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Microplastics in mussels along the coastal waters of China☆

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ABSTRACT

Microplastic has been confirmed as an emerging pollutant in marine environments. One of the primary environmental risks of microplastics is their bioavailability for aquatic organisms. Bivalves are of particular interest because their extensive filter-feeding activity exposes them directly to microplastics present in the water column. In the present study, we investigated microplastic pollution in mussels (*Mytilus edulis*) from 22 sites along 12,400 mile coastlines of China in 2015. The number of total microplastics varied from 0.9 to 4.6 items/g and from 1.5 to 7.6 items/individual. *M. edulis* contained more microplastics (2.7 items/g) in wild groups than that (1.6 items/g) in farmed groups. The abundance of microplastics was 3.3 items/g in mussels from the areas with intensive human activities and significantly higher than that (1.6 items/g) with less human activities. The most common microplastics were fibers, followed by fragments. The proportion of microplastics less than 250 μm in size arranged from 17% to 79% of the total microplastics. Diatom was distinguished from microplastics in mussels for the first time using Scanning Electron Microscope. Our results suggested that the numbers of microplastic kept within a relatively narrow range in mussels and were closely related to the contamination of the environments. We proposed that mussels could be used as a potential bioindicator of microplastic pollution of the coastal environment.

1. Introduction

Microplastics, defined as plastic materials or fragments <5 mm, are most likely the most numerically abundant plastic debris in the ocean today (Law and Thompson, 2014). The quantities of marine microplastics will inevitably increase due to the degradation of plastic items, ultimately breaking down into millions of microplastic pieces (Cózar et al., 2014). The widespread use and persistent nature of plastic has made microplastics ubiquitous in marine waters, sediments, organisms and even sea salts (Yang et al., 2015). Therefore, research regarding plastic pollution has focused on sources, fate and ecological effects of microplastic particles in recent years (Cole et al., 2015; Hall et al., 2015; Rocha-Santos and Duarte, 2015).

The primary environmental risks associated with microplastics is their suspected bioavailability for marine organisms (Wright et al., 2013; Desforges et al., 2015). Bivalves are of particular interest because their extensive filter-feeding activity exposes them directly to microplastics present in the environment. Mussels are representative benthic filter feeders and the diet of many intertidal species. Bivalves have been widely used in biomonitoring of marine environments due to several advantages such as broad geographical distribution, easy accessibility and high tolerance to a considerable range of salinity. One famous example is the Mussel Watch Program in which environmental contaminants are measured in a field-exposed marine bivalve species, creating a database of contamination level in space and time (Bricker et al., 2014).

Mussels are the most common benthic species used for studying the fate and toxic effects of microplastics in laboratory (Browne et al., 2008; von Moos et al., 2012; Farrell and Nelson, 2013; Watts et al., 2014; Avio et al., 2015). Microplastics have also been found in farmed and wild mussels from several European countries and a fishery market of China (Mathalon and Hill, 2014; Van Cauwenberghe and Janssen, 2014; Li et al., 2015; Van Cauwenberghe et al., 2015). Uptake of microplastics has been used as one of the marine health status parameters in mussels from Belgium (De Witte et al., 2014). Microplastic levels in mussels have even been added to Europe database on environmental contaminants of emerging concern in seafood (Vandermeersch et al., 2015a). Mussel stands for one of the most vulnerable species to

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microplastic pollution and is also one of important vectors to transfer microplastics into human food chain. However, there is still a lack of large-scale investigations on microplastic pollution in mussels along the coastal waters.

Several recent studies showed that the coast of China is a hotspot of microplastic pollution (Zhao et al., 2014; Fok and Cheung, 2015; Qiu et al., 2015). High numbers of microplastics have been found in the commercial bivalves from the fishery market of China (Li et al., 2015). In the present study, we conducted a large scale survey on microplastic pollution in mussels along the coastal waters of China. Our aims were to determine the features of microplastic pollution in mussel communities as well as its relationship with the living environments of mussels.

2. Materials and methods

2.1. Sample collection

Mytilus edulis was collected from 22 sites along the coastal waters of China from July to October 2015. The investigated coastline covers approximately 12,400 mile coastlines, accounting for approximately 65% of the total length of coastline of mainland China (Fig. 1). Two aspects were considered in selecting the sampling sites. One aspect was the types of mussels, i.e., wild and farmed mussels. Wild mussels were collected at 12 sites, and farmed mussels were collected at 10 sites. The other aspect was the degree of the general contamination at the sampling sites. The sites were defined as highly and slightly contaminated sites according to the previous regular monitoring data of water quality available, the location and the environments nearby (Supplementary Material Table 1). In brief, the sites in the relative closely bay with intensive anthropic activities were generally considered as highly contaminated sites; the sites in the open area with less anthropic activities were considered as slightly contaminated sites. Approximately 50 mussels were collected at each site. Wild mussels were directly collected with tweezers in intertidal zone during low tide; farmed mussels were collected underwater with the help of fishermen. The mussels were put in the plastic bags, transferred to the laboratory and kept in −20 °C refrigerator for microplastic analysis. The exact information for the sites and mussels were also recorded (Supplementary Material Table 1).

2.2. Hydrogen peroxide treatment of soft tissue

The analysis of microplastics in mussels followed our previous methods for bivalves (Li et al., 2015). In brief, one blank extraction group without tissue was performed simultaneously to correct the potential procedural contamination, one positive control with known polyester and polyethylene fibers (0.3–1.6 mm in length and 10–40 μm in diameter) was used to test the digestion effects of H_2O_2 on the features of microfibers. All of the liquid (freshwater, salt water and hydrogen peroxide) was filtered with 1 μm filter paper prior to use. All of the containers and beakers were rinsed three times with filtered water. The soft tissues of 2–5 mussels were emptied into a 1 L glass bottle with a height of 35 cm and regarded as a replicate, and six replicates with 12–30 mussels were prepared at each site. Approximately 200 mL of 30% H_2O_2 was added to each bottle to digest the organic matter of the soft tissue in each bottle. The bottles were covered and placed in an oscillation incubator at 65 °C at 80 rpm for 24 h and then at room temperature for 24–48 h.

2.3. Floatation and filtration of microplastics with saline (NaCl) solution

A prefiltered (<1 μm) saturated saline solution (1.2 g/mL) was used to separate the microplastics from dissolved liquid of the soft tissue via floatation (Li et al., 2015). Approximately 800 mL of filtered NaCl solution was added to each bottle. The liquid was mixed, and the bottle stood overnight. The overlying water was directly filtered over a 5 μm pore size, 47 mm diameter cellulose nitrate membrane filter (Whatman AE98) using a vacuum with a pump. Next, the filter was placed into clean petri dishes with a cover for further analysis. All of the experimental procedures were finished as soon as possible.

2.4. Observation and validation of microplastic

The filters were observed under a Carl Zeiss Discovery V8 Stereo microscope (MicroImaging GmbH, Göttingen, Germany), and images (25–80 magnification) were taken with an AxioCam digital camera. A visual assessment was applied to identify the morphotypes of microplastics according to the physical characteristics of the particles (Hidalgo-Ruz et al., 2012). Microplastics were classified into four morphotypes including fiber, sphere, flake and fragment. In brief, fiber was defined as microplastic with a slender and greatly elongated appearance; sphere was round microplastic and looked like a ball in shape; flake was a small and very thin layer or piece of large plastic debris; fragment was an isolated or incomplete part of large plastic debris, especially that could not be classified as fiber, sphere and flake.

A number of common and undeterminable particles were selected and verified with a micro-Fourier Transformed Infrared Spectroscope (μ-FT-IR, Thermo Nicolet iN10 MX) and Scanning Electron Microscope (SEM, Hitachi S-4800, Japan)/Energy Dispersive Spectrometer (EDS, EMAX).

In the identification with a μ-FT-IR, the transmittance mode was applied following the method of Yang et al. (2015). All the spectra were directly compared with the library of different polymers provided by Thermo Fisher Scientific in their software (OMNIC Picta) based on the methods of Vianello et al. (2013). No transformation or postprocessing of the spectra were carried out. For the SEM, samples were spread on double-sided adhesive tape, coated with a thin film of evaporated gold. The morphology of the samples was examined under a SEM, and the images were taken with an optimized acceleration voltage of 3 kV and detector working distance of about 2 mm. During the SEM observation, qualitative elemental composition of particles was confirmed using an EDS. According to the morphological structure and chemical composition of detected items, some particles were verified as or excluded from microplastics.

2.5. Data analysis

Statistical analyses were performed using SPSS. The mean differences of the abundance of microplastics in mussels between two groups were determined by independent-samples T test. Statistical significance was accepted at $^{*} = p < 0.05$, $^{**} = p < 0.01$.

3. Results

3.1. Abundance of microplastics in mussels

Contamination with airborne microplastics was prevented during handling the samples, and the procedural blanks only contained 0.67 ± 0.82 items/filter of microplastics. In the positive control, 76 of 80 selected microfibers were detected under the microscope. No changes of physical structure were observed, and partial of the fibers were discolored (Supplementary Material Fig. 1). In mussel samples, the number of total encountered microplastics varied from 0.9 to 4.6 items/g (wet weight) and from

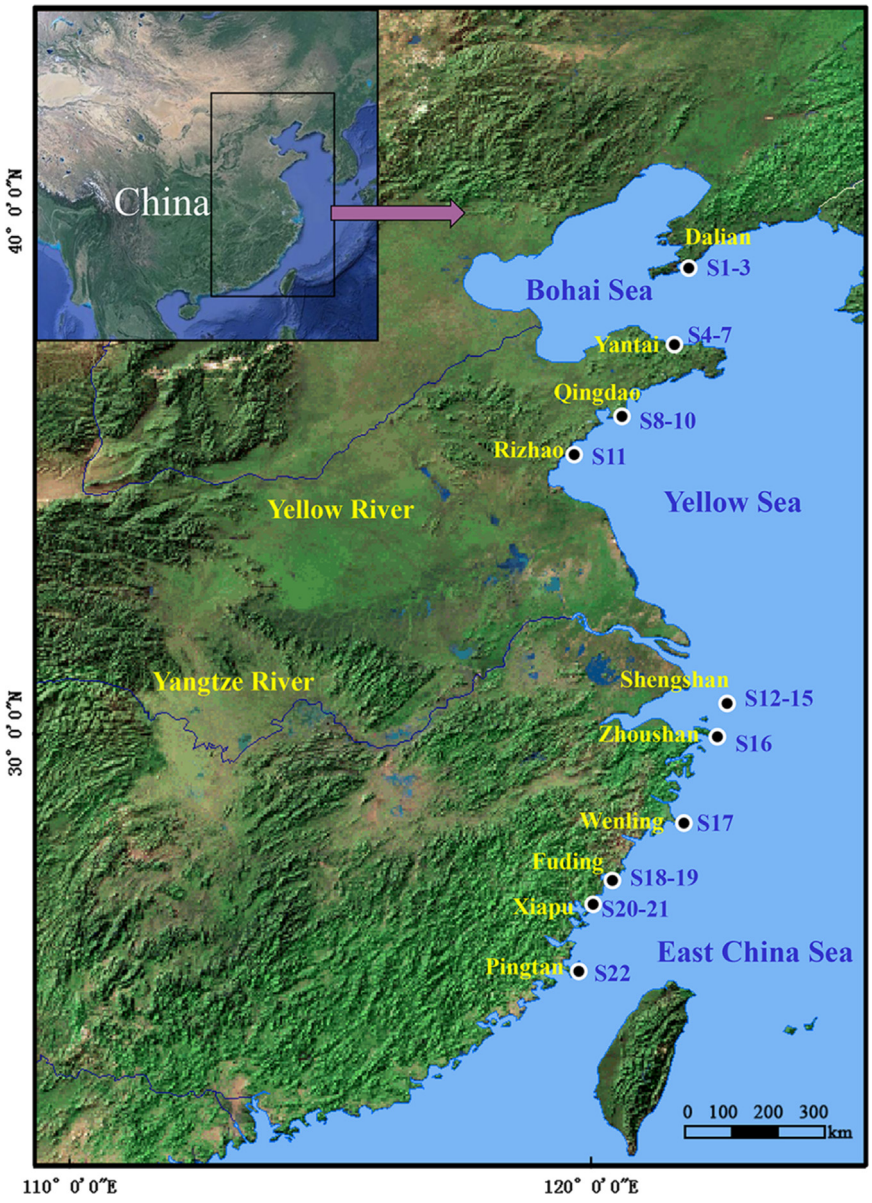

Fig. 1. Sampling sites of mussels along the coastal waters of China.

1.5 to 7.6 items/individual (Fig. 2). The average abundance of microplastic was 2.2 items/g and 4 items/individual in mussels from all sites. The mussels from S2 contained 4.6 items/g (7.6 items/individual) microplastics and showed the highest levels of microplastic contamination either by weight or by individual (Fig. 2).

The average abundance of microplastics was 2.7 items/g (4.6 items/individual) in wild mussels and 1.6 items/g (3.3 items/individual) in farmed mussels (Fig. 3A, B). An average of 3.3 items/g (5.3 items/individual) in mussels from heavily contaminated areas and 1.6 items/g (3.3 items/individual) in mussels from slightly contaminated areas was found (Fig. 3C, D). The abundance was significantly higher in mussels from heavily contaminated areas than those from slightly contaminated areas ($p < 0.01$).

3.2. Sizes and shapes of microplastics in mussels

The sizes of microplastics showed great variations in mussels from different sites. The proportion of microplastics less than 250 μm in size ranged from 17% to 79% of the total microplastics, and that of microplastics more than 1 mm arranged from 1% to 34% (Fig. 4A). Multiple morphotypes of microplastics, including fibers, fragments, spheres and flakes, occurred in the tissue of mussels (Fig. 4B). The most common microplastics were fibers, followed by fragments. Fibers accounted for more than 65% of the total microplastics at 18 sites, and the proportion of fragments ranged from 5% to 67% of the total microplastics (Fig. 4B). Fibers ranged from 33 μm to 4.7 mm in length and 10 to 65 μm in diameter.

3.3. Composition of microplastics in mussels

One hundred and twenty-nine plastic-like particles were selected from 1519 visually identified particles. These selected particles were further validated using μ-FT-IR (Supplementary Material Table 2). Various polymer types were identified, including cellophane (CP), polyethylene terephthalate (PET) and polyester (PES) (Supplementary Material Fig. 2). Some non-plastic

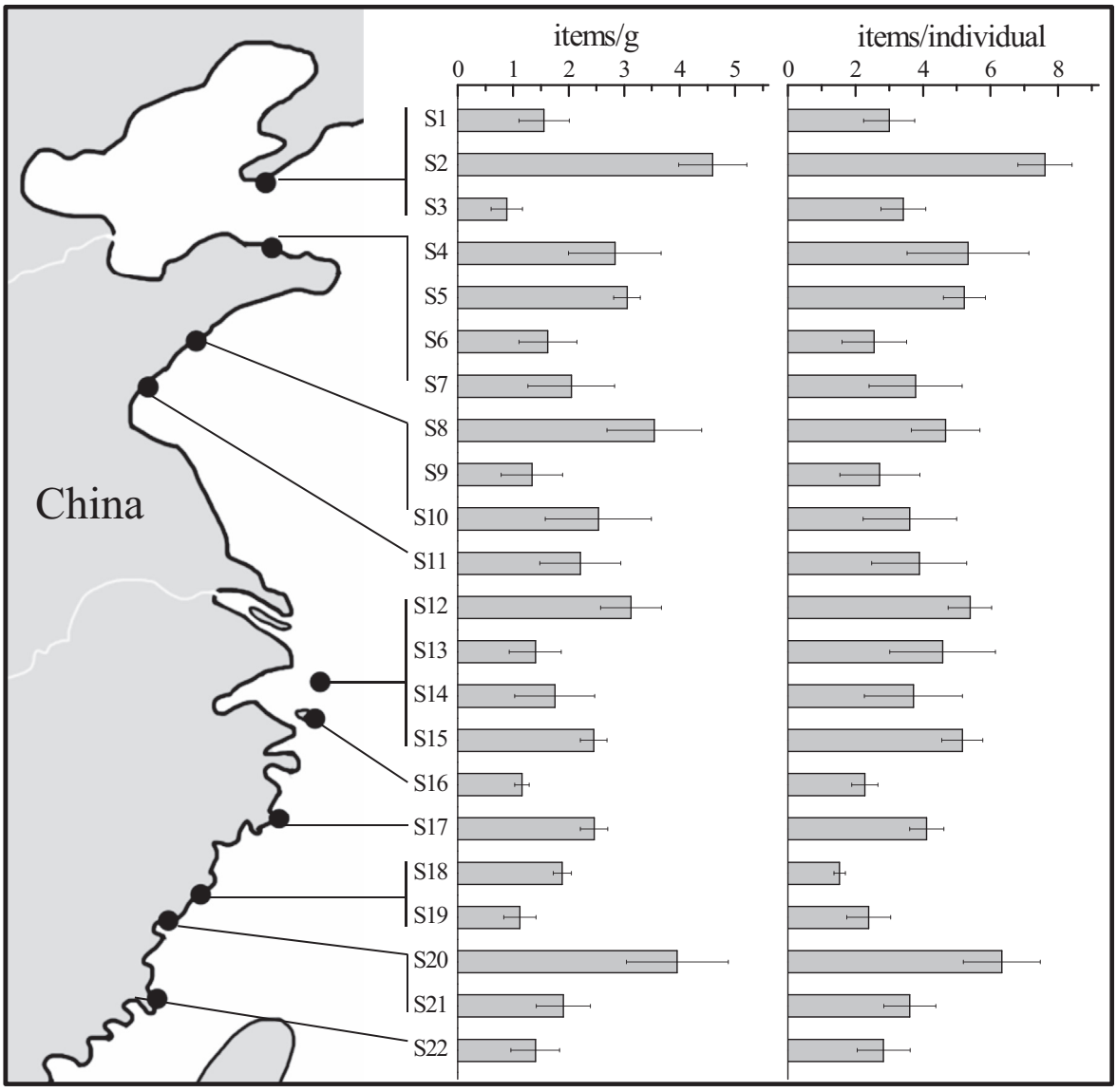

Fig. 2. Abundance of microplastics in mussels along the coastal waters of China. Six replicates were set for mussels at each site (n = 6), and 2–5 individuals were pooled as one replicate.

particles, such as diethanolamine and selenious acid, were also identified (Supplementary Material Fig. 2). The spectrum matches were at least 80% for most of the identified particles (Supplementary Material Table 2).

Some plastic-like particles were selected and further identified using SEM. Generally, polymers showed smooth or unregulated surface (Fig. 5A, B). A large amount of uniform transparent spheres, which had been proven to have aluminum silicate using μ-FT-IR, showed regular holes on the surface and were confirmed as diatoms (Fig. 5C). Some dark blue particles showed regular crystal array and were determined as $CaCO_3$ (Fig. 5D), which was in accordance with the result identified using μ-FT-IR (Supplementary Material Fig. 2).

4. Discussion

4.1. Microplastic in mussels along the coastal waters of China

In the present study, we investigated microplastics in mussels in a large geographical area along the coastal waters for the first time. The investigated coastline covers approximately 2/3 of the total coastline of mainland China. Our results suggested that microplastic pollution was widespread in both wild and farmed mussels along the coastal waters of China. The average abundance of microplastics (2.2 items/g) in the present study was highly in accordance with our previous reports on mussels (2.4 items/g) from a fishery market (Li et al., 2015). Compared to the abundance reported worldwide (Supplementary Material Table 3), the numbers of microplastics were significantly higher than those documented in mussels from Belgium, Germany, French and Dutch coastal waters (De Witte et al., 2014; Van Cauwenberghe and Janssen, 2014; Van Cauwenberghe et al., 2015). However, the numbers of microplastics reached 34–178 items/individual in mussels from Canada (Mathalon and Hill, 2014). Our results might be comparable to their study if the contamination of airborne microplastics is excluded from the research (Mathalon and Hill, 2014).

A previous study suggested that farmed mussels contain more microplastics than wild mussels due to the fact that the farmed mussels grow on polypropylene plastic lines (Mathalon and Hill, 2014). However, we got a reverse result to that. Other researchers

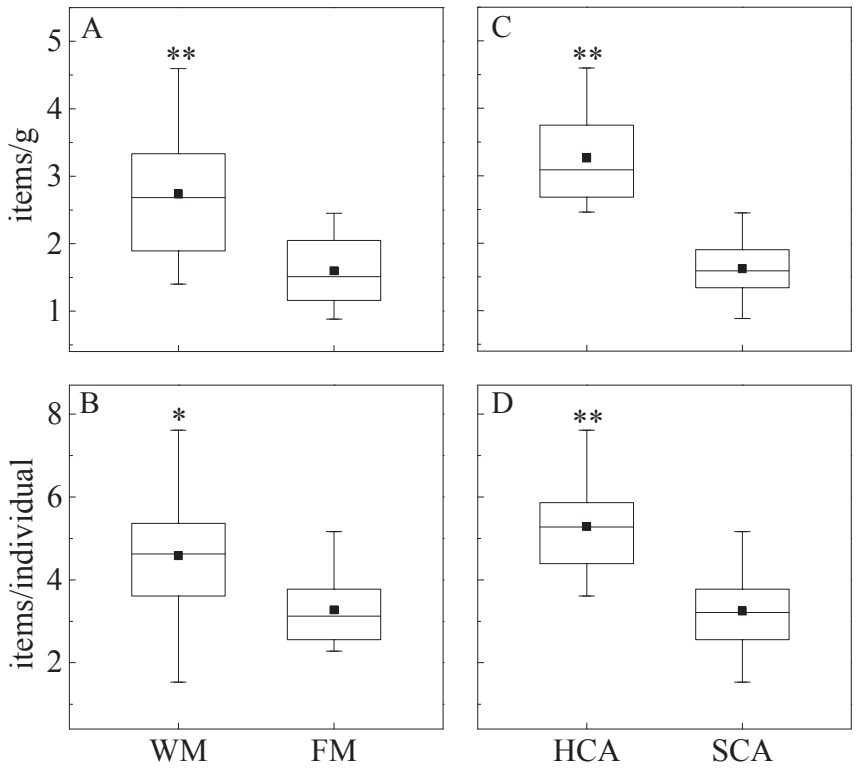

Fig. 3. Comparison of abundance of microplastics in wild mussels (WM, n = 12) and farmed mussels (FM, n = 10) as well as in mussels from heavily contaminated areas (HCA, n = 8) and slightly contaminated areas (SCA, n = 14). One site was regarded as a replicate. * means $p < 0.05$, and ** means $p < 0.01$.

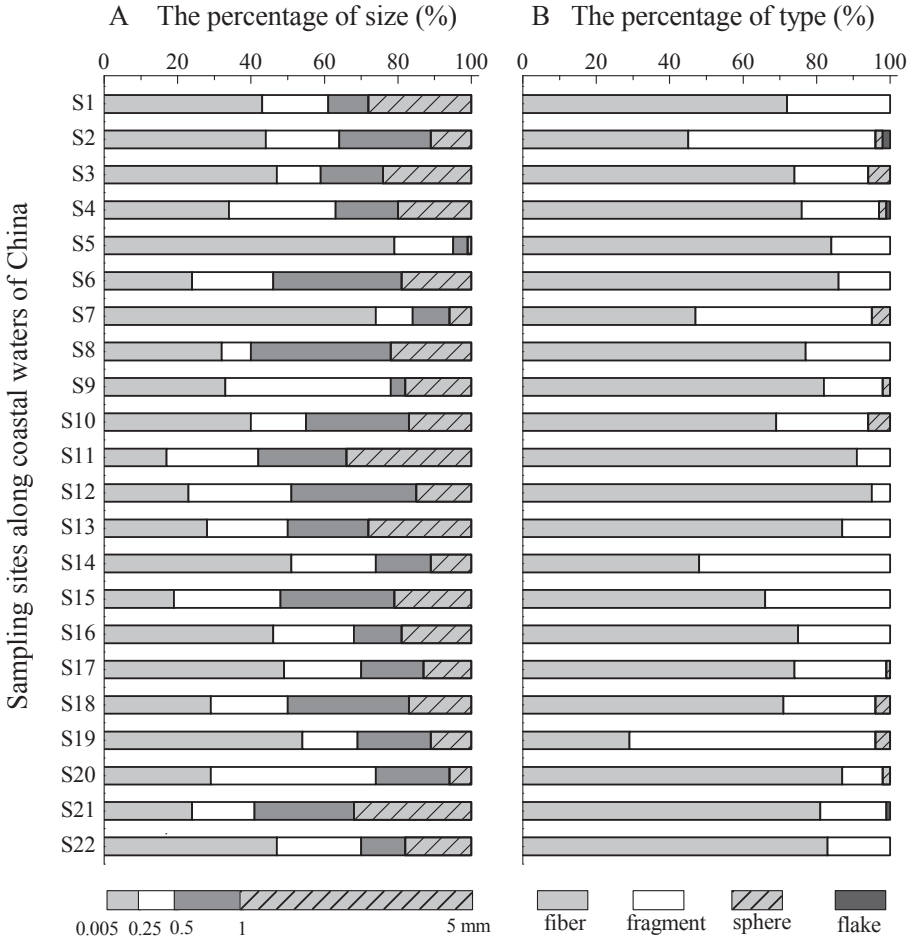

Fig. 4. The sizes and shapes of microplastics in mussels along the coastal waters of China. Six replicates were set for each species (n = 6), and 2–5 individuals were pooled as one replicate.

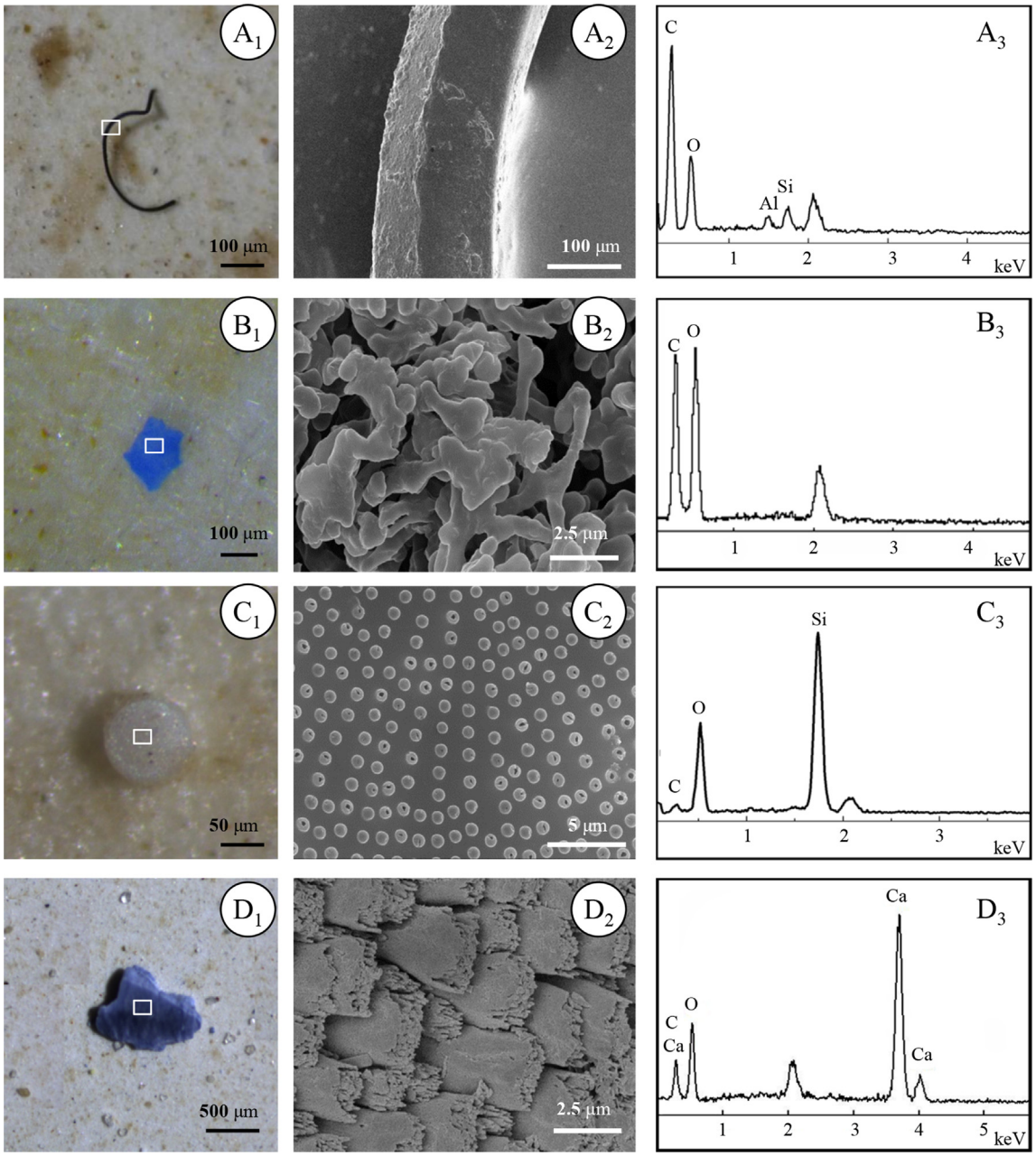

Fig. 5. Identification of microplastics with SEM/EDS. The left photos were taken under microscopes, the middle photographs were taken under SEM for the white box areas of the left ones, and the right photographs were the spectra of EDS for particles in the middle photographs. Some particles were identified as microplastics (A, B), and the others were identified as non-plastics such as diatoms (C) and $CaCO_3$ (D).

found no significant differences in the microplastic levels between farmed and wild mussels (De Witte et al., 2014; Vandermeersch et al., 2015a). In China, the farmed mussels are cultured in areas with high quality water, which are generally less affected by human activities. The analysis of composition suggested that no polypropylene was identified in the present study (Supplementary Material Table 2). In addition, our analysis suggested that the abundance of microplastics was significantly higher in areas with intensive human activities than that with less human activities. The high concentrations of microplastics in mussels should be closely related to the plastic contamination of their living environments. In recent years, severe microplastic pollution has been reported in the water column and sediments along the coastal waters of China (Zhao et al., 2014; Fok and Cheung, 2015; Qiu et al., 2015). Therefore, our results further confirmed that it should be the level of total pollution of microplastic in the environments rather than the single factor (e.g. plastic lines in farm) that determined the abundance of microplastics in mussels.

4.2. Identification and validation of microplastics

In the present study, contamination with airborne microplastics was prevented during handling the sampling, and the procedural blanks only contained 0.67 ± 0.82 items/filter. If we transferred it to the unit of items/g using the average weight of mussel tissue (5.47 ± 1.14 g) for each sample, the number of microplastics was only 0.12 items/g, accounting for less than 5% of the average number of microplastics in mussels. The abundance of

microplastics varied in a relatively narrow range (0.9–4.6 items/g). In our previous study, we also found that the microplastics change in a range of 2.1–10.5 items/g in 9 commercial bivalves from a fishery market (Li et al., 2015). However, the abundance of microplastics shows great variations in mussels among different researches worldwide (Supplementary Material Table 3). Does the difference come from the real levels of microplastic pollution or methods of analysis?

Actually, different methods have been used in the isolation, identification and calculation of microplastics in mussels in previous studies (Supplementary Material Table 3). Generally, fibers are the most common microplastics in organisms. However, fibers are not reported due to the detrimental effect of concentrated HNO_3 on fibers during the isolation process of microplastics in the study of Van Cauwenberghe and Janssen (2014), Van Cauwenberghe et al. (2015). The abundance of microplastics is 0.26–0.51 items/g in mussels in the study of De Witte et al. (2014). They use an acid mixture method (HNO_3:$HClO_4$) to isolate microplastics. Vandermeersch et al. (2015b) suggest that the acid mix method detects a higher fiber content and a lower particle content than the nitric acid method.

Nuelle et al. (2014) suggest that the treatments of H_2O_2 successfully remove more than 90% biogenic material whereas the tested polymers are resistant. Our results suggest that 95% recovery efficiency could be obtained after H_2O_2 digestion. The losses might result from the sticking of the microfibers to the wall of the bottle. Whereas, H_2O_2 bleached the microplastics and made them discolored, which affected the determination of colors rather than numbers of microfibers. Therefore, different isolation methods led to great variations in the abundance of microplastics (Supplementary Material Table 3).

In addition, the ambiguous characteristics of non-plastics and plastics make it difficult to accurately identify microplastics (Song et al., 2015). In a previous study, we have identified a large number of uniform transparent spheres as aluminum silicate but not microplastics using μ-FT-IR (Li et al., 2015). In the present study, we further identified these aluminum silicate spheres as diatom using SEM. It is well-known that diatom can be isolated from the gut of mussels using HNO_3 and identified using SEM (Seethalakshmi and Selvakumar, 2015). Zettler et al. (2013) suggest that diatoms are one of common microbial communities on marine plastic debris. Nevertheless, these aluminum silicate spheres could not be accurately identified until SEM method was used due to H_2O_2 digestion in the present study. Therefore, it is the very important to apply different methods to further valid those plastic like particles after visual identification of microplastics under microscopes.

In all, the differences in methods of isolation and identification must contribute a lot to the difference in the abundance of microplastics in the same type of tested samples, including microplastics in mussels worldwide (Supplementary Material Table 3). Such differences make it highly difficult to compare the levels of microplastic pollution between different studies (Vandermeersch et al., 2015b; Phuong et al., 2016). Internationally uniform and more effective methods should be developed to isolate and identify microplastics in the same type of samples in future.

4.3. Mussel used as a potential bioindicator of microplastic pollution

Long term monitoring of plastics in fulmars stomachs has become one of the Ecological Quality Objectives (EcoQOs) set by the Oslo-Paris Convention (OSPAR) for the North Sea (OSPAR, 2008; Van Franeker et al., 2011). However, there is no bioindicator for microplastic pollution along the coastal waters worldwide. Mussel has been widely used as sentinels in “Mussel Watch Programme” to describe the current status of pollution and to detect changes in the environmental quality of estuarine and coastal waters.

Our results suggested that microplastics could be found not only in wild mussels but also in farmed mussels, which indicate risk of both ecological system (Bouwmeester et al., 2015). The abundance of microplastics in mussels was closely related to human activity. Bivalves keep quick ingestion and clearance of microplastics (Setälä et al., 2016). The abundance of microplastics showed great variations not only in water bodies but also in organisms. The more microplastics the mussels ingest from the environment, the more microplastics the mussels will exclude through excretion. The amount of accumulating microplastics should vary within a stable range, which makes it possible to build an assessment system. Therefore, our study further confirms that microplastic pollution was widespread in mussels and that mussels could be used as indicators of microplastic pollution in coastal waters (Li et al., 2015).

Nevertheless, more studies are required to build a valid biomonitoring system using mussels. First of all, further research should be conducted to develop an international and standardized protocol for the quantification and monitoring of microplastics in mussels (Vandermeersch et al., 2015b). These methods should not only include sampling and isolating methods but also include identification and calculation methods. In addition, more large scale investigations should be conducted to find the quantitatively relation of microplastics in mussels and their living environments.

In brief, we successfully distinguished diatom from microplastics in mussels. Our results suggest that microplastic pollution was widespread in mussels along the coastal waters of China. The abundance of microplastics was significantly higher in wild mussels than in farmed mussels, and the numbers of microplastic kept within a relatively narrow range in mussels. We proposed that mussels could be used as a potential indicator of microplastic pollution of coastal waters.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2016.04.012.

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