A review of microplastics aggregation in aquatic environment: Influence factors, analytical methods, and environmental implications

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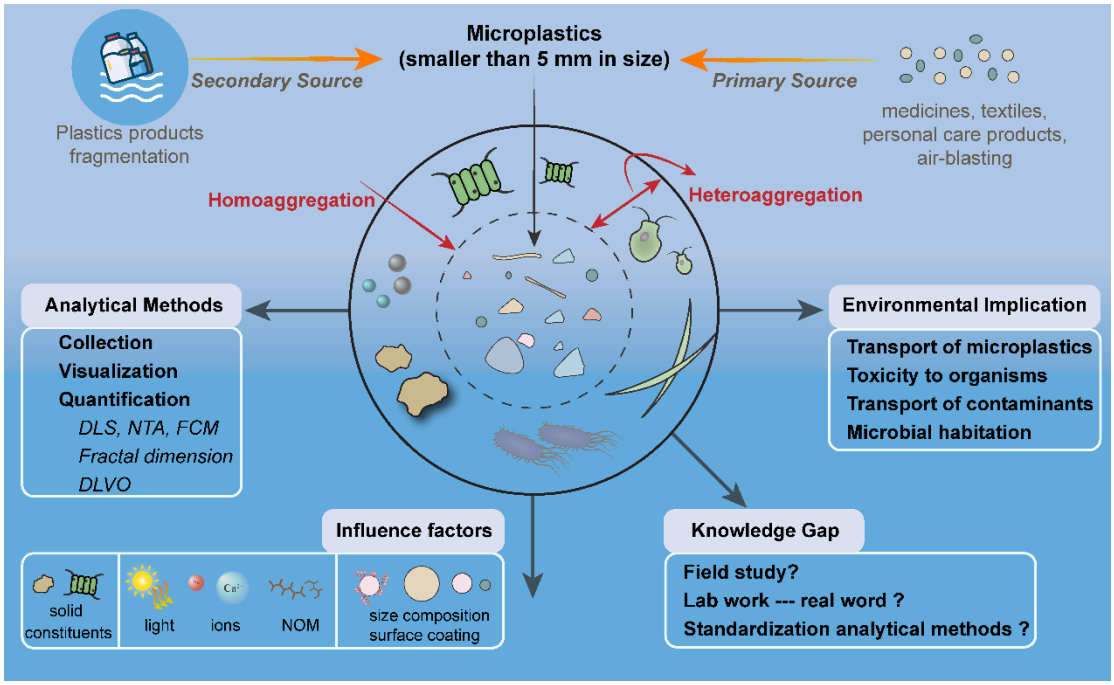
Highlights

- The aggregation of MPs in real aquatic environment is far from fully understood.
- Sampling and analyzing methods for studying MP aggregation were summarized.
- Influence factors and environmental implications of MP aggregation were reviewed.
- MP aggregation in the field and their temporal stability deserve extensive research.
- Laboratory studies should use MPs representing those in real aquatic environment.

Graphical abstract



Abstract: A large amount of plastic waste released into natural waters and their demonstrated toxicity have made the transformation of microplastics (MPs; < 5 mm) and nanoplastics (NPs; < 100 nm) an emerging environmental concern. Aggregation is one of the most important environmental behaviors of MPs, especially in aquatic environments, which determines the mobility, distribution and bioavailability of MPs. In this paper, the sources and inputs of MPs in aquatic environments were first summarized followed by the analytical methods for investigating MP aggregation, including the sampling, visualization, and quantification procedures of MP' particle sizes. We critically evaluated the sampling methods that still remains a methodological gap. Identification and quantification of MPs were mostly carried out by visual, spectroscopic and spectrometric techniques, and modeling analysis. Important factors affecting MP aggregation in natural waters and environmental implications of the aggregation process were also reviewed. Finally, recommendations for future research were discussed, including (1) conducting more field studies; (2) using MPs in laboratory works representing those in the environment; and (3) standardizing methods of identification and quantification. The review gives a comprehensive overview of current knowledge for MP aggregation in natural waters, identifies knowledge gaps, and provides suggestions for future research.

Keywords: Microbead, Stability, Detection methods, Microbial habitation, Contaminant vector

Abbreviations

nanoplastics (NPs)

microplastics (MPs)

polystyrene (PS)

polyethylene (PE)

polypropylene (PP)

polyvinyl chloride (PVC)

poly(methyl methacrylate) (PMMA)

amino-modified PS NPs (PSNPs-NH_2)

carboxyl-modified PS NPs (PSNPs-COOH)

ultraviolet (UV)

humic acid (HA)

laser diffraction (LD)

fractal dimensions (D_f)

flow cytometry (FCM)

attachment efficiency (α)

hydrodynamic diameter (D_h)

exopolymeric substances (EPS)

field flow fractionation (FFF)

dynamic light scattering (DLS)

atomic force microscopy (AFM)

dissolved organic matter (DOM)

extended DLVO-theory (eDLVO)

nanoparticle tracking analysis (NTA)

scanning electron microscopy (SEM)

energy dispersive spectroscopy (EDS)

wastewater treatment plants (WWTPs)

hydrodynamic chromatography (HDC)

transmission electron microscopy (TEM)

critical coagulation concentration (CCC)

Derjaguin-Landau-Verwey-Overbeek (DLVO)

Fourier-transform infrared spectroscopy (FTIR)

asymmetrical flow field-flow fractionation (AF4)

1. Introduction

Microplastics (MPs) and nanoplastics (NPs) are defined as plastic particles with diameters < 5 mm and < 100 nm, respectively (Feng et al., 2020). Scientists agree that the pollution of MPs in marine and freshwater environments have potential adverse impacts on aquatic organisms, marine ecosystems and even human health (Castelvetro et al., 2020; Wang and Wang, 2018). Regulators and researchers currently assess the environment risk of MPs relying upon their ecotoxicity tests and environmental fate modeling (Karbalaei et al., 2018). Aggregation of MPs is an important physical-chemical process dominating the transport behavior and overall fate of MPs in aquatic environments (Alimi et al., 2018). Recent investigations on MP aggregation have focused on simulated natural waters in laboratory, while the process in complex heterogeneous media or in natural waters is largely unknown (Singh et al., 2019). Adequate experimental protocols and environmental fate models are needed to develop for theoretically understanding and quantifying the aggregation process of the MP pollutants (Praetorius et al., 2020).

Aggregation involves the transport of two particles toward each other to collide, followed by attachment (Zhang, 2014). This can occur between the same type of MPs (homoaggregation) or different types of particles (heteroaggregation) (Alimi et al., 2018). Compared with the homoaggregation of MPs, the heteroaggregation of MPs with other solid constituents such as natural minerals and seaweeds is more prone to occur in natural waters driving the floating, sedimentation, and resuspension processes of MPs (Long et al.,

2017; Oriekhova and Stoll, 2018; Singh et al., 2019). The organism-associated aggregation of MPs/NPs have been widely reported, as shown in **Fig.1** a-e. Plastic surfaces commonly contain hydrophobic functional groups that facilitate the adsorption of dissolved organic matter (DOM) and organisms, especially algae and bacteria (Zhang et al., 2020). Microbial habitation and biofilm formation are common on MP surface in natural waters (Harrison et al., 2018). Apart from organism-associated MP aggregates, organic matter (e.g., alginate) (**Fig. 1** f), layered clay minerals (e.g., suspended sediment (**Fig. 1** g), Fe_2O_3), and nanoparticles also could aggregate with MPs (Li et al., 2020). Because of the inherently higher system complexity, studies on heteroaggregation of MPs are still scarce (Oriekhova and Stoll, 2018).

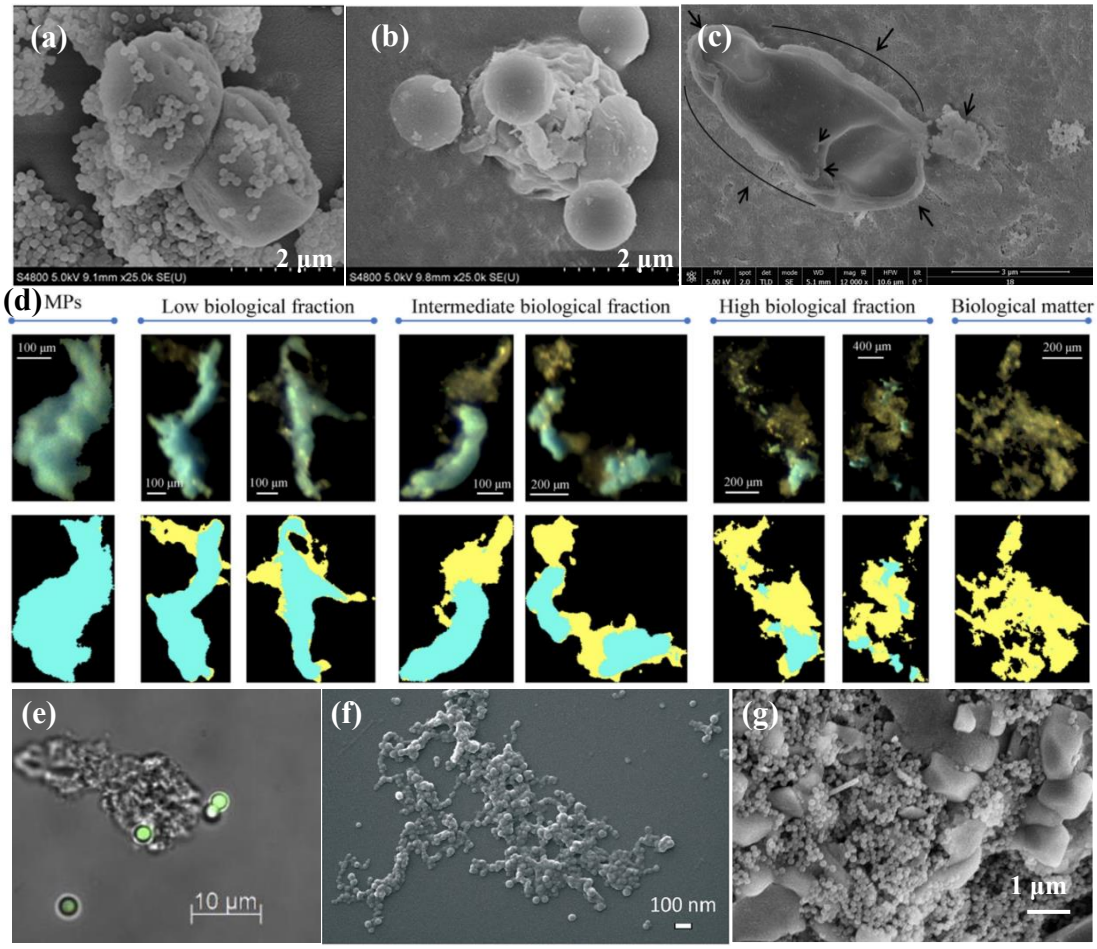


Fig. 1. Major types of aggregates formed by microplastics (MPs)/nanoplastics (NPs). (a) 0.1 mm polystyrene MPs (PS MPs) aggregate with *Chlorella pyrenoidosa* (Mao et al., 2018); (b) 1.0 mm PS MPs aggregate with *Chlorella pyrenoidosa* (Mao et al., 2018); (c) 100 nm PS NPs aggregate with marine microalgae *Phaeodactylum tricornutum* (Sendra et al., 2019); (d) Samples of microbial-associated MP aggregates obtained by optical measurement of cell colonization (Bäbler et al., 2020); (e) Micrograph of PS MPs (green) aggregate with bacteria (Long et al., 2017); (f) SEM image of NPs in the presence of alginate (Oriekhova and Stoll, 2018); (g) SEM image of suspended sediments (100-500 µm) associated PS NPs heteroaggregates (Li et al., 2019). Figures are adapted from references mentioned above with permission.

The aggregation of MPs in aquatic environments has mainly been studied in laboratories with simulated water samples due to the limitation of collection and detection techniques in studying in the field (Oriekhova and Stoll, 2018). Recently, a number of review papers summarized the sampling methods of natural water samples to investigate

the mass and distribution of MPs in water columns (Cutroneo et al., 2020). However, some sampling methods such as the density separation method using electrolyte might change the particle size of MPs (Cutroneo et al., 2020; Prata et al., 2019). Therefore, these methods are not quite applicable for investigating the stability of MPs in water. During characterizing the size distribution of MPs using light-scattering methods, the detection might be hampered by the low concentration of MPs in environmental samples or by the complexity of real environmental media (Praetorius et al., 2020). Only a few studies exist to investigate the aggregation of plastic particles at the micro- and nanometer size fractions in complex matrices, because of the practical and fundamental challenges for isolation and analysis of particulate plastics smaller than 100 μm (Gigault et al., 2016; Nguyen et al., 2019). Therefore, the limitations and advantages of various sampling and detection methods have been compared in this review to facilitate the investigation of MP stability in real waters, which has not been well summarized before.

The distribution and toxicity of MPs has been the focus of numerous recent works (Wu et al., 2019b). Only a few review papers have paid attention to the research progresses on MP aggregation in water, which are of particular concern, because their changed particle sizes might influence their own transformation, co-transport with other contaminants, and the toxicity to organisms (Yang et al., 2018). It has been demonstrated that the toxicity of PS NPs was higher than that of PS MPs toward *Brachionus koreanus*, *Paracyclopina nana*, *Daphnia magna*, and *Tigriopus japonicus* (Choi et al., 2019). Till now, studies were primarily focused on the aggregation of spherical polystyrene (PS) MPs with various particle-specific properties (e.g., size and surface coating) (Dong et al., 2018; Romero-Cano et al., 2001; Yu et al., 2019). There remain little data on the aggregation of MPs with

other compositions or shapes. In addition, the water chemical conditions play dominant roles in MP aggregation, but often the effect of a single water chemical condition was taken into consideration in laboratory experiments (Singh et al., 2019). Few reviews discussed the key factors governing MP aggregation and the impact of MP aggregation on the transport of contaminants (Alimi et al., 2018; Gigault et al., 2018; Huffer et al., 2017). Thus, it is necessary to conduct a systematic and comprehensive literature review summarizing the quantitative information and modelling of MP aggregation and environmental impacts of MP aggregation.

This paper provides a critical overview of the recent progress in investigating the aggregation of MPs in aquatic environment, especially in the last three years. First, we review the sources and inputs of MPs in aquatic environments. Next, we critically delineate the sampling, visualization and quantification methods for studying MP aggregation. In addition, the main influencing factors of MP aggregation including their own physicochemical properties and environmental conditions are summarized. The potential environmental implications of MP aggregation in water, particularly in the toxicity to organisms and microbial habitation are discussed. Finally, the current gaps in knowledge and suggestions regarding future research on MP aggregation are discussed. The main objectives of this review paper are to: (1) obtain a clear understanding of MP aggregation; (2) propose to establish standardized sampling programs and field studies for investigating MP aggregation; and (3) facilitate a better understanding of potential environmental risks related to MPs aggregation.

2. Sources and inputs of microplastics in aquatic environments

MPs are a ubiquitous water contaminant, present in lakes, oceans, and even arctic ice

(Zobkov and Esiukova, 2018). Once in the aquatic environment, wind, run-off, and ocean currents aid in their transport, allowing them to travel well beyond their source. Many of the behaviors (e.g., degradation, weathering, and adsorption of contaminants) associated with MPs are influenced by their pathways of transport and surrounding environment (Chamas et al., 2020). One important behaviour is the aggregation process of MPs upon entering the aquatic environment (Li et al., 2018). To fully understand this behavior and associated interactions, the sources and inputs of MPs first need to be discussed.

2.1 Primary sources of microplastics in aquatic environments

Primary MPs are the manufactured plastic particles with diameters < 5 mm that perform a specific function within or enhance the requirements of a product (Lei et al., 2017). Examples of primary MPs include those used for or found within personal care products, air-blasting, medicines, and textiles (Fendall and Sewell, 2009). Of all the primary MPs, cosmetic microbeads have received the most attention (Guerranti et al., 2019). Natural exfoliating agents such as crushed fruit stones were originally used within facial scrubs until the cosmetic industry found that exfoliation could be achieved by adding small plastic fragments or beads with low cost (Fendall and Sewell, 2009). Unfortunately, facial scrubs are 'rinsed-off', and consequently, wastewater treatment plants (WWTPs) became a sink for MPs (Murphy et al., 2016). The deluge of research condemning microbeads has resulted in their slow phase-out. Although the Netherlands was the first country to state their intent to ban cosmetic microbeads by 2016, it was within the United States that the first national legislation, the *Microbead-Free Waters Act* of 2015, was passed (United States Congress, 2015; Xanthos and Walker, 2017). Air-blasting has also been reported as a source of primary MPs when a less abrasive material is required for

cleaning or paint stripping (Auta et al., 2017). MPs and polymeric film coatings also aid medical drug delivery by acting as a vector and increasing control (Cole et al., 2011). Therefore, the medical industry is also a source of plastic particles (Chamas et al., 2020). Finally, some MPs arise from textile and clothing fibers (Galafassi et al., 2019). However, their classification is somewhat of a grey area as they can be either primary or secondary MPs. The fibers are a result of fabric shredding during manufacture (primary), machine washing of clothes, or after the clothes are discarded to landfill (secondary) (Cole et al., 2011).

2.2 Secondary sources of microplastics in aquatic environments

Alternatively, secondary MPs originate from large plastic material. Human activities or natural weathering processes have resulted in plastics breaking down to micro- or nano-sized particles (Chamas et al., 2020). Tire wear and tear is a stealthy source of MPs in the environment (Capolupo et al., 2020). Photo-oxidative and thermo-oxidative degradation, mechanical degradation, hydrolysis, and microbial activity are all examples of natural processes that enhance the formation of secondary MPs (Auta et al., 2017). Ultraviolet (UV) light causes photo-oxidative degradation (which can lead to thermo-oxidative degradation), and is a significant factor in the formation of MPs (Andrady, 2011). However, these processes are location dependent, as MPs on land have greater degradation than those in the water (Anderson et al., 2016). Differences in photo-oxidative degradation are primarily due to the reduced temperature and oxygen content caused by the surrounding water. Furthermore, water conditions promote biofilm accumulation onto the surface of the plastic (Andrady, 2011; Bäbler et al., 2020). Although biofilm may reduce UV degradation by forming a barrier to sunlight, certain microorganisms can contribute to the

biodegradation of plastics (Oberbeckmann et al., 2015). Some MPs can breakdown by hydrolysis, but it is not considered a significant mechanism in oceans (Andrady, 2011). Marine conditions will form secondary MPs through wave action (e.g., surf or swash zone), or sediment and wind abrasion (Efimova et al., 2018). Storm conditions further amplify the degradation of plastics in the marine environment.

2.3 Inputs of microplastics in aquatic environments

Pathways in which MPs enter the aquatic environment include direct input, or from terrestrial or atmospheric routes (Auta et al., 2017). **Table 1** provides examples of the average concentrations of MPs sampled from aquatic environments, and their suggested sources. Many products contain plastic, and these can directly end up in waterways, lakes, and oceans through careless handling and littering, especially from recreational water products (e.g., boats, fishing gear, or plastic toys used at the beach) (Sun et al., 2019). They can also indirectly enter aquatic environments through littering, illegal dumping, and poor management of landfills (Cole et al., 2011). Paint chips from boats or ships, and fibers from marine ropes or nets for aquaculture also contribute to water MP pollution (Duis and Coors, 2016). Besides serving as a sink for MPs, WWTPs can also be a potential point source of MP exposure for aquatic environments through effluent discharge (Kazour et al., 2019). A study by Edo et al. (2020) predicted that 300 million MP debris were released into a nearby river per day through effluent discharge. Furthermore, MPs can be transported from terrestrial to aquatic environments through run-off, erosion, and wind (Rezaei et al., 2019). Terrestrial inputs of MPs usually contained land applications of biosolids and soil mulching (Campanale et al., 2020). Storm events can increase the concentration of land-based MPs that reach aquatic environments (Eerkes-Medrano et al., 2015). Another input that is

receiving more attention in recent years is the atmospheric deposition of MPs (Murphy et al., 2016). Although higher concentrations of airborne MPs are reported in urban environments, the proximity of coastal cities to water sources could lead to increased levels of MPs in rivers and oceans (Dris et al., 2016). Furthermore, long-range atmospheric transport has been reported as an important factor for the concentration of MPs in the Arctic region (Bergmann et al., 2019). Overall, the extent of MP contamination is evident by the abundance of these particles, even in remote locations such as the Arctic (Bergmann et al., 2019; Peeken et al., 2018).

Table 1 Sources and concentrations of microplastics in aquatic environments.

Continent	Location	Suggested Sources	Average MP Concentration	Sample Year	Reference
Antarctica	Ross Sea	WWTPs, ship traffic, research activities, ocean currents, and unknown	0.17 MPs·m^{-3}	2010	(Cincinelli et al., 2017)
Asia	29 rivers in Japan	Urban living, WWTPs, airborne (e.g., winds), and rainfall run-off	0 to 4.94 MPs·m^{-3}	2015-2018	(Kataoka et al., 2019)
	Nakdong River	Atmospheric fallout, heavy rain, run-off, urban living, and WWTPs	1555 MPs·m^{-3}	2017	(Eo et al., 2019)
Australia	GMA / Goulburn River Catchment	Natural processes	0.40 MPs·L^{-1}	2018	(Nan et al., 2020)
Europe	Henares River	WWTPs	350 MPs·m^{-3}	2019	(Edo et al., 2020)
	Le Havre harbor	WWTPs	~0.1-1.8 MPs·L^{-1}	2018	(Kazour et al., 2019)
	Lake Bolsena	Airborne (e.g., winds), storms, land-based sources, and WWTPs	3.02 MPs·m^{-3}	2014	(Fischer et al., 2016)
	Lake Chiusi	Water circulation, WWTPs, and local fisheries	2.22 MPs·m^{-3}	2014	(Fischer et al., 2016)
	Ofanto River	Land-based sources (e.g., agricultural activities, run-off, and erosion)	~6 MPs·m^{-3}	2017-2018	(Campanale et al., 2020)
	Rhine River	Urban living, WWTPs, and ship traffic	892,777 MPs·km^{-2}	2014	(Mani et al., 2015)
	River Clyde	WWTPs	0.25 MPs·L^{-1}	Not mentioned	(Murphy et al., 2016)
North America	Lake Winnipeg	Synthetic textiles, secondary MPs, atmospheric fallout, urban living, and WWTPs	193,420 MPs·km^{-2}	2014-2016	(Anderson et al., 2017)
	Raritan River	WWTPs	~30 MPs·m^{-3}	2015	(Estahbanati and Fahrenfeld, 2016)
	Chicago River	WWTPs	17.9 MPs·m^{-3}	2013	(McCormick et al., 2014)

Note: Microplastics (MPs), Greater Melbourne Area (GMA), Wastewater Treatment Plants (WWTPs)

3. Analytical methods for studying microplastic aggregation in aquatic environment

The physiochemical properties of MPs including color, shape, density (0.9-2.3 $g \cdot cm^{-3}$), and chemical constituents vary significantly in the environment, which increase the difficulty to detect and quantitatively estimate the aggregation state of MPs in real water environment (Stock et al., 2019). Generally, the analytical procedures for MP aggregation in water environments include collection of samples to cover the full-size range of MPs, their visualization, and quantification, as shown in **Fig. 2**. A convincing analysis of MP aggregation requires standardized methods, which can be useful for understanding the aggregation of MPs in water environments and maximize comparability of investigations. A number of systematic methodologies for this purpose has been compared and summarized in this section.

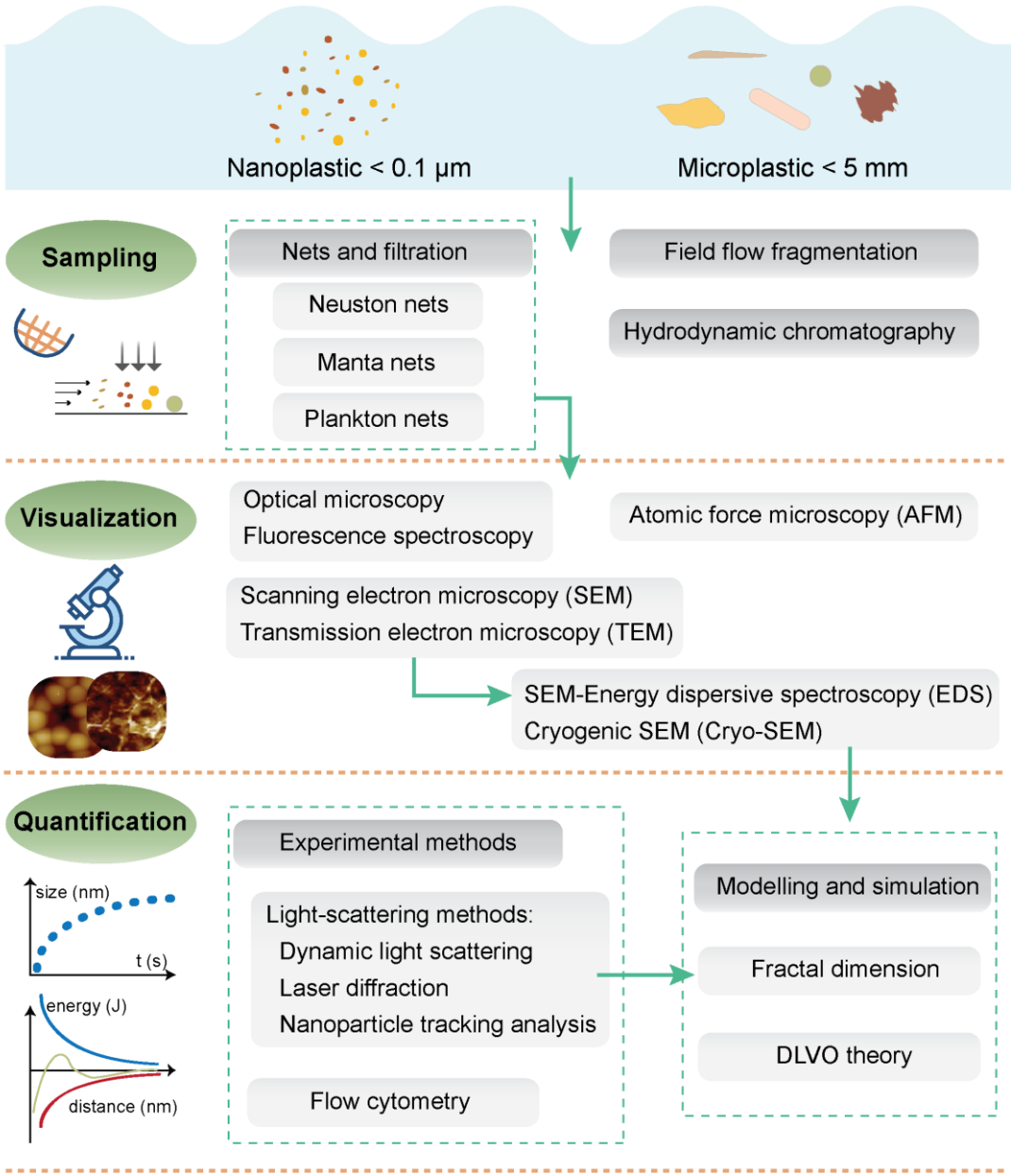


Fig. 2. Analysis processes for the aggregation of microplastics (MPs) in water including the sampling, visualization and quantification of MP' particle sizes.

3.1 Microplastic sampling methods

Sampling MPs in water environment is the first step for investigating the aggregation of MPs (Pico and Barcelo, 2019). To the best of our knowledge, most studies regarding water sample collection focused on quantifying the content of MPs in natural waters (Cutroneo et al., 2020). Some sampling and separation methods were also suitable for

investigating the aggregation state of MPs in natural waters.

The sampling methods of MPs in water can be divided into the selective, bulk sampling, and volume-reduced methods (Wang and Wang, 2018; Zobkov and Esiukova, 2018). Selective sampling refers to direct collection of plastic particles, and they are recognizable by naked eyes (particles between 1 and 5 mm) (Silva et al., 2018; Zobkov and Esiukova, 2018). This technique is simple but misses some MPs when they are mixed with other debris, or when they have no characteristic shapes, or with ultra-small size (Wang and Wang, 2018). Bulk sampling refers to collecting the entire volume of water samples containing the whole size range of MPs (Silva et al., 2018). Bulk sampling captures relatively small amount of a sample that probably have low concentration of MPs for subsequent processing and detection (Zobkov and Esiukova, 2018). Volume-reduced sampling refers to the condensation of volume of sample, preserving only the portion of interest for further processing (Silva et al., 2018). Therefore, the condensation of MPs improves their mass concentration for stability assessment by instruments (Zobkov and Esiukova, 2018). Selective and bulk sampling methods are usually used to collect sediment samples or water samples, and the volume-reduced method seems to be the most frequently applied approach for sampling water samples (Wang and Wang, 2018).

287 **Table 2** Advantages and disadvantages of various sampling methods for microplastics (MPs) from water

Method	Size range	Advantages	Disadvantage	Ref.
Neuston nets and Manta trawls	64-5000 µm	• Sample large volumes of water quickly • Easy to operate • Surface water is collected for floating MPs • Collect large number of MPs	• Clogging of nets by organic or mineral materials • Potential contamination by tow ropes or containers	(Cutroneo et al., 2020; Hidalgo-Ruz et al., 2012; Pico et al., 2019)
Plankton nets	50-500 µm	• Sample medium volumes of water • Easy to operate • Water column is collected • Quick to use	• Clogging of nets by organic or mineral materials • Expensive and time-consuming	(Cutroneo et al., 2020; Estahbanati and Fahrenfeld, 2016; Pico et al., 2019)
Filtration	100, 20 and 5 µm	• Easy to operate • Known water volume • Choice of filter pore sizes	• Obstruction by organic or mineral matter • Sample low volumes of water • Potential contamination by the apparatus • Time consuming and laborious depending on pore sizes	(Stock et al., 2019)
Field flow fractionation	1 nm-100 µm	• No stationary phase improved separation resolution • Coupled detectors • Cover the whole nanometer range	• Hard to operate • Expensive (advanced instruments) • Low recovery and analysis speed • Hard to analyze environmental samples (need pretreatment) • Membrane limitation	(Shendruk and Slater, 2012; Shendruk et al., 2013)
Hydrodynamic chromatography	5 nm-1.2 µm	• Rapid separation of particles • Higher recovery than field flow fractionation • Coupled detectors • Less interaction with stationary phase	• Hard to operate • Expensive (advanced instruments) • Low separation resolution	(Blom et al., 2003; Schwaferts et al., 2019)

288

3.1.1 Nets and filtration

Nets are the most frequently used devices for sampling MPs from waters (Hidalgo-Ruz et al., 2012; Pico et al., 2019). This method is advantageous for covering large sampling areas, filtering large volumes of water rapidly and concentrating MPs directly during sampling (Stock et al., 2019). However, this method is difficult to apply in narrow spaces such as pot basins, little channel, or in the presence of obstacles (Cutroneo et al., 2020). Besides, the mesh size results in the omission of lower size fractions of MPs (Zobkov and Esiukova, 2018). Nets with a mesh width of 50-3000 μm were often used, and ~300 μm was the most commonly employed size (Stock et al., 2019). Various types of nets were used across studies (Cutroneo et al., 2020). For example, manta nets and neuston nets with mesh sizes ranging from 64-5000 μm are used for surface water sampling (Cutroneo et al., 2020; Hidalgo-Ruz et al., 2012; Pico et al., 2019). Plankton nets with smaller mesh sizes (c.a. 50-500 μm) are used for water columns, and can have 30 times higher recovering concentration than manta nets (Prata et al., 2019). For riverbed or seabed sampling, drift or benthic nets that can be attached to the ground were used, while bongo nets were used for sampling from mid-water levels (Stock et al., 2019). Manta nets, neuston nets and plankton nets are the most frequently used devices, and a summary of their comparative performances is given in **Table 2**.

For the filtration method, MPs are separated by passing the water samples over a filter, usually aided by a vacuum. To sort out larger particles before the filtration step, water samples can first be passed through a sieve with 500 μm mesh size. The size of MPs retained and the filterable volume are a direct consequence of the filter pore size used (Sun et al., 2019). A promising method is filtering of large volumes of water samples via

cascades of 100, 20 and 5 μm filters by directly fractionated pressure filtering (Stock et al., 2019). This method facilitates the simultaneous collection of different size fractions of MPs down to < 5 μm, and enables a comprehensive resolution of the size spectrum of MPs (Stock et al., 2019; Sun et al., 2019).

Nets and filtration methods can maintain the original aggregation state of MPs (Lenz and Labrenz, 2018). However, when these two methods are used, we must balance between the filters' ability to obtain MPs and potential clogging of pores by organic matter, mineral matter and microorganisms (Pico et al., 2019; Stock et al., 2019). The sequential filtration using filters with smaller pore sizes might minimize the clogging (Pico and Barcelo, 2019). In addition, the above two methods would lose the nanometer-sized particles because of their sorption on filters or pore limitations (Sun et al., 2019).

3.1.2 Field flow fractionation (FFF)

Beyond the above fractions with large particle sizes, there are smaller plastic fragments that are < 1 μm (Pico and Barcelo, 2019). Chromatographic techniques including passive and active separation are typically applied to collect the micro- or nano-sized plastics (Fu et al., 2020). Field flow fractionation (FFF) is a representative active separation technique that can be used for separating MPs with size of 1 nm - 100 μm from water samples (Shendruk and Slater, 2012; Shendruk et al., 2013). Particles such as colloids and macromolecules are size-separated in a channel with parabolic and laminar flow under a perpendicular external field (Shendruk and Slater, 2012). The nature of external fields (i.e., thermal, sedimentation, flow, gravitational, electrical, and magnetic) defines the type of FFF (Messaud et al., 2009). Thermal FFF is suitable for separating polymers based on both molecular weight and composition differences (Runyon and Williams, 2011).

Sedimentation FFF is an alternative to density separation of MPs by electrolytes (Huppertsberg and Knepper, 2018). Flow FFF is most commonly applied method in the separation and analysis of MPs with two modes: normal mode where larger particles have longer retention, whereas this is reversed for larger particles in the steric mode (Gigault et al., 2017; Huppertsberg and Knepper, 2018; Mintenig et al., 2018). Flow FFF technique does not require a stationary phase, which reduces variations or errors caused by the interaction with stationary phase (Shendruk and Slater, 2012). However, low recovery of MPs exists for the flow FFF technique and an advanced instrument is indispensable (Mintenig et al., 2018).

Asymmetrical flow field-flow fractionation (AF4) that belongs to flow FFF is primarily used to characterize polymers (Fraunhofer et al., 2004). AF4 was applied to characterize the size distribution of PS NPs and separate them in fish (Correia and Loeschner, 2018; Gigault et al., 2017). AF4 was superior when tissues had auto-fluorescence property that might interfere with the results of fluorescence microscopy and flow cytometry (FCM) (Correia and Loeschner, 2018). However, AF4 cannot detect polyethylene (PE) NPs in fish due to the interference of light-scattering background, indicating that AF4 requires further adjustment for analyzing different types of NPs (Correia and Loeschner, 2018). AF4 coupled to pyrolysis gas chromatography-mass spectrometry can determine particle sizes and polymer types of MPs, which contributed to a framework development for standardizing the measurement method of MP sizes (Mintenig et al., 2018). Furthermore, hollow fiber flow field-flow fractionation coupled with UV-Vis detector can characterize the particle size distribution of poly(vinyl acetate) NPs in aqueous suspensions (Xiao et al., 2018).

3.1.3 Hydrodynamic chromatography (HDC)

Hydrodynamic chromatography (HDC) is a passive separation technique that can be applied for MP separation from water samples for particle sizes in the range from 10 nm to 1 μm (Lespes and Gigault, 2011). The HDC separation principle relies on the size-dependent exclusion from the wall in a channel where a pressure-driven flow is applied (Lespes and Gigault, 2011). Separating components of sample mixtures is accomplished by parabolic flow velocity gradients that develop within the column between a packed bed filled with media grains (Shendruk and Slater, 2014). The movement of particles with size range from 10 nm to 1 μm under the Brownian motion can be disturbed by media grains (Fu et al., 2020). Small particles receive increasing hydrodynamic effect and van der Waals interactions with media grains and migrate closer to the channel wall, which is subjected to a lower flow velocity (Cejas et al., 2018). Larger particles stay close to the center of the channel where the higher flow velocity causes them to move faster (Liu et al., 2013). Thus small particles elute slower than large particles (Shendruk and Slater, 2014).

HDC has been employed to separate MPs and NPs from sediment, seawater and food (Bouwmeester et al., 2015; Chain), 2016). HDC coupled with other detectors has also been used for separation (Philippe et al., 2014; Pirok et al., 2017). For example, HDC coupled with UV-vis detector or ICP-MS was employed to separate PS NPs and several metal nanoparticles in aqueous suspensions (Philippe et al., 2014). HDC combined with size-exclusion chromatography was used to obtain information on the two-dimensional particle size distribution of the mixed suspensions between PS NPs and polyacrylate NPs (Pirok et al., 2017). HDC showed rapid separation and better recoveries than AF4, but the separation resolution was lower than that of FFF (Revillon, 2000).

3.2 Microplastic visualization methods

After sampling MPs, various visual sorting methods are applied to provide the stability information of MPs as single particles, homoaggregates or heteroaggregates with other materials, such as suspended sediments, algae, and bacteria (Bäbler et al., 2020; Sendra et al., 2019; Sun et al., 2018). This is frequently conducted by direct observation by naked eyes or assisted by a microscope (Silva et al., 2018). For example, the homoaggregation of PE MPs with diameters of 1-5 mm was observed by naked eyes and recorded with a digital camera (Li et al., 2019). To obtain more detailed information on MP aggregation in a water sample, optical microscopy, electron microscopy and scanning probe microscopy are utilized. These microscopes based on different operation modes offer direct access to the geometry and surface characteristics of MP samples (Schwaferts et al., 2019).

3.2.1 Optical microscopy

Optical microscopes are usually applied for providing the surface texture, structural information and number of particles with micrometer size (Silva et al., 2018). The heteroaggregates between suspended sediments and PE MPs with diameters ~2 mm were directly observed by optical microscopes (Li et al., 2019). Techniques such as fluorescence spectroscopy can analyze fluorescently marked particles or materials emitting sufficient fluorescence signal, which could facilitate the investigation of the homoaggregation of MPs or their heteroaggregation with other particles in natural waters (Cunha et al., 2019).

For example, fluorescence spectroscopy was applied to compare the homoaggregation of fluorescent PS NPs and their heteroaggregation with suspended sediment in different water chemical conditions (Li et al., 2019). In 500 mM NaCl solution, fluorescent PS NPs

with diameter of 100 nm were found to attach onto the surface of suspended sediment with sizes greater than plastic particles (100~500 μm) in the presence or absence of humic acid (HA). By contrast, no homoaggregation or heteroaggregation of PSNPs with suspended sediment was observed in 10 mg·L^{-1} HA solutions. This was primarily because HA could be adsorbed onto PS NP and sediment surfaces, imparting electrostatic repulsion and hinderance forces, and thus increased the stability of PS NPs (Tallec et al., 2019). PS and poly(methyl methacrylate) (PMMA) MPs were stained with Nile Red, and applied to characterize their heteroaggregation with microalgae through fluorescence microscopy (Cunha et al., 2019). As shown in **Fig. 3**, two freshwater microalgae (*Scenedesmus sp*. and *Microcystis panniformis*) and two marine microalgae (*Gloeocapsa sp.* and *Tetraselmis sp.*) excreted exopolymeric substances (EPS) and colonized MPs. The heteroaggregation degree was related to MP' type, size and density as well as the yield of EPS that was species-specific.

However, fluorescence spectroscopy is not applicable for environmental MPs because the dye or fluorophores are not plastic-specific (Schwaferts et al., 2019). Further studies are suggested to focus on the interaction between dyes and MPs, which may facilitate the application of plastic-specific and plastic-sensitive dyes and dye combinations. Another drawback of visual sorting is the size limitation, i.e., particles below a micrometer is difficult to visually discriminate from other materials. Smaller MPs and NPs should generally be sorted out using electron microscopy or scanning probe microscopy (Hale, 2017).

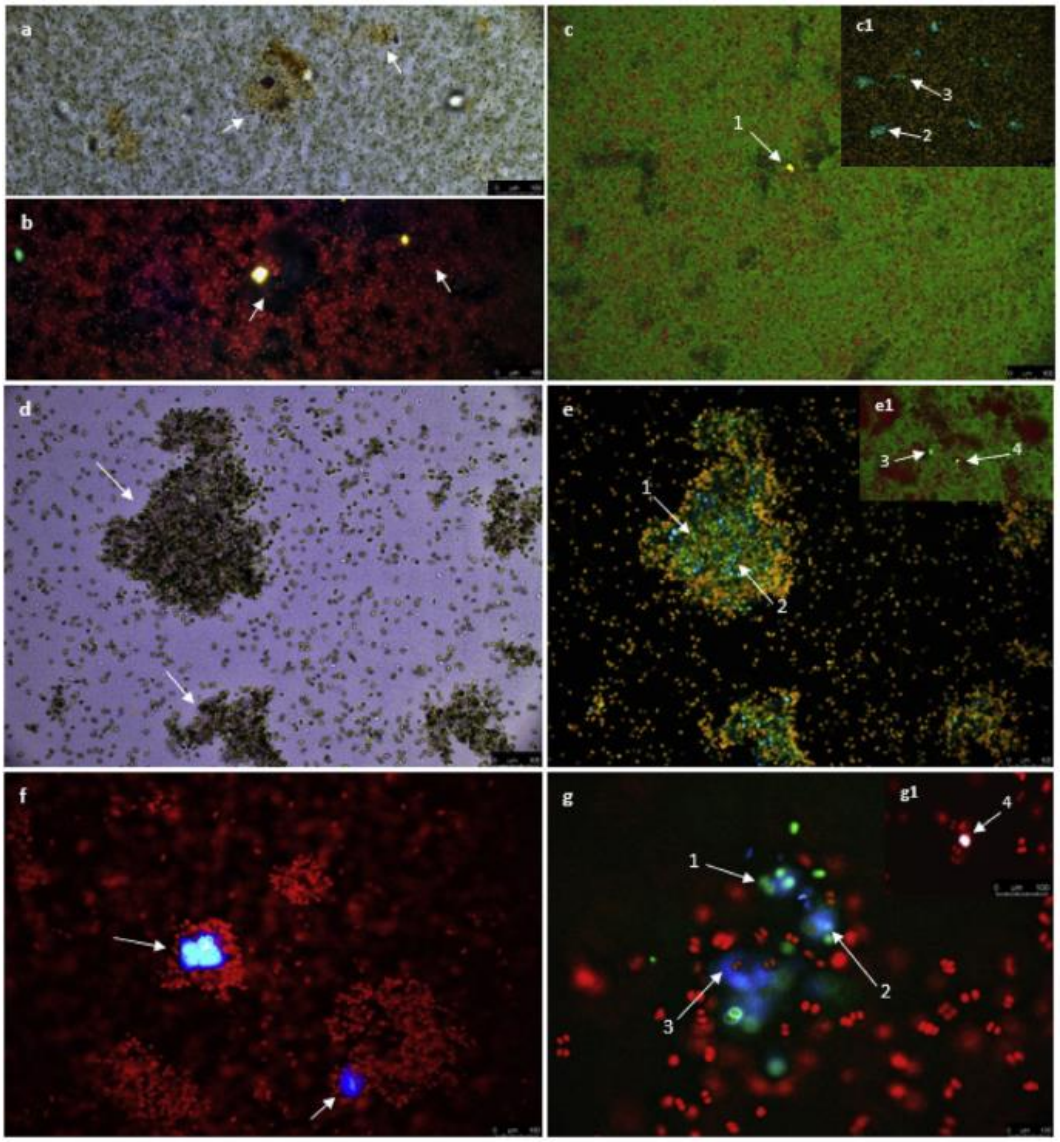


Fig. 3. (a) Bright field micrograph of *Microcystis panniformis* heteroaggregates; (b) Same micrograph seen in (a), but under DAPI filters, being visible in the incorporation of the fluorescent microplastics (MPs) in the heteroaggregates; (c) Micrograph of *Scenedesmus sp*., under I3 filters, showing the abundance of exopolymeric substances (EPS) and the MPs aggregation; (c1) Micrograph of *Scenedesmus sp*. under DAPI filters, exhibiting aggregation of different MPs. Each arrow pointing at yellow (1) and blue (2) represents polystyrene (PS). Each arrow pointing at the green (3) and purple (4) represents poly(methyl methacrylate) (PMMA); (d) Bright field micrograph of *Tetraselmis sp*. Heteroaggregates; (e) Same micrograph seen in (d), but under DAPI filters, showing heteroaggregates composed of microalgae (orange) and MPs (blue/green) and EPS; (e1) Micrograph of *Tetraselmis sp.* heteroaggregates, under I3 filters; (f) Micrograph of *Tetraselmis sp.* showing the colonization of MPs by the microalga, under DAPI filters; (g/g1) Micrographs of MPs aggregate with *Gloeocapsa sp.*, under DAPI filters. Graph was cited and reproduced from ref. (Cunha et al., 2019) with permission.

3.2.2 Electron microscopy

By means of electron beams, the resolution of electron microscopy is much higher than optical microscopy, spanning the range from 1 nm to millimeters (Schwaferts et al., 2019). Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are often applied to provide high-resolution images, characterize the precise size, and facilitate the differentiation of MP homoaggregates and heteroaggregates with Fe_2O_3, alginate, soil particles, and other solid particles (Liu et al., 2019a; Oriekhova and Stoll, 2018).

The interaction between MPs and microalgae was mostly analyzed by SEM and TEM, as shown in **Fig. 1**. For example, microalgae (*Chlamydomas reinhardtii*) colonized MPs (polypropylene (PP) and high-density polyethylene), and the heteroaggregates constituted by microalgae, MPs and EPS were observed by SEM (Lagarde et al., 2016). The formation of heteroaggregation between TiO_2 nanoparticles and PS MPs in water was confirmed by SEM, which decreased the toxicity of TiO_2 nanoparticles to marine algae *Chlorella sp*. (Thiagarajan et al., 2019). TEM was applied to characterize the particle size distribution and mean sizes of polyvinyl chloride (PVC) MPs (Zhang et al., 2017). TEM images showed that PS beads attached on the surface of marine bacterium *Halomonas alkaliphila* led to cellular membrane damage and death (Sun et al., 2018). Although most MPs with a wide size range could be observed by TEM or SEM, these two methods require dry sample preparation, which may induce deposition and interfere with the aggregation state of MPs (Schwaferts et al., 2019). These two methods also need a long analysis time and limited sample sizes or numbers to be studied, which make the results random and thus unreliable (Fu et al., 2020).

TEM and SEM can be coupled with other instruments to provide details about the MP aggregation state. Energy dispersive spectroscopy (EDS) provides the elemental information of samples via detecting the characteristic X-rays emitted from the elements within the sample by the electron beam (Schwaferts et al., 2019). The SEM-EDS was able to distinguish the homoaggregation of MPs, and heteroaggregation between MPs and DOM or inorganic minerals in real water samples (Fu et al., 2020). Cryogenic SEM (Cryo-SEM) was used to directly observe the aggregation state of PS NPs in the mixture of DOM and salt solution (Cai et al., 2018). As shown in **Fig. 4,** Cryo-SEM images clearly show the formation of PS NPs-DOM clusters through the bridging effect in the mixture of DOM and $FeCl_3$. Cryo-SEM method maintained the morphology of samples in their native environment by rapid freezing, which is a promising tool to reveal the precise information of aggregation state (Kaberova et al., 2020). However, Cryo-SEM may face challenges including low image contrast and low signal to noise ratio.

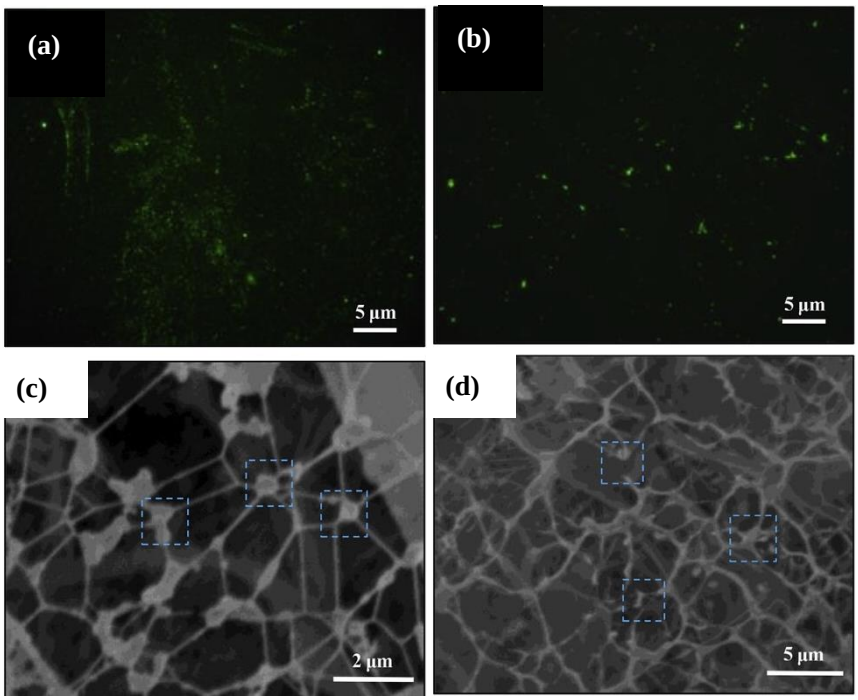


Fig. 4. Fluorescence images (a, b) and Cryo-SEM images (c, d) of polystyrene nanoplastics in 1 mM $FeCl_3$ and Suwannee River humic acid (a, c) or Suwannee River fuvic acid (b, d) solutions. Graph was cited and reproduced from ref. (Cai et al., 2018) with permission.

3.2.3 Scanning probe microscopy

Scanning probe microscopy, especially atomic force microscopy (AFM), has been identified as one of the most powerful techniques for providing images at nanoscale resolutions (Stawikowska and Livingston, 2013). AFM has been proven useful in the assessment of in-situ surface properties of particles at both microscale and nanoscale (Fu et al., 2020). More importantly, the original dispersed states can be preserved during the AFM analysis process as samples can be placed in vacuum, gaseous, or aqueous environments with desirable conditions, thereby reducing potential experimental artifacts on samples during the preparation process (Fu et al., 2020). AFM provides an effective and non-destructive means for nanoparticle characterization and surface properties analysis such as force profiles, which could also be applied for investigating the stability of MPs (Fu and Zhang, 2018). For example, AFM images indicated that the surface morphology

of particulate plastics was altered by the formation of PP-bacteria heteroaggregates (Kumari et al., 2018). Ruiz-Cabello et al. (2013) employed AFM to measure direct force profiles between carboxyl-modified latex particles (diameter 1.0 μm) in different electrolyte solutions (KCl, $MgCl_2$, $LaCl_3$, $ZrCl_4$) and used the observed force profiles to predict their aggregation rates. The force profiles fitted well with Derjaguin-Landau-Verwey-Overbeek (DLVO) theory even though some non-DLVO forces also existed (Trefalt et al., 2017).

Visualization methods are commonly applied by most researchers to identify MP aggregation in natural waters. However, visual examination may provide inaccurate information on MP aggregation because it is difficult to differentiate plastic particles from other organic or inorganic particles with similar sizes or shapes (Hale, 2017). Additional techniques such as Raman spectroscopy, Fourier-transform infrared spectroscopy (FTIR), liquid chromatography, and pyrolysis gas chromatography coupled to mass spectrometry are required to be applied to identify the chemical composition of MPs, in addition to the use of visualization methods (Schwaferts et al., 2019).

3.3 Microplastic aggregation quantification methods

3.3.1 Experimental methods

3.3.1.1 Light-scattering methods

Light-scattering methods are widely used to quantify the in-situ aggregation behavior of MPs since it is non-destructive (Yu et al., 2019). The most frequently applied light-scattering method, dynamic light scattering (DLS) instrument, determines the time-dependent hydrodynamic diameter (D_h) of sample with size range from 0.6 nm to 6 μm under the Brownian motion (Kastner and Perrie, 2016). The zeta potential and

electrophoretic mobility of MPs in an applied electric field can be measured by detecting frequency shifts in the scattered light, which is important for predicting MP aggregation behavior (Kaszuba et al., 2010). So far, there have been a large amount of studies using DLS to measure the aggregation kinetics, aggregate size, and zeta potentials of MPs/NPs in various water chemical conditions (Cai et al., 2018; Yu et al., 2019). Although DLS is fast and simple operation, it has disadvantages such as low resolution and low accuracy for large particles (Wang et al., 2015). DLS is more appropriate for measuring monodisperse suspensions, and has limitation in heteroaggregation studies (Praetorius et al., 2020).

Laser diffraction (LD) analyzer based on the static light scattering is used for measuring particles with sizes ranging from submicron (500 nm) to millimeter (10 mm), i.e., particles can have sedimentation process and their movement cannot only be governed by Brownian motion (Kastner and Perrie, 2016; Schwaferts et al., 2019). LD relies on time-averaged intensity measurements and uses volume mean diameter to represent the size of particles (Praetorius et al., 2014). The distribution percentiles D(10), D(50) and D(90) can be reported to analyze variation in particle sizes, representing the 10th, 50th, and 90th percentile of particle size, respectively (Praetorius et al., 2014). The effects of monovalent (KNO_3, $NaNO_3$ and NaCl) and divalent ($CaCl_2$ and $BaCl_2$) electrolytes with and without HA in water on the aggregation kinetics of PS MPs (diameter of 17.9 μm) were investigated by LD (Li et al., 2018). The results showed that divalent electrolytes were more efficient in destabilizing PS MPs as compared to monovalent electrolytes due to the higher charge neutralization effect of divalent cations (Qu et al., 2010). LD has been proposed as a method to study heteroaggregation kinetics of nanoparticles with larger μm-sized

suspended particulate matters, which enables it to measure heteroaggregation of MPs (Praetorius et al., 2020).

DLS and LD measurements probe the polydispersity index, of which the value >0.2 indicates a very broad size distribution, or heterogeneous or multimodal particle size distributions (Li et al., 2019). The aggregation kinetics at an early stage and initial aggregation rate were calculated using the DLS and LD data in most studies (Summers et al., 2018). The initial aggregation rate constant of MPs (k) is proportional to the inverse of MPs concentration (N_0) and the initial increase rate of the D_h with time (t), which is calculated by eq 1 (Li et al., 2018).

$$k \propto \frac{1}{N_0}\left(\frac{dD_h(t)}{dt}\right)_{t\to 0} \tag{1}$$

The attachment efficiency (α) is defined as the reciprocal of the stability ratio (W) and is calculated by eq 2 (Praetorius et al., 2020):

$$\alpha = \frac{1}{W} = \frac{k}{k_{fast}} = \frac{\frac{1}{N_0}\left(\frac{dD_h(t)}{dt}\right)_{t\to 0}}{\frac{1}{(N_0)_{fast}}\left(\frac{dD_h(t)}{dt}\right)_{t\to 0,\, fast}} \tag{2}$$

where, the subscript "fast" represents a favorable aggregation condition. The numerator and denominator represent the aggregation rate constants under the reaction-limited aggregation regime (α<1) and diffusion-limited aggregation regime (α=1), respectively (Qu et al., 2010). The critical coagulation concentration (CCC) values of MPs can be obtained from α. For instance, the CCC values of PS NPs were around 300 mM to 450 mM in monovalent electrolytes (e.g., NaCl and KCl) and around 30 mM in divalent electrolytes (e.g., $CaCl_2$ and $BaCl_2$) (Liu et al., 2019b; Yu et al., 2019). While for micro-sized PS MPs, CCC value was smaller compared to PS NPs, such as around 15 mM in NaCl, KNO_3, $NaNO_3$, and around 3 mM in $CaCl_2$ and $BaCl_2$ (Li et al., 2018). Besides, the addition of

HA resulted in CCC values of 1.03-2.15 times higher than that without HA, and the CCC values were positively correlated to the HA concentrations (Li et al., 2018). This can be attributed to the fact that HA could readily cover the MP surface via adsorption or hydrophobic interaction, which might enhance the stability of MPs due to steric stabilization and/or electrostatic repulsion (Qu et al., 2010).

LD or DLS is becoming a preferred technique for particle size measurement compared to SEM, due to their short analytical time, high precision, reproducibility, flexible operation, and easy application (Shekunov et al., 2006). However, significant error in estimating the hydrodynamic particle size for non-spherical particles, such as films and fibers exists in LD or DLS (Shekunov et al., 2006). By contrast, due to direct visualization, SEM is more reliable in analyzing the particle size of non-spherical particles. But characterizing particle size by SEM may have statistical biases associated with particle aggregation during the drying process of sample preparation (Klang et al., 2012).

572 **Table 3** Comparison of light-scattering methods for investigating microplastc aggregation
573 in water

Method	DLS	LD	NTA
Size range	0.6 nm ~ 6 μm	500 nm ~ 10 mm	10 nm ~ 1000 nm
Concentration range	Maximum 40 % w/v	Not defined; need low concentration of sample	10^7 ~ 10^{10} particle·mL^{-1}
Principle	Scattered light and Brownian motion	Laser diffraction	Scattered light and Brownian motion
Key parameters	• Average hydrodynamic diameter • Intensity, volume and number-based particle size distribution • Polydispersity index	• Volume mean diameter • D(10), D(50) and D(90) • Polydispersity index	• Particle concentration • Hydrodynamic diameter
Advantages	• Fast and simple operation • Aggregation rate measurement • Zeta potential measurement • Stability ratio measurement	• Wide size range • Suitable for sedimentation • Aggregation rate measurement • More accurate in heterogeneous system • Stability ratio measurement	• Provide particle number concentration • Provide size of individual particles • Video of particle motion
Limitations	• Low accuracy and resolution for large particles • Less suitable for heteroaggregation studies • Affected by light absorption of the medium	• Affected by light absorption of the medium	• The motion trail reduces accuracy • Operation difficult • Not suitable for particles that are too polydisperse or too close in size

574 Nanoparticle tracking analysis (NTA) comes to the forefront when studying poly-
575 dispersed samples where a range of sizes is present, because the NTA data clearly indicate
576 different sizes of particles in suspension (Fu et al., 2020). The detailed comparison of the
577 three light-scattering methods are summarized in **Table 3**. NTA can simultaneously analyze
578 the particle concentrations and particle size distributions of a sample (Gross et al., 2016).
579 The individual particles from 10 to 1000 nm can be detected to give particle size
580 distribution data under NTA (Fu et al., 2020; Gross et al., 2016). The formation of NP
581 particles during the degradation of a PS coffee cup lid was observed, and the time-resolved

evolution of particle size distribution of PS NPs was measured by using NTA (Lambert and Wagner, 2016). Additionally, NTA was combined with dark field microscopy to measure the particle size distribution of nanoparticles with the resolution of *ca.* 20 nm (Wagner et al., 2014).

3.3.1.2 Flow cytometry (FCM)

FCM detect the light scattering and fluorescence by laser excitation of samples with fluorescence characteristics (natural or staining) in suspension when they pass through a light beam (Adan et al., 2017). FCM is highly sensitive to detect samples with diameter of 0.5 - 20 μm and can be applicable to analyze particles as large as 100 μm in diameter (Green et al., 2003). FCM has been widely used in characterizing and distinguishing different cell types in heterogeneous cells, analyzing the size and volume of cells, and analyzing the expression of intracellular molecules (Adan et al., 2017). Some functions are capable of quantifying and characterizing MP aggregation in seawater and study their distribution in the water column (Arias-Andres et al., 2018; Long et al., 2017). For example, the aggregation state of MPs (diameter of 500 nm) in seawater was depicted by FCM (Summers et al., 2018). The 500 nm MPs dispersed and slightly aggregate with the portion of 39.9%. After 24 h seawater treatment, obvious aggregation could be detected because the proportion of aggregated increased to 93.9%. FCM was also used to observe the heteroaggregation between MPs and organisms such as phytoplankton, bacteria and algae (Arias-Andres et al., 2018; Long et al., 2017). For instance, Long et al. (2017) used FCM to verify the heteroaggregation between fluorescent MPs and phytoplankton (e.g., diatom *Chaetoceros neogracile* and algae *Rhodomonas salina*), and measure the concentration of MPs in the aggregates. The FCM approach was also used to quantify the amount of PS

MPs adsorbed on algal surfaces (Bhattacharya et al., 2010).

3.3.2 Modelling and simulation for studying MP aggregation

3.3.2.1 Fractal dimension

Apart from the aggregation kinetics of MPs, an in-depth study of the morphology and structure of MP aggregates is required to determine the toxicity and fate of the aggregates in aquatic environments (Quik et al., 2014). The aggregation of colloids leads to fractal structures that exhibit fractal dimensions (D_f) from zero to three (Li and Logan, 2001). D_f is defined by a power-law relationship between fractal aggregate mass (m) and aggregate radius (r) (Quik et al., 2014).

$$m \propto r^{D_f} \tag{3}$$

D_f can be determined from LD measurement, or optical sampling and digital image analysis (Meng et al., 2013). D_f describes the geometry of aggregates, aggregation rate, and various physical properties such as density and settling velocity of MP aggregates (Li and Logan, 2001; Long et al., 2015). The lower the aggregation rate, the more time particles have to form a denser and more compact structure, and lead to a higher fractal dimension (Meng et al., 2013). Long et al. (2015) used D_f to understand the structure of aggregates between PS MPs and phytoplankton or algal species. They found that the heteroaggregate structure turned less fractal after exposure to PS MPs. The correlation between sinking rates and fractal aggregates can be illustrated by a fractal scaling model. In addition, D_f has been applied to explain the compactness of MP homoaggregates and chitosan or alginate-associated MP heteroaggregates in salt solution (Ramirez et al., 2016).

3.3.2.2 Derjaguin-Landau-Verwey-Overbeek (DLVO) theory

DLVO theory has been widely employed to understand the relationships between

628 colloids and their aggregation behavior, and it can be used to explain the stability of MPs
629 in water (Zhang, 2014). The classical DLVO theory considers van der Waals forces and
630 electrostatic forces for yielding the total interaction energies between the material surface
631 in contact (Wang et al., 2015). A particle-particle geometry was employed for
632 homoaggregation between MPs in aqueous environments because most studies used
633 commercial MPs with spheres shape (Cai et al., 2018). When investigating the
634 heteroaggregation between layered clay minerals and NPs in electrolyte solutions, a
635 particle-plate geometry was used because NPs behaved as small particles that deposited on
636 the surface of a large flat plate (Li et al., 2019). For the interaction energies between micro
637 or milli-sized MPs in water, a plate-plate geometry fitted well and elucidated the
638 aggregation behavior, such as the homoaggregation of PE MPs (diameter of 1 mm). DLVO
639 theory could also be employed to quantify the aggregation kinetics of MPs and estimate
640 their Hamaker constants (Romero-Cano et al., 2001). For example, the Hamaker constants
641 of PS NPs at different aging status derived from DLVO theory were found to decrease from
642 3.5 to 1.5×10^{-18} mJ after 3-day of UV irradiation (Liu et al., 2019b).

643 However, after release into aquatic environments, MPs are likely to undergo aging
644 process and as well as heteroaggregation with DOM, bio-colloids, and inorganic colloids,
645 which modify the surface properties of MPs and introduce non-DLVO forces between
646 particles (Alimi et al., 2018). DLVO theory failed to predict the stability of MPs when non-
647 DLVO forces (e.g., hydration forces, osmotic pressure, depletion attraction, or steric forces)
648 existed between two approaching particles or in multivalent electrolytes with high
649 concentrations (Wang et al., 2015). In this way, extended DLVO (eDLVO) theory was
650 proposed to model the complex systems. For example, eDLVO theory well explained the

aggregation of UV-aged PS NPs due to aging-induced increase in hydrophilicity (Liu et al., 2019a).

4. Factors affecting aggregation of microplastics

The physicochemical properties (e.g., hydrophobicity, surface areas, and surface charge) of MPs may change to some extent under the influence of their own properties, and surrounding pH, light irradiation, solid constituents, and other factors (Jódar-Reyes et al., 2006a; Wang et al., 2020). Therefore, the environmental conditions, coexisting solid constituents as well as their own properties could affect the aggregation behavior of MPs in aquatic environments (Jódar-Reyes et al., 2006a; Wu et al., 2019b). Few recent studies pointing out the factors affecting the aggregation of MPs are summarized in **Table 4**.

661 **Table 4** Review of recent studies regarding the aggregation of microplastics

Parameter	Main findings	Ref
Particle size	Smaller MPs are more prone to aggregation at the same conditions. Larger PSNPs-COOH (200 nm) have higher stability than smaller PSNPs-COOH (50 nm) in $CaCl_2$ solution. Smaller PS MPs (0.1-0.6 μm) aggregated rapidly than larger PS MPs (0.8-1.5 μm) in seawater.	(Dong et al., 2018; Song et al., 2019)
Composition	MP composition affects their homoaggregation and heteroaggregation with microalgae. PS NPs are more stable in water than PE NPs. The CCC values of PS NPs were 10 mM for $CaCl_2$, 25 mM for $MgCl_2$, and 800 mM for NaCl, whereas those of PE NPs were lower (e.g., 0.1 mM for $CaCl_2$, 3 mM for $MgCl_2$, and 80 mM for NaCl). The heteroaggregation process between MPs and microalgae was influenced by MP type and EPS excreted by microalgae.	(Cunha et al., 2019; Shams et al., 2020)
Surface modification	Surface modification changed the steric hinderance, hydrophilicity and electrostatic force of plastic particles, and further influenced the aggregation kinetics. The stability decreased in the order of bare PS NPs< PSNPs-NH_2< PSNPs-COOH due to the hydrophilic surface modifications.	(Yu et al., 2019)
Electrolyte type	Regardless the type of plastic particle (PS, PE), the CCC of MPs increased in the order of trivalent cation ($FeCl_3$), divalent cations (e.g., $CaCl_2$, $BaCl_2$, $MgCl_2$), and monovalent cations (e.g., NaCl, KCl). This phenomenon was similar to other colloids and followed the Schulze-Hardy rule.	(Liu et al., 2019b; Shams et al., 2020)
pH	pH influenced the surface charge and electrostatic repulsion of particles via affecting the ionization of surface groups and adsorption of ions. The aggregation rate of PE NPs remain constant from pH 2 to 9. The aggregation of PS NPs with or without surface group modified as the function of pH was different among previous studies.	(Romero-Cano et al., 2001; Shams et al., 2020; Skaf et al., 2020)

DOM	The concentration, size, and surface physiochemical property of DOM determine its impact on the plastic particle stability and mobility. DOM is one of the most significant factors affecting plastic particle aggregation in waters and may overwhelm the effect of physicochemical properties of plastic particle. DOM (e.g., humic acid and fulvic acid) destabilized MPs through the bridging effect or surface charge reverse and stabilized MPs through increasing steric repulsion and electrostatic repulsion. In NaCl solution, DOM stabilized PS NPs and PS-COOH. In $CaCl_2$ solution, DOM (< 5 mg·C·L^{-1}) inhibited the aggregation of PS NPs and PS-COOH and accelerated their aggregation when DOM concentration was higher than 5 mg·C·L^{-1}. In 1 mM $FeCl_3$ solution, DOM promoted the aggregation of PS NPs.	(Song et al., 2019; Wang et al., 2020; Yu et al., 2019)
Surfactant	Plastic particles (e.g., PE fibers and PS particles) were stabilized by surfactants. Different types of surfactants had different stabilization mechanisms. Anionic/cationic surfactants can add sufficient charges to the particle surface and shield the particle from the effect of solution pH changes. Nonionic surfactants rarely shifted the surface charges of particle, but provided steric hindrance force to stabilize particles.	(Jódar-Reyes et al., 2006b; Romero-Cano et al., 2000)
Light	Light can affect the aggregation of plastic particles through aging process. UV irradiation improved the stability of PSNPs in NaCl solution and promoted PSNP aggregation in $CaCl_2$ solution.	(Liu et al., 2019b)
Other factors	Phytoplankton, microorganisms (e.g., bacteria and algae), and inorganic colloids (e.g., clay, suspended sediments and Fe_2O_3) easily adhere to the surfaces of plastic particles and form heteroaggregates. EPS and the size of suspended sediment play an important role in the heteroaggregation processes of MPs. The MP- algae aggregation depends on the species and the physiological state of the algae.	(Long et al., 2015; Mao et al., 2018; Singh et al., 2019; Zhang et al., 2017)

Note: microplastics (MPs), critical coagulation concentration (CCC), dissolved organic matter (DOM), exopolymeric substances (EPS), polystyrene (PS), polyethylene (PE)

4.1 Physicochemical properties of microplastics

4.1.1 Size

Surface charge behavior, electronic structure, surface energy, and surface reactivity are dependent on particle size, which can change the interaction forces between two approaching surfaces (Zhang, 2014). For example, smaller particles are easy to aggregate under a given condition than larger particles because of the higher surface energy of the smaller particles (He et al., 2008). In artificial seawater (35 practical salinity units (PSU)), smaller PS MPs (0.1, 0.4, 0.6 μm) aggregated rapidly with sand, while no heteroaggregation occurred between larger PS MPs (0.8 or 1.5 μm) and sand (Dong et al., 2018). This was primarily because larger PS MPs had higher negative surface charges (~ -30 mV) compared to the smaller MPs (~ -25 mV). DLVO interaction profiles demonstrated that the energy barriers between larger PS MPs and sand (161 k_BT and 296 k_BT) were 1.5-18.5 times higher than that between smaller PS MPs and sand (16 k_BT to 108 k_BT). In low salinity seawater (0 - 3.5 PSU), neither larger nor smaller PS MPs had interaction with sand because all PS MPs were highly negatively charged with zeta potentials of -40~-50 mV (Wang et al., 2020). The DLVO interaction profiles showed high interaction energy barriers (≥ 218 k_BT) existed between all PS MPs and sand (Dong et al., 2018). Larger carboxyl-modified PS NPs (PSNPs-COOH) (diameter 200 nm) with high electrical forces showed higher stability than smaller PSNPs-COOH (diameter 50 nm) in a $CaCl_2$ solution, because increasing particle sizes led to the lower Gibbs free energy and reduced adsorption rate of Ca^{2+} (Song et al., 2019).

4.1.2 Composition

Homoaggregation of MPs could differ due to MP chemical composition probably because the Hamaker constant determining van der Waals forces was composition-dependent (Zhang, 2014). Previous studies showed that PS NPs were more stable than PE NPs in a variety of electrolyte solutions (NaCl, $CaCl_2$, and $MgCl_2$) (Shams et al., 2020). The effect of MP composition on their

heteroaggregation behaviors with microalgae was also investigated. Microalgae could excrete viscous EPS which favored the aggregation with MPs (Long et al., 2015). Cunha et al. (2019) compared the interactions of PMMA MPs and PS MPs with four microalgae, including *Microcystis panniformis*, *Scenedesmus sp*, *Tetraselmis sp*, and *Gloeocapsa sp*. They found that the formation of MPs-microalgae heteroaggregates depended not only on MP type but also on the content and viscosity of EPS. Since *Microcystis panniformis* produced the lowest amount of EPS with low viscosity, they were able to form heteroaggregates with PMMA MPs. *Gloeocapsa sp*. had the capability to aggregate with both PMMA MPs and PS MPs due to the abundant production of viscous EPS. Compared to PE MPs, microalga *Chlamydomas reinhardtii* were more inclined to form heteroaggregates with PP MPs after 20 days because the EPS produced by the alga had a stronger adhesion to PP MPs than PE MPs (Lagarde et al., 2016).

4.1.3 Surface coatings

Functionalization and/or incidental surface coatings are frequently used to stabilize MPs via increasing the electrostatic, steric, or hydrophilic repulsion forces among the particles (Saavedra et al., 2019). For instance, linear poly(ethylene imine) and poly(diallyldimethyl ammonium chloride) improved the stability of sulfate-modified PS MPs in NaCl solution (Shams et al., 2020). The negatively charged PSNPs-COOH rapidly formed aggregates of 1764 ± 409 nm size because of the charge neutralization effect of cations in seawater, whereas positively charged amino-modified PS NPs (PSNPs-NH_2) formed aggregates of only 89 ± 2 nm size (Alimi et al., 2018). The aggregation rate of amidine-modified PS MPs was much faster than sulfate-modified PS MPs in NaCl solution because the former had a weaker electrostatic repulsion than the latter (Montes Ruiz-Cabello et al., 2015). Furthermore, HA was found to have greater destabilizing effects on PSNPs-NH_2 with positive charges than PS NPs and PSNPs-COOH with negative surface charges (Wu et al., 2019a). This was mainly because the negatively charged HA was absorbed on the particle surface, and thus the neutralization

712 effects were greater for positively charged PSNPs-NH_2 (Qu et al., 2010).

713 **4.2 Environmental conditions**

714 **4.2.1 Electrolyte concentration and valence**

715 Electrolyte concentration strongly affects the aggregation behavior of MPs in water by
716 influencing the surface charge of particles (Alimi et al., 2018). For instance, PS NPs of 100 nm size
717 remained stable in 0.01 mM $FeCl_3$ solution, but the particle size of PS NPs increased rapidly up to 350
718 nm with the $FeCl_3$ concentrations increasing to 0.1 and 1 mM (Cai et al., 2018). The enhanced
719 aggregation was mainly due to the compression of the electric double layer and charge shielding on
720 the surface of the particulate plastics, which weakened the repulsive forces between particles (Wu et
721 al., 2019a; Xiao et al., 2018). When the electrolyte concentration exceeded the CCC value, the
722 repulsive interactions were insignificant or absent (Li et al., 2018). As a result, the aggregation rate of
723 MPs changed slightly due to the fact that the aggregation changed from the reaction-limited
724 aggregation regime to the diffusion-limited aggregation regime (Xiao et al., 2018).

725 The valence of ions is also a key factor that controls the aggregation behavior of MPs in water
726 (Wang et al., 2020). For example, the CCC value of PS NPs in NaCl solution (800 mM) was 80-fold
727 and 32-fold higher than those in $CaCl_2$ (10 mM) and $MgCl_2$ (25 mM) solutions, respectively (Shams
728 et al., 2020). The CCC value of PE NPs in NaCl solution (80 mM) was 27-fold higher than that in
729 $MgCl_2$ solution (3 mM). The ion-valence-dependent effect usually follows the Schulze-Hardy rule,
730 which suggests that the aggregation state mainly depends on the ionic valence of the opposite charge
731 to that of colloids (Wu et al., 2019a; Xiao et al., 2018). The aggregation rates of sulfonate-modified
732 PS NPs with the diameter of 115 nm increased in the order of $K^+ < Mg^{2+} < La^{3+}$ (Schneider et al., 2011).

733 **4.2.2 Effect of pH**

734 The pH of the solution can influence the ionization of surface functional groups and surface
735 charge of MPs, which then determines the magnitude of the electrostatic repulsion, and thus affects

the aggregation behavior (Li et al., 2018). The pH in the aquatic environment typically remains between 5 and 9 (Chowdhury et al., 2013). Isoelectric point or zero point of charge is the pH at which the surface of particles has a net neutral charge (Bolan et al., 1999; Hierrezuelo et al., 2010). Most MPs have no isoelectric point, or have isoelectric point below 3, which are away from pH values in natural waters (Li et al., 2018; Skaf et al., 2020; Wang et al., 2020). Thus MPs can be stabilized by electrostatic repulsive forces in the aquatic environment (Hierrezuelo et al., 2010). For example, sulfate-modified PS NPs (100 nm diameter) had no isoelectric point and the particle sizes changed slightly from pH 2.3 to 11 due to strong electrostatic repulsion between particles in ultrapure water (Wang et al., 2020). Similarly, Shams et al. (2020) found that the particle size of PE NPs remained statistically constant from 284.92 ± 140.56 nm to 310.29 ± 165.58 nm from pH 5 to 9 in 10 mM NaCl solution due to strong electrostatic repulsive forces. The hydrodynamic diameters of PS NPs were constant (c.a. 900 nm) in these conditions. While some studies found that the stability of PS NPs (100 nm diameter) and PSNPs-COOH (303 nm diameter) increased with increasing pH in NaCl solution, because more hydroxyl groups could be adsorbed on particle surfaces making the zeta potential values more negative and the electrostatic repulsion higher (Romero-Cano et al., 2001). The discrepancy among these studies may rely on the different production processes, which resulted in the differences of physicochemical properties of the particles such as size, surface chemistry and heterogeneity (Praetorius et al., 2020).

4.2.3 Effect of DOM

DOM is ubiquitous in natural waters and can adsorb onto MP surfaces by hydrophobic interaction, ligand exchange, and electrostatic interaction (Tallec et al., 2019). DOM may change the stability of MPs and make them aggregated or dispersed variably in natural waters depending on the valence of ions and functional groups on MP surface (Cai et al., 2018). Adsorbed DOM can impart a negative surface charge and steric forces among particles in aquatic systems, which subsequently improves the

stability of PS MPs and PSNPs-COOH in the absence of electrolytes (Li et al., 2018; Wu et al., 2019a). HA and fulvic acids (FA) are main fractions of DOM, and are often used as a model DOM (Cai et al., 2018). In ultrapure water, high concentration of HA (30 mg·L^{-1}) induced the aggregation of PSNPs-NH_2 with positive charges because the addition of HA significantly (ANOVA, F= 1497, $p < 0.001$) decreased the zeta potential (from > 50 mV to 24.3 mV) (Tallec et al., 2019). No aggregation of PSNPs-NH_2 was observed at low concentration of HA (1 and 10 mg·L^{-1}) due to the insufficient neutralization effect.

The electrolyte valence played important roles in the aggregation of MPs in DOM (i.e., HA and FA) (Singh et al., 2019). In monovalent electrolyte solutions, DOM provided steric hindrance forces and electrostatic repulsion to stabilize PS NPs and PSNPs-COOH (Yu et al., 2019). Both HA and FA had negligible effects on the aggregation of negatively charged PS NPs in NaCl solution due to high electrostatic repulsive forces between particles (Cai et al., 2018). HA in the concentration range of 0-50 mg·L^{-1} promoted the aggregation of positively charged PSNPs-NH_2 in NaCl solution owing to the charge neutralization effect (Wu et al., 2019a). In the presence of divalent or trivalent metal ions, DOM destabilized MPs through the bridge attraction and intermolecular bridging between metal ions and surface functional groups of DOM (Li et al., 2018). For example, in $CaCl_2$ solution, DOM at low-concentration (< 5 mg·C·L^{-1}) inhibited the aggregation of PS NPs through steric repulsion, but accelerated the aggregation at high DOM concentrations (> 5 mg·C·L^{-1}) through the complexation between Ca^{2+} and carboxyl groups of DOM (Singh et al., 2019; Yu et al., 2019). Similarly, in 1 mM $FeCl_3$ solution, the complexion reaction between Fe^{3+} and carboxyl groups of DOM (i.e., HA and FA) adsorbed on PS NP surfaces decreased their stability (Cai et al., 2018).

In the mixture of artificial seawater and HA, the hydrodynamic diameters of PSNPs-COOH and PS NPs increased significantly ($p < 0.001$) with sizes exceeding 1 μm in 24 h due to the charge neutralization effect of divalent cations and bridging effect (Tallec et al., 2019). However, HA

significantly decreased the hydrodynamic diameters of PS NPs ($p < 0.001$) in artificial seawater in 24 h to 48 h, causing a stabilizing effect and partial disaggregation presumably due to the increase of steric repulsion forces (Qu et al., 2010). In freshwater, high concentration of nature organic matter, such as alginate and HA, can alleviate the toxicity to zooplankton through increasing stability of MPs or forming an eco-corona on MP surface (Saavedra et al., 2019; Wu et al., 2019a).

4.2.4 Effect of surfactant

Surfactants are widely used in domestic and industrial products, and they are frequently used reagents to disperse MPs in water (Skaf et al., 2020). Electrostatic repulsion played a dominant role in increasing colloidal stability, and thereby varying aggregation rates when ionic surfactants covered MPs (Jódar-Reyes et al., 2006b). A cationic surfactant, domiphen bromide, resulted in the aggregation of negatively charged PSNPs-COOH particles due to the charge neutralization effect (Jódar-Reyes et al., 2006b). Similarly, an anionic surfactant, sodium dodecylbenzenesulfonate, promoted the aggregation of positively charged amphoteric PS MPs (diameter around 350 nm). In both the above cases, electrostatic repulsion forces were reduced among the plastic particles due to the presence of surfactant molecules (Jódar-Reyes et al., 2006b). Additionally, the adsorption of non-ionic surfactants on latex particles at high surface coverage could act as a steric stabilizer, and thus improve the stability of PS MPs (Romero-Cano et al., 2000).

4.2.5 Effect of light

Solar irradiation, especially the UVA fraction of sunlight, was found to change the physicochemical properties (surface polarity and functional groups) of NPs and alter their aggregation behavior in water (Liu et al., 2019b). The stability of PS NPs was improved in NaCl solution but decreased in $CaCl_2$ solution after exposure to UV light. The high stability of PS NPs in NaCl solution was likely because of the following three reasons: (1) lowered van der Waals attraction between aged PS NPs because of their decreased Hamaker constants; (2) increased electrostatic forces due to

deprotonation of oxygen-containing functional groups generated on aged PS NPs; and (3) enhanced steric hindrance forces between UV-exposed PS NPs rendered by the leaching organic matter (Liu et al., 2019b). The aggregation of PS NPs was promoted in $CaCl_2$ solution primarily because Ca^{2+} could complex with oxygen-containing functional groups such as carbonyl and carboxyl groups, that were formed on UV-irradiated PS NP surfaces (Qu et al., 2010).

4.3. Other factors

Microorganisms, phytoplankton and suspended sediments widely existing in natural waters can remarkably interfere with the aggregation states of MPs (Long et al., 2015; Zhang and Chen, 2019). The negatively charged algae *Chlorella sp*. was found to form heteroaggregates with positively charged PSNPs-NH_2 particles but not with negatively charged PSNPs-COOH (Thiagarajan et al., 2019). Cellulose is a component of the cell wall of many algal species such as *Chlorella* and *Scenedesmus* (Bhattacharya et al., 2010). If suspended in water, cellulose can initiate heteroaggregation between algal cells and MPs. Similarly, EPS enhanced the heteroaggregation of PS MPs and PVC MPs with *Chlorella pyrenoidosa* and *Skeletonema costatum* cells (Mao et al., 2018; Zhang et al., 2017). In addition to microorganisms, Li et al. (2019) indicated that suspended sediment formed heteroaggregates with PS NPs and promoted settling of plastic particles in NaCl solution. However, the interaction between PE MPs and suspended sediments was minor, and PE MPs floated on the water surface for 8 months after the addition of 500 $mg \cdot L^{-1}$ suspended sediments.

5. Environmental implications of MP aggregation

Investigating the aggregation behavior of MPs is of great importance to elucidate their potential environmental implications after their release into natural waters (Rummel et al., 2017). Significant consequences concerning the aggregation of MPs are their altered particle sizes and specific surface areas, which subsequently impact their toxicity toward organisms, their own transformations, co-transport with other pollutants, and formation of biofilm.

5.1 Transport and transformation of microplastics

The aggregation of MPs determines their distribution in natural waters (Bhattacharya et al., 2010). Nano- and micro-sized particulate plastics floating on the water surface can form aggregates with microbial communities or plankton, which may affect the density of the plastics and change their depth in natural waters (Alimi et al., 2018). Aggregates of MPs located at different water depths undergo different degrees of weathering from solar irradiation, biological degradation, mechanical wearing, and pyrolysis, which results in different mass losses or degradation rates of the plastic aggregates (Alimi et al., 2018). Solar irradiation plays a primary role in the photodegradation and pyrolysis of plastic aggregates in the upper surface of natural waters (Chatani et al., 2014). The plastic aggregates can absorb sunlight and generate free radicals through impurities, which lead MPs to break into small fragments with low molecular weights (Zhao et al., 2018). Plastic aggregates settling to the seabed are not affected by illumination, but the complex hydrodynamic processes and microorganisms significantly affect the physicochemical properties and fates of the bottom plastic aggregates (Zhao et al., 2018).

5.2 Toxicity to organisms

The toxicity of MPs to organisms depends on their aggregate size (Fan et al., 2019). The aggregated MPs could be less bioavailable to aquatic organisms because the toxicity of particles was inversely proportional to size in general (Choi et al., 2019). In natural seawater, PSNPs-NH_2 formed nano-sized aggregates (< 200 nm) inducing death of brine shrimps in 14 d (LC_{50} = 0.83 μg·mL^{-1}) and inhibition of algal growth (EC_{50} = 12.97 μg·mL^{-1}) (Bergami et al., 2017). By contrast, PSNPs-COOH rapidly formed micro-sized aggregates (> 1 μm), thereby greatly reduced the toxicity to brine shrimps (LC_{50} > 10 μg·mL^{-1}) or microalgae (EC_{50} > 50 μg·mL^{-1}). It should be noted that MP aggregates outside the organisms could exert negative effect (Wu et al., 2019a; Zhu et al., 2019). For instance, MP aggregates inhibited the photosynthesis process and limited the transfer of nutriment and energy of

microalgae in marine ecosystem (Zhu et al., 2019). In addition, the MP-biota heteroaggregates could cause physical damage such as split and oxidative stress toward organisms (Choi et al., 2019; Zhu et al., 2019). The positively charged PS NPs were more prone to aggregate with marine bacterium (*Halomonas alkaliphile*) via electrostatic attraction, which induced higher toxicity than negatively charged PS NPs (Sun et al., 2018).

The aggregate dimensions of MPs in natural waters fall in the range from nanometers to centimeters or larger, and this could lead to their toxicity toward organisms in different layers of water column (Browne et al., 2008; Ward and Kach, 2009). If MPs aggregated slightly, Brownian motion can keep them suspended on the water surface for months or even longer (Li et al., 2019). The suspended or floated plastic aggregates pose negative effect on zooplankton, planktivory, filter feeders or suspension-feeders. Plastic particles with a large degree of aggregation settle quickly and accumulate in the seabed and sediments (Bergami et al., 2017). Colonization of MPs with organisms results in their higher densities and sinking to the benthos (Galloway et al., 2017). They eventually exert toxic effect on deposit feeders and detritivores that inhabit in the seabed. Because many toxic pollutants could be adsorbed on the surface of sinking plastic aggregates, the detoxification effect for organisms living in the water surface could occur, but toxic effects would transfer to the benthic organisms (Zhang et al., 2017).

The regional variation in biotoxicity of MPs can be assessed by toxicity assay of organisms exposed to MPs in the laboratory through three steps (Bergami et al., 2017; Saavedra et al., 2019). First, water samples or aquatic organisms from different layers of natural waters are collected (de Sá et al., 2018). Second, the type, shape, size and concentration of MPs in those field samples are analyzed (Nan et al., 2020). Third, MPs collected in the field or pristine MPs having similar physicochemical properties with those in the field are selected to examine the ecotoxicity of MPs toward model organisms in the laboratory (Sendra et al., 2019; Zhu et al., 2019). In general, the most frequently

applied model organisms include planktonic crustacean (*Daphnia magna*) as zooplankton, fish (*Danio rerio* and *Pomatoschistus microps*) as organisms in water column, and mollusc (blue mussel *M. edulis*) as benthic organisms (de Sá et al., 2018).

5.3 Transport of contaminants

MPs can transport pollutants in the following two ways. Firstly, the additives, monomers, and non-intentionally added substances (e.g., flame retardants, phthalate, plasticizers and heave metals) can be leached by MPs (Suhrhoff and Scholz-Böttcher, 2016; Xu et al., 2020). Some of these contaminants are extremely harmful toward organisms (Suhrhoff and Scholz-Böttcher, 2016). Secondly, MPs may act as a transport vector for organic pollutants and heavy metals because MPs may adsorb these pollutants on their surfaces (Cole et al., 2011). Aggregation of MPs may alter their particle sizes and surface areas, which is a key factor determining the transport of the released additives and the adsorbed contaminants (Yang et al., 2018). Compared to large aggregates, small aggregates of MPs with larger surface areas and more reactive sites may release more contaminants and degrade faster (Chen et al., 2019). For example, organotin compounds (dimethyltin and dibutyltin) were released from PVC MPs under UV and visible light irradiation, and the released content of organotin by small sized PVC MPs (10 μm) was nearly 1000-fold higher than that by large sized PVC MPs (300 μm) after 56-h UV or visible light irradiation (Chen et al., 2019).

5.4 Microbial habitation and biofilm formation

Plastic surfaces exhibit hydrophobicity that facilitates DOM adsorption in aquatic ecosystems. Biofilms of microorganisms can then form on plastic surfaces due to abundant carbon and nutrient sources in the DOM (Harrison et al., 2018). Microbial habitation of MPs and subsequent biofilm formation facilitates the aggregation of MPs, which has several implications that include (**Fig. 5**): (1) degradation of MPs and the associated contaminants; (2) horizontal gene transfer between microorganisms; (3) toxicity of contaminants associated with MPs; and (4) migration or spreading of

microorganisms especially in aquatic ecosystems (Oberbeckmann et al., 2015; Rummel et al., 2017). Biodegradation of MPs and consequent release of associated organic contaminants are accelerated by certain organisms present in the biofilm. Moreover, there is growing evidence that the microbial habitation in the biofilm can promote gene exchange (Jacquin et al., 2019); so determining the potential of biofilms for providing the surface for anti-microbial resistance gene transfer is important. Adsorption and desorption processes of pollutants on MP surface in water are influenced by factors such as microbial activity, MP weathering and surface area, and interaction with DOM. MPs provide a more robust vehicle than biodegradable biotic substrates for the transport of organisms over long distances, which can facilitate the movement of microorganisms to different ecosystems and potentially introduce invasive and exotic species (Viršek et al., 2017).

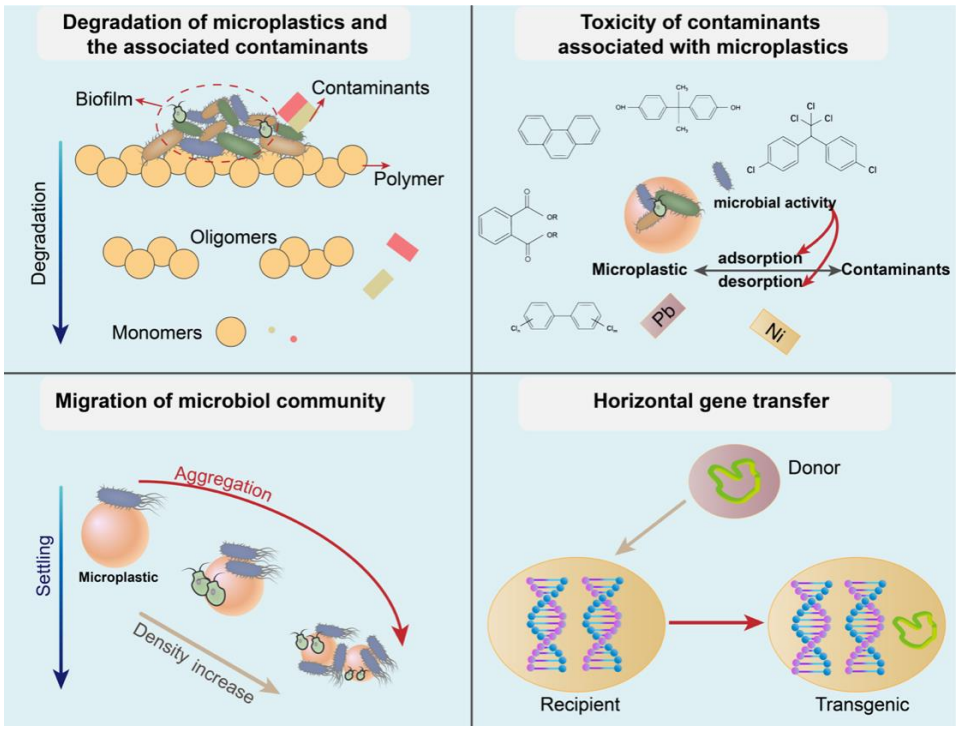


Fig. 5. Implications of microbial habitation and biofilm formation on microplastics.

5.5 Environmental management

The wide distribution and ecological risk of MPs in aquatic environment are a symptom of mismanagement of plastic wastes, particularly MP debris (Karbalaei et al., 2018). Despite the policies

and regulations in many countries are implemented to reduce the risk of MPs, the action plans have not considered the potential impacts resulting from the aggregation behaviors of MPs (Auta et al., 2017; Praetorius et al., 2020). In addition, studies have not been conducted extensively on MP aggregation in the field (Li et al., 2019). Therefore it is imperative to investigate the aggregation of MPs in environmentally relevant conditions and/or in the field. On the other hand, the need for MP cleaning-up strategy should be considered. Based on systematic investigations of MP aggregation, the removal efficiency of MPs in WWTPs and drinking water treatment via flocculation processes can be promoted (Skaf et al., 2020; Zhang and Chen, 2019). Studies have shown that MP surfaces are suitable substrates for microbial growth, thus biodegradation can be harnessed to degrade MPs (Auta et al., 2017). Manufacturers are encouraged to produce plastics with biodegradable materials (Gallo et al., 2018).

6. Knowledge gaps and future recommendations

From numerous studies concerning MPs in the environment, it is evident that although our understanding of MP aggregation in water environments is advancing, currently no standard methods exist for MP separation and identification. Similarly, studies concerning the mobility of MPs in real environments are far from fully understood. To fill these knowledge gaps and fully reveal the influences of plastic pollution on the environment, following most important research issues deserve immediate attention:

6.1 More field studies

Most published works focused on the investigations of stability and mobility of MPs in laboratory scales with only a few works studying the aggregation of MPs in environmentally relevant systems, especially in natural surface waters, groundwater, or WWTPs. The natural water system is complex and contains DOM, microorganisms, mixtures of contaminants, and the laboratory research using simulated natural waters may misestimate MPs aggregation behaviors. There is an urgent need to study

the homoaggregation and heteroaggregation behaviors of primary and secondary MPs in the field to understand how UV weathering and biological processes impact the stability of MPs in natural waters. In addition, river sediments are important sinks for MPs. Further work is therefore needed to better understand the aggregation of MPs in these complex heterogeneous media. Most of the laboratory aggregation studies have been conducted over one hour, but this only provides an initial snapshot of the aggregate. Therefore, it is necessary to perform temporal stability experiments which would allow to evaluate long-term impact of MPs.

6.2 Use microplastics in laboratory works representing those in the environment

Notably, the majority of existing laboratory studies on the aggregation of MPs have used commercial and spherical PS particles with sulfate, amine or carboxyl groups, which are less likely to exist in natural waters (Wang et al., 2020). Most MPs in the environment are expected to have non-spherical shapes (e.g., fibers, strings, pellets, films, thin sheets) and varying compositions (e.g., PE, PP, PVC, polyethylene terephthalate). Therefore, future aggregation research should consider the diversity of MPs to better elucidate their mobility and associated behaviors. In addition, the aggregation behaviors of environmentally relevant secondary MPs comprised of fragments, fibers, films, and rods warrant further examination. Many previous studies on the aggregation of MPs in water have applied extremely high doses of MPs which do not reflect real-world conditions, and resulted in misinterpretation of the results. Researchers are suggested to use environmentally realistic concentration of MPs to yield more realistic estimates of MPs' impacts in aquatic environments.

6.3 Standardization of identification and quantification methods

Visual observation is one of the most frequently used techniques for distinguishing between the homoaggregation and heteroaggregation with other solid constituents in water. However, nearly 70% of small particles, which are characterized visually as MPs, are not confirmed as plastics by chemical detection methods. Therefore, the spectroscopic techniques such as EDS, FTIR, Raman spectroscopy

are strongly recommended to combine with visual observations for a reliable characterization of MP stability in water environments. In addition, the commonly applied visual observations are insufficient to investigate the aggregation of MPs in environmental samples. Small-sized MPs, especially for the case of MPs <500 μm size, remain poorly understood because of the limitations of existing detection methods and their low resolution for particles at nanoscale. Because the standardized methods for sample collection, identification, and quantification are still in their infancy, comparison of results among different works becomes difficult. Therefore, new research methods should be carefully collated together to formulate standard protocols.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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