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RESEARCH ARTICLE

Occurrence and distribution of micro- and mesoplastics in the high-latitude nature reserve, northern China

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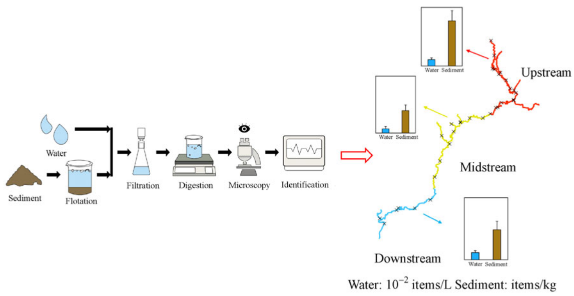
HIGHLIGHTS

- The first study on micro(meso)plastics (MMPs) in the Liaohe River Reserve is reported.
- Diverse MMP were detected in surface water and sediment at all 32 sites.
- The abundance of MMPs decreased in the course of the river.
- The MMPs abundance in water is significant association with the county population.

GRAPHIC ABSTRACT



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ABSTRACT

Microplastics pollution has received growing attention worldwide in recent years. However, data on microplastics in the freshwater environment are still limited, especially in high-latitude nature reserves in Northern China. The first study on microplastic pollution in the Liaohe River Reserve in Northern China is reported here, and mesoplastics were also incorporated. Surface water and sediment samples were collected from 32 sites along the nature reserve. The abundance, type, shape, color, and size of micro- and mesoplastics were measured using density extraction, optical microscopy, and FTIR spectroscopy. The data showed that diverse micro- and mesoplastics were found widespread in the 32 sites, and the average abundance of these plastics was 0.11 ± 0.04 10^{-2} items/L in surface water and 62.29 ± 54.30 items/kg in sediment. Moreover, 70% and 66% were smaller than 2000 µm in surface water and sediment, respectively. Fiber accounted for 91.86% in surface water and 43.48% in sediment, indicating that the major source of micro- and mesoplastics in the Liaohe River Reserve may be domestic sewage and aquaculture. A total of 16 and 27 polymers were identified in surface water and sediment, respectively, and mostly consisted of rayon, polyester, polystyrene, and poly(ethylene terephthalate). Moreover, both the risk index and the pollution load index demonstrated a low risk of micro- and mesoplastics in surface water and sediment in the Liaohe River Reserve.

1 Introduction

Plastic products are widely used in daily life due to their lightweight, anticorrosion and inexpensive properties (Thompson et al., 2009). The current annual global production and consumption of plastics has been reported to exceed 368 million tons (Plastic Europe, 2020), accompanied by extreme quantities of plastic waste released into the environment. Plastics have a very long residence time in the environment and during this period, these polymers may undergo fragmentation and degradation resulting from physical, chemical, and biological processes, which generate mesoplastics with a size larger than 5 mm but smaller than 2 cm and microplastics with a size smaller than 5 mm (Thompson et al., 2004). Certainly, the monomers, oligomers, and additives may release in this process and may have negative effects on organisms (Wright and Kelly, 2017). Therefore, micro- and mesoplastics have become an emerging persistent contaminant

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of global concern.

The occurrence, origin, and fate of microplastics have been extensively studied in seawater, marine sediments, beaches, and shorelines (Van Cauwenberghe et al., 2013; van der Hal et al., 2017; Xu and Ren, 2021). Due to the high intensity of human activities near the inland freshwater rivers, microplastic pollution in freshwater has attracted growing attention in recent years (Sun et al., 2022). More importantly, freshwater environments were suggested to be an important source of marine microplastics (Horton et al., 2017a).

China is both a large producer and consumer of plastic materials in the world, accounting for 31% of the global plastic production (Plastic Europe, 2020). Therefore, a considerable amount of plastic waste is released into the environment, which has been a major source of micro- and mesoplastics. The first report on microplastic pollution in freshwater (Three Gorges Reservoir) in China was published by Zhang et al. (2015). Since then, a series of related articles focusing on the microplastics distribution in freshwater systems in southern China have been reported, including research in the Taihu Lake (Su et al., 2016), the Yangtze River (Xiong et al., 2019), the Poyang Lake (Yuan et al., 2019), the Dongting Lake (Hu et al., 2020), the Pearl River (Lam et al., 2020), and the Yulin River (Mao et al., 2020), etc. However, current research on freshwater is mainly concentrated on the river systems in southern China, while relevant research has not been common in the high-latitude northern regions.

In this study, the spatial distribution of micro- and mesoplastics in the water and sediment phase in the Liaohe river reserve in north-east China was studied. The area was chosen because the Liaohe River is one of the most important aquatic ecosystems in China, being an important base of industrial and agricultural production and one of the most developed regions in north-east China. Serious pollution has been experienced in this basin for the last few decades because of the fast economic development and lack of effective control of sewage discharged from domestic and production (Ke et. al., 2017). After the establishment of the Liaohe River Reserve in 2010, the environment in the basin has gradually recovered, mainly due to the reduction of anthropogenic disturbance. Conventional contaminants (like N, P, and heavy metals) were effectively controlled (Ke et. al., 2017), but information on microplastics remains unclear. Therefore, the objectives of this study are 1) to investigate the micro- and mesoplastics distribution and characteristics in the water surface and sediment of the Liaohe River reserve, and 2) to assess the environmental risks of micro- and mesoplastics in the reserve based on risk assessment models. The data provided by this study provides important information on micro- and mesoplastics pollution in high-latitude freshwaters and also serves as a reference for a better understanding of the microplastic distribution regularity in different basins.

2 Materials and methods

2.1 Study area

The Liaohe River is one of seven big rivers in China. It serves the economic and social development of north-east China and it is an important center of economic and community activities (Liu, 2013). The Liaohe River Reserve was established in 2010 (43°02′–40°47′N, 123°55.5′–121°41′E) with an area of 1869.2 km^2, originating from the confluence of the East and West Liaohe River, meandering through 4 cities, 14 counties (districts), and emptying into the Bohai Sea in Panjin city (Fig. 1). The climate is warm-temperate with a semi-humid continental monsoon climate, and the topography is predominately flat with a single landform, namely, alluvial plain.

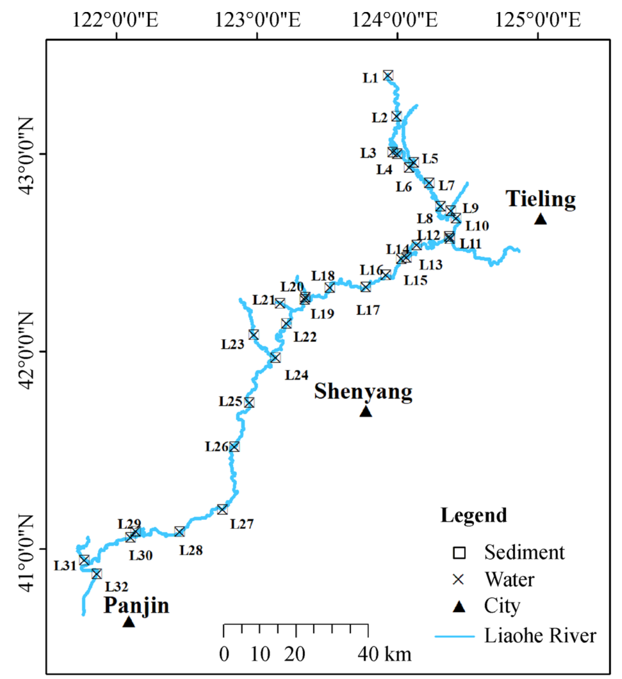

Fig. 1 Sampling sites in the Liaohe River Reserve (sites L1–L15 are located in the upstream region from Tieling City; sites L16–L26 are located in the midstream region from Shenyang City; sites L27–L32 are located in the downstream region from Anshan and Panjin City).

2.2 Sample collection

Surface water and sediment samples were collected from 32 sites along the Liaohe River Reserve in September 2018 (Fig. 1). At the same time, the flow rate and turbidity at each site was measured during each sampling. Spacial distance from the sampling site to the city center was obtained using GPS coordinates, and the county populations of each site were obtained from Liaoning statistical yearbook 2018. Data details are presented in the Supplementary Information (Table. S1).

At each site, 20 L of river surface water was collected using a 5 L clean steel bucket four times and then filtered using a 50-μm stainless steel standard sieve. The particles remaining in the sieve were washed with DI water and stored in clean bottles prepared in advance. Sediment samples on the surface layer (0–5 cm) at each site were randomly sampled using a five-point sampling method in a 50 cm × 50 cm quadrat. All samples were transferred to the laboratory and stored in pre-labeled glass jars at 4°C until further analysis. Three replicates were taken in parallel at each site.

2.3 Microplastic extraction

Water samples were filtered through a membrane (47 mm diameter, 5 μm aperture, cellulose nitrate filter) by vacuum extraction (negative pressure ≤ 40 kPa). The filtered samples were transferred into glass bottles. Hydrogen peroxide (H_2O_2, 30%, v/v) was added for the digestion of organic matter at room temperature (25°C) for 72 h. Then, the samples were dried in an oven at 40°C until constant weight was obtained. Finally, they were analyzed under a stereoscopic microscope (Olympus SZ61, Japan).

For sediment, all samples were dried at 60°C for 72 h. To extract microplastics from sediment, the samples were treated using a density separation method from Su et al. (2016). Briefly, zinc chloride solution (ρ = 1.5 g/mL) was added to dried sediment samples. The suspension was stirred for 30 min and left standing undisturbed for 12 h. Then, the supernatant was filtered through a 5 μm cellulose nitrate filter by vacuum extraction. The above filter film containing the suspected microplastics was washed with H_2O_2. When all the particles on the filter film were transferred to the beaker, 100 mL of 30% H_2O_2 was used to eliminate impurities such as organic matter. The digestion conditions and subsequent operations were the same as those of the water samples.

2.4 Microplastic identification

The material on the filters was visually inspected by a stereomicroscope with a digital camera (Z61, Olympus, Japan). The suspected microplastics were placed on a 1 mm × 1 mm grid paper for easy counting and statistical analysis. The abundance unit of micro- and mesoplastics in water is expressed as items/L, and that in sediments is expressed as items/kg. Based on the classification criteria established previously (Hidalgo-Ruz et al., 2012), the particles were characterized with regards to their size (longest particle diameter), shape (fiber, film, fragment, pellet, and foam), and color (transparent, white, yellow, blue, green, red, black, purple, and multicolor).

A subsample of the micro- and mesoplastics was manually isolated and analyzed by a Fourier Transform Infrared spectrometer (PerkinElmer, Spectrometer 400, USA) fitted with an ATR accessory (ATR-FTIR) to determine the polymer type. The particle spectra were compared to the Agilent Sadtler infrared spectrum database. Considering that micro- and mesoplastics have been eroded by the aquatic ecosystem to different degrees, a spectrum that could be matched with a quality index of > 60% was accepted. The typical infrared spectra of the randomly selected plastics and the matching results are shown in Supplementary Fig. S1 of the Supplementary Information.

2.5 Quality assurance and quality control (QA/QC)

During the experiment, every step should avoid any experimental error caused by the pollution of microplastics in the environment. Sampling tools and all experimental consumables were cleaned by ultra-pure water for three times before use and rinsed with river water before collecting the samples. The sample pretreatment and analysis process was carried out in a clean laboratory using non-plastic materials and avoiding direct contact with consumables made of plastic materials. Cotton laboratory coats and nitrile gloves were worn during sampling and analysis processes. Blank controls were analyzed to correct microplastic background pollution in the air during the experiment, and the results showed that no microplastics were found in the blank controls.

2.6 Statistical analysis

The results were analyzed using SPSS Statistics (SPSS v 22.0, IBM, New York, NY, USA) and are presented as mean values±SD as indicated. One-way ANOVA and Tukey's HSD post hoc test were used to examine the difference of microplastic abundance between the different sampling sites, when normality of data (Shapiro-Wilks test) and homoscedasticity (Levene's test) conditions were respected. When at least one condition was rejected, the Kruskal–Wallis test for pairwise comparison was used. Furthermore, the Person correlation analysis was used to identify the correlation between microplastic abundance and the county populations, distance from the sampling site to the city center, flow rate, and turbidity. A p-value of less than 0.05 was considered to indicate statistical significance. Microplastic pollution risk in the Liaohe River Reserve was analyzed based on risk assessment models relevant to both the abundance and the chemical composition of the microplastics (detailed in Supplementary Information and Table S5). Graphs were compiled using the Origin Pro 9.0 software (OriginLab Inc., Northampton, MA, USA). Mapping of the sampling sites was performed using ArcGIS10.2 (ESRI, Redlands, CA).

3 Results

3.1 Abundance of micro(meso)plastics in surface water and sediment

Micro- and mesoplastics were detected along the Liaohe River Reserve (Fig. 2). All the particles were analyzed together because the amount of mesoplastic was much lower than that of microplastic. Therefore, they will be referred to as micro(meso)plastic hereof. In total, 221 micro(meso)plastic particles were isolated from the surface water, with the abundance ranging from 0.02 to 0.42 items/L and an average of 0.11±0.04 items/L. Micro (meso)plastics were also found in all sediment samples, with abundance ranging from 6.67±1.15 to 240.00±52.92 items/kg and an average of 62.29±54.30 items/kg. From the 32 sampling sites, the abundance of micro(meso) plastics was the highest in surface water at site L18 (0.42 items/L) and in sediment at site L19 (240 items/kg), both in Shenyang city. On the other hand, abundance was the lowest in surface water at site L10 (0.02 items/L) in Tieling city and sediment at site L27 (6.67 items/kg) in Anshan city.

3.2 Properties of the micro(meso)plastics

3.2.1 Shapes

Four particle shapes (fiber, fragment, film, and pellet) were found both in surface water and sediment samples, while foam was only found in sediment (Fig. S2). Typical images of the five shapes of microplastics under a dissecting microscope are presented in Fig. S3. The percentages of the differently-shaped micro(meso)plastics in surface water and sediment is shown in Fig. 3. On average, the percentage of fiber was predominant (91.86%), followed by film (4.07%), fragment (3.17%), and pellet (0.97%) in surface water. The detection rate of fiber in all sites was 100% and the amount of fiber was significantly higher than that of other microplastics ($p < 0.05$) (Fig. S2). As seen from Fig. 4(a), an increase in abundance with decreasing particle size is evident for micro(meso)plastics in surface water, regardless of shapes.

In sediment, the average percentage of fiber was still the highest (43.48%), followed by fragment (24.41%) and film (20.40%), then foam (10.03%) and pellet (1.67%). The detection rate of differently-shaped micro(meso)plastics was different at each site (Fig. S2). The detection rate of fiber in sediment of all sites was 100%, while fragment were only detected at L03, L11, L17 and L32, and foam were only detected at L06, L19, L23 and L25. In addition, the dominant shape of micro(meso)plastics in each site was not the same. For example, the site L19 had the largest amount of foam (360 items/kg), the site L20 had the largest amount of fragment (260 items/kg), and the site L17 had the largest amount of fiber (180 items/kg), film (240 items/kg) and pellet (40 items/kg). Similarly, the abundance of fiber, fragment, film, and pellet in sediment

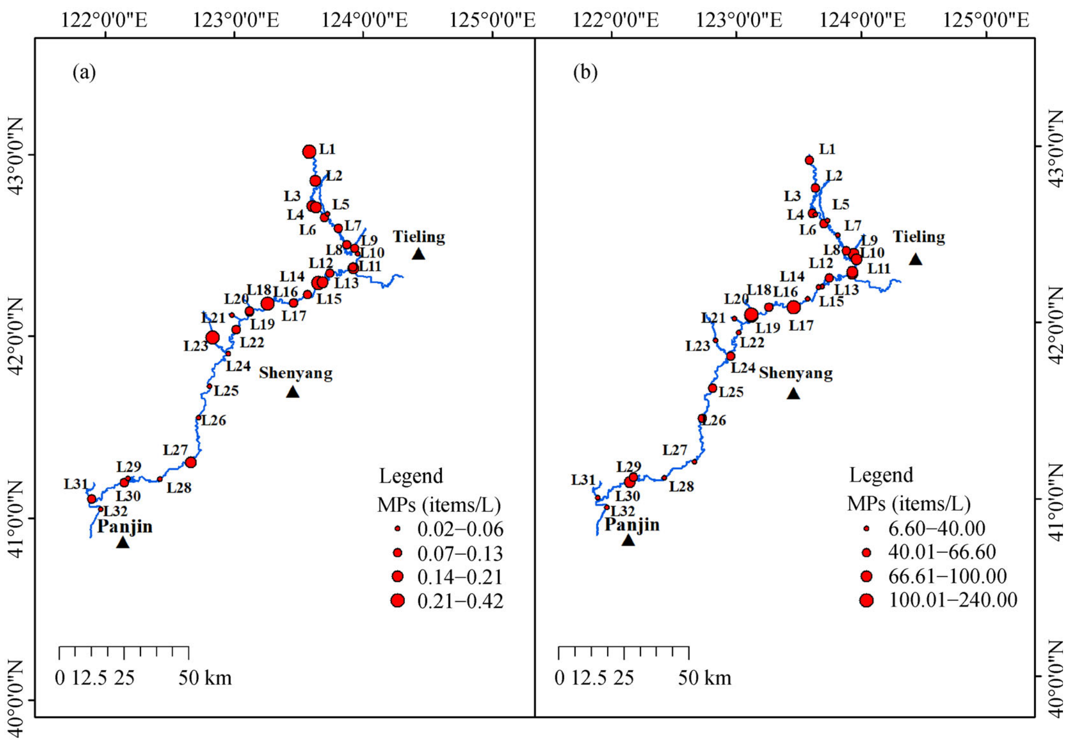

Fig. 2 Abundance and distribution of micro(meso)plastics in surface water (a) and sediment (b).

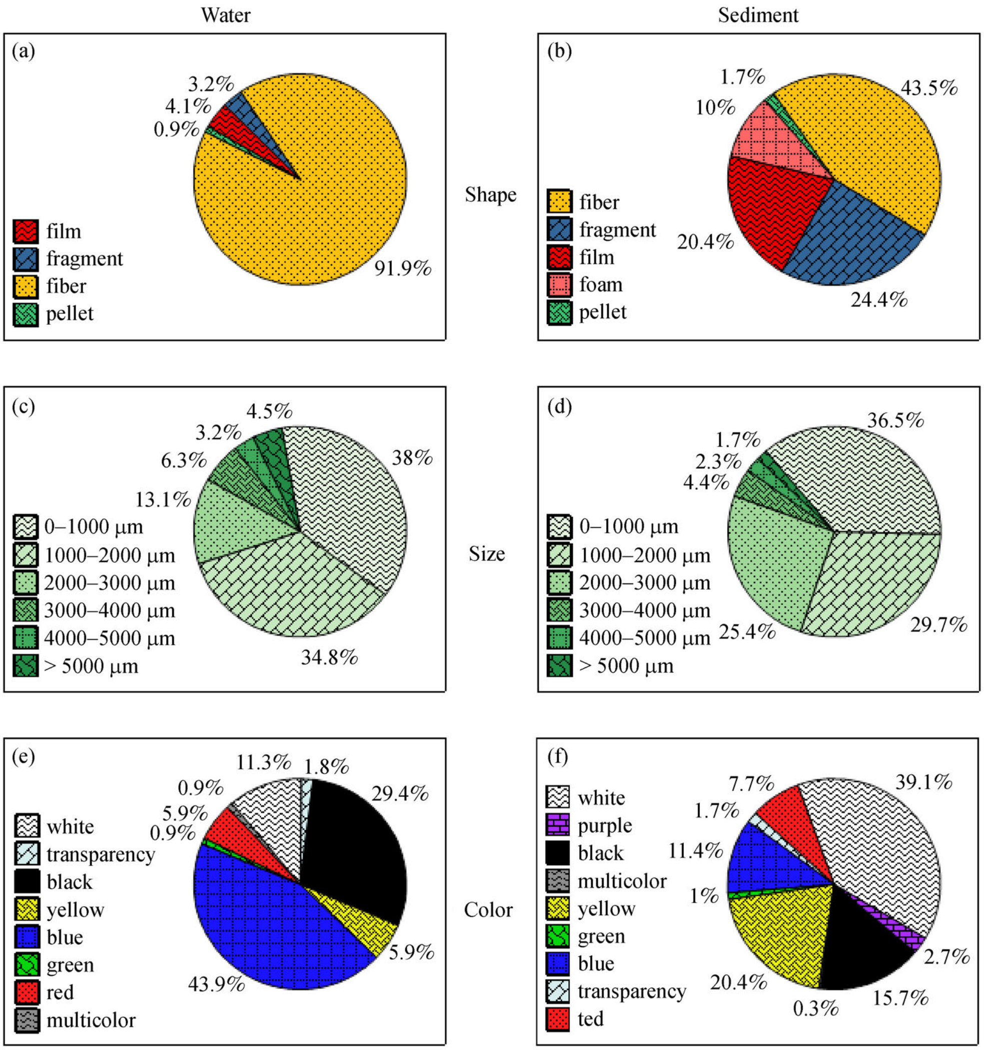

Fig. 3 Percentage of micro(meso)plastics in surface water and sediment (a) shape in water; (b) shape in sediment; (c) size in water; (d) size in sediment; (e) color in water; (f) color in sediment).

were also increased with the decrease of particle size, while the particle size of foam mainly fell within the range of 2000–3000 μm (Fig. 4(b)).

3.2.2 Size

The size of microplastics ranged from 143 to 5000 μm while that of mesoplastics was greater than 5000 μm. The proportion of micro(meso)plastics in each site is shown in Fig. 3. In the total of micro(meso)plastics from surface water in the 32 sites, 95.48% belonged to microplastics while 4.52% belonged to mesoplastics. Similarly, 97.66% of particles found in sediment in the 32 sites corresponded to microplastics while 2.34% corresponded to mesoplastics. Generally, the size distribution of micro(meso) plastics in the surface water and sediment increased exponentially with decreasing particle size (Fig. 4), with small microplastic particles (< 2000 μm) being predominant, accounting for 72.85% of micro(meso)plastics in surface water and 66.22% in sediment (Fig. 3).

3.2.3 Colors

Surface water and sediment samples showed different distributions of plastic colors (Fig. 3). Blue (43.89%) was the predominant color found in surface water, followed by black (29.41%) and white (11.31%). Other colors accounted for less than 10%. The color characteristics of micro(meso)plastics in surface water at each site were basically the same, except for L07 and L31 where yellow was the predominant color (Fig. S2). Differently, the predominant micro(meso)plastics were white (39.13%), followed by yellow (20.40%), black (15.72%), and blue (11.37%) in sediment samples. The color characteristics of

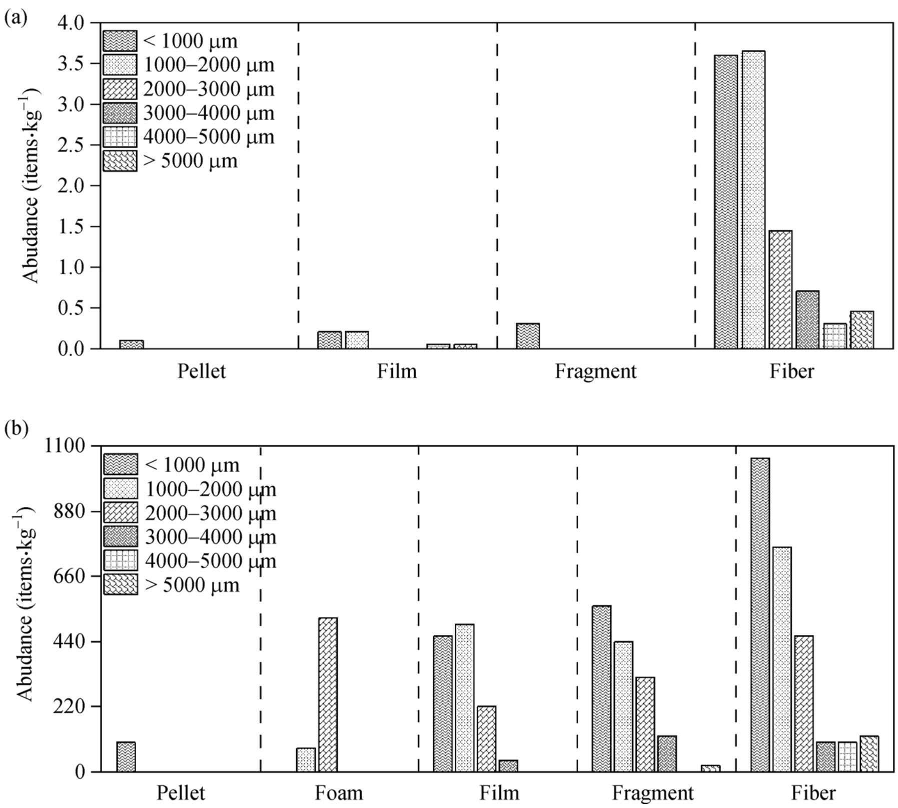

Fig. 4 Size frequency and typology of the different micro(meso)plastics in water (a) and sediment (b) as an average of all sampling sites.

micro(meso)plastics in sediment at each site were basically the same, except for L22 where purple was the predominant color.

3.3 Identification of micro(meso)plastics

The polymer composition of the visually identified micro (meso)plastics in the water and sediment samples was further determined by ATR-FTIR. In the surface water samples, 16 types of polymers were identified, mostly corresponding to rayon (56.11%), polyester (19.46%), and Poly(ethylene terephthalate) (PET, 7.69%), while each of the remaining polymer types accounted for less than 5% (Supplementary Table S2). A more diverse set of polymers was identified in the sediment samples, where 27 types of plastics were identified. Besides rayon (32.44%) and polyester (5.02%), plastic polymers in the sediments included polystyrene (17.06%), styrene-butadiene-styrene (7.69%), polyamide (6.69%), and cellophane (6.69%).

In addition, the shapes of the different micro(meso) plastics varied greatly (Supplementary Table S3 and S4). Rayon particles were predominant in fibrous micro(meso) plastics (water: 56.11%, sediment: 43.08%) as well as in fragmented micro(meso)plastics (water: 33.33%, sediment: 28.77%). Moreover, film-like micro(meso)plastics from surface water were mainly made of poly(octadecyl acrylate) (30.00%) and poly(ethylene:4-methyl-1-pentene) (20.00%), whereas those from sediment samples were mainly made of rayon (32.79%) and polyamide (13.11%). Comparatively, the component of pellet-shaped micro (meso)plastics were rayon (50.00%) and poly(N-methyl acrylamide) (50.00%). The foamy micro(meso)plastics were only detected in sediment samples, and all of them were polystyrene.

3.4 Correlation of micro(meso)plastics abundance and typical factors

Correlations between micro(meso)plastics abundance in surface water and typical factors like the county population, distance from the sampling site to the city center, flow rate, and turbidity, were performed. The micro(meso) plastics abundance in surface water showed significant association with the county population ($r = 0.57, p < 0.05$). However, there was no significant correlation between micro(meso)plastics abundance and distance from the city center ($p = 0.159$), flow rate ($p = 0.610$) or turbidity at each site ($p = 0.266$) probably due to the small sample size.

3.5 Microplastic-induced risk index (H) and pollution load index (PLI)

Risk levels of micro(meso)plastic in surface water and sediment from the Liaohe River Reserve were analyzed based on the microplastic-induced risk index (*H*) and the pollution load index (*PLI*) (Table S6). For surface water, the risk index *H* of almost all sites was category I except for sites L6 and L7, and the *PLI* of 28% and 59% of sites were categories I and II, respectively. For sediment samples, the risk index *H* varied from category I to V, whereas the *PLI* of almost all sites were categories I except for site L19. The average values of *H* and PLI_{zone} in surface water for the entire reserve were 14.9 and 13.4, respectively, which both fall into risk category II. Whereas the indexes in sediment were 8.9 and 4.9, respectively, and both of them fall into risk category I.

4 Discussion

4.1 Abundance and distribution of micro(meso)plastics in the Liaohe River Reserve

Micro(meso)plastics were prevalent in surface water and sediment in all 32 sites from the Liaohe River Reserve. The abundance of micro(meso)plastics in sediment samples of the Liaohe River Reserve (6.67 to 240 items/kg) were on average 500-fold higher compared to that in surface water (0.02 to 0.42 items/L), indicating that sediments are key for micro(meso)plastic entrapment in aquatic ecosystems. This tendency is in good agreement with previous reports (Zhou et al., 2020). Moreover, differences were observed between the different sampling sites. Although microplastics and mesoplastics abundance showed an opposite trend at some individual sites, such as L18 and L16, there were no statistically significant relationships ($p > 0.05$). Overall, the abundance of micro(meso)plastics decreased in the course of the river. A relatively lower amount of micro(meso)plastics was found in the downstream region than in the other sites. In this study, sites L18 and L1 exhibited the highest micro(meso)plastics abundance, about 2–25 times more than the other sites, and are located in the midstream (Shenyang city) and upstream (Tieling city) of the Liao River, respectively. Similarly, sites L19, L17, and L20, which were the top three sites with the highest micro(meso)plastics abundance in sediments, about 3–36 times more than the other sites, were all taken from the midstream (Shenyang city). Shenyang, the capital of Liaoning province, is one of the largest heavily industrialized cities in China, with a total population of 8.228 million. The higher micro(meso)plastics abundance might be a result of the intensive discharge of wastewater from industry, fishing, and residential activities (Pan et al., 2020). Indeed, we found that the micro(meso)plastics abundance in surface water were significantly and positively associated with the county population (r = 0.57, $p < 0.05$), suggesting a great impact of human activities.

4.2 Microplastic levels compared with other studies

Compared with other freshwater systems of the world, the microplastic level in the surface water of the Liaohe River Reserve (average: 0.11±0.04 items/L) was comparable to that in Lake Geneva in Switzerland (Faure, 2012) and Western Lake Superior in Italy (Hendrickson et al., 2018), while it was significantly higher than that in the Austrian Danube in Austria (Lechner et al., 2014), and the Laurentian Great Lakes in USA (Eriksen et al., 2013). However, microplastic levels in the surface water of the Liaohe River Reserve were 1–2 orders of magnitude lower than that in other southern regions in China. For example, our data was far lower than that in the Changjiang Estuary (4.137±2.462 items/L) reported by Xu et al. (2018), the Taihu Lake (3.4–25.8 items/L) reported by Su et al. (2016), urban surface waters of Wuhan (8.93±1.59 items/L) reported by Wang et al. (2017). For sediments, the microplastic level in the Liaohe River Reserve (average: 62.29 items/L) was also comparable to that in other sediment systems of the world, including in other areas of China, e.g., the Vitória bay estuarine system in SE Brazil (Baptista Neto et al., 2019), the River in Portugal (Rodrigues et al., 2018), and the Taihu Lake in China (Su et al., 2016). Other studies reported 1–2 order of magnitude higher microplastic level in other regions than in Liaohe River Reserve, such as the Rhine River in Germany (Klein et al., 2015), the River Thames in the UK (Horton et al., 2017b), the Atoyac River in Mexico (Shruti et al., 2019), the Nakdong River in South Korea (Eo et al., 2019), and the Pearl River (Lin et al., 2018) and the Wei River (Ding et al., 2019) in China. Collectively, the levels of microplastic abundance found in the Liaohe River Reserve are currently low in all freshwater environment in China. A reason for this relatively low microplastic abundance could be attributed to the lower anthropogenic disturbance after the establishment of the Liaohe River Reserve.

4.3 Characterization of micro(meso)plastics in the Liaohe River Reserve

More studies have demonstrated that the source of microplastics is related to human activities, such as wastewater treatment plants (WWTPs), agricultural activities like plastic mulch usage, fishery, cargo shipping, and harbors (Fahrenfeld et al., 2019; Wagner et al., 2014). Characteristics like particle shape, size distribution, colors, and polymer types could potentially provide useful information to elucidate the source of microplastics (Fahrenfeld et al., 2019). A predominance of fiber was observed in surface water (91.86%) from the Liaohe River Reserve, which was composed majorly of rayon and polyester, 59.11% and 20.69%, respectively (Table S3 and

S4). Rayon is often used in textile and clothing, and industrial fields, while polyester which accounts for more than 50% of the global fiber output, is mainly used for packaging, fishing nets, ropes, clothing, and decorative items (Geyer et al., 2017). It, therefore, can be inferred that these large percentages of fiber may be attributed to domestic wastewater streams and the broad use of fishing nets and lines in aquaculture. Fibers were also predominant in sediment (43.48%) from the Liaohe River Reserve, however, fragments (24.41%) and films (20.40%) were more common in the sediments than in the water phase. This is probably due to biofilm formation on their surface or their low surface-to-volume ratio (Corcoran, 2015; Wang et al., 2017). In addition, color is often perceived as an index of weathering degree and is associated with residence time in the environment (Gewert et al., 2015). In this study, the high proportion of white micro(meso) plastics (11.31% in surface water and 39.13% in sediment) and the apparent faded color indicated that a substantial proportion of micro(meso)plastics have remained in the river for a certain time and underwent various aging processes (Pan et al., 2019; 2020).

4.4 Risk of micro(meso)plastics in the Liaohe River Reserve

There are different risk levels of micro(meso)plastics pollution in different environmental media in the Liaohe River Reserve. Collectively, large differences in risk category were observed among the sampling sites, and the risks of micro(meso)plastics pollution in surface water were slightly higher than that in sediment. As a composition, the risk of microplastics in surface water from the Liaohe River Reserve was lower than those in the Changjiang Estuary and the adjacent East China Sea (Xu et al., 2018) and the Tuojiang River Basin (Zhou et al., 2020), whereas it was comparable to the risks in the Dongshan Bay (Pan et al., 2021). Both the risk index H and the pollution load index PLI in the sediment from the Liaohe River Reserve were low, which was comparable to that in the sediments of the mangrove ecosystem of Southern China (Li et al., 2020). However, the risk model developed in the present study has several notable limitations. The lack of unified quantification models and well-defined background values makes it difficult to compare results from different studies. Also, the toxicity index is based on plastic monomer toxicity, which is mainly polymer dependent. However, we believe different types (pellet, fiber, fragment, etc.) and different sizes will exert obvious distinct toxic effects. More efforts are needed to involve more parameters or indicators and establish unified quantification models as part of future risk assessment studies.

5 Conclusions

This study provides data on microplastic and mesoplastic pollution in the Liaohe River Reserve in Northern China for the first time. Our results showed the ubiquitous presence and widespread distribution of micro(meso) plastics in surface water and sediment of the Liaohe River Reserve. Compared to other studies of global freshwater systems, the Liaohe River Reserve exhibited lower levels of microplastic abundance and lower ecological risks relevant to both the abundance and the chemical composition of microplastics. Our results provide basic information on the status of microplastic pollution in the nature reserve and indicate the control of anthropogenic disturbances might be a sustainable and effective way to manage microplastic pollution following the establishment of a natural reserve.

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