Microplastic pollution in Chinese urban rivers: the influence of urban factors

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Abstract

Microplastics are being widely discussed as an emerging global environmental contaminant. Microplastic pollution usually originates from land-based sources, which are then mainly transported through hydrological and atmospheric pathways and accumulated in terrestrial, freshwater and marine ecosystems. Urban environments represent a condensed area of human activities (including the production and use of plastic materials), and urban rivers may therefore be a key transporter of microplastic pollution. Understanding microplastic abundances in urban rivers is potentially important in finding effective means of reducing fluvial microplastic discharge. This study quantified microplastic abundances in surface waters along the Fenghua River, Ningbo, a coastal megacity in East China. Microplastic pollution was distributed unevenly along the river, with concentrations ranging from 300 n/m^3 to 4000 n/m^3 (0.3 – 4.0 n/L). Average concenterations were 1620.16 ± 878.22 n/m^3 (1.62 ± 0.88 n/L) in summer (43 sampling points) and 1696.08 ± 983.52 n/m^3 (1.70 ± 0.98 n/L) in winter (17 sampling points). The most common microplastic shapes, sizes, colors and types of polymers were fiber, <0.5mm, transparent and polypropylene, respectively. Using multidimensional scaling analysis, microplastic distribution patterns were related to seasonal factors and levels of urbanization. No clear relationships were found, with implications for site selection when studying microplastics and the challenges of attributing sources to microplastic pollution in urban rivers.

Keywords: freshwater, river, microplastics, surface water, urban, China

1. **Introduction**

Microplastics refer to plastic debris smaller than 5 mm in diameter. Today, microplastics have been widely documented in global aquatic (Horton et al., 2017), atmospheric (Liu et al., 2020; Zhang et al., 2020) and terrestrial environments (Scheurer and Bigalke, 2018), as well as in biota (Yuan et al., 2019). Ingested and inhaled microplastics may have negative physical and chemical impacts on digestive and respiratory systems associated with abrasion and blockages, as well as leaching toxic monomers and/or additives and other associated pollutants (Di et al., 2019; Rochman et al., 2019; Zou et al., 2017). Microplastics may also accumulate through the food web and could eventually contaminate human food items (Rochman et al., 2019; Zou et al., 2017).

The production and consumption of plastic products, as well as mismanaged plastic waste, are the main sources of microplastic pollution (Xu et al., 2020b). By studying these sources, microplastics can be divided into primary (plastic materials produced in micron size, e.g. plastic microbeads) and secondary microplastics (debris physically worn or photodegraded from larger pieces of plastics) (Eerkes-Medrano et al., 2015). Microplastic particles can enter aquatic environments from terrestrial environments via numerous routes including rainfall runoff, sewage discharge, garbage dumping and soil erosion (Horton et al., 2017; Zhang et al., 2018). Fluvial (riverine) environments are usually conduits for the transport of terrestrial microplastics to marine environments (Horton et al., 2017; Pan et al., 2020; Xu et al., 2020b). Globally, marine environments receive and accumulate most of the microplastic pollution discharged from freshwater environments (Caruso, 2019; Eerkes-Medrano et al., 2015; Horton et al., 2017).

China is the largest global producer and consumer of plastic materials (Garside, 2019). The large scale of production and usage of plastic products (including agricultural mulch film, disposable tableware, plastic bags, and synthetic fabrics) has generated large quantities of land-based microplastics (Xu et al., 2020b). So far, the abundance of microplastics has been reported in some major fluvial freshwater environments in China, such as the Yangtze River (Hu et al., 2018; Li et al., 2020; Zhao et al., 2014), Poyang Lake (Liu et al., 2019; Yuan et al., 2019), Taihu Lake (Su et al., 2016), Pearl River (Lam et al., 2020; Ma et al., 2020) and Yellow River (Han et al., 2020), and adjacent oceans of China (Fraser et al., 2020; Wu et al., 2019; Zhao et al., 2014). These show that major Chinese freshwater environments are discharging microplastics into global oceans, with global implications. With China likely to be the leading emitter of microplastics in the world (van Wijnen et al., 2019), paying attention to Chinese freshwater microplastic pollution is important to provide a scientific basis for the discussion of the relationship between microplastic pollution and large-scale human activities.

Cities provide multiple sources of microplastics in spatially concentrated areas (Xu et al., 2020a), which are readily transported to other ecosystems, particularly by rivers (Xu et al., 2020b). However, studies on microplastic abundances in urban river catchments remains limited, especially relative to the marine environment (Xu et al., 2020a, 2020b; Zhang et al., 2018), yet critically important in understanding the processes and characteristics of microplastics entering aquatic environments. Thus, this manuscript investigates microplastic pollution in the Fenghua River, which flows through the Chinese coastal megacity Ningbo, in order to quantify concentrations, morphologies and material properties of microplastic pollution, and to relate these variables to surrounding urban land use.

2. Methods

2.1. Research area and study sites

Ningbo (Fig. 1), Zhejiang Province, is a mega-port city along the eastern coast of China and a new economic center on the southern branch of the Yangtze River Delta (Tang et al., 2015). By 2019, the total population of Ningbo exceeded 8.5 million and the Ningbo Port has become the third largest port in the world, in terms of annual container throughput (Lloyd's List, 2019). In the past two decades, rapid urbanization has brought considerable economic development to Ningbo, while at the same time modifying the local land-use planning, economic and population structures (NDRC, 2020). The development of Ningbo City district (population > 4 million) has also led to the urbanization of surrounding satellite cities of Ningbo (GOSC, 2020). Among them, the development of Fenghua district (population: 0.2-0.5 million) and Yuyao district (population: 0.5-1 million) is closely related to the major urban rivers of the Ningbo City district (NDRC, 2020; Xu et al., 2020a).

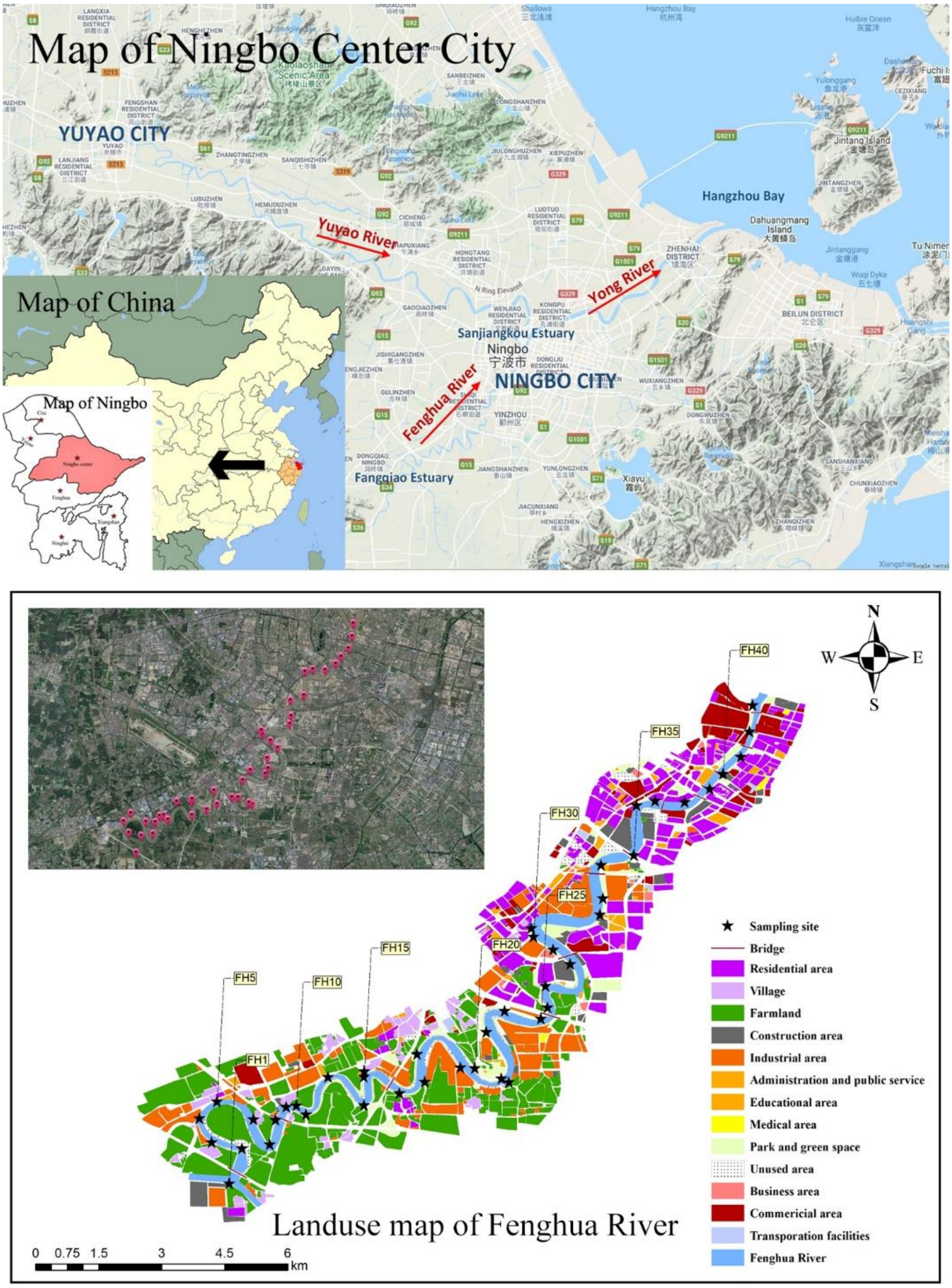

Fig. 1 Satellite Map of Ningbo Center City and land-use map of Fenghua River

Ningbo City Center has three major rivers, namely the Yuyao River, Fenghua River and Yong River. The Fenghua River (27-km river length) is an important waterway from south to north, connecting Fenghua City and Ningbo City Center (Fig. 1). It meets the Yuyao River in the middle of Ningbo City Center (Sanjiangkou Estuary), in the commercial center of Ningbo, and then joins into the Yong River downstream. The Yong River flows eastward into Hangzhou Bay, northeast of Ningbo Center City. The upper reaches of Fenghua River are on the outskirts of Ningbo City Center, which are mostly covered by farmlands, villages and industrial areas. The lower reaches of Fenghua River flow through the city center, with high-rise residential buildings, businesses and commercial buildings and other facilities. The variation of the urbanization patterns (from semi-urban to urban) along Fenghua River provides quantifiable variables for investigating the significance of urban factors to microplastic pollution.

To consider the influence of urban factors (especially land-use types, population density and GDP) on microplastic concentrations, a spatially dense sampling network was deployed on the urban section of Fenghua River. The sampling was carried out twice; once in July 2019 (summer) and once in January 2020 (winter). In July 2019, 43 approximately equidistant sampling points were selected along the river, shown in Figure 2. 31 points were located on the left bank of the river and 12 points were on the right bank because of access limitations. In January 2020, 17 sites were sampled again (Fig. 1), in order to observe the impacts of seasonal factors on urban fluvial microplastic abundance conditions.

2.2. Sampling and extracting microplastics

The procedures of microplastic sampling and extracting methods in this research are similar to those in previous research, which have been reviewed by Zhang et al. (2018). The viability of equipment parameters has been verified by Stanton et al. (2020). At each sampling site, stainless-steel buckets were used to collect 30 L of surface water (0-20 cm depth) of the Fenghua River near the bank. The river water was poured from the buckets into a 30L water tank through a stainless-steel sieve (pore size: 0.063 mm) on the bank of the river. This was repeated until the water tank was full. Microplastics suspended or floating in surface water usually have relatively low density and, thus are meaningful in attempting to understand the transportation and of microplastic particles through waterways. The solid residues left on the sieve were washed into a brown glass bottle (250 ml) using deionized water, representing a sample of particulate matter at that site. The glass bottle was sealed by aluminized paper to avoid contamination and then brought back to the laboratory for microplastic extraction. If necessary, the samples were preserved for a short time (< 14 days) under the conditions of low temperature and avoiding light inputs.

In the laboratory, 30% hydrogen peroxide (H_2O_2) solution was mixed with the water sample in a larger transparent glass bottle (500ml). The mixed solution was heated in a water bath for 5 hours at a temperature of 80°C. The purpose of this step is to digest the bio-organic matter in the sample. Subsequent to the solution cooling, the sample was filtered again with a stainless-steel sieve (pore size: 0.063 mm). The solid residue left on the screen was washed into a centrifugal tube by saturated sodium chloride solution (1.2 g/ml). Then the centrifugal tube was centrifuged at a speed of 4000 rpm for 5 minutes to separate impurities (e.g., sediments) from microplastics through density differences. With the help of a vacuum pump, the upper layer of centrifuged liquid, was filtered through a nitrate cellulose membrane with a pore diameter of 0.45μm (114H6-47-ACN, Sartorius, Germany). The residues on the filter membrane were suspected to be microplastics. The filter membrane containing suspected microplastics was stored in a covered glass dish, protected from light, for later observation and identification.

2.3. Identification of microplastics

A stereomicroscope (S9D 170x, Leica, Germany) equipped with a digital camera (MD170, Leica, Germany) was used to visually identify suspected microplastics on each filter membrane, to distinguish obvious impurities (such as minerals, diatom skeletons and freshwater sponge spicules). Meanwhile, the size, color, shape and number of suspected microplastics were recorded.

Confirmation of suspected microplastics, as well as their polymer composition was identified, using *Fourier Transform Infrared Spectrometer* (FTIR) (Vertex 70, Bruker, Germany) equipped with Attenuated *Total Reflection* (ATR) accessory (A225/Q Platinum ATR Diamond F. Vacuum, Bruker, Germany). *OPUS 7.0.129 software* (Bruker, Germany) installed with *BrukerOptics SH spectrum* libraries were used to compare the detected and standard spectrums for polymers. Due to the limitations of the technique, suspected microplastics with sizes less than 0.5 mm were too small to be identified using ATR-FTIR. Therefore, the evaluation of the accuracy of microplastic identification could only be achieved on larger particles, a limitation shared with other studies that used a similar approach (e.g. Stanton et al., 2019; Zhao et al., 2020).

In this study, microplastics were divided into four shapes: fragment, fiber, film and pellet/foam (Fig. 2). It is difficult to identify fibrous microplastics using ATR-FTIR because of their small diameters. Whilst confidence in the visual identification of fibers was increased through the use of *FZT01057.3-2007* national standard document of China for identifying natural and synthetic fibers (NDRC, 2007), their polymer composition could not be identified. As a result, the polymer type of fibrous microplastics were not recorded in this study.

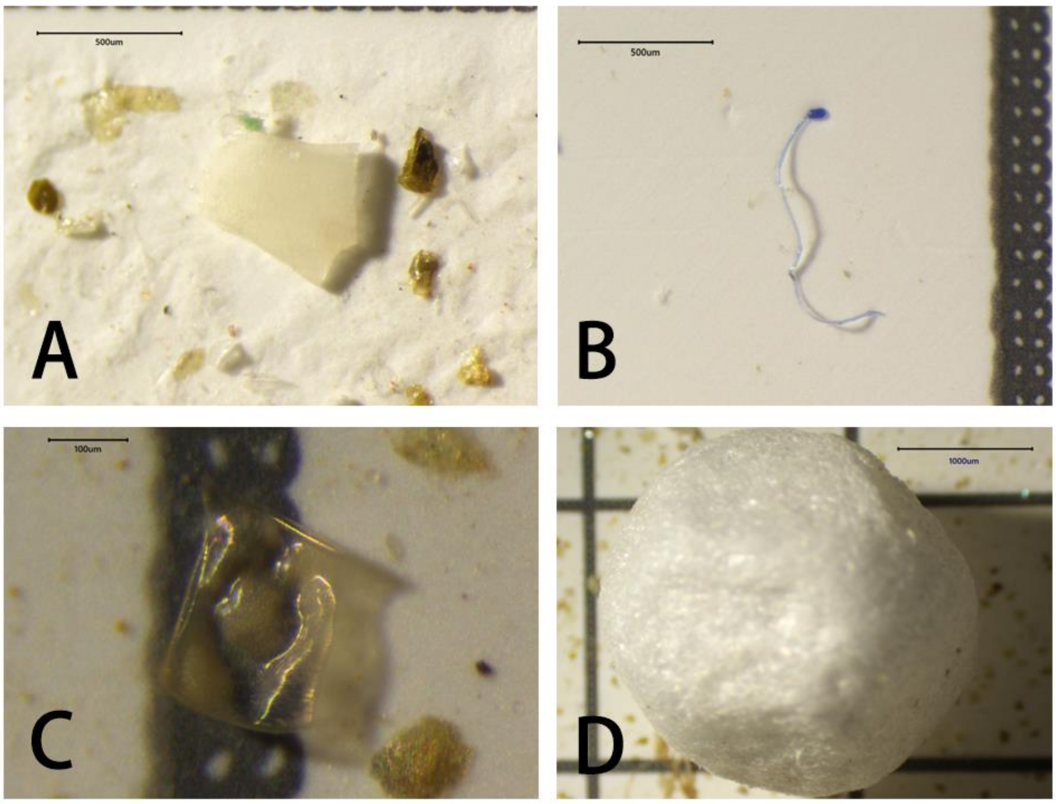

Figure 2 Hard plastic debris with irregular shapes (A), linear or wire-like synthetic materials (B), soft and flat plastic debris (C) and spherical or nearly spherical plastic materials (D) observed in this study were classified as microplastic fragments, fibers, films and pellets/foams

2.4. Quality assurance and quality control (QA/QC)

In this study, a total of 77 suspected microplastics were identified by ATR-FTIR. 65 of those were identified as artificial polymers while another 12 pieces as natural or non-polymer materials. Therefore, the recognition accuracy of microplastics by microscopic identification could be up to 84.4%. However, considering that most of the suspected microplastics with smaller sizes and those with fibrous morphology were only identified with microscopy, it is possible errors in identification are large. Potential overestimation or underestimation of the results will be discussed in following sections.

To avoid contamination, non-plastic sampling tools and sample storage items were preferred. For tools that were made of plastic, products with obvious colors and high material density were selected because plastics with density higher than 1.2g/cm^3 were excluded during the process of centrifugal flotation and plastic debris from contamination could be distinguished according to its color. For example, orange plastic spray cans were used to carry deionized water during fieldwork, and no orange microplastics were found in samples.

In the laboratory environment, samples were sealed or protected by aluminum foil or a glass cover when stored. During the microscopic examination of suspected microplastics, non-plastic particles in small size (<1mm) were also excluded by observing whether cellulose texture or cell veins exist on the particle surface and whether the particle is easily broken under slight pressure by a metal tweeze or dissecting needle. Meanwhile, three blank samples with deionized water went through the same pretreatment and laboratory work process before observation, to evaluate background contamination levels.

2.5. Statistical approaches

From past studies of urban microplastic abundance patterns, (a) the distance from sampling sites to city center, (b) land-use types, (c) population density and (d) local economic structure (usually with gross domestic product (GDP) as an index) have been considered as potential factors (Fan et al., 2019; Peng et al., 2018; Wang et al., 2017). This paper employed linear regression and Kruskal-Wallis tests to analyze the above four factors, statistically. The order of sampling points from upstream to downstream and the straight-line distance from sampling points to city center were both considered in the distance analysis. A map of the land-use conditions within 1 km of each bank was drawn according to a combination of the "*Essential Urban Land Use Categories*" map of Ningbo (Gong et al., 2020), records taken during fieldwork, and satellite images. According to this map (Fig. 1), we classified the land-use type of each sampling site (see sampling site geographic information in Tab. S1). A raster map with the population density and GDP density data (for 2015) were used to determine the population and GDP size of each site (Geographical Information Monitoring Cloud Platform, 2020a, 2020b).

It is worth noting that the unit population density and unit GDP density of 43 sampling sites have similar trends (Fig. 3). According to this pattern, we classified FH1-FH22 into semi-urban areas, FH23-FH33 as transition area and FH34-FH43 as urban center area and applied Kruskal-Wallis analysis to evaluate the differences of microplastic concentrations with varying urbanization level.

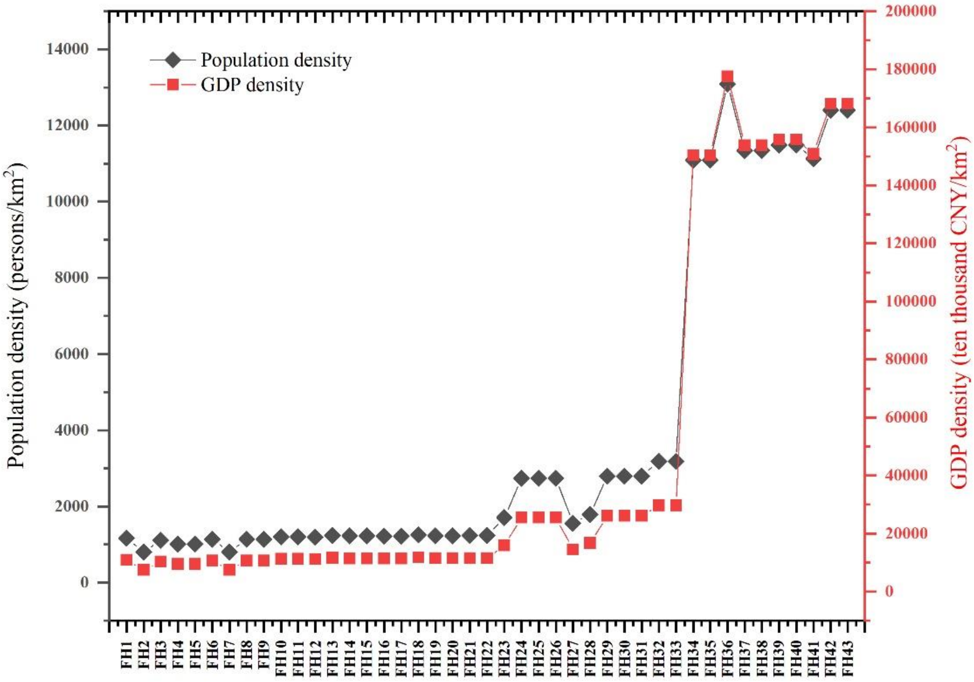

Figure 3 Population density (Black) and GDP density (Red) of 43 sampling sites

In Ningbo, the urban inland river network covers sub-catchments of different city blocks and flows into the urban mainstream through sluice gates. 18 of 43 sampling points were adjacent to those sluices in this study. Those sluices could be point sources of microplastic pollution along Fenghua River because they regulate the water level and flow. A Mann-Whitney U test was used to evaluate whether the sluices contribute to microplastic discharge events. In addition, a Wilcoxon test was used to detect the seasonal difference in microplastic pollution in the Fenghua River. All statistical works were conducted in *IBM SPSS Statistics 23*.

Because microplastics concentrations and morphologies may not vary in response to any single dominant variable, this study also utilized non-metric multidimensional scaling analysis (NMDS) to assess the Bray-Curtis dissimilarities of microplastic concentrations and typology between sampling sites at the same time, which was completed under the environment of *RStudio 1.4*.

3. Results

3.1. Microplastic concentration

Microplastics were detected in all 43 summer samples (July 2019) and 17 winter samples (Jan 2020) (Fig. 3). The average microplastic concentration in the 43 summer samples was 1620.16 ± 878.22 n/m^3, the maximum value was 4000.00 n/m^3 at site FH16, and the minimum value was 633.33 n/m^3 at FH15 and FH42. The 17 sites sampled in winter had an average microplastic conentration of 1696.08 ± 983.52 n/m^3, the maximum value was 4000.00 n/m^3 at FH12, and the minimum value was 300.00 n/m^3 at FH20. The equivalent summer values for the same 17 sites were : average 1698.04 ± 863.23 n/m^3 , maximum 2966.67 n/m^3 at FH40, and minimum 633.33 n/m^3 (FH15) (Fig. 4 & 5). There were two outliers during summer, which had very high concentrations; FH2 (3933.33 n/m^3) and FH16 (4000.00 n/m^3) (Fig. 4 & 5). In winter, there were also 2 outliers, with similarly high concentrations but at different sites; FH12 (4000.00 n/m^3) and FH38 (3733.33 n/m^3) (Fig. 4 & 5).

The concentrations of microplastics detected in the three blank samples were 100.00 n/m^3, 100.00 n/m^3 and 166.67 n/m^3, respectively. This is likely derived from airborne contamination in periods of collection and identification between samples between covered. As these values were consistent and small relative to mean and minimum values, they were not used to correct sample concentrations.

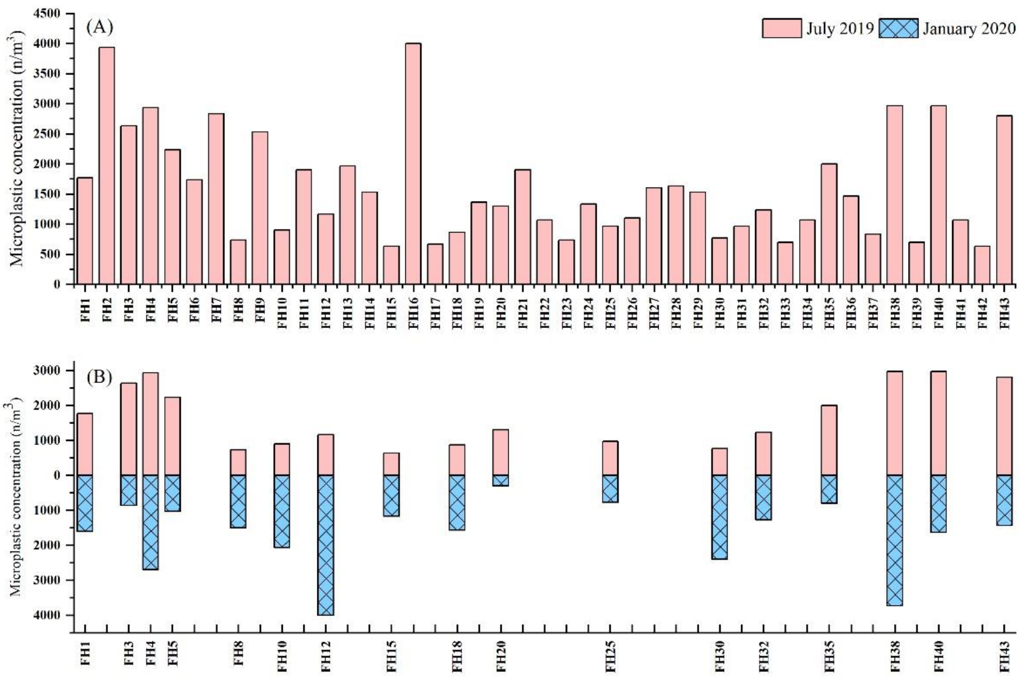

Fig. 4 Microplastic concentrations at 43 summer sampling sites (A) and the 17 sampling sites investigated during both July 2019 (B, red) and January 2020 sampling sites (B, blue)

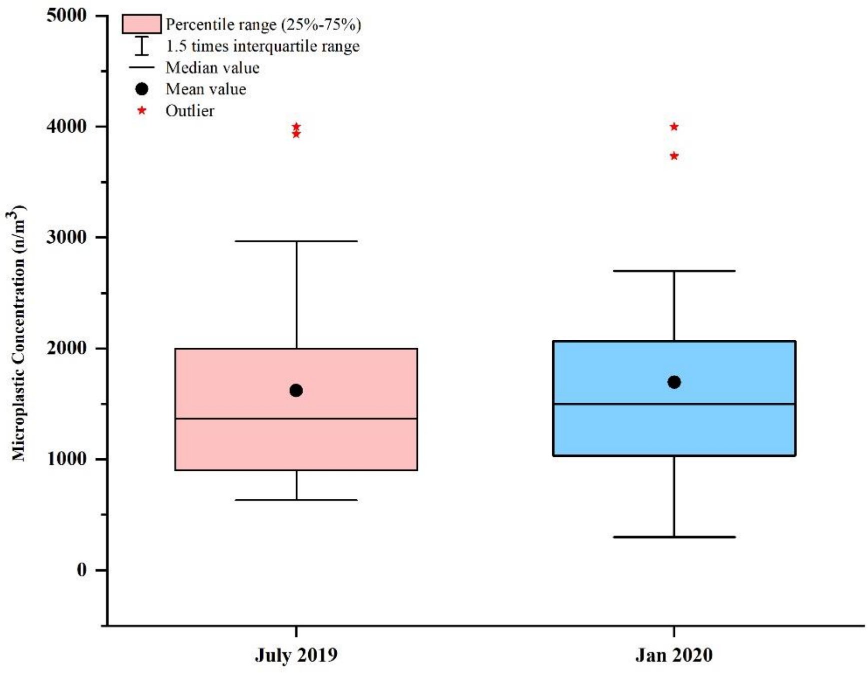

Fig. 5 Quartile box chart of 43 summer (red) and 17 winter samples (blue) of Fenghua River, Ningbo

3.2. Microplastic properties

Among the four recorded shapes of microplastics, fiber was, on average, the most common across the summer and winter samples (Fig. 6). Of the 43 summer samples, the percentage of fibers ranged from 9-83%; fragments ranged from 4-57%; films were 0-42%; and pellet/foam ranged from 0-23% of the total particles. Among the 17 winter samples, the range in the percentage of total for fibers, fragments, films and pellets/foams were 38-89%, 4-56%, 0-33% and 0-11%, respectively.

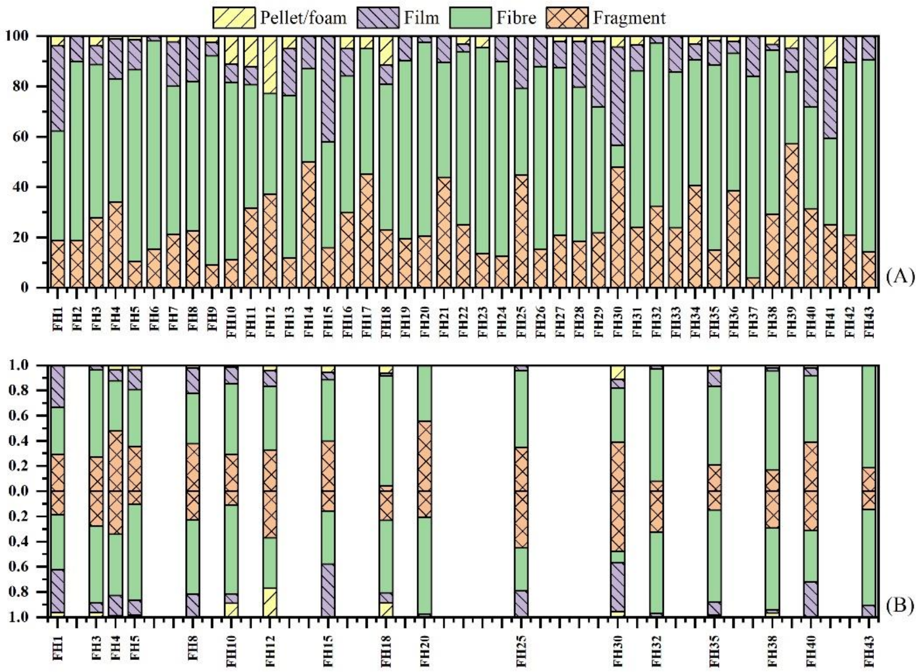

Figure 6 The proportion of four microplastic shapes between 43 sampling sites during summer (July 2019) (A) and 17 sampling sites during summer (B, above zero scale) and winter (January 2020) (B, below zero scale).

According to size, microplastics were divided into six groups (0 – 0.5mm, 0.5 – 1mm, 1 – 2 mm, 2 – 3 mm, 3 – 4 mm, 4 – 5 mm). Microplastics with diameters smaller than 0.5 mm were the most common in samples, accounting for 14-70% of 43 summer samples and 26-67% of 17 winter samples, with an average of 72.19 ± 10.88% below 1 mm (74.19 ± 10.30% for winter) (Fig. 7). More than 10 colors of microplastics were observed. Transparent microplastics were the dominant type, accounting for 13-74% during summer and 16-55% in 17 winter samples (Fig. 8). In additon, blue, black and white were also observed frequently.

Three major types of polymers were identified using ATR-FTIR; polypropylene (PP, 57%), polyeheylene (PE, 35%) and copolymer (8%). Detected PP and PE also contained different types of materials, such as polypropylene with different molecular weights, Low Density Polyethylene (LDPE) and ultra-high-molecular-weight polythylene. The compositions of the copolymers were also diverse, including PE-Ceresin copolymer, PP-PE-acrylonitrile-styrene copolymer and styrene-allylalcohol copolymer. This means although the types of polymers were similar, the uses of them are likely to be varied.

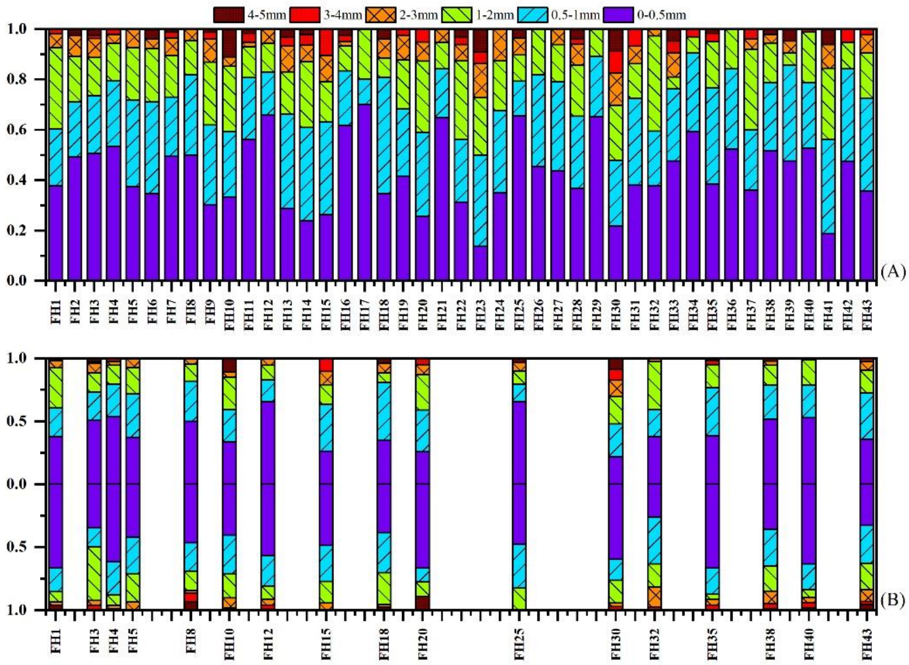

Figure 7 The proportion of microplastic size groups between 43 sampling sites during summer (July 2019) (A) and between 17 sampling sites in summer (B, above zero scale) and winter (January 2020) (B, below zero scale).

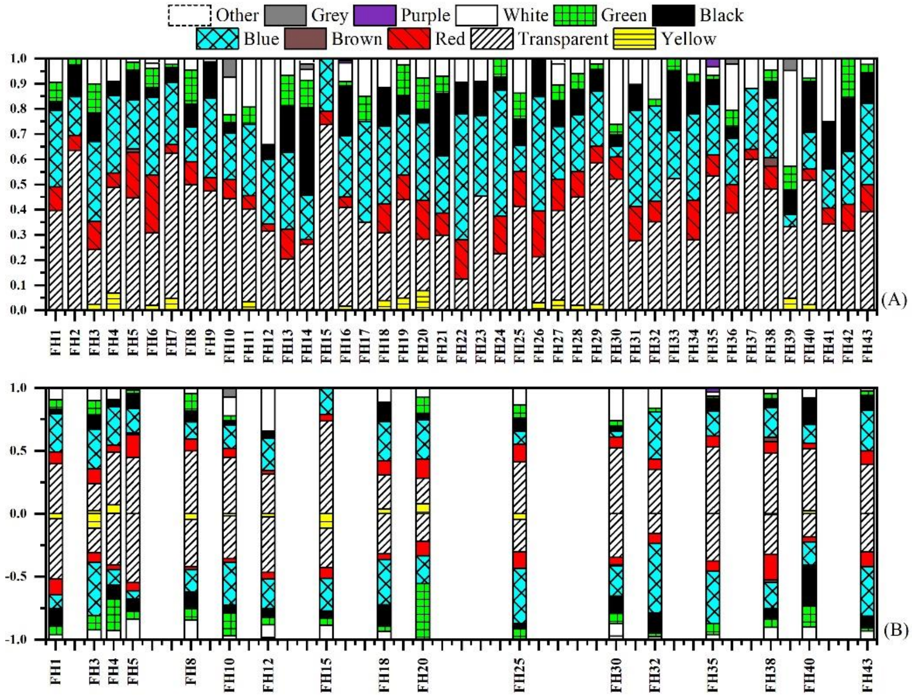

Figure 8 The proportion of microplastic colors between 43 sampling sites during summer (July 2019) (A) and between 17 sampling sites in summer (B, above zero scale) and winter (January 2020) (B, below zero scale).

3.3.Statistial analysis results

There was no significant linear correlation between microplastic concentration and the straight-line distance from sampling sites to the city center (R^2 of 43 summer samples = 0.054; R^2 of 17 winter samples = 0.103). The order of 43 sampling points (from upstream to downstream) had no linear relationship with microplastic abudance conditions (see detailed R^2 values in Tab. S2). There was also no significant relationship between microplastic concentration and the population density, or between microplastic concentration and GDP density (Tab. S2). Kruskal-

Wallis tests failed to find significant statistical differences of microplastic concentration among different land-use types (total microplastic concentration, $p = 0.717$) and different urbanisation levels (total microplastic concentration, $p = 0.171$) (Tab. S3&S4).

Mann-Whitney tests similarly did not distinguish significant differences between total microplastic concentrations at 18 sampling sites with water sluices and the 25 sampling points without sluices ($p = 0.325$, Tab. S5). However, the concentrations of microplastic films were found have significant diffences between sites with and without sluices ($p = 0.048$, Tab. S5). Microplastic concentrations of the 17 winter and summer samples were statistically similar (Wilcoxon test; $p = 0.723$, Tab. S6).

Non-metric multidimensional scaling resutls of microplastic concentrations in different categories are shown in Fig. 9 & S1. Stress values of n-MDS graphs are are between 0.039 and 0.140, which represents high scaling quality. In the same coordinate system, as the distance between two points decreases, the similiarity between them increases. In Fig. 9 & S1, X-axis (NMDS1) indicates a changing trend of microplastic concentration (black arrow shows positive direction) whereas the evenness of microplastic types vertically changed (black arrow shows positive direction) along Y-axis (NMDS2) (i.e. sites dominanted by a single type of microplastic have low evenness). The results of the NMDS indicate no clear relationships or explanatory drivers of the between-site pattern in microplastic presence, supporting earlier, linear analysis.

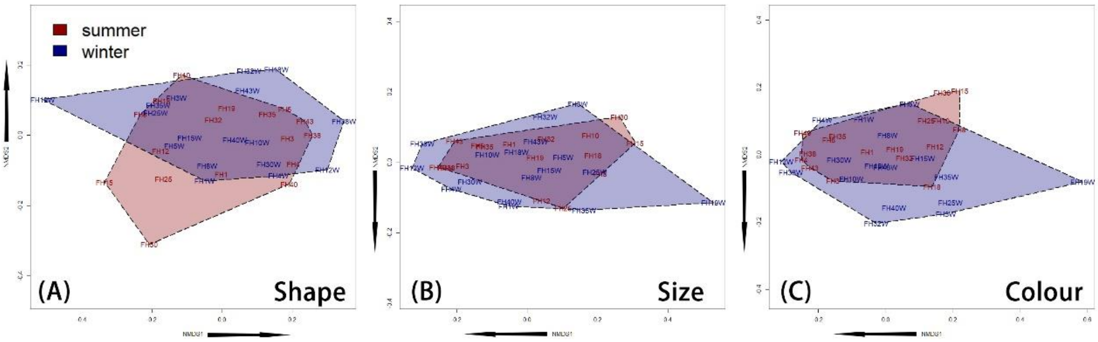

Figure 9 NMDS coordinate graphs of 17 repeated sampled sites for microplastics in four shapes (A) (Stress = 0.106), six size ranges (B) (Stress = 0.081) and eleven colors (C) (Stress = 0.120). Horizontal axis of each graph is the first non-metric dimension scale (NMDS1), and the vertical axis is the second non-metric dimension scale (NMDS2). Summer points are in red and winter points are in navy. Hull polygons seal the points of different seasons.

4. Discussion

4.1. Overall microplastic pollution condition

4.1.1. Microplastic concentration level

The microplastic concentration range in the Fenghua River was 300-4000 n/m^3 (refer Fig. 4 & 5). When compared to the 19 previous studies on microplastic pollution in Chinese freshwater environments, the microplastic concentrations in the Fenghua River are relatively mild and similar to concentrations recorded in the urban sections of Tuojiang River, Qiantang River, Dongting Lake and Hong Lake (Wang et al., 2018; Zhao et al., 2020; Zhou et al., 2020) (Fig. 10). The Qiantang River flows through Hangzhou City, Zhejiang, which is the neighboring catchment to Ningbo with broadly similar geographical conditions. It is therefore noteworthy that the characteristics of microplastic pollution (including shape, size and color distributions) in the Qiantang River were similar to those in Fenghua River (Xu et al., 2020a; Zhao et al., 2020). Microplastic concentrations were lower in the Fenghua River than those recorded in the Pearl River Basin and Yangtze River Estuary (Fig.10). This may be because the population size in Ningbo (> 8 million) is relatively small compared to the cities in Pearl River Basin (e.g. Guangzhou: > 15 million; Shenzhen > 13 million) and in the Yangtze River Basin (e.g. Shanghai: >24 million) (National Bureau of Statistics of China, 2020). The microplastic concentration in the Fenghua River is also lower than that recorded in Taihu Lake, Poyang Lake and other large lakes (Su et al., 2016; Yuan et al., 2019) (Fig. 10). This is likely because lakes act as sinks, accumulating microplastic through time.

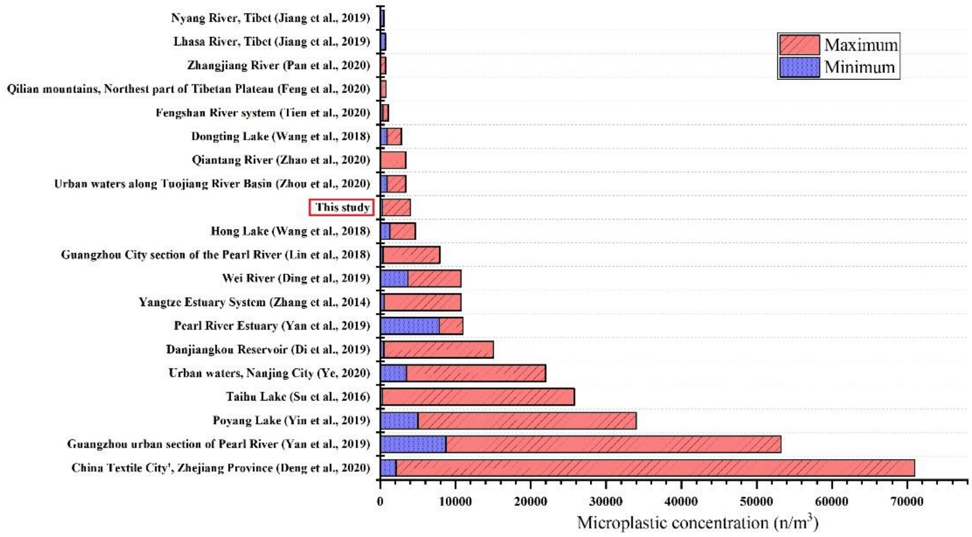

Figure 10 Microplastic concentration ranges of this study and previous publications about microplastic pollution in surface water of Chinese freshwater environments (Deng et al., 2020; Di et al., 2019; Ding et al., 2019; Feng et al., 2020; Jiang et al., 2019; Lin et al., 2018;

Pan et al., 2020; Su et al., 2016; Tien et al., 2020; Wang et al., 2018; Yan et al., 2019, 2019; Ye, 2020; Yuan et al., 2019; Zhao et al., 2014, 2020; Zhou et al., 2020)

4.1.2. Microplastic characteristics

Fibers were the most common microplastic shape along the Fenghua River, which is consistent with the results of previous research in China (Fig. 6) (Lin et al., 2018; Xu et al., 2020b; Yan et al., 2019), indicative of domestic sewage, waste fabrics, and fishery activities (Xu et al., 2019; Xue et al., 2020). Numerous clothing/garment factories in Ningbo may be an important source of detected microplastic fiber pollution (Tang et al., 2015; Xu et al., 2020a). It is also worth noting that during the visual identification of microplastics in this study, the amounts of natural fibers (including cotton and wool) was much higher than that of synthetic fibers, supporting recent recognition of the prevalence of these non-plastic anthropogenic particles in freshwater (Stanton et al., 2019), marine (Suaria et al., 2020) and biota (Guen et al., 2019) microplastic surveys. The widespread abundance of natural fibers may impact the accuracy of microplastic fiber identification in this, and other, studies.

In terms of size, most of microplastics detected in the Fenghua River were smaller than 1 mm (Fig. 6). This result is consistent with previous studies on freshwater basins in China (Xu et al., 2020b; Zhang et al., 2018; Zhao et al., 2020), although direct comparison of size ranges is challenging because different studies have used different mesh sizes when extracting plastics. Microplastics of smaller size will have higher bioavailability, which may increase ecological risks (Zou et al., 2017). It takes time for macro-plastics to breakdown into smaller plastic particles through physical wear or degradation in the environment (Horton et al., 2017; Lehtiniemi et al., 2018). Therefore, the abundance of smaller microplastics (<1 mm) in Fenghua River may indicate that plastic pollution in Chinese freshwater environments has a relatively long history. Further observations and research is required on the implications of the microplastic size range in Chinese freshwater environments on potential ecological risks (Dong et al., 2020).

Transparent microplastics were observed most frequently along the Fenghua River, which is similar to previous studies (Fig. 7) (e.g. Zhang et al., 2018; Zhao et al., 2020). Some studies used color as an indicator of possible sources (Ding et al., 2019; Eerkes-Medrano et al., 2015). For example, transparent film microplastics may come from agricultural mulch of plastic bags, while brightly colored microplastic fragments may come from the decomposition of plastic industrial products (Jiang et al., 2019; Tien et al., 2020). The digestion of organic matter with hydrogen peroxide solution may also digest some dye additives in plastic materials, resulting in the potential discoloration of microplastics (Crawford and Quinn, 2017). In this research, a number of microplastics showed signs of discoloration, such as fibers faded from blue to transparent and fragments from green to light blue. Therefore, we do not recommend using microplastic colors as a basis for speculating microplastic sources. Nonetheless, the diversity of colors found in this study is likely to imply a diversity of pollution sources.

PP and PE were the most common polymer types in the surface water along the Fenghua River, consistent with other publications of microplastics in Chinese freshwaters (Li et al., 2019; Xu et al., 2020b; Zhang et al., 2018). PP and PE are the two most widely used artificial polymer materials in China, involved in plastic packaging and agricultural mulch (Lam et al., 2020). Most of the co-polymers found along the Fenghua River were plastic foam materials, most likely derived from plastic packaging associated with e-commerce and express delivery services (Industry, 2019). As a coastal megacity with rapid economic development, Ningbo has seen rapid increases in the fields of e-commerce and express business (Li and Yang, 2016). This may explain the dominant distribution of PP and PE in surface water. Compared to other studies, fewer polymers types were detected in Fenghua River, for example, polyethylene terephthalate (PET) has been commonly found in surface waters in other Chinese studies (e.g. Zhang et al., 2018). The dominance of PP and PE in our surface water samples is also likely to partially relate to them having lower density than some other plastic materials, which would be more likely to sink and be present deeper in the water column or in the sediments.

4.2. Microplastic distribution pattern

4.2.1. Seasonal variation

First, although there were differences between the sampling points in terms of microplastic concentration, shape, size, color and polymer type, the variations between seasons were not significant according to Wilcoxon tests (Tab. S6). Nonetheless, according to n-MDS analysis, it is possible to tentatively describe some patterns (Fig. 9). On the aspect of shape, size and color, winter polygons in Fig. 9 (A, B & C) have a larger range along the horizontal axis, which means a larger distribution range of winter points in terms of microplastic concentration.

Similarly, winter samples have a larger variation in the size and color of microplastics (Fig. 9 B & C). If the summer outlier FH30 is removed, the vertical distance of the summer polygon is also smaller than that of winter polygon (Fig. 9A). These features indicate that microplastic concentrations and types between the 17 sampling sites were more similar in summer than in winter. July and January are respectively the wet and dry seasons of Ningbo. Therefore, the frequent rainfall events and larger upstream flow may reduce the significance of microplastic point sources along Fenghua River urban section in winter, but increase the influence of non-points source discharge (Xu et al., 2020b). To further explore these relationships, sampling over multiple days in summer and winter to generate seasonal averages at each individual site was likely necessary; however, sampling at both a high spatial and temporal resolution was not plausible here and raises important questions about the resolution of data needed to make meaningful estimates of microplastic concentrations and fluxes at sites (Stanton et al., 2020).

4.2.2. Urban factors

Urban factors are usually thought to shape microplastic distribution patterns. For example, Wang et al. (2017) noticed microplastic concentrations declined with the distance from urban centers. The concentration of microplastics in waterbodies was found to positively correlate to the unit gross domestic product (GDP) or the unit population density where the sampling site was located (Fan et al., 2019; Peng et al., 2018). In addition, studies have reported that microplastic pollution in urban waterbodies is related to local land-use functions (Peng et al., 2018). Despite microplastic concentrations being spatially heterogeneous in this study, there were no obvious trends from the upstream (FH1) to downstream (FH43) in microplastic concentration according to linear regression analysis (Tab. S2), supported by the NMDS results (Fig. S1). Therefore, the distance from the sampling site to city center was not a determining factor in microplastic pollution in the surface water in this study river.

Land-use types were grouped but, again, no significant differences in microplastic concentrations were found according to Kruskal-Wallis tests (Tab. S4). This study defined land-use types within 1 km of both banks of the Fenghua River; however, the actual use of the land is difficult to quantify; land-use classification can include a range of potential microplastic sources (e.g. a textile factory in comparison to a different industry); and land-use conditions may integrate and overlap, such as industrial workshops on agricultural land and business offices in residential buildings (Liu et al., 2008). Also, land use from further away may still provide sources of microplastic to the Fenghua River, further complicating relationships between land-use and microplastic concentrations.

No significant linear relationships were found between GDP density or population density and microplastic concentrations (Tab. S2). We interpret the lack of strong relationship between microplastic concentration and land-use and socio-economic variables as being due to the inherent complexity and spatial heterogeneity of urban environments. We suspect that land-use and socio-economic factors do influence microplastic concentrations but the over-lapping nature of these variables and accumulation of microplastics downstream complicates the ability to determine clear relationships. The semi-urban area tends to have a larger diversity of microplastics compared to those found in the transitional and center-urban areas (Fig. S1). This may be explained by the fact that the semi-urban area includes both primary and secondary industries (e.g. agricultural and industrial production) while the city center is occupied more by business and commercial services.

Although the 18 sluices along Fenghua River were suspected to be the point sources of microplastic pollution in this study, the Mann-Whitney test results of sluice factors showed that the presence or absence of sluices made no significant influences (Tab. S5). One explanation is that the sampling in this study did not capture the opening time of sluices, which may lead to a short duration increase in concentrations that are rapidly washed downstream. Another explanation is that sluices do not represent a major factor of microplastic abundance patterns in Fenghua River.

4.3. Insights and suggestions

Our research found that the influence of urban factors on microplastic pollution in fluvial surface water was complex, with no statistical significance emerging. Statistically significant patterns in microplastic distributions in urban environments from other studies tend to be where the environmental matrix is relatively stable. For example, Wang et al. (2017) documented microplastic concentrations decreased with the distance from the urban center in the surface water of urban lakes linearly ($p < 0.001$). Moreover, Fan et al. (2019) reported the linear relationship between microplastic concentration and population density as well as GDP ($p < 0.01$) in river sediment. In contrast, the high potential for diffusion and transportation of microplastics in surface waters associated with waves, currents and other hydraulic and anthropogenic factors, are likely to lead to the lack of obvious relationship between microplastic concentrations and urban factors. This also demonstrates the complicating factor that fluvial transport is likely to have when trying to attribute a source to microplastic pollution. It also has implications for site selection when studying microplastics, especially where sampling networks are less dense than that used here, with estimates of microplastic concentrations in fluvial surface water potentially varying over several orders of magnitude depending on the location selected (Stanton et al., 2020). The findings of this work further support the need for comprehensive sampling campaigns when microplastic concentrations are being investigated, and especially when microplastic fluxes are calculated.

5. **Conclusion**

In this study, the microplastic pollution of surface waters was quantified along the Fenghua River, a major urban river of Ningbo City, Zhejiang Province. The results showed that microplastic pollution was detected in all samples in summer and winter, and concentrations varied widely from 300 n/m^3 to 4000 n/m^3 (0.3 – 4.0 n/L), with an average of 1620.16 ± 878.22 n/m^3 (1.62 ± 0.88 n/L) in summer and 1696.08 ± 983.52 n/m^3 (1.70 ± 0.98 n/L) in winter. Fibers were the most common microplastic shape, while microplastics smaller than 1 mm were the most numerous. Transparency was the most common color and PP was the most common polymer type. Statistical trend is difficult to be observed from the spatial heterogeneity of microplastic concentrations and typology along the river, which might be led by the combination of the spatially dense sampling network and the complex transport of microplastics through urban river environments. Future research work should focus on distinguishing microplastics from point and non-point sources to determine the relative significance of contributions, and the quantification of microplastic typologies.

Acknowledgement

This work was supported by funding from the Ningbo Social Development Project, Ningbo Science and Technology Bureau, China (grant no. 2014C50011/201401C5008005), the National Natural Science Foundation of China (NSFC) (grant no. 41850410497) and the Faculty of Science and Engineering Postgraduate Research Grant 2018/21– University of Nottingham Ningbo China; the National Key R&D Program of China (2019YFC1510400). The authors would like to acknowledge their funders for enabling this research to take place.

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Appendix: Supplementary document

Table S1. Location and land-use condition of all sampling sites

Site	Closest land-use condition	Location	Distance to city center (km)
FH1	Construction area	N 29°46‘11.88"/E 121°26‘52.20"	15.8
FH2	Farmland	N 29°46‘37.97"/E 121°27‘02.85"	15.1
FH3	Village	N 29°46‘43.14"/E 121°26‘39.40"	15.4
FH4	Industrial area	N 29°47‘01.66"/E 121°26'29.89"	15.2
FH5	Administration area	N 29°47'14.28"/E 121°26'43.70"	14.7
FH6	Village	N 29°47'00.68"/E 121°27'11.20"	14.4
FH7	Unused area	N 29°46'40.86"/E 121°27'23.00"	14.6
FH8	Farmland	N 29°47'00.22"/E 121°27'28.62"	14.1
FH9	Village	N 29°47'10.40"/E 121°27'36.68"	13.7
FH10	Farmland	N 29°47'11.38"/E 121°27'44.29"	13.5
FH11	Farmland	N 29°47'12.68''/E 121°27‘35.89''	13.6
FH12	Administration area	N 29°47'24.06''/E 121°28‘08.53''	12.6
FH13	Farmland	N 29°47'19.75''/E 121°28‘20.71''	12.6
FH14	Administration area	N 29°47'32.98''/E 121°28‘36.14''	12.1
FH15	Village	N 29°47'38.09''/E 121°28‘37.74''	12.0
FH16	Residential area	N 29°47'20.19''/E 121°28‘02.64''	11.9
FH17	Farmland	N 29°47'28.49''/E 121°29‘22.26''	11.3
FH18	Farmland	N 29°47'50.82''/E 121°29‘17.06''	10.9
FH19	Industrial area	N 29°47'40.89''/E 121°30‘01.56''	10.6
FH20	Farmland	N 29°47'39.93''/E 121°29‘49.49''	10.5
FH21	Greenspace	N 29°47'28.32''/E 121°30‘28.29''	10.4
FH22	Farmland	N 29°47'31.56''/E 121°30‘22.02''	10.4
FH23	Greenspace	N 29°48'06.48''/E 121°30‘10.62''	9.6
FH24	Greenspace	N 29°48'24.14''/E 121°30‘23.25‘	9.0
FH25	Greenspace	N 29°48'18.32''/E 121°30‘51.70''	8.7
FH26	Greenspace	N 29°48'25.77''/E 121°30‘56.06''	8.5
FH27	Greenspace	N 29°48'43.42''/E 121°30‘54.49''	8.1
FH28	Greenspace	N 29°48'57.44''/E 121°31‘15.51''	7.3
FH29	Greenspace	N 29°49'19.11''/E 121°30‘44.69''	7.3
FH30	Greenspace	N 29°49'21.00''/E 121°30‘46.65''	7.3
FH31	Greenspace	N 29°49'36.33''/E 121°30‘29.58''	7.1
FH32	Administration area	N 29°49'46.96''/E 121°31‘46.96''	6.0
FH33	Greenspace	N 29°49'59.32''/E 121°31‘24.98''	5.7
FH34	Greenspace	N 29°50'25.11''/E 121°31‘23.58''	5.0
FH35	Greenspace	N 29°50'32.88''/E 121°31‘48.76''	4.4
FH36	Residential area	N 29°51'11.32''/E 121°31‘50.82''	3.5
FH37	Residential area	N 29°51'15.01''/E 121°31‘5.22''	3.2
FH38	Residential area	N 29°51'14.67''/E 121°32‘27.46''	2.8
FH39	Residential area	N 29°51'23.88''/E 121°32‘47.79''	2.3

FH40	Greenspace	N 29°51'35.43''/E 121°32'57.57''	1.9
FH41	Greenspace	N 29°51'48.64''/E 121°33'11.35''	1.4
FH42	Greenspace	N 29°52'7.97''/E 121°33'17.89''	0.7
FH43	Greenspace	N 29°52'28.41''/E 121°33'20.36''	0.1

Table S2 Linear regression analysis results (R^2 value) of the relationships between microplastic concentrations and (a) straight-line distance from sampling sites to city center, (b) the order of sampling sites, (c) population density and (d) GDP density

R^2 value of linear regression for 43 summer samples	Distance from city center	Order of sampling points	Population density	GDP density
Total concentration	0.054	0.063	0.002	0.000
Fragment concentration	0.011	0.017	0.001	0.002
Fiber concentration	0.055	0.087	0.003	0.001
Film concentration	0.011	0.022	0.000	0.000
Pellet/foam concentration	0.058	0.061	0.019	0.15
Microplastics in size of 0-0.5mm	0.040	0.059	0.001	0.000
Microplastics in size of 0.5-1mm	0.009	0.210	0.213	0.231
Microplastics in size of 1-2mm	0.009	0.026	0.009	0.014
Microplastics in size of 2-3mm	0.162	0.197	0.120	0.111
Microplastics in size of 3-4mm	0.020	0.017	0.009	0.007
Microplastics in size of 4-5mm	0.031	0.032	0.022	0.020
Microplastics in yellow	0.080	0.081	0.051	0.045
Microplastics in transparent	0.025	0.050	0.001	0.002
Microplastics in red	0.036	0.053	0.002	0.001
Microplastics in brown	0.022	0.015	0.048	0.050
Microplastics in blue	0.090	0.110	0.025	0.021
Microplastics in black	0.003	0.007	0.000	0.000
Microplastics in green	0.054	0.070	0.018	0.015
Microplastics in white	0.000	0.000	0.011	0.012
Microplastics in purple	0.011	0.013	0.035	0.036
Microplastics in grey	0.000	0.000	0.014	0.015
Microplastics in other colors	0.029	0.026	0.039	0.037

Table S3 Kruskal-Wallis test results for investigating the microplastic concentration variations among semi-urban group, transition group and city center group of sampling sites

Kruskal-Wallis for 43 summer samples in three different GDP/population groups	***p* value**
Total concentration	0.171
Fragment concentration	0.356
Fiber concentration	0.306
Film concentration	0.905
Pellet/foam concentration	0.238
Concentrations of microplastics in 0-0.5mm	0.305
Concentrations of microplastics in 0.5-1mm	0.148

Concentrations of microplastics in 1-2mm	0.154
Concentrations of microplastics in 2-3mm	0.036
Concentrations of microplastics in 3-4mm	0.424
Concentrations of microplastics in 4-5mm	0.683
Concentrations of microplastics in yellow	0.387
Concentrations of microplastics in transparent	0.373
Concentrations of microplastics in red	0.802
Concentrations of microplastics in brown	0.546
Concentrations of microplastics in blue	0.143
Concentrations of microplastics in black	0.550
Concentrations of microplastics in green	0.296
Concentrations of microplastics in white	0.529
Concentrations of microplastics in purple	0.546
Concentrations of microplastics in grey	0.315
Concentrations of microplastics in other colors	0.477

Table S4 Kruskal-Wallis analysis results for investigating land-use factors on microplastic concentrations

Kruskal-Wallis test for land-use factors on 43 summer samples	***p* value**
Total concentration	0.717
Fragment concentration	0.551
Fiber concentration	0.741
Film concentration	0.229
Pellet/foam concentration	0.593
Concentrations of microplastics in 0-0.5mm	0.732
Concentrations of microplastics in 0.5-1mm	0.883
Concentrations of microplastics in 1-2mm	0.327
Concentrations of microplastics in 2-3mm	0.331
Concentrations of microplastics in 3-4mm	0.692
Concentrations of microplastics in 4-5mm	0.440
Concentrations of microplastics in yellow	0.082
Concentrations of microplastics in transparent	0.307
Concentrations of microplastics in red	0.967
Concentrations of microplastics in brown	0.349
Concentrations of microplastics in blue	0.596
Concentrations of microplastics in black	0.852
Concentrations of microplastics in green	0.881
Concentrations of microplastics in white	0.400
Concentrations of microplastics in purple	0.834
Concentrations of microplastics in grey	0.271
Concentrations of microplastics in other colors	0.723

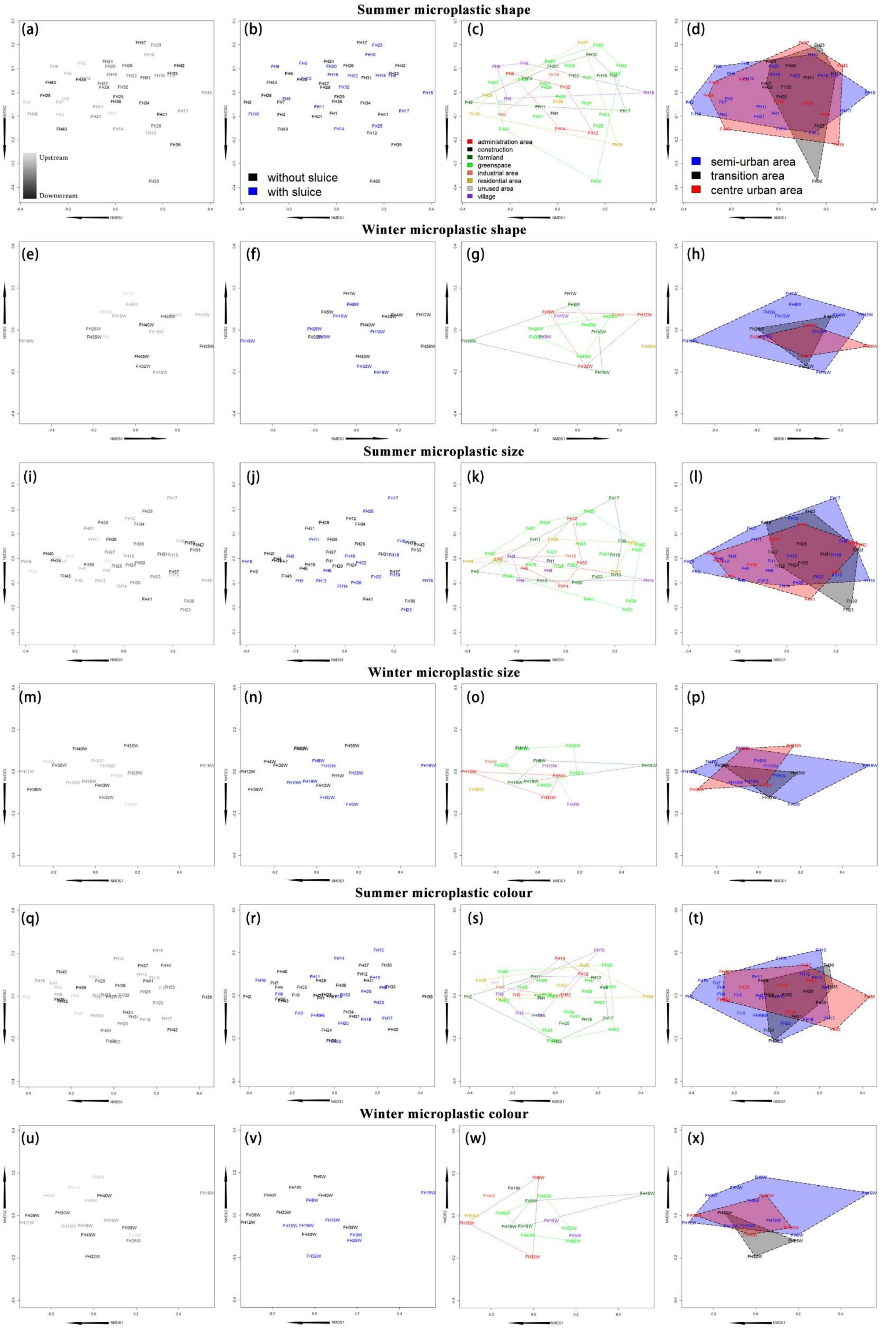
Table S5 Mann-Whitney test for investigating the influences of water sluice on local microplastic concentration

Mann-Whitney test for sluice factor on 43 summer samples	***p* value**
Total concentration	0.325
Fragment concentration	0.283
Fiber concentration	0.631
Film concentration	0.048
Pellet/foam concentration	0.688
Concentrations of microplastics in 0-0.5mm	0.115
Concentrations of microplastics in 0.5-1mm	0.444
Concentrations of microplastics in 1-2mm	0.892
Concentrations of microplastics in 2-3mm	0.803
Concentrations of microplastics in 3-4mm	0.473
Concentrations of microplastics in 4-5mm	0.058
Concentrations of microplastics in yellow	0.611
Concentrations of microplastics in transparent	0.204
Concentrations of microplastics in red	0.406
Concentrations of microplastics in brown	0.225
Concentrations of microplastics in blue	0.961
Concentrations of microplastics in black	0.193
Concentrations of microplastics in green	0.238
Concentrations of microplastics in white	0.901
Concentrations of microplastics in purple	0.840
Concentrations of microplastics in grey	0.696
Concentrations of microplastics in other colors	0.163

Table S6 The Wilcoxon signed ranks test (paired samples non-parametric test) results of the seasonal differences between 17 summer samples and 17 winter samples

Wilcoxon test for summer and winter samples from 17 sampling sites	***p* value**
Total summer and winter microplastic concentrations	0.723
Summer and winter fragment concentrations	0.434
Summer and winter fiber concentrations	0.925
Summer and winter film concentrations	0.103
Summer and winter pellet/foam concentrations	0.878
Summer and winter concentrations of microplastics in 0-0.5mm	0.868
Summer and winter concentrations of microplastics in 0.5-1mm	0.704
Summer and winter concentrations of microplastics in 1-2mm	0.538
Summer and winter concentrations of microplastics in 2-3mm	0.887
Summer and winter concentrations of microplastics in 3-4mm	0.813
Summer and winter concentrations of microplastics in 4-5mm	0.915
Summer and winter concentrations of microplastics in yellow	0.528
Summer and winter concentrations of microplastics in transparent	0.227
Summer and winter concentrations of microplastics in red	0.280
Summer and winter concentrations of microplastics in brown	0.785

Summer and winter concentrations of microplastics in blue	0.906
Summer and winter concentrations of microplastics in black	0.538
Summer and winter concentrations of microplastics in green	0.065
Summer and winter concentrations of microplastics in white	0.691
Summer and winter concentrations of microplastics in purple	0.317
Summer and winter concentrations of microplastics in grey	0.655
Summer and winter concentrations of microplastics in other colors	0.180



Summer microplastic shape
Winter microplastic shape
Summer microplastic size
Winter microplastic size
Summer microplastic colour
Winter microplastic colour
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(j)
(k)
(l)
(m)
(n)
(o)
(p)
(q)
(r)
(s)
(t)
(u)
(v)
(w)
(x)
Upstream
Downstream
without sluice
with sluice
administration area
construction
farmland
greenspace
industrial area
residential area
unused area
village
semi-urban area
transition area
centre urban area
NMDS1
NMDS2

Figure S1 (Previous page continued) n-MDS coordinate images. The horizontal axis of each graph is the first non-metric dimension scale (NMDS1), and the vertical axis is the second non-metric dimension scale (NMDS2). Taking Bray-Curtis dissimilarities as the proximity calculation standard, 43 summer sampling points were scaled in coordinates (a-d), coordinates (i-l) and coordinates (q-t) by regarding microplastics in different shapes (*Stress* = 0.121), size ranges (*Stress* = 0.087) and colors (*Stress* = 0.140) as different microplastic 'species'. In the same way, 17 winter sampling sites were scaled in coordinates (e-h), coordinates (m-p) and coordinates (u-x) by regarding microplastics in different shapes (*Stress* = 0.053), size ranges (*Stress* = 0. 039) and colors (*Stress* = 0.082) as different microplastic species. In coordinates (a, e, i, m, q & u), the color of sampling site changes from light to dark from upstream to downstream. In coordinates (b, f, j, n, r & v), the sampling sites in blue are affected by sluice factor while the points in black are not. In coordinates (c, g, k, o, s & w), different colors were used to distinguish the land-use types and dotted polygons to envelop the sampling sites in the same land-use types. In coordinates (d, h, l, p, t & x), hull polygons in different colors were used to cover the sampling sites in three urbanization levels.