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Microplastic quantification affected by structure and pore size of filters

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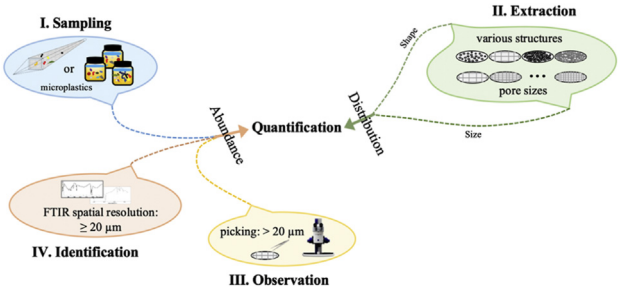
HIGHLIGHTS

- Filter structure can affect abundance of retained microplastics of different shapes.
- Small pore size filter leads to high abundance and wide size range of microplastic.
- Filtering with 20 μm filter are efficient for field investigation of microplastic.

GRAPHICAL ABSTRACT



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ABSTRACT

Filters of various structures (filter by pore depth or pore width) and pore sizes are used to extract microplastics (<5 mm) in researches. In present study, we demonstrate that filters with different structures and pore sizes can lead to different outcomes in microplastic filtering. Our results showed that when filtering large-sized microplastics, nylon filter (double-layer-hole type) retained nearly 100% of fibers, while polycarbonate filter (single-layer-hole type) only retained 61.7%. Polycarbonate filter retained the most fragments (80.8%), while cotton fiber filter (multilayer-hole type) retained the least (54.4%). Pellets were retained on different layers of nylon and cotton fiber filters, and could not be quantified accurately. Additionally, the sizes of some fibers and fragments captured were not within the expected ranges by lattice-knitting filters. Large fiber (3568.0 μm) was not filtered out after 1000 μm pore-size filtration. Small fragment (37.2 μm) was found on 50 μm pore-size filters. To validate laboratory results, filed waters containing microplastics (~90% in form of fibers) were filtered through different pore-size filters. As expected, the relationship between abundance and pore size followed a same trend as that in laboratory fiber samples. Thereby, our results indicated that filter structure and pore size could affect the abundances of microplastics with different shapes. To obtain more accurate abundance of microplastics in a wide size range, and to consider filtration duration, size limitation of observation, and spatial resolution of identification instrument, we recommend that water samples should be filtered using 20 μm pore-size filters with a double-layer-hole type of structure.

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1. Introduction

Microplastics are emerging pollutants widely found in many environmental systems and organisms (Auta et al., 2017; Hu et al., 2018; Lares et al., 2018; Li et al., 2019; Simon et al., 2018; Su et al., 2019). In field investigations, filtration including sampling and pretreatment is commonly used to extract microplastics from environmental matrices (Cincinelli et al., 2017; Lourenço et al., 2017; Schymanski et al., 2018). After filtration, particles in field samples have to be retained on the filter for qualitative (color, shape, and component identification) and quantitative analysis (abundance and size distribution/range) (Hidalgo-Ruz et al., 2012; Tagg et al., 2015; da Costa Araújo et al., 2020a; da Costa Araújo et al., 2020b). However, there is no uniform protocol describing a sampling method and especially, the types of mesh/membrane filters to use. In some investigations, large-volume water samples were mass-filtered or -sieved *in situ* (e.g., manta-trawls, neuston nets, and continuous intake on large research vessels) (GESAMP, 2015; Steer et al., 2017). In others, small-volume water samples were collected, and then filtered *in situ* or in the laboratory (Löder and Gerdts, 2015; Miller et al., 2017). After sampling, diverse filters were used for extracting microplastics and were made of nylon, nitrocellulose, glass fiber, polycarbonate, or stainless steel (Cincinelli et al., 2017; Barrows et al., 2018; Hu et al., 2018; Long et al., 2019; Vermaire et al., 2017). Based on the ways particles being retained, membrane filters are classified into two types, according to pore depth and pore width. The pore-depth filters include stainless-steel mesh, nylon, glass fiber/cotton fiber, and nitrocellulose/mixed cellulose filters. These filters are also different in terms of structure. The structure of nylon membrane and of stainless-steel mesh is like lattice knitting; the one of mixed cellulose/nitrocellulose is a multilayer-hole type; and the one of glass/cotton fiber is formed with high pressure. Their pore canals are deep and curvy; their pore size is an average value measured with the Bubble Point Method, which uses bubbles as the model spheres (Yu et al., 2010). The pore-width structure type has actual pore size (e.g., polycarbonate membrane), which means their pores on filters are circular, and the canals are shallow and straight. The pore size (i.e., the diameter of circular pore) measured using a microscope is the actual pore size of membrane filter (Wyart et al., 2008). However, in field samples, the prevalent shapes of microplastics are fibers and fragments, which are different from the shape of the model spheres used for pore size measurement (Hendrickson et al., 2018).

In addition to the structure of filter, the pore size of filter is also an important factor for filtration. Researchers generally use large size meshes (300–350 μm) which allow investigations for large size fraction of microplastics, but loss of small size fraction (Bouwmeester et al., 2015). Large pore size mesh/membrane filter will allow small particles and long fibers to pass through (due to relatively small widths of these fibers), resulting in underestimated concentration of microplastics (Barrows et al., 2017; Kang et al., 2015; Sighicelli et al., 2018). Moreover, the majority of microplastics ingested by marine invertebrates and fish are often smaller than 300 μm in diameter (Leslie et al., 2017; Wieczorek et al., 2018). For small size filters (pore size <1 μm), impurities in water samples are likely to accumulate during filtration and make microplastics nearly invisible on the filter surface. Especially, the duration of filtration and difficulties during sample observation will greatly increase in case of samples with a high turbidity and/or a rich biota. Lost and invisible microplastics will lead to an underestimated abundance and a misrepresented size distribution in the field samples; this effect is in discordance with the original consideration of using small pore size filter.

In the present paper, filters with different structures and pore sizes were tested in laboratory, on samples containing microplastics with different shapes. The results were validated using field samples. We propose a hypothesis that the structure types and pore sizes of membrane/mesh filters could have effects on the quantification of microplastics. We also recommend more efficient extracting pore size for field investigation.

2. Materials and methods

2.1. Preparation and observation of original microplastic samples

2.1.1. New filter holder used in experiments

The filter holder used in this study was made with stainless steel (Fig. S1A). The pore size of this filter holder is larger than that of the filter holder made with glass (Fig. S1C). During the filtration, all used containers were flashed three times with Milli-Q water to ensure that microplastics were all removed to the membrane filters.

2.1.2. Preparation of original microplastic samples

Plastic items used in this study were purchased from the market. Fiber and sheet plastics were washed with Milli-Q water for three times, and then were dried in an incubator at 60 °C for 24 h. For fiber preparation, 2 g of red polyacrylonitrile (PAN) fibers were cut into tiny pieces using scissors in beakers (Cai et al., 2019). Then, 50 mL of ultra-pure water (Milli-Q) was poured into the beaker for following screening. To prepare fragments, 2 g of yellow polyvinyl chloride (PVC) plastic sheets were firstly scissored into small pieces (about 1 cm long) in a beaker with 200 mL of Milli-Q water. Afterwards, an immersion blender (Bosch ErgoMixx 600W (E-nr: MSME6110CN), Robert Bosch Hausgeräte GmbH, Germany) was used to mix the plastic pieces with water for 10 min at room temperature (25 °C) (Ekvall et al., 2019). The obtained suspension was poured into a new beaker for later use. Beakers with smaller plastic pieces were covered with aluminum foil to avoid microplastic contamination from air.

The preparation of microplastics included two steps. Firstly, the fiber/fragment suspension was filtered through a large pore size membrane (100 μm) or mesh (1000 μm). Secondly, the filtrate was filtered again through a small pore size membrane (50 μm), the plastic pieces retained on the membrane were washed and poured into a beaker. Then, Milli-Q water was added for a total volume of 500 mL. Hence, theoretically, the particles used in the membrane filtration, including the structure experiment and pore size experiment were in the range of 50–100 μm. The particles used in the mesh filtration experiments were in the range of 50–1000 μm (Table S1). Standard and fluorescently polystyrene (PS) microspheres (green 468 nm excitation/508 nm emission and 1.05 g/cm^3 in density, G1000) with a diameter of 10 μm were purchased from the Duke Scientific Corporation. PS microspheres were only used in structure experiment. One hundred microliters of PS were added to 500 mL Milli-Q water as the original solution for later use. To sum up, we prepared red PAN fibers, yellow PVC fragments, and green fluorescent PS pellets in Milli-Q water as our original samples for following experiments.

2.1.3. Observation and quantification of original samples

The original samples were firstly ultrasound dispersed in an ultrasonic bath (KQ-500E, Shumei, China) at room temperature (25 °C) for 10 min, just before filtration. Twenty milliliters of the original solution were filtered through a 0.45 μm nitrocellulose membrane with grids (Table S1), and the abundance (item/L) of particles was counted in each of these grids. The beakers containing

original samples were put back into ultrasonic bath after each filtration to ensure optimal dispersion of microplastics. The microplastics were counted directly based on their different morphologies with typical contaminants on filter using a Carl Zeiss Discovery V8 Stereomicroscope (MicroImaging GmbH, Göttingen, Germany) (Fig. S2), and images were taken with an AxioCam digital camera. The PS pellets were observed under an Olympus BX53 fluorescence microscope (Olympus Optical Co., Ltd, Tokyo, Japan), and the images were taken with an Olympus DP 80 camera (ex: 460–500 nm, em: 510–560 nm). When we obtained the abundance, the visible light was turned on to make sure that the grids can be seen and that the PS pellets were still green under the fluorescence microscope. Each abundance is the average value of triplicate samples.

Images of all particles on membrane filters were taken, and then the sizes of the largest and the smallest particles on each image were measured with ImageJ 1.48 software (Rasband, 2014). Generally, the largest dimension of a microplastic particle is defined as its size (Hartmann et al., 2019). The size range of the original samples was determined by the recorded sizes of the particles.

2.2. Filtration, observation, and quantification of laboratory samples

Considering accessibility of membranes on the market, four membrane filters with the same pore size of 8 μm, but different structures were used for structure experiment. We used Scanning Electron Microscopy (SEM, S-4800, HITACHI, Japan) operating at 3.0 kV to obtain the surface structure of these four filters. They were lattice-knitting/double-layer-hole type (nylon, NY), multilayer-hole type (mixed cellulose with the same structure as nitrocellulose, MC), pressure-formed type (cotton fiber with the same structure as glass fiber, CF), and single-layer-hole type (polycarbonate, PC) (Fig. 1). In structure experiment, the original samples were ultrasound dispersed at 25 °C for 10 min just before filtration. Twenty milliliters of the original solution with micron-sized fibers, fragments, and pellets were filtered in triplicate through each structure.

In addition, nine pore sizes of membranes and meshes (NY membranes: 0.45, 5, 20, 50 μm; stainless-steel meshes: 63, 100, 200, 300, 500 μm) were used for pore size study. The selected NY membranes and stainless-steel meshes have the same structure. More detailed information about membrane and mesh filters is presented in Table S1. Twenty milliliters of the fiber and fragment original solution were filtered through these nine membranes and meshes in triplicate. After filtration, each membrane was put on a piece of paper with grids. The top light of stereomicroscope was turned on to observe the morphology of fibers and fragments (Fig. S3A$_{1\text{-}2}$), and the bottom light was turned on to obtain the abundance of particles by counting them in each grid (Fig. S3B$_{1\text{-}2}$).

The retention efficiency (%) was calculated according to Eq. (1) and presented the ability of membrane/mesh to retain particles and the modification in terms of particle abundance during the filtration:

$$\text{Retention efficiency } (\%) = \frac{A1}{A2} \times 100\% \quad (1)$$

In Eq. (1), A1 is the abundance of particles on membrane/mesh after filtration, and A2 is the abundance of particles on membrane/mesh before filtration. The size ranges of fibers and fragments after each filtration were recorded using the same method as that for the original samples in section 2.1.3.

2.3. Validation using field samples

2.3.1. Duration of filtration

The duration of filtration was recorded by a timer (PC 3860, Tianfu Co., Ltd), using two decimal points. The duration included the process of changing membranes, flushing membranes/meshes and filtration bottles during the filtration. In field sample filtration, the time was recorded every 500 mL of sample (with a calibrated filter flask, Fig. S1A) passing through the filter to study the relationship between volume of filtered water and duration.

2.3.2. Filtration, observation, and identification of field samples

Field water samples were collected from the same site by the Yingtao River shore in Shanghai (121°26′41" E, 31°1′45" N) to validate the relationship between the abundance of microplastics and the pore size of filters. This river is an urbanized river which is a tributary of Huangpu River going through the campus. Briefly, 5 L glass bottles were filled with surface water (0–10 cm in depth) using a steel bucket. Afterwards, we transferred buckets to laboratory within 20 min without cold storage. The water samples were poured through NY membranes of different pore sizes (5, 20, 50 μm) and through stainless-steel meshes (63, 100, 200, 300, 500 μm) (Table S1). To avoid the particles on the membrane filters being covered by sand and clay, an adjusted filtration method was used in 5 and 20 μm membrane filtration, namely, a new membrane was used once the sand and clay on the membrane were too thick and particles were difficult to spot. The particles on the meshes were washed into glass flasks and then poured through 5 μm SMWP membranes for later observation (Table S1). To quantify the air contamination, procedural laboratory blanks were run in parallel for the field samples. In brief, 1 L of Milli-Q water filtered through same filters as those used for parallel field samples. Triplicate samples were processed and analyzed as described for filed water samples (Simon et al., 2018).

All samples were observed under the stereomicroscope. The chemical compositions of the original products and the field samples were confirmed by μ- Fourier Transform Infrared spectroscopy (FTIR, Nicolet iN 10, Thermo Fisher, USA) under transmittance mode. The spectral resolution was 4 cm^{-1} and the scan time was 12 s. The polymer types of particles were acquired on this instrument according to a commercial library using the spectral range of 4000–600 cm^{-1}. The lowest acceptable Hit Quality was set as 70% (Renner et al., 2017).

2.4. Statistical analysis

Statistical analysis was conducted using SPSS 23.0 software (IBM Corp, Armonk, NY, USA). Data were first tested with a normality test (ShapiroeWilk test) and analyzed by a one-way ANOVA followed by an LSD post-hoc test for paired comparisons, $p < 0.05$ was considered significance. The correlation was conducted using Origin 9.0 software (OriginLab Corp, Northampton, Massachusetts, USA). Coefficient of determination (R^2) was calculated for the relationship between abundances/filtration duration and pore sizes (pore size as X, abundance/filtration duration as Y); for the relationship between filtration duration and volume of water samples (volume as X, filtration duration as Y).

3. Results

3.1. Different positions of microplastics captured by different structures of membrane filters

During membrane structure study, some fibers penetrated into

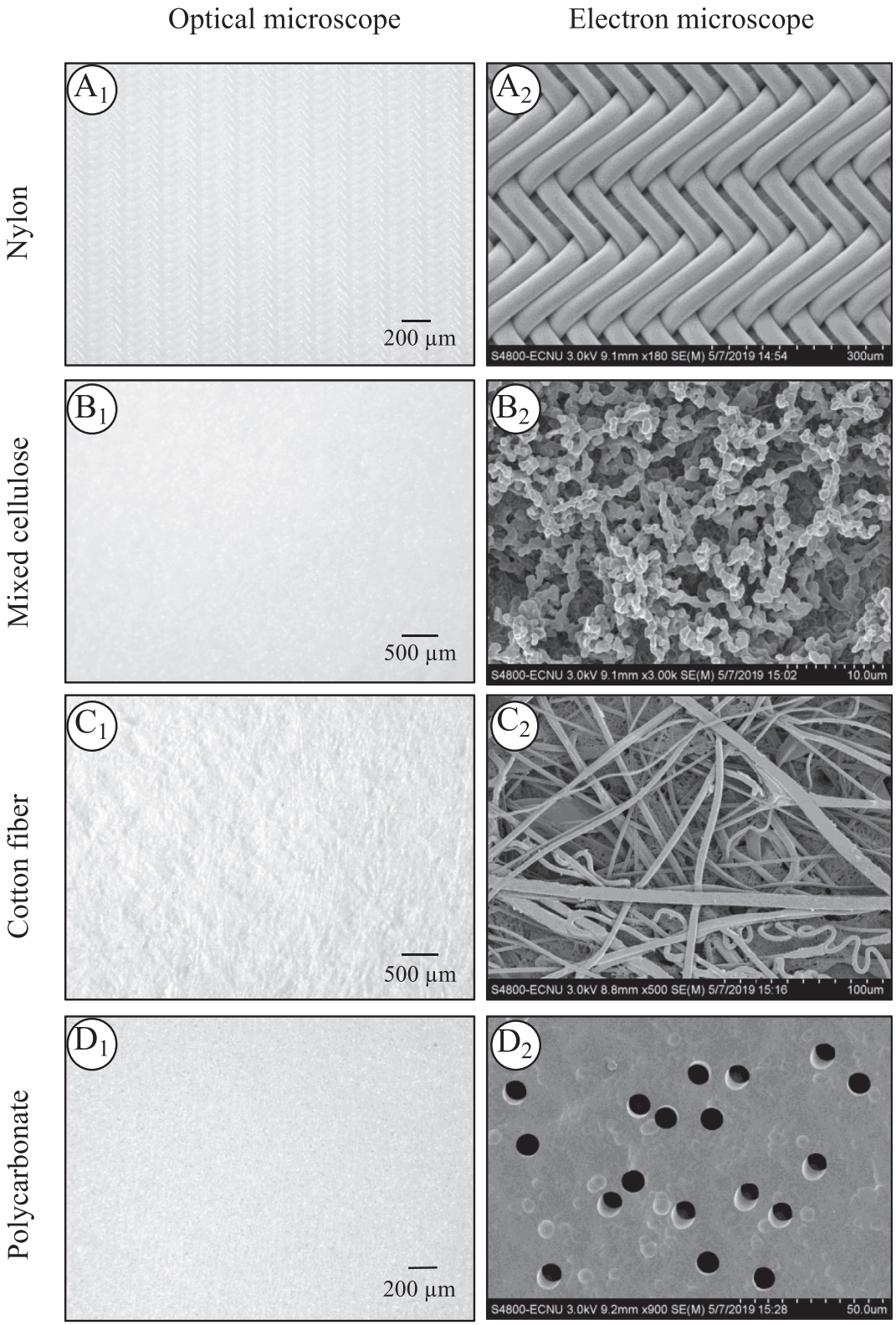

Fig. 1. Surface morphology of four structures of membrane filters (A: nylon; B: mixed cellulose; C: cotton fiber; D: polycarbonate) under optical microscope (A_1-D_1) and electron microscope (A_2-D_2).

the pores of the filters. The upper part of the fiber was observed under the stereomicroscope, but the lower part was not on the same focal plane (Fig. 2$A_{1\text{-}2}$). Small PVC fragments were attached to the mesh pores, which were larger than the fragments (Fig. 2$B_{1\text{-}2}$).

Some PS pellets clustered in the nets of the upper and lower layers in NY membrane after filtration (Fig. 3A_2); some pellets clustered in many focal planes of CF membrane and looked like they were buried in the multilayers of CF (Fig. 3B_2). The

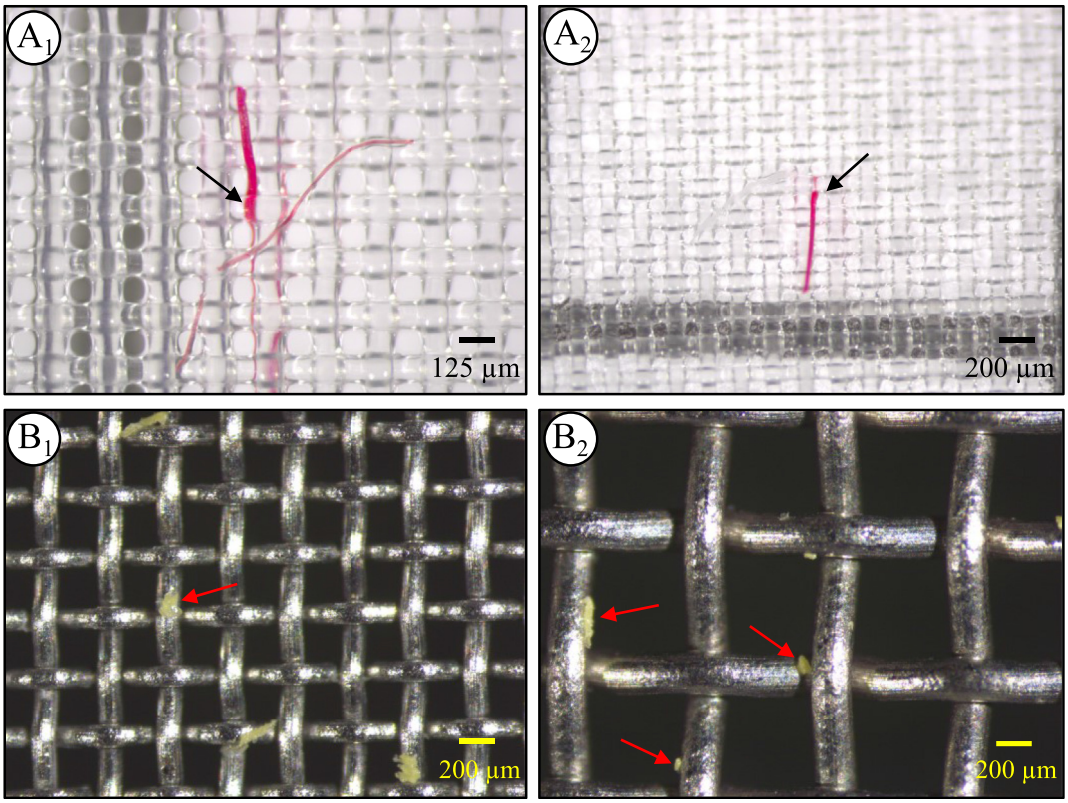

Fig. 2. Various results shown by filtration. Note that fibers can go through pores smaller than their sizes (A_1 and A_2), and fragments can be retained on mesh larger than their sizes (B_1 and B_2). The scale bar is at the lower right corner of each panel.

distributions of PS pellets on NY and CF reflected the structures of the membrane filters (Fig. 3$A_{1\text{-}2}$, Fig. 3$B_{1\text{-}2}$). The pellets were mostly on the same focal plane in a microdomain on the PC membrane, and the shape of the pellet agglomeration area matched the pore shape of the stainless-steel filter holder (Fig. S1C, Fig. 3C_2). The pellets on MC were mostly on the same focal plane.

3.2. Retention efficiency and size range of microplastics in laboratory samples

The retention efficiencies of fibers, fragments, and pellets were different. In the fiber groups, NY showed the highest retention efficiency (99.2% on average), and PC showed the lowest (61.7% on average). PC also showed a significant difference from NY ($p = 0.003$), MC ($p = 0.009$), and CF ($p = 0.011$). The other three types of membranes were similar in retention efficiency (Fig. 4A). In the fragment groups, PC showed the highest retention efficiency (80.8% on average), and CF showed the lowest (54.4% on average). The retention efficiency of PC significantly differed from those of NY ($p = 0.043$), MC ($p = 0.038$), and CF ($p = 0.07$). Like the fiber groups, the three types of filters, NY, MC, and CF, were similar in retention efficiency (Fig. 4B). In the pellet groups, the retention efficiencies of PC and MC did not show any significant difference (Fig. 4C). Because NY and CF have the double-layer/multilayer structure, the number of pellets could not be counted accurately under the microscope (Fig. 3A_2, B_2).

For the pore size study, the retention efficiency of fiber groups decreased with increasing pore size following an S-curve, which was substantially a logistic model ($R^2 = 0.980$, Fig. 5A). When the pore size was in the range of 100–300 μm, the retention efficiency decreased rapidly with increasing pore size. The retention efficiency of fragment decreased with increasing pore size, following a power function. The curve reached a plateau when the pore size was smaller than 50 μm ($R^2 = 0.989$, Fig. 5A).

The sizes of some fibers and fragments were not within the expected size ranges after filtration. When the original samples were filtered, the size ranges of captured particles were beyond the limits (50–100 μm for membrane filtration, and 50–1000 μm for mesh filtration). The actual size range of original fiber samples was 39.7–2176.7 μm, and that of fragment was 44.2–334.8 μm for membrane filtration. The actual size range of fibers was 48.7–3977.9 μm, and that of fragments was 33.0–596.5 μm in original samples for mesh filtration. After gradient pore size filtration, the size range of fibers was supposed to be 39.7–2176.7 μm in the 0.45, 5, and 20 μm filter groups; this size range is the same as that of the original fiber sample (theoretical size range). It was supposed to be 50.0–2176.7 μm in 50 μm membrane group; to be pore size-3977.9 μm in the rest of the filter groups. After filtration, the largest fiber was found in the 63 μm mesh filtration group (3568.0 μm), and the smallest (37.2 μm) was found in the 50 μm membrane filtration group (Fig. 5B). The size range of fragments was supposed to be 33.0–334.8 μm in the 0.45, 5, and 20 μm filter groups, 50–334.8 μm in the 50 μm membrane group, and pore size-596.5 μm in the rest of the filter groups (theoretical size ranges). The size of fragments after filtration was smaller than the pore size of corresponding filter with the exception of the 63 μm mesh filtration group (Fig. 5C). The smallest one was 33.0 μm in the 0.45 μm membrane filtration group.

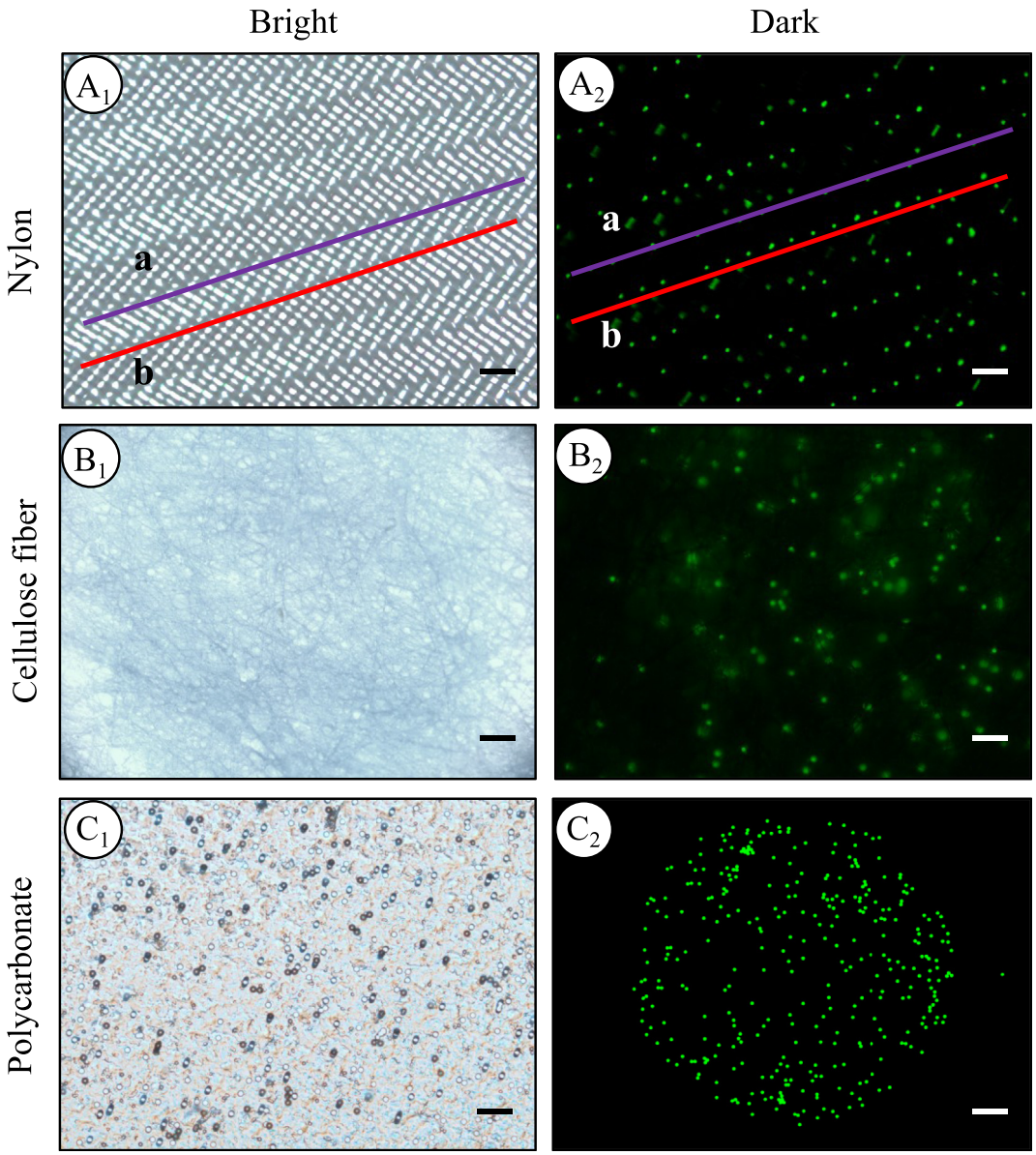

Fig. 3. Polystyrene (PS) pellets on membrane filters after filtration in bright field (A_1-C_1) and dark field (A_2-C_2). The pellets are on two different focal planes of a nylon filter (A_2, line a, line b), or on many different focal planes of a cotton fiber filter (B_2), or on one focal plane of a polycarbonate filter (C_2). The scale bar is 100 μm.

3.3. Abundance, size distribution, and the duration of filtration in field samples

The abundance of microplastics in field samples was verified with FTIR results. Most of them were polyester (33.3%), polypropylene (23.1%), and rayon (20.5%). Before the adjusted filtration method used in the 5 and 20 μm groups, the curve followed a Gaussian function ($R^2 = 0.860$, Fig. 6A). With the adjusted filtration method, however, the curve followed a logistic model ($R^2 = 0.940$, Fig. 6B). The abundance of microplastics increased logarithmically when the pore size became smaller than 200 μm. The abundance of microplastics in the 5 μm filtration group was 13.9 items/L on average (Fig. 6B), nearly five times more than before the adjustment (2.2 items/L, Fig. 6A). The abundance of the 20 μm filtration group was 9.4 items/L on average (Fig. 6B), and nearly twice of that before the adjustment (4.2 items/L, Fig. 6A). Fiber was the dominant shape (91.8%) of the microplastics in field samples of this study.

The number of particles smaller than 100 μm increased with decreasing pore size in field samples, and no particles smaller than 100 μm were found in the 200, 300, or 500 μm filtration group (Fig. S4). In the 5 μm filtration group, 29 particles found were smaller than 100 μm, while two particles were smaller than 100 μm in the 100 μm filtration group. The size range of particles in the 5 μm filtration group was 33.0–4249.0 μm, whereas that in the 500 μm filtration group was 256.0–3750.0 μm.

In the field sample study, the filtration duration through gradient pore size filters was curved. The duration of mesh filtrations was too short to be recorded, so we only studied the duration of membrane filtrations. The curves of 5 μm ($R^2 = 0.996$), 20 μm ($R^2 = 0.990$), and 50 μm ($R^2 = 0.966$) groups followed a power function (Fig. 7A–C). The relationship between the cumulative time and the pore size followed a logistic model ($R^2 = 0.940$, Fig. 7D). The filtration duration increased quickly when the pore size was smaller than 50 μm. The duration of the 5 μm group was nearly 20 min and was twice the duration of the 20 μm group for one parallel field water sample (5 L).

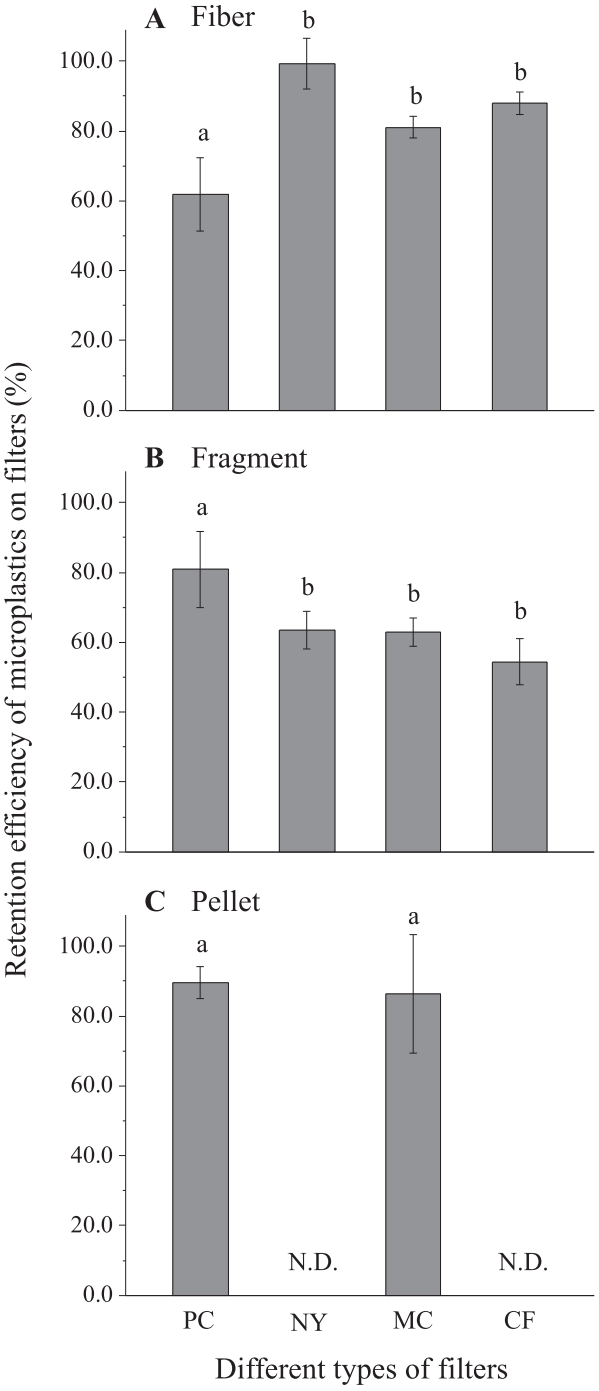

Fig. 4. Retention efficiency of fibers (A), fragments (B), and pellets (C) by 8 μm membrane filters of polycarbonate (PC), nylon (NY), mixed cellulose (MC), and cotton fiber (CF; filter paper) membrane filters. The retention efficiency of pellets by NY and CF membrane filters cannot be counted (no data, N.D.). Letters a and b indicate significant differences ($p < 0.05$) in retention efficiency by different filters (more information is given in section 3.2). The whisker indicates error bar.

4. Discussion

4.1. Effect of different filter structures on microplastic quantification

Different membrane filters have different filtration principles. However, little is known about the effect of membrane structure on microplastic quantification. In this study, pellets clustered in the pore canals of double-layer-hole and multilayer-hole types of filters (nylon and cotton fiber membrane filters), although the size of PS pellets was larger than the membrane pore size. It is possible that the nylon and cotton fiber membrane filters were easily deformed under the same vacuum pump pressure. For this reason, it was hard to observe pellets under the microscope. It implied that using multilayer-hole types of filters might change the ratio between different shapes of microplastics in field samples. However, glass fiber membrane filters, which have the same structure as cotton fiber filters used in this study, are commonly used for filtration in field investigations (Cincinelli et al., 2017; Frère et al., 2017; Hurley et al., 2018; Peng et al., 2017; Tsang et al., 2017; Xiong et al., 2018; Zhang et al., 2017). Hence, there is a possibility that pellets may show higher abundance in field samples than those in reported results using glass fiber filters.

Different from pellets, fibers and fragments have a higher prevalence in field investigations (Cincinelli et al., 2017; Hurley et al., 2018). In this study, nylon, mixed cellulose, and cotton fiber membrane filters retained more fibers than the polycarbonate membrane filter. The pore canals of polycarbonate membrane filter are shallow and straight, allowing fibers to go through easily. The deep and curvy pore canals in the other three membrane filters prevented the fibers from passing through smoothly. The fibers are usually long in one dimension. Even when fibers were clogged in the pore canals, a large part of the fiber body could still be observed. In this situation, the curvy canals of nylon, mixed cellulose, and cotton fiber membrane filters may become an advantage for fiber retainment. When fiber-shaped microplastics are used during laboratory studies, membrane filters with structures of double-layer-hole type, multilayer-hole type or pressure-formed type will be a better choice. In the case of fragments, the dimensions are similar, at least on two sides. The single-layer-hole type filter (PC membrane filter) has circular pores, and its diameter is the actual pore size. The other three filter types are different. The lattice-knitting type filter pore has diagonal pores, which are larger than the defined pore size (edge of lattice). Consequently, fragments can pass through, even if their sizes are slightly larger than the pore size. Multilayer-hole and pressure-formed type filters (MC and CF) have uneven pore sizes; only large pores will allow fragments to pass through. As a result, the single-layer-hole type filter is recommended in fragment filtration because of its significantly higher retention efficiency compared with the other filters. Considering unknown shape ratio in field samples, a filter should retain fibers, fragments, and pellets during filtration. We calculated the total retention efficiencies of the four structures on the three shapes of microplastics. Single-layer-hole type (232.0%) and multilayer-hole type (230.1%) structures had better performance.

In summary, filter structure could have a direct effect on the dominant shape of retained microplastics during the filtration. For laboratory studies, a suitable filter should be used for known microplastic shapes. We recommend double-layer-hole type filters for fiber filtration, single-layer-hole type filters for fragment filtration, and single-layer-hole/multilayer-hole type filters for pellet filtration. Based on the results of the structure experiment, single-layer-hole type and multilayer-hole type filters have higher retention efficiency on microplastics like fibers, fragments, and pellets during field investigations.

4.2. Effect of pore size of membrane/mesh filter on microplastic quantification

According to the results from our laboratory and field experiments, the pore size of filters has a direct effect on the abundance and the size distribution of identified microplastics. However, in

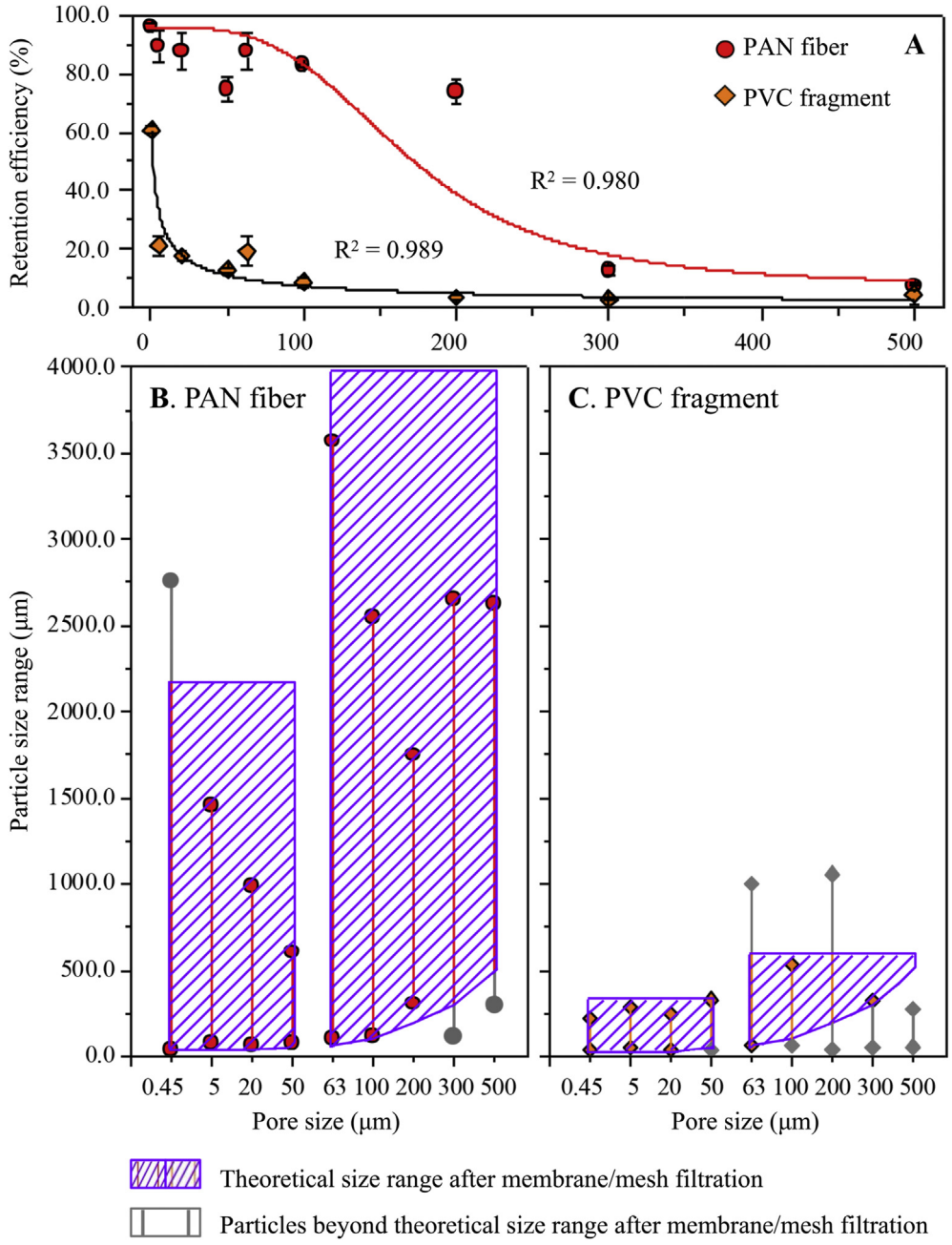

Fig. 5. A: Retention efficiency of fiber decreasing with increasing pore size following the logistic model, and that of fragment decreasing following the power function. Whiskers indicate error bars. B: Size range of polyacrylonitrile (PAN) fiber after gradient pore size membrane/mesh filtration. Red line is for membrane filtration, and orange line is for mesh filtration. Purple hatching indicates theoretical size range after particle filtration, and gray area stands for particles beyond the theoretical size range after filtration. C: Same as B, except for polyvinyl chloride (PVC) fragment. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

filed investigations, there is no uniform procedure for the filtration which could help make the results comparable. A wide range of pore size filters (350−0.22 μm) were used for extracting microplastics from field samples filtered *in situ* (using large-volume water samples) and filtered in laboratory (small-volume water samples) (Ghosal et al., 2018; Sagawa et al., 2018). Covernton et al. (2019) analyzed 41 scientific papers and concluded that microplastic concentrations decrease exponentially with greater mesh size. This means using large pore size mesh will lead to underestimated microplastic concentration.

Different from Covernton et al. (2019), we used a gradient pore size experiment with two prevalent shapes of microplastics (fiber and fragment). The fiber group showed a logistic relationship between retention efficiency and pore size. When the pore size was smaller than 300 μm, more microplastics were found on the filter. It means that more microplastics were obtained with a membrane filter of <300 μm pore size than those with large pore sizes. For the fragment group, however, the inflection point was at about 20 μm. When the pore size was smaller than 20 μm, the number of microplastics on the filter increased rapidly. When the pore size was larger than 20 μm, the number of fragments reached a plateau. For fibers and fragments, the smaller the pore size of the filter, the closer the retention efficiency is to 100%. The relationship between retention efficiency and pore size in the field study followed a

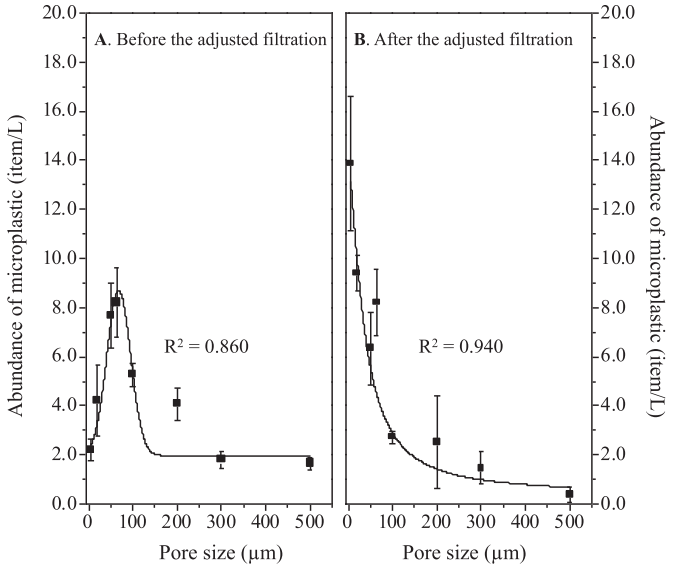

Fig. 6. The fitted curve of the relationship between microplastic abundance (item/L) and pore size in the field investigation. A: The curve follows the Gaussian function before the adjusted filtration method is used. B: The curve follows the logistic model after the adjusted filtration method is used. Whiskers indicate error bars.

Balcer, 2019). Hence, the relationship that was obtained between retention efficiency and the pore size of filters could be applicable to most of field investigations.

Besides abundance, the size distribution/size range of microplastics also played an important role during quantification analysis in field investigations. Unexpectedly, the size range of the original samples was not within the theoretical range obtained by filtration. The upper end of size is much bigger than the filter pore size. Although the nylon filter is supposed to retain more fibers than the other filters, large fibers can go through the pore canals and be collected by smaller pore-size filters randomly. Only if the pore size of a filter is much smaller than the smallest dimension of fiber, can the filter retain most fibers. This is the reason that large fibers were observed in the 0.45 μm group of filters but were not observed in the original samples although the origin solution was evenly ultrasound-dispersed just before filtration. For the fragment groups, the size range was slightly deviated from the theoretical range. Unlike for fibers, the difference of dimensions for fragments was smaller. Therefore, the size deviation was probably due to the pressure of the vacuum pump, which helped the fragments pass through the small filter pores. After filtration, some small fragments were observed on mesh filters as well. These small fragments were attached to the stainless-steel mesh, which means the mesh prevented them from passing through along with the water.

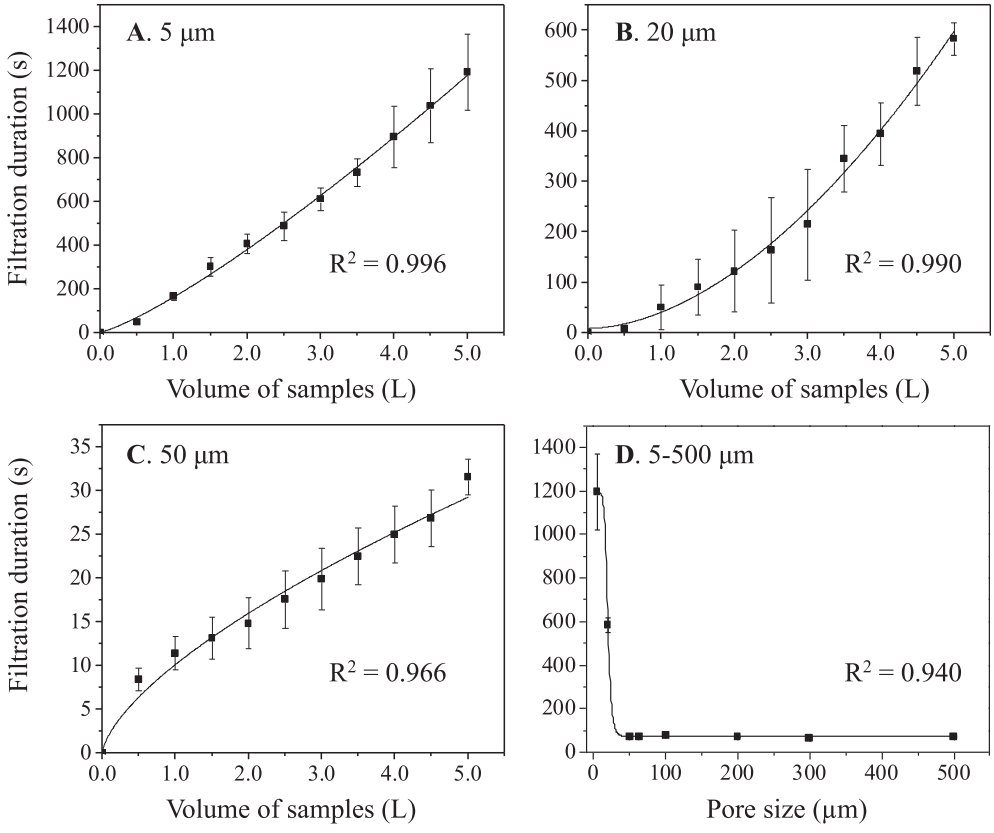

Fig. 7. Relationship between the volume of field water samples and corresponding filtration duration using different pore size membranes (A–C). Panel D shows that the relationship between different pore sizes of membrane/mesh filters and the total filtration duration follows the logistic model.

logistic model and showed the same trend as the fiber filtration in the laboratory study. It is highly probable due to that most of the microplastics were fibers (91.8%) in our field samples. Indeed, fiber is the dominant shape in most field investigations (Mendoza and

The laboratory experiment and field validation demonstrated that using a smaller pore size membrane filter could retain more particles; specifically, 20 μm pore size retained more fibers and fragments based on the results of the laboratory simulation.

Membrane/mesh filtration is not a good choice for preparing a specific size range of fibers or fragments.

4.3. Recommendation for filtration during microplastic quantification

In a field investigation, we usually do not know the dominant shape of microplastics in samples. Although we cannot choose a suitable structure of membrane filter for extraction, we should consider the intensity of filter and pump pressure carefully. In addition, the pore size of membrane/mesh is an important factor for determining the abundance of microplastics obtained in the environment. In many reported investigations, large pore size trawls or nets were used for sampling and much smaller pore size membrane filters for particle extraction from samples (Frère et al., 2017; Kanhai et al., 2018; Lin et al., 2018; Schmidt et al., 2018; Tang et al., 2018; Xiong et al., 2018). It is reasonable to use large pore size net in large-volume water sampling, but the sampling process has "filtered out" small size fraction of the particles. After such sampling, using much smaller pore size membrane for extraction is a "waste" of time, because small pore size filtration will increase the filtration duration. Besides, the loss of microplastics that are <300 μm makes up a dominant proportion found in fish and invertebrates. Therefore, large pore size trawl/net sampling fails to adequately measure a biologically relevant class of microplastics, subsequently undermining our ability to assess ecological risk related to microplastics (Covernton et al., 2019).

In field investigations of microplastics, we recommend to collect water samples with a Niskin bottle/jar or pump sampling directly and filter water through small pore sized filters. In this way, a wider size range of microplastics will be obtained for more comprehensive understanding of microplastic pollution and more accurate ecological risk study. At the same time, we should consider filtration duration. In some field samples, impurities like sand or clay commonly exist and are likely to clog filters. This will extend filtration duration. For this reason, we used an adjusted filtration method in the 5 and 20 μm filter groups, but the number of membrane filters for one parallel sample depends on the pre-experiments. Using the adjusted filtration method, less time will be used and more microplastics will be observed under the microscope. It is more accurate for quantification analysis to use the adjusted filtration method when dealing with high-turbid water samples. Much longer filtration duration in the 5 μm group than that in the 20 μm group suggests that filtration duration is more acceptable when using the 20 μm filter.

The selected pore size of the filter should also be consistent with the demand for later observation, selection, and identification. In most investigations, stereomicroscope is used for the observation of particles, and the largest magnification is 80 or 100 (Barrows et al., 2018; Lares et al., 2018; Simon et al., 2018). In the process of observation, suspectable particles should be picked up with a tweezer from a membrane filter for instrument identification (Shim et al., 2017). These particles need to be transferred onto a transparent substrate in transmittance mode or onto a cleaner substrate to avoid impurity interfering in ATR/reflection mode. Under 80 or 100 magnification, empirically, it is hard to observe particles smaller than 20 μm, let alone to pick them up. The size limit of picking up particles with a tweezer is about 30 μm under microscopy. Meanwhile, the spatial resolution of the popular instrument (e.g., FTIR) is about 20 μm for the identification of microplastics in field samples (Imhof et al., 2016). For the above size limits, we find that a 20 μm pore size filter is small enough for microplastic investigation. Among present membrane products, due to the intensity and stability with 20 μm pore size, we could only find nylon, polyethylene terephthalate membrane, and stainless-steel mesh filters. In future research, a stainless-steel membrane filter may be a better choice for field investigations considering the portability of filter. Although single-layer-hole type and multilayer-hole type structures had better performance on retaining microplastics such as fibers, fragments, and pellets, their intensity are not strong enough to have 20 μm pore size, which is the most efficient for field investigation. These types of filters usually have small pore sizes; they can only be used for clean water samples with little impurity to obtain small-sized microplastics, i.e. sea water.

Tamminga et al. (2019) suggested that a Niskin bottle or jar sampling method might not be representative enough due to its small volume, especially in great rivers/lakes and ocean sampling. Nevertheless, we should avoid large mesh size net/trawl sampling because of the underestimating and the misunderstanding of microplastic pollution. In future field investigation, continuous water intake (with non-plastic vessels and gentle pumps) followed with filtration equipment (which has different pore size filters) should be designed and used. It will avoid the contradiction between sample representative and particle size range.

At present, the Niskin bottle or jar sampling method followed with filtration by 20 μm pore size membrane filters made in stainless steel (double-layer hole type of structure) is more suitable for conventional field investigations on microplastic, such as environmental monitoring survey. A better method is needed for actualizing large-volume water sampling and small pore size filtering.

5. Conclusions

The structure and pore size of membrane/mesh filters for studying microplastic pollution were assessed in this study. The results we obtained were consistent with our initial hypothesis. In a laboratory experiment, the structure of filter should be considered for different retention efficiencies of fibers, fragments, and pellets. Besides the structure, the pore size of membrane/mesh can influence the quantification as well. Small pore size filters can lead to higher abundance and wider size range of microplastics than large filters. In field investigations, we should consider the time cost of filtration, the size limitation of observation, and the spatial resolution of identification instrument. Hence, we recommend to collect water samples directly and to filter them with a 20 μm pore size membrane in field investigations. At present, double-layer hole type of structure is strong enough with a 20 μm pore size. To develop a valid and widely accepted method, more studies are needed to balance large-volume sampling and small pore size filtration.

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.

CRediT authorship contribution statement

Huiwen Cai: Writing - original draft, Methodology, Formal analysis. **Mengdi Chen:** Methodology, Writing - review & editing. **Qiqing Chen:** Formal analysis, Writing - review & editing. **Fangni Du:** Data curation, Writing - review & editing. **Jingfu Liu:** Writing - review & editing. **Huahong Shi:** Supervision, Writing - review & editing.

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Appendix A. Supplementary data

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