biota samples—fish samples: <i>Alburnus alburnus</i> , <i>European Perca Fluviatilis</i> , <i>Rutilus rutilus</i> , <i>Leuciscus leuciscus</i> , bird samples: <i>Ardea cinerea</i> , <i>Ygnus olor</i> <i>Anas</i> , <i>platyrhynchos</i> )	Faure et al. (2015)
	Middle-Lower Yangtze River Basin, China	Filtered onto net 20 μm pore size, 30% H <sub>2</sub> O <sub>2</sub> , filtered again (water samples), NaCl, filtered onto nylon net 20 μm pore size, 30% H <sub>2</sub> O <sub>2</sub> , filtered again (sediment samples), H <sub>2</sub> O <sub>2</sub> filtered onto nylon net 20 μm pore size (biota samples—Asian clams: <i>Corbicula fluminea</i> )	Su et al. (2018)
Pond	Storm water pond, Viborg, Denmark	Filtered on several 10-μm filters, sodium dodecyl sulfate solution, enzymatic digestion, 50% H <sub>2</sub> O <sub>2</sub> + 0.1 M Fe (II), sieved through below 80 μm mesh size (water samples), sieved through a 2 mm pore size sieve by sodium dodecyl sulfate solution, filtered onto a stainless steel filter with a mesh size of 10 μm, 50% H <sub>2</sub> O <sub>2</sub> , filtered onto several 10-μm filters, ZnCl <sub>2</sub> , filtered on a 0.8-μm filter (sediment samples), KOH, filtered onto a 10-μm filter (biota samples—three-spined sticklebacks and newts: <i>Gasterosteus aculeatus</i> and <i>Triturus vulgaris</i> )	Olesen et al. (2019)
Small water bodies	Yangtze River Delta, China	Filtration with a 20 μm pore size filter paper, 30% H <sub>2</sub> O <sub>2</sub> , filtered again using a filter with a pore size of 5 μm (water samples), NaCl, 30% H <sub>2</sub> O <sub>2</sub> (sediment samples), digested using 30% H <sub>2</sub> O <sub>2</sub> , filtered through 5 μm a pore size filter paper (biota samples—tadpoles: <i>Microhyla ornata</i> , <i>Rana limnochari</i> , <i>Pelophylax nigromaculatus</i> , <i>Bufo gargarizans</i> )	Hu et al. (2018)
River, Canal, WWTPs, Sea	Dutch river delta and Amsterdam canals, wastewater treatment plants, North Sea sediments and biota, Netherland and Germany	NaCl, passed through 0.7-μm glass filters (water and wastewater samples), NaCl, passed through 0.7 μm glass filters (suspended particulate matter, sludge, sediment samples), nitric acid and microwave destruction step, neutralized with a sodium hydroxide (NaOH) solution, 30% H <sub>2</sub> O <sub>2</sub> , passed through 0.7-μm glass filters (biota samples—five benthic species: common shore crab ( <i>Carcinusmaenas</i> ), sand hopper ( <i>Gammarus spp.</i> ), periwinkle ( <i>Littorina littorea</i> ), blue mussel ( <i>Mytilus edulis</i> ) and Pacific oyster ( <i>Crassostrea gigas</i> )	Leslie et al. (2017)

Microplastics in these studies were separated from water, sediment and biota matrix. Order of the steps may be different in some studies or may be omitted in the others

**Fig. 2** Frequency of chemicals used for microplastics separation in freshwater studies



**Table 6** Advantages and disadvantages of some commonly used salts for microplastic isolation using density separation method

Separation salt	Advantages	Disadvantages
NaCl	Ease in access Cheap price Less potential for negative environmental impacts	Low efficiency to isolate microplastics with higher density (e.g., polyvinylchloride or polyethylene terephthalate) Possibility of crystal production which may interfere with the detection of particles under the microscope
ZnCl <sub>2</sub>	High efficacy to separate both low- and high-density microplastic particles Appropriate price	Negative environmental impacts More expensive compared to NaCl Possibility to extract interfering particles from sample matrix Possibility of white deposit or crystal production which may interfere with the detection of particles under the microscope Reaction with aluminum covers (which is used to prevent the entry of atmospheric microplastics)
NaI	High efficacy to separate both low- and high-density microplastic particles	Negative environmental impacts More expensive compared to NaCl and ZnCl <sub>2</sub> Possibility to extract interfering particles from sample matrix Reaction with digestion step chemicals and producing blackish-brown color if not washed between laboratory steps

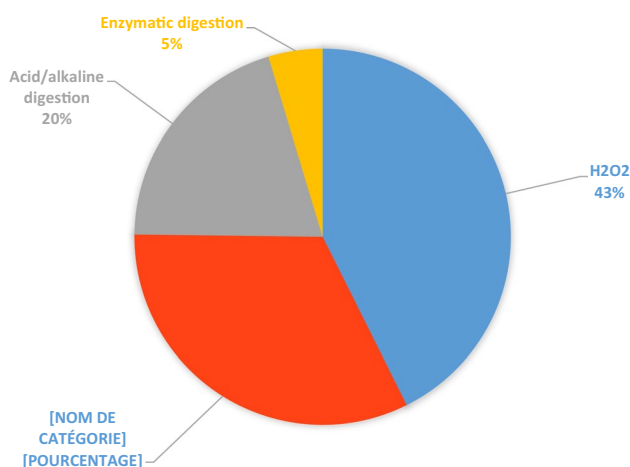
cost and more interfering substances in the sediments that might be extracted as well (Wang et al. 2017a). Zinc chloride has offered adequate density for separation of most polymer types and aids in effective extraction of microplastics. Also, lower cost of ZnCl<sub>2</sub> makes it suitable for large-volume samples compared to other high-density separators, such as sodium polytungstate and sodium iodide (Shruti et al. 2019). Advantages and disadvantages of three salts with the highest frequency of use in density separation solution in freshwater studies are listed in Table 6.

The use of oil, CaCl<sub>2</sub>, and KF has been recently reported. Although liquids with higher density help to better recover microplastic particles, they may be expensive and toxic to environment. Some researchers have used methods, such

as elutriation and froth flotation to reduce sample size and consequently, reducing costs (Crawford and Quinn 2016).

Hydrogen peroxide and Fenton's reagent are the most frequently used chemicals in digestion step and more than 73% of the freshwater studies have reported the use of these chemicals (Fig. 3). Some researchers have reported that this method may potentially digest some polymers (Anderson et al. 2017), while other researchers have addressed the lack or minimal effect of it on micro-sized plastic particles (Hurley et al. 2018a; Tagg et al. 2017).

Acid–base digestion using different chemical compounds like H<sub>2</sub>SO<sub>4</sub>, HNO<sub>3</sub>, H<sub>2</sub>SO<sub>5</sub>, KOH, NaClO, and NaOH has been also reported. Acid digestion can degrade some polymers. For example, polystyrene (PS) particles



**Fig. 3** Frequency of digestion methods used in freshwater studies

are pH-sensitive polymers (Erni-Cassola et al. 2017). Base digestion seems to have less effect in altering microplastics, but it may be more time-consuming. Advantages and disadvantages of different digestion methods/materials in microplastic studies are discussed in Table 7.

Generally, the smaller the size (particularly particles lower than 1 mm) of plastic particle, the more difficult it is to separate them from sample matrix. It seems that the current experimental methods are still insufficient to deal with this fact. It is often challenging to quantify microplastics in complex matrices, such as fine-grained organic-rich samples and biological tissues. Currently employed density and size separation techniques to isolate plastic particles from aquatic environmental samples are not well suited and information cannot easily be compared. Quality control procedures, including blanks and spike recovery should be employed and related results should be reported

in experimental studies. Another important issue is the use of high amounts of salts to achieve appropriate density in the density separation step. Therefore, new methods should move toward less chemical use and reusing of solutions. The current methods need to be optimized to increase efficiency, to reduce contamination potential, and to avoid color and structure changes in plastic particles during sample processing.

## Conclusion

Size selection, digestion, density separation, and filtration are the main steps in microplastic separation from environmental samples. Among the papers with clearly determined experimental methods, sodium chloride was the most commonly used salt in separating microplastics in freshwater studies. Though, it seems that, sodium chloride solution might not fully isolate high-density microplastic polymers and may lead to false negative errors. Hydrogen peroxide and Fenton's reagent were the most prevalent chemicals used in digestion step. The preliminary results call for more research efforts to better characterize the microplastics in inland waters. Continuous research is needed to develop efficient methods for separating particles from different substrates. For this purpose, it is necessary to develop standard protocols for better comparison of data and results at different times and places. New methodologies are still emerging and their ability to separate out a wide range of micro-sized polymers with appropriate shapes and sizes found in environment needs to be investigated. Exploring new techniques like centrifugation and anticoagulant use along with common methods is suggested for achieving better results.

**Table 7** Advantages and disadvantages of different digestion methods/materials in microplastic studies

Digestion methods/chemicals	Advantages	Disadvantages
Hydrogen peroxide	Ease in access Inexpensive	Unable to fully digest all types of biogenic material (e.g., lignin) Time consuming Loss of its properties after a while (especially when exposed to air)
Fenton's reagent	Higher digestion speed due to catalyst presence	Reaction with digestion step chemicals and producing blackish-brown color if not washed between laboratory steps
Acid-alkaline digestion	Higher digestion speed	Possibility to alter microplastics shape or color Loss of particle fluorescence
Enzymatic digestion	Effective performance in digestion	Expensive Selective performance

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## Declarations

**Conflict of interest** The authors have no conflict of interests to disclose.

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