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Occurrence of microplastics in the water column and sediment in an inland sea affected by intensive anthropogenic activities☆

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ABSTRACT

Microplastics may lose buoyancy and occur in deeper waters and ultimately sink to the sediment and this may threaten plankton inhabiting in various water layers and benthic organisms. Here, we conduct the first survey on microplastics in the water column and corresponding sediment in addition to the surface water in the Bohai Sea. A total of 20 stations covering whole Bohai Sea were selected, which included 6 stations specified for water column studying. Seawater was sampled every 5 m, with maximal depth of 30 m in the water column using Niskin bottles coupled with a ship-based conductivity, temperature and depth sensor (CTD) system and surface sediment samples were collected using box corer. The results indicated that higher microplastic levels accumulated at a depth range of 5–15 m in the water column in some stations, suggesting the surface water survey was not sufficient to reflect microplastics loading in a water body. Fibers predominated microplastic types in both seawater and sediment of the Bohai Sea, which accounted for 75%–96.4% of the total microplastics. However the relatively proportion of the fibers in the deeper water layers and sediment was lower than that in the surface water. Microplastic shapes are more diverse in the sediment than in the seawater in general. The microplastic sizes changed with depth in the water column and the proportion of the size-fraction < 300 μm increased with depth, probably as a result of rapid biofouling on the small microplastics due to their higher specific surface area. Such depth distribution also implied that sampling with manta net (>330 μm) that commonly used in the oceanographic survey might underestimate microplastics abundance in the water column. Further studies are recommended to focus on the sinking behavior of microplastics and their effects on marine organisms.

1. Introduction

Plastic particles <5 mm are defined as microplastics (Thompson et al., 2004). In addition to the degradation of larger plastic debris, some plastics are manufactured in the form of microplastics (Van Cauwenberghe et al., 2013). Microplastics are readily transported long distances from source areas by wind and water and are distributed widely across coastal beaches, waters and sediments in the marine environment (Ryan et al., 2009). The ubiquity and persistence of microplastics pose a threat to marine wildlife as their small size makes them available to a wide range of marine organisms (Barnes et al., 2009; Doyle et al., 2011). In addition, owing to their large specific surface area, microplastics tend to adsorb a number of pollutants including trace metals, persistent organic pollutants and chemical additives (Liedermann et al., 2018; Rios et al., 2007; Teuten et al., 2007). Microplastics coated with pollutants may be transported, thus contaminating otherwise pristine ecosystems or injected by marine organisms (Teuten et al., 2007).

Microplastics may become negatively buoyant upon fouling, resulting in their sinking and distribution in various water layers or accumulation on the seafloor (Kaiser et al., 2017; Katija et al.,

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2017; Lusher et al., 2015; Van Cauwenberghe et al., 2013; Woodall et al., 2014). Although field surveys on pollution by microplastics or plastic debris in water has mainly focused on their occurrence on the surface water (Setala et al., 2016; Su et al., 2016; Zhang et al., 2017; Zhao et al., 2014), more studies indicate that monitoring microplastics in the water column and benthic sediment might improve our ability to predict mass budgets in the ocean, size distribution, drifting pattern, and impact on marine species and habitats (Bagaev et al., 2017; Katija et al., 2017; Koelmans et al., 2017; Lattin et al., 2004; Reisser et al., 2015). For instance, the observational study conducted by Reisser et al. (2015) in the North Atlantic Gyre indicates that smaller pieces exhibit lower rise velocities and are more susceptible to vertical transport. Bagaev et al. (2017) found that the accumulation of anthropogenic fibers at near-surface and near-bottom layers (defined as one tenth of the local depth) was 3–5 times greater than intermediate layers in the Baltic Sea water column. As most plastics are surveyed in the water column by sampling with 150–505 μm nets, knowledge gaps still exist regarding the distribution of smaller pieces in the water column.

The Bohai Sea is a semi-enclosed inner sea and is surrounded by one of the most densely populated and industrialized zones in China, thus putting it under great environmental pressure (Gao et al., 2014). Recent field surveys indicate that plastic fragments, lines, films and resin pellets are ubiquitous in the surface waters of the Bohai Sea (Zhang et al., 2017) and its surrounding sandy beaches (Yu et al., 2016; Zhou et al., 2018). However, the vertical distribution of plastics in the marine water and benthic sediments remain unknown in this ocean. In this context, the present study aimed to obtain information on the abundance and vertical distribution of microplastics in the water column of the Bohai Sea, and their relationships with the types and sizes of microplastics with sampling to a depth of 30 m. Surface sediments beneath the water column were also investigated to identify the relationships between microplastic pollution in sediments and waters.

2. Materials and methods

2.1. Description of the study area

The Bohai Sea is a shallow, semi-enclosed marginal sea of the western Pacific Ocean. As shown in Fig. 1, it is composed of Liaodong Bay, Bohai Bay, Laizhou Bay and a central basin. The Bohai Sea is connected with the North Yellow Sea through the Bohai Strait at the eastern extension. A semi-diurnal tidal current dominates in the Bohai Sea with a current speed of 20–80 cm s^{-1}, while the wind-driven circulation exhibits a strong southward current along the western shore with a current speed of 8–20 cm s^{-1} in winter (Corcoran, 2015). Riverine inputs have an important impact on the marine coast, and in particular the Yellow River is the second largest river in the world in terms of sediment load (Long et al., 2015).

2.2. Water and sediment sampling

The marine water and sediment samples were collected during summer cruises deployed by the Research Vessel- *Chuangxin* I in the Bohai Sea for multidisciplinary oceanographic survey at 20 locations in September 2016. The sampling sites were distributed in the nearshore and offshore zones, covering the Liaodong bay, Bohai bay, Laizhou Bay, central Bohai basin and Bohai strait as shown in Fig. 1. The vessel stopped sailing when water and sediment sampling were conducted. Surface water samples were collected using a stainless steel bucket from all the stations. Six of the twenty stations were selected for studying the depth distributions of microplastics in water columns. The selected six stations are all located at the nearshore or the area with low hydrodynamic exchange, where plastic debris are assumed to have a relatively higher accumulation. The seawater samples in the water columns were collected every 5 m from a depth of 5 m to the bottom using a 12-bottle Rosette sampler system (SBE 32 Carousel Water Sampler) (See supplementary Fig. S1 online). The system consists of a conductivity, temperature and depth sensor (CTD) system (SBE 911plus, USA) at the bottom of the Rosette and is equipped with 10 L Niskin bottles. This equipment allows an operator to remotely actuate a sequence of up to 12 water sampling bottles. Each Niskin bottle has a stopper at the top and the bottom. The Rosette sampler was then lowered at a determined depth with both ends of each Niskin bottle in the open position. There is a depth meter on the CTD that relays its position to computers on board the vessel. Two Niskin bottles were closed and captured a water sample inside at each sampling depth of the water column by remotely controlled from the deck of the vessel. Therefore, a total of 20 L seawater was collected for each sample and 5 L of this was filtered for monitoring microplastics. Altogether 46 water samples were collected for the study. Physicochemical parameters (water salinity, temperature, chlorophyll a, turbidity and pH) were measured simultaneously *in situ* by CTD.

Surface marine sediments (top 3 cm depth) of the water column stations were collected simultaneously using a deployed stainless steel box corer having a sampling area of 0.1 m^2 with minimal disturbance. Each sediment sample was collected in polyethylene bags after packaged with aluminum film. A dry weight of 500 g sediment sample was used for extraction of microplastics. All the samplers and sample containers were washed with Milli-Q water three times prior to sampling.

2.3. Microplastic extraction from water and sediment samples

Extraction of microplastics from the water sample was conducted using the method of Su et al. (2016). Briefly, each water sample was filtered through a 47-mm-diameter cellulose nitrate filter with 5 μm porosity (Whatman AE 98). All the residues on the filters were washed into a glass breaker containing hydrogen peroxide (30%, v/v) and the beaker was immediately covered with a watch glass to avoid spillage. The glass breakers were placed on an electric heating plate at 120 °C for approximately 48 h in order to completely remove organic matter from the residues. After standing for 24 h, the mixture in the beakers was transferred onto a 20-μm nylon net filter. The filters were then stored for further identification.

Extraction of microplastics from the sediment samples was conducted using a continuous air floating separation method developed by our group (Zhou et al., 2018). Briefly, 500 g of wet sediment was dispersed thoroughly with 0.5 mol L^{-1} sodium hexametaphosphate solution and then transferred to a sample cup which was placed inside a 5-L container. Saturated sodium chloride solution was pumped into the sample cup at a flow rate of 1.0 L min^{-1} and air was blown into the cup simultaneously using a bubble stone. Low density materials including microplastics overflowed in suspension into the outside container due to the air flotation. All the suspension in the container was then pumped into a vibrating sieve (50 μm) after overflowing was completed. This procedure was repeated three times and the 500 g original sediment sample was ultimately concentrated into approximately 100 g of residue. The residue on the sieve was collected and settled in a zinc chloride solution (1.5 g cm^{-3}) for 48 h for microplastic separation. The floating materials were collected by filtration using a 5-μm cellulose nitrate filter (Whatman AE 98).

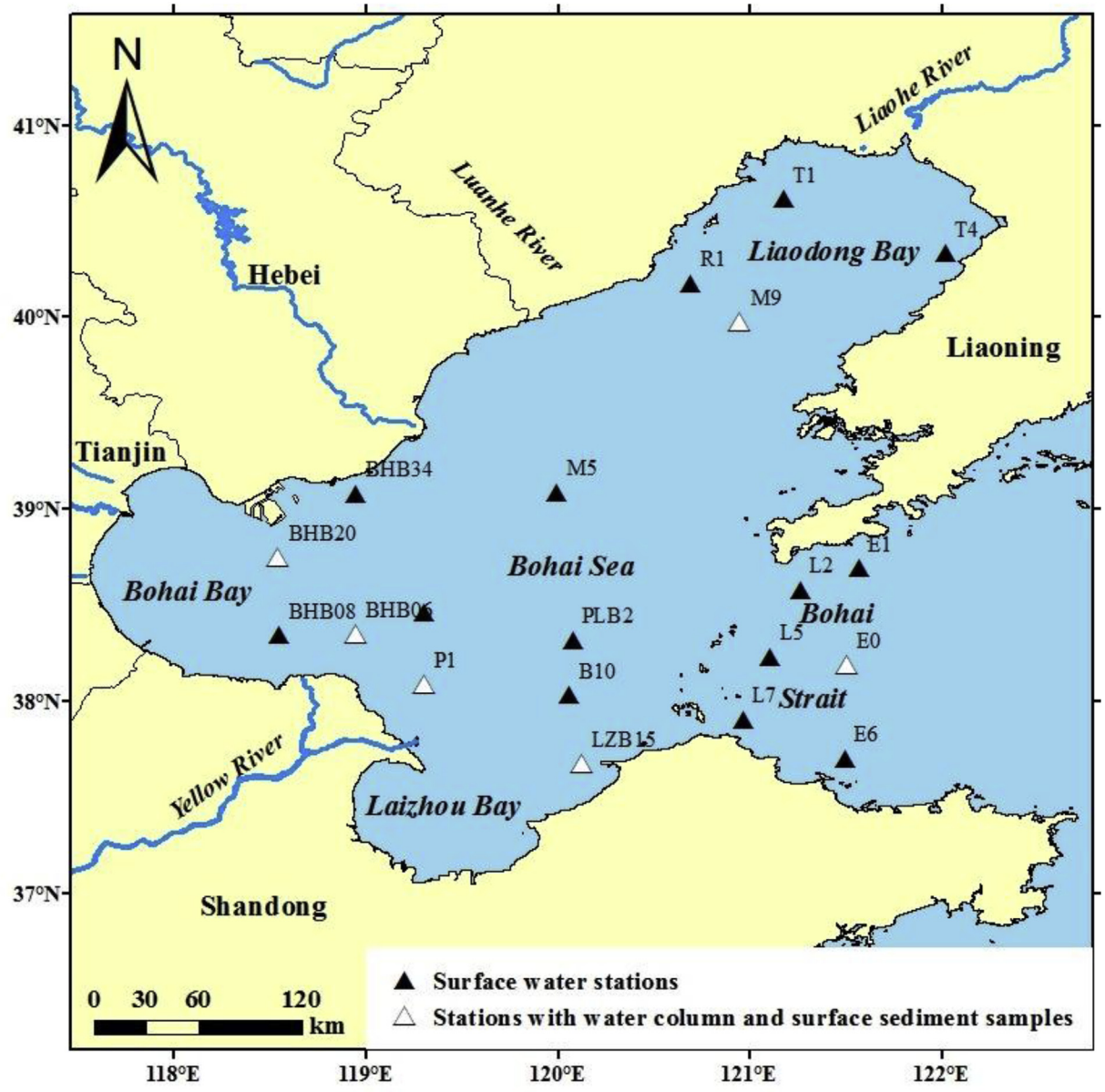

Fig. 1. Water and sediment sampling stations in the Bohai Sea.

2.4. Identification, quantification and characterization of microplastics

Preliminary identification of micropolastics in the filtered residues was conducted by visual examination under a Nikon SMZ25 microscope. Three rules were setup to avoid mis-identification of the microplastics based on the methods of Mohamed Nor and Obbard (2014) and Peng et al. (2017) as follows. First, particles that cannot be torn apart with a tweezer. Second, particles that do not have cellular or organic structures. Three, fibers that are equally thick throughout their entire length and are not tapered. All the potential microplastics were picked up and sorted based on their shapes and colors. Suspected microplastics were also picked up and sorted separately. Photographs were taken of the potential and suspected microplastics. A further polymer identification was carried out using Nicolet 6700 μ-FT-IR (Thermo Scientific, Waltham, MA) for all the suspected and some selected potential microplastics covering all the shapes and colors (See supplementary Fig. S2 online). Overall, a total of 98 microplastics samples were analyzed. Quantification and size measurement of the identified microplastics were performed using the program Nano Measurer 1.2.

The microscopic surface features of the microplastics were observed using a Hitachi S-4800 field emission scanning electron microscope equipped with a Horiba Emax Energy EX-350 energy dispersive x-ray microanalyzer (Hitachi, Tokyo, Japan).

2.5. Quality assurance (QA) and quality control (QC)

All instruments and containers were cleaned with MilliQ water and considered sterile before use. Sampling and analysis devices were covered with aluminum foil when not in use. Sample separation and identification were carried out in a laboratory specified for microplastic studies. Non-textile robes, caps and gloves were worn in order to prevent contamination from fibers and hairs when conducting the experiments. Procedural blanks (absence of

biological material or microplastics) were run in parallel with water samples on the ship and in the laboratory. Fibers were monitored in the blanks and ranged from 2 to 5 pieces L^{-1}. Thus, quantification of fibers in the samples was corrected with blanks.

3. Results

3.1. Abundance and spatial distribution of microplastics in the surface waters

Microplastics were detected in all 20 surface water samples collected from the Bohai Sea. The abundance of the microplastics ranged from 0.4 to 5.2 pieces L^{-1} and averaged 2.2 pieces L^{-1} (Table 1). As shown in Fig. 2, spatial heterogeneity in microplastic abundance was found in the study area. The E0 station in the Bohai Strait was detected highest microplastic abundance in the surface waters, followed by the BHB20 station in Bohai Bay. While the T1 station in the Liaodong Bay shows the lowest abundance of microplastics. In addition, other sampling stations with abundances >3.0 pieces L^{-1} occurred throughout Bohai Bay and Liaodong Bay. As a whole, the microplastic abundance in Bohai Bay was highest on average, however that of the Central Basin was the lowest (Table 1).

3.2. Microplastic abundance in the water column and surface sediments

There was no consistent trend of microplastics abundance change with depth among the six water column stations in the Bohai Sea as shown in Table 2. The two stations named of BHB06 and BHB20 in Bohai Bay exhibited microplastics accumulation in the surface water. While other four stations had higher accumulation of microplastics in the depth ranged of 5–15 m. For instance, station M9 in Liaodong Bay and station P1 in Laizhou Bay had the highest microplastic abundance at the depth of 5 m, while station E0 in the Bohai strait and station LZB15 in the Laizhou Bay had the highest microplastic abundance at the depth of 10 m and 15 m, respectively. However, microplastics abundance at deeper layer (>20 m) of the water column was lower in general or even not detected in the 5 L water sample. The abundance of microplastics in surface sediments was inconsistent with that in the water column among the six stations. The highest abundance of the microplastics in the sediment was found at station BHB06 in Bohai Bay, in contrast to the lowest average abundance in its water column. However, the lowest abundance in the sediment was found at station E0 in Bohai Strait among the six stations, in contrast to the highest abundance in its water column.

3.3. Changes in types and size-fractions of microplastics in the water column and sediments

The types of microplatics found in the water column and sediments based on shape and color are shown in Fig. 3. Fibers were the dominant components in both the water and the sediments in general, followed by fragments (Fig. 3a). However, slight changes were found in the vertical distribution of microplastic types in the water column. The proportion of fibers was lower in the surface layer than in the deeper layers of the water column. Particles and films were only found at some specific layers of the water column, which probably resulted from the small water sampling volume. Microplastic types in the sediments were more diverse than in the water column, which all four shapes occurred. White, blue and black were the three dominant colors of microplastics in the water column and sediments, with white accounting for >50% in most of the water layers and sediments (Fig. 3b).

Fig. 4 shows the change in plastic size fractions with depth in the water column and in the sediments. Microplastics were dominant in the water column and sediments and the size fraction >5 mm accounted for a very small percentage (Fig. 4 and Supplementary Table S2). Most microplastics were within the size range of 100–3000 μm. A notable increase in the percentage was observed in the size fraction <300 μm which increased from the surface water to a depth of 30 m in the water column.

Table 1
Microplastic abundances (pieces L^{-1}) in the surface water of different regions of the Bohai Sea.

Region	*N*	Mean	Range
Liaodong Bay	4	1.7 ± 1.2	0.4–3.4
Bohai Bay	4	3.0 ± 1.6	0.8–4.6
Laizhou Bay	2	2.9 ± 1.8	1.6–4.2
Central basin	4	0.9 ± 0.2	0.8–1.2
Bohai Strait	6	2.6 ± 1.4	1.0–5.2
The whole Bohai Sea	20	2.2 ± 1.4	0.4–5.2

3.4. Polymer identification and surface microscopic features of the microplastics

The microplastic polymers consisted of polypropylene (PP), polyethylene (PE), polyvinyl chloride (PVC), polystyrene (PS), polyethylene terephthalate (PET), acrylonitrile butadiene styrene (ABS) and cellulose as shown in Fig. 1S of the supplementary online material. Copolymer with PE and PP was also identified in the microplastic samples. In the surface waters, the microplastic polymer detected with the highest density was polystyrene which has a density of 1.06 g cm^{-3}. In contrast, in the deeper water polyethylene terephthalate (density 1.39 g cm^{-3}) and polyvinyl chloride (density 1.56 g cm^{-3}) were found. Polyethylene microplastics were detected in the sediments despite their densities being lower than that of seawater. Fig. 5 shows the microscopic features of the microplastic surfaces in the water column and sediment with a same magnification scale. Severe erosion of the surfaces could be observed on the micro-fibers in the deeper water layers than in the surface water or 5 m water layer. Biofouling and particle adhering could also be observed on the microplastic surface as shown in the sample from the 10 m depth and sediment, respectively (Fig. 5).

4. Discussion

Microplastic abundances reported here are nearly three orders of magnitude higher than found in a previous survey conducted by Zhang et al. (2017) and the big difference was mainly due to the different sampling methods used in the two studies. Manta nets with 330 μm mesh were used for water sampling in the survey of Zhang et al. (2017) and most fibers of diameter 10–50 μm may have easily escaped from the trawl net (Setala et al., 2016). As shown in Table 3, similar differences in the data can be found in a survey of the Yangtze estuary and East China Sea (Zhao et al., 2014). It was noticeable that the percentage of microplastics with sizes <330 μm increased dramatically with increasing depth of the water column in our study (Fig. 5). The use of a Manta net with 330 μm mesh might therefore have underestimated the microplastic loadings.

The microplastic abundance reported here for the Bohai Sea is higher than that found in the open ocean, for example in the northeast Pacific Ocean (Desforges et al., 2014), but lower than found in the Yangtze estuary, another coastal water greatly influenced by anthropogenic activities (Table 3). In the Bohai Sea the stations approaching the coastline had higher microplastics abundances than the central basin (Fig. 2). In particular, in Bohai Bay the

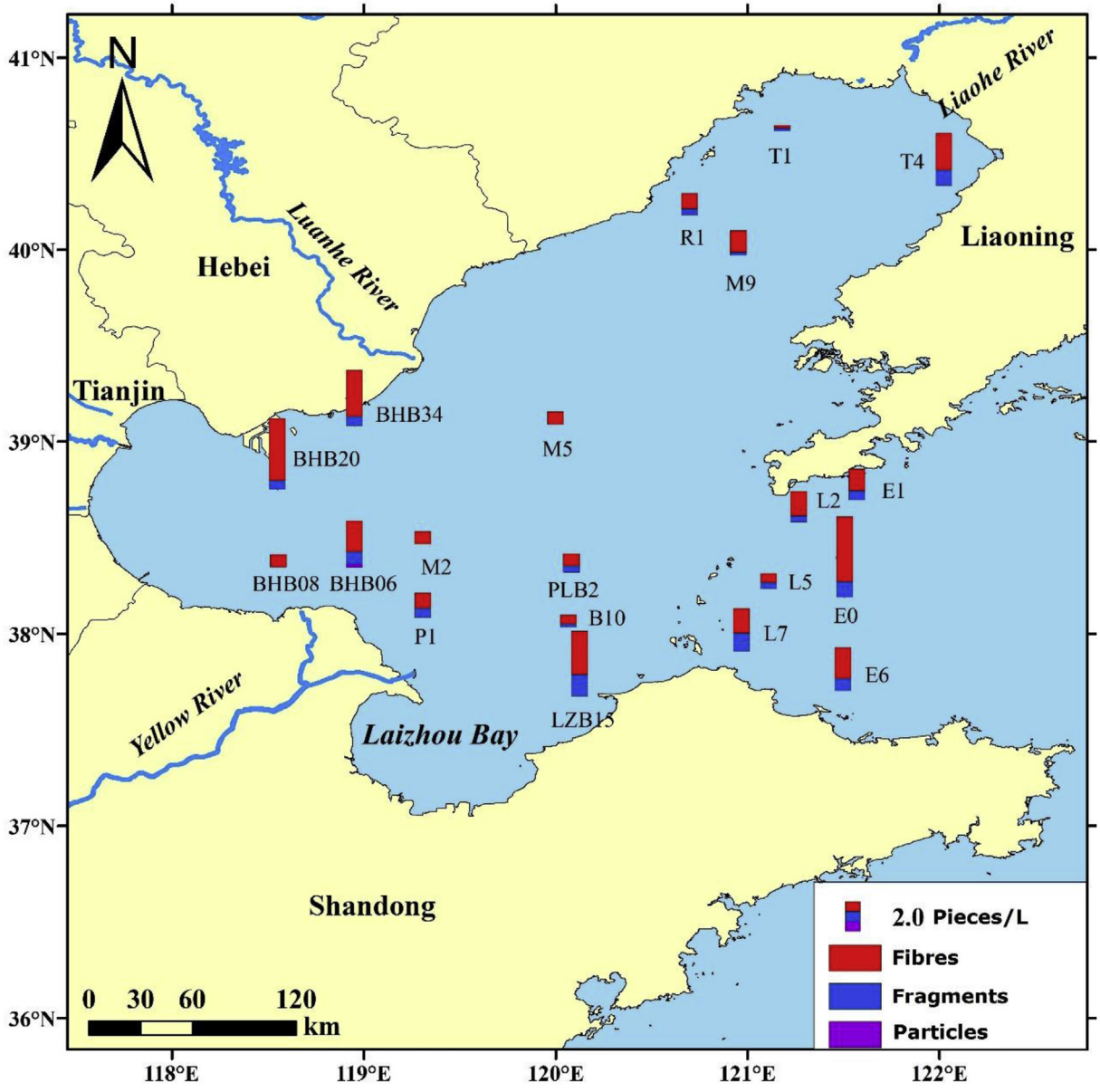

Fig. 2. Spatial distribution of different microplastics in surface waters of the Bohai Sea.

Table 2
Microplastic abundance in water columns (pieces L^{-1}) and surface sediments (pieces kg^{-1}) of the Bohai Sea.

Water column depth (m)	Liaodong Bay	Bohai Bay		Laizhou Bay		Bohai Strait
	M9	BHB20	BHB06	P1	LZB15	E0
0	1.6	4.6	3.0	1.6	4.2	5.2
5	23.0	2.8	1.2	7.6	3.6	7.8
10	3.0	2.0	2.2	3.6	4.4	21.6
15	1.6	3.6	0.6	2.0	6.2	3.4
20	0.8	3.8	1.2	n.a.	n.a.	6.6
25	0.2	3.6	n.a.	n.a.	n.a.	2.2
30	n.a.	n.a.	n.a.	n.a.	n.a.	1.2
Average in water column	5.0	3.4	1.6	3.7	4.6	6.9
Surface sediment	105.4	72.6	256.3	95.8	50.9	31.1

Note: n.a. indicates no data available.

abundance was significantly higher than in the central basin (Table 1). Spatial heterogeneity was also found in the survey conducted by Zhang et al. (2017) in this area. We found station E0 had the highest abundance of microplastics in the surface water and in the water column. This sampling station was on the cruise route between Yantai and Dalian, two tourist cities located at either end

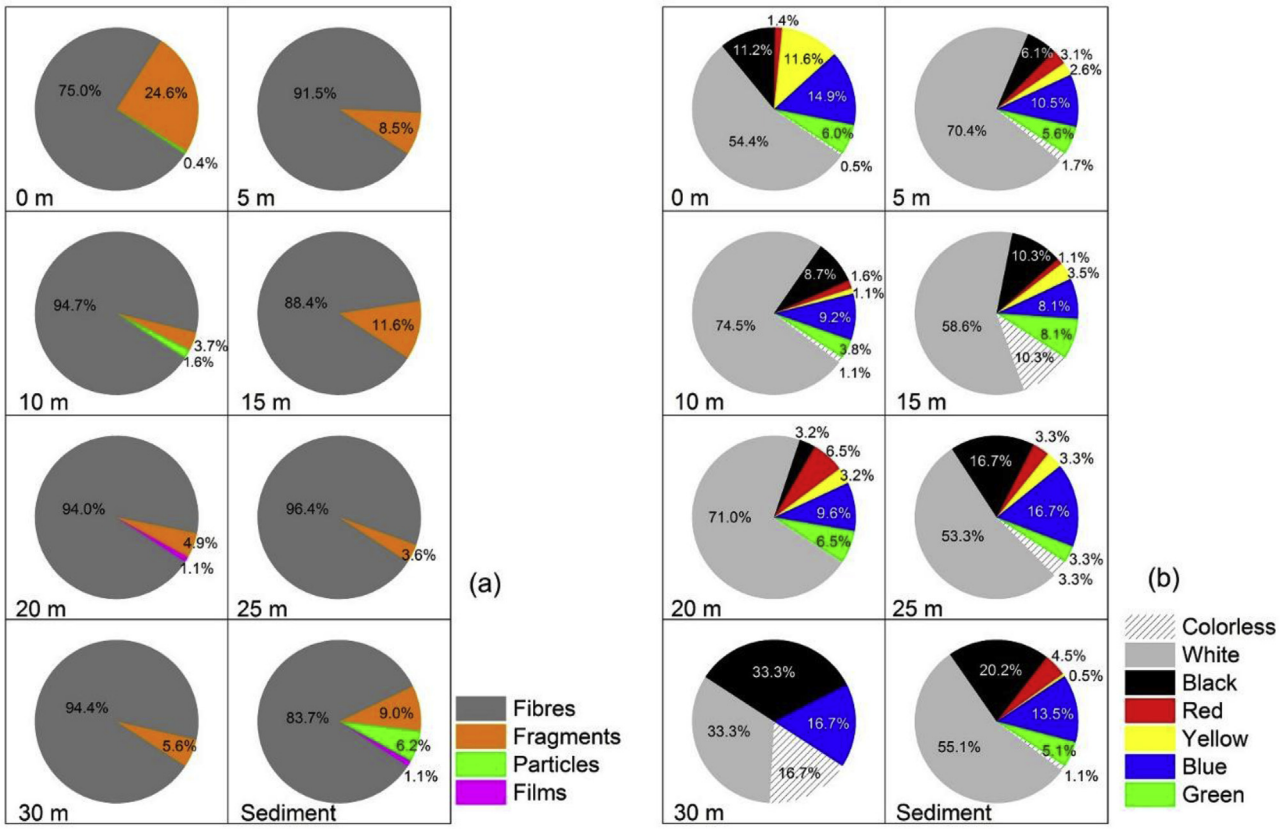

Fig. 3. Microplastic (a) shapes and (b) colors in the water column and sediments of the Bohai Sea. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

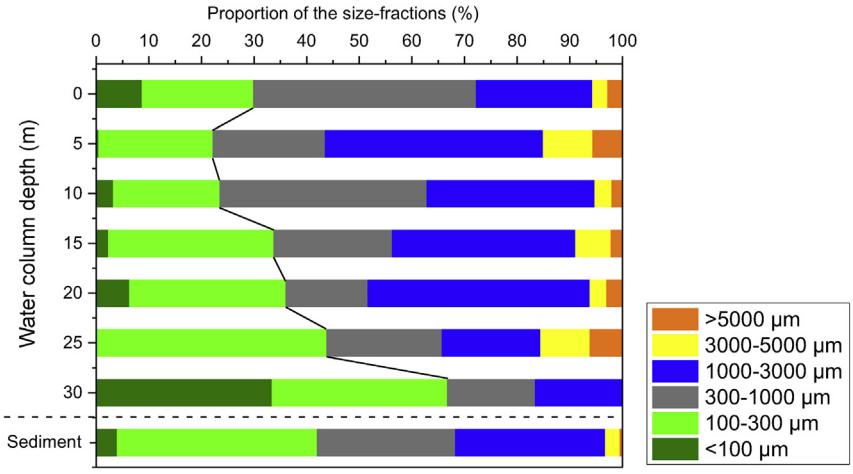

Fig. 4. Bar chart showing the changes in size-fractions of the plastics with increasing depth of water and their differences from the sediments.

of the Bohai strait. The high abundance of microplastics might therefore be associated with higher shipping activity (Lusher et al., 2015).

Monitoring of multiple water layers rather than surface water merely was more reliable to obtain the spatial distribution and loading of microplastics in a water body since the microplastics might accumulate in the deeper water (Liedermann et al., 2018; Woodall et al., 2014). Our results show higher abundance of microplastics accumulation at depths of 5–15 m than in the surface water (Table 2). Lusher et al. (2015) also observed more microplastics in the water column (6 m depth) than in the surface water in the Arctic Ocean. One possible explanation for the depth distribution may be the different composition of different polymers. Polyvinyl chloride (PVC) has a higher density than polystyrene or polyethylene and hence PVC was found only at the bottom layer in the water column of the Bohai Sea in the present study. Turbulence due to water currents might also affect the redistribution of microplastics in the water column. Surface currents in the Bohai Sea have a higher velocity than the sub-surface ocean current and bottom current (Corcoran, 2015) and this may

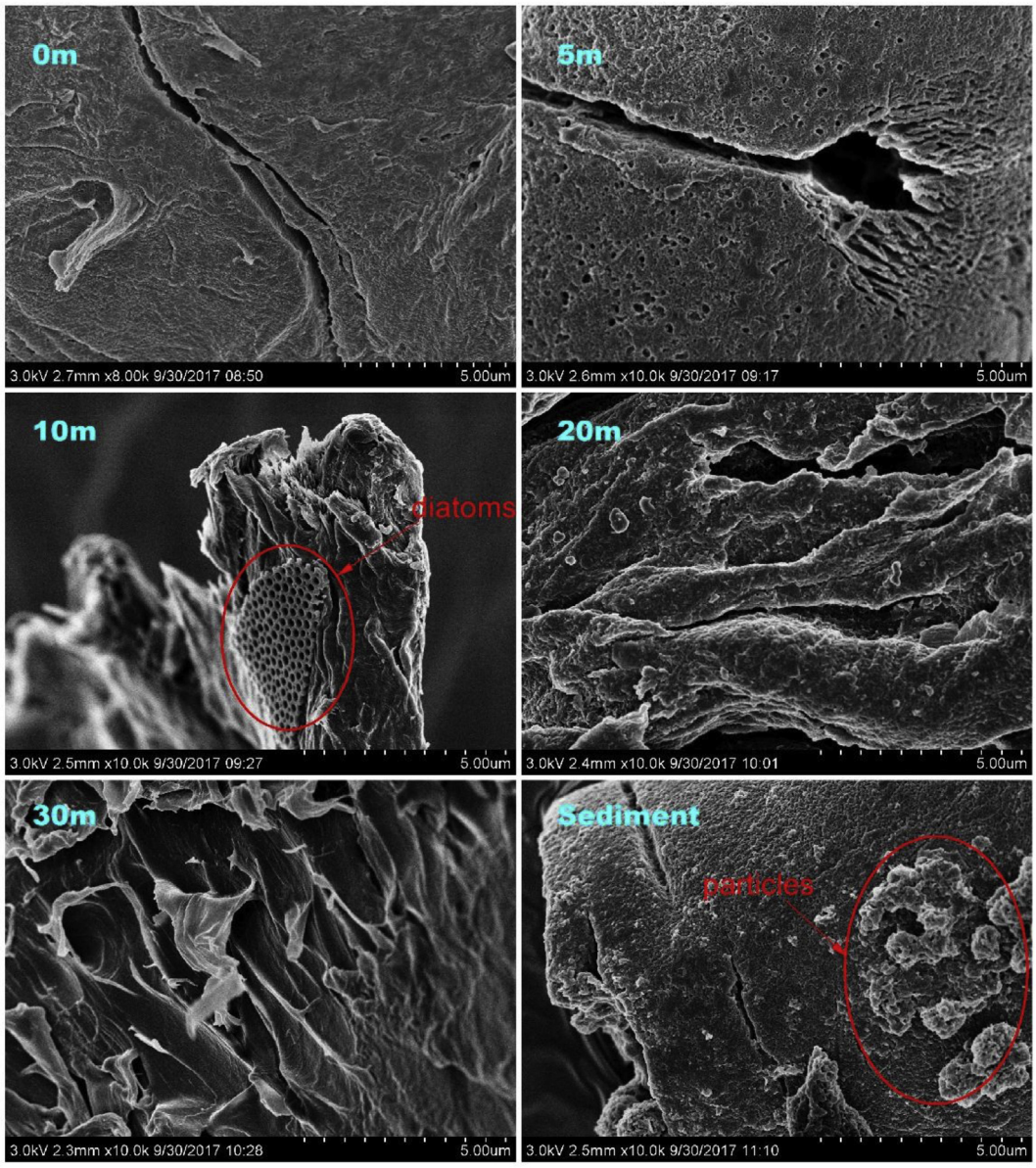

Fig. 5. Scanning electron microscope (SEM) images showing microscopic surface features of the micro-fibers from the different depth of the water column and surface sediment of the Bohai strait, ten thousands magnification was used in the scanning.

Table 3
Comparison of microplastic abundance in the Bohai Sea with other studies.

Study area	Depth (m)	Sampling method	Mesh size (μm)	Abundance (pieces m^{-3})	References
NE Pacific Ocean	4.5	saltwater intake system	62.5	2080 ± 2190	Desforges et al., 2014
Arctic Ocean	0.16	manta net	333	0.34 ± 0.31	Lusher et al., 2015
	6	seawater pump	250	2.68 ± 2.95	Lusher et al., 2015
North Atlantic subtropical gyre	0–0.5	multi-level trawl	150	0.68	Kooi et al., 2016
	0–5	multi-level trawl	150	0.11	Kooi et al., 2016
	4.5–5	multi-level trawl	150	0.02	Kooi et al., 2016
Yangtze Estuary	1	DC Teflon pump	32	4137.3 ± 2461.5	Zhao et al., 2014
East China Sea	surface water	neustonic trawls	330	0.167 ± 0.138	Zhao et al., 2014
Bohai	surface water	manta net	330	0.33 ± 0.34	Zhang et al., 2014
Bohai	surface water	stainless steel bucket	20	2200 ± 1387[a]	This study
	5	CTD (Seabird 911 plus)	20	7667 ± 7271[a]	This study

[a] The unit conversion from pieces L^{-1} to pieces m^{-3} is obtained by multiplying by a factor of 1000.

easily lead to transport of microplastics from the surface to the sub-surface with long residence times at the sub-surface. In addition, different shapes of plastic particles between the surface and the sub-surface layers in the water column (Fig. 3a) suggest that

physical shapes might also affect the depth distribution of the microplastics in the water column (Kooi et al., 2016). Bagaev et al. (2017) found that fibers were the prevalent type of microplastics in the water column, especially in the near-surface and near-bottom layers.

Sinking behavior which is influenced by surface properties and biofouling of microplastics is important in determining their vertical distribution. Rough surfaces with irregular cracks and pores were observed in the microplastics collected in the current study (Fig. 5). This may increase the probability of attachment or incorporation of foreign particles such as clay minerals or quartz grains (Corcoran, 2015; Zhou et al., 2018), decreasing the buoyancy of the particles (Kowalski et al., 2016). Moreover, weathered surfaces may favor the adhesion of microorganisms and ultimately the formation of biofilms, hence the biofouling process may affect the sinking of buoyant microplastics from the sea surface by increasing their densities (Corcoran, 2015; Fazey and Ryan, 2016; Harrison et al., 2018; Kaiser et al., 2017; Kowalski et al., 2016; Long et al., 2015). Here, we found an evidence of diatoms adhering on the surface of a fiber sample from the 10 m depth of water column (Fig. 5). Correlation analysis indicated an inverse relationship between the microplastic abundances and chlorophyll *a* concentrations in the surface water (See supplementary Table S3 online). Both of the above results suggested that algal aggregates might have an effect on the sinking of floating microplstics from the surface water in this area (Bergmann et al., 2017). However, more studies are still required to be conducted for confirming the assumption.

In addition, an elevated proportion of smaller size (<300 μm) microplastics with increasing water depth presented in the water column (Fig. 4). This distribution of size-fractions in the water column indicates that microplastics of smaller size are more likely to sink than large ones. This is consistent with the observations of Fazey and Ryan (2016) who found that small microplastic particles lost buoyancy much more rapidly than larger ones as a result of biofouling in False Bay, South Africa over a study period of 12 weeks.

However, sinking of microplastics may not ultimately result in incorporation in the benthic sediments (Rummel et al., 2017). As shown in the current study, microplastic abundance in the water column was not consistent with their abundance in the sediments among the six water column stations (Table 2). Microplastics settle with higher probability in areas with very calm environments, i.e., in the deepest parts of the sea and bottom depressions, where the finest sediments also settle (Bagaev et al., 2017). Microplastic abundance in the sediments might therefore be assumed to be affected by rate of sinking of the particles as well as the physical conditions of the benthic sediments.

In summary, we have indicated microplastic abundance and vertical distribution in the water column of the Bohai Sea for the first time. This study also reminds us that surveys of surface waters are far from sufficient in determining the contamination status of microplastics in a water body. Higher levels of microplastics might accumulate in the Bohai Sea to depths of 5–15 m rather than in the surface water. Furthermore, the shapes and sizes of the microplastic particles also change from the surface to the bottom of the water column. This may result in different ecological effects on marine organisms inhabiting different water layers. More attention therefore needs to focus on microplastics pollution in the whole water column and the associated environmental risks.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2018.07.131.

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