Characterizing Microplastic Pollution and Microbial Community Status in Rice Paddy Soils Across Varied Environmental Settings in Songjiang, Shanghai: An Analysis of Morpho-Chemical Characteristics

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Abstract:- Microplastic (MP) pollution poses a huge threat to rice fields, but the distribution characteristics of MPs in farmlands of different types of areas are still uncertain. In this work, 24 samples from 12 rice fields of four different land-use types (Factory, highway, greenhouse/mulching and normal fields) were collected from Songjiang, Shanghai. From our selected sites, it was found that MP abundances were in the range of 233.33-173.33 particles/kg in rhizosphere and bulk soil. MP distribution results showed that over 40% of particles were less than 1 mm and MP sizes ranging between 1 and 5 mm represented the greatest proportion. According to our study, MP in rhizosphere soil has the highest abundance (233.33 \pm 57.73 particles/kg) than bulk soil.

The particle shape classified as fragment (with edges and angular) was the most frequent shape found near factory areas, with an abundance of approximately 37.10%. Copolymers of polypropylene- polyethylene (PP/PE) at 24.30% were the most abundant polymers in rice lands in both bulk and rhizosphere soils; following is polystyrene (PS) at 21.40%, respectively. Most of the particles found in soils were white. Statistical analyses showed that fields near factories and fields where plastic mulching (mulch film and greenhouse crops) was used had a significantly higher particle abundance for bulk and rhizosphere soils, hence identifying plastic mulching as a major contributor to MP pollution in paddy soils. In industrial areas, MP can also be generated by released waste or by air. Microbial studies in rice roots, rhizosphere soil, and bulk soil show variation in the abundance of different species and genera. The dominant bacterial phyla in rice roots are Proteobacteria, Actinobacteriota, Firmicutes, and Bacteroidota. These microbes have been observed and can be impacted by the presence of MPs. Rhizosphere soil and bulk soil have an abundance of Chloroflexi, Actinobacteriota, Proteobacteria, Firmicutes, and Acidobacteriota. The specific effects on the microbial community structure depend on factors like MP type, concentration, and exposure duration. As our study was field-based, the significant effect of a specific type or concentration of MP was difficult to identify.

Variation analyses of MP characteristics revealed that paddy lands were more likely to contain fragment shapes and large MP particles (1-5 mm). Also, rhizosphere soils were likely to contain fragment shapes and pony-size MPs (0.02-0.2 mm). Differences among rice fields may depend on various reasons, such as using slowrelease fertilizers, mulching plastic application, irrigation, atmospheric fallout, etc. This study provides some proper evidence about the characteristics of MP pollution in rice fields of Songjiang and explores some probable conditions and predominant MP sources in rice fields.

Keywords:- Microplastics, Songjiang City, Soil, Paddy Field, Microplastic Classification.

I. INTRODUCTION

The global number of plastic wastes produced is expected to nearly triple by 2060, with the majority going to landfills and less than one fifth being recycled (OECD, 2022). As the plastic waste is predicted to rise, microplastics (MPs) become a major environmental problem worldwide. MPs have been detected in various environmental media such as aquatic and terrestrial environments and even in the atmosphere (Evangeliou et al., 2020; Rillig, 2012; Hurley et al., 2018). With different external forces such as biological degradation, collision wear and UV radiation over time, plastics in the soil can be gradually broken down into fragments smaller than 5 mm in size (de Souza Machado et al., 2018; Bouwmeester et al., 2015; Thompson et al., 2004). It is predicted that the median level of MPs in soil in the world will be 1167 particles/kg in the coming years (Büks and Kaupenjohann, 2020). As a vector for various anthropogenic contaminants that transferring into soil and water, MPs can pose a great threat to the ecological security and sustainable development of human society (Hartmann et al., 2017; Bradney et al., 2019; Guo et al., 2020).

https://doi.org/10.38124/ijisrt/IJISRT24MAR2137

In agricultural fields, MPs can originate from instruments, plastic film waste from mulching, greenhouses, discarded pesticide bottles, discarded fishing nets, household plastic waste, insulated covers, sewage sludge containing MPs as fertilizers, organic fertilizers, and slow-release fertilizers, which can be transferred to the soil (Henseler et al., 2019; Sommer et al., 2018; Choi et al., 2021; Heuchan et al., 2019; Weithmann et al., 2018; Medyńska-Juraszek and Szczepańska 2023; Cai et al., 2023; Lwanga et al., 2023). Due to the complexity of the soil environment, various pollutants (e.g., persistent organic pollutants, heavy metals, and antibiotics) may adhere to plastic surfaces, accelerating the transfer of MPs up to the food cycle (Hartmann et al., 2017; Hodson et al., 2017; Sun et al., 2018). The presence of MPs may alter the physical and chemical properties of soil, such as water-holding capacity (Wang et al., 2022) and dissolved organic matter composition (Zhou et al., 2020). The study by Chen et al. (2020) found that the abundance of MPs ranged from 320 to 12,560 particles kg^{-1} in the vegetable farmlands of a suburb of Wuhan, China. MPs also have potential impacts on soil sustainability through affecting soil microbial structure (Zhang et al., 2020) and soil fauna (Wang et al., 2021). To document MPs in different sectors, a number of literatures have been published in recent years (Yang et al., 2021b). Among all research papers on microplastics published in the Web of Science from 2010 to 2020, the majority were focused on microplastics in marine and biological contexts and 7.3% of the papers dealt with issues of MPs in soils (Kim et al., 2021). However, MPs in agricultural fields are less studied as it takes a lot of time and effort to separate and identify them from soils (Li et al., 2021). Although the paddy fields have received less attention (Lambert & Wagner, 2018), the field soils are an important "sink" for MPs. (Li et al., 2023a).

Rice provides food for more than half of the global population (Wu et al., 2018), which is one of the most important crops in China. The productivity of rice fields is threatened by natural and anthropogenic stressors, including global warming, land use change, biodiversity decline, and environmental pollutants (Bhowmik, 2020). With the development of city and suburban areas, more and more industries are growing up near agricultural lands. MPs generated from different sources like tire abrasion and wastes from the highway, fertilizers, plastic mulch, and plasticproducing factories can also work as stressors to farmlands. In Shanghai city, Songjiang is one of the rice-growing areas among other districts due to the relatively higher soil fertility (Li et al., 2023b). It is essential to account for the pollution caused in rice lands and find the effect and abundance of MPs. In comparison, studies related to microplastic pollution in the paddy soils on an area basis are still insufficient, which may lead to an underestimation of the ecological impact of MPs in different areas.

The aims of this work are to provide original evidences on the pollution status of MPs in rice fields in the Songjiang district of Shanghai. The possible sources and the distribution of MPs are delineated along with microbial community study of rice roots, rhizosphere soil and bulk soil from the fields. The number of MPs in Songjiang's rice fields is unknown, and potential contamination routes have rarely been compared. According to our hypothesis, rice fields in Songjiang have different sources of MPs. Factories and highways may be a source for MPs in paddy fields, and mulching in fields is also a common practice in the district. Subsequently, we hypothesize that MPs can be particularly abundant in fields receiving direct or indirect waste. Normal paddy fields are compared to the test as well. Moreover, the following paper provides innovative insights concerning MPs interaction with various land characteristics as well as the abundance and distribution characteristics (including shapes, sizes, and components) of MPs. This study may provide field evidence for our understanding of the sources and accumulation of MPs in farmlands as well as the effect on microbial community. Finally, the study calls into attention the necessity of efficient soil management of MPs pollution to conserve or possibly enhance the quality of rice fields.

II. MATERIALS AND METHODS

A. Study Area

The study area was selected based on various types of land use. Songjiang District in Shanghai is selected due to the availability of a large number of agricultural, residential, roadside, park, and industrial areas in the region. A total of 4 designated sites were selected. Three fields were randomly selected from each site. Near Factory area (plastic factory) (31° 4' 6"N, 121° 17' 24"E), Near Highway area (31° 59' 28"N, 122° 17' 39"E), Near Greenhouse/ Mulching area (30° 56' 44"N, 121° 17' 55"E), and Normal Field area (30° 57' 4"N, 121° 15' 20"E) were the sites. Most of the soil was welldrained and dry at sampling time as the rice plant was fully grown. Usually, the soil of Songjiang is rich in silt loam retaining enough nutrients and allowing good drainage. The Huangpu River flows through Songjiang and most of the agricultural lands are connected by small canals and lakes. The river is the main source of water for irrigation drainage.

B. Sample Collection

Topsoil samples (0-15 cm) were collected from rice paddy fields in Songjiang, Shanghai, in November 2023. The fields were located near various land use types, including fields near plastic factories, highways, greenhouses/mulching areas, and normal fields, where no detected disturbance present (Fig. 1). The rice plants were fully grown at the sampling time. To ensure minimal contamination, the bulk soil and rhizosphere soil samples were collected using the sterilized glass jars. The rhizosphere soil samples were prepared by gently shaking the roots to remove the closely adhering soil particles and storing them in glass jars. Additionally, mesoplastic samples found near rice roots and soil were collected. A total of 24 samples, comprising 12 bulk soil samples and 12 rhizosphere soil samples, were collected and transported to the laboratory for further analysis. The soil samples were stored in glass jars at a temperature of 4°C in a freezer until analysis. Plant samples with roots were collected in aluminium foil, transported in zip-lock bags, and immediately brought to the lab. Root samples were A designated portion of all the raw samples for DNA sequencing were subsequently transferred to the freezer at -20°C for microbial community analysis.

International Journal of Innovative Science and Research Technology https://doi.org/10.38124/ijisrt/IJISRT24MAR2137



4 Normal Fields

Fig 1: Sampling Sites Near Factory (1), Highway (2), Plastic Mulch Using (3), and Normal Fields (4).

C. Sample Preparation for Analysis

> Sample Preparation

Initially, an appropriate method for testing MPs in soil was selected based on previous reports (Liu et al., 2018; Besley et al., 2017; Imhof et al., 2012). To prevent contamination from the air, the soil samples were placed in a drying oven at 30 °C for 48 h. The dried soil samples were sieved using a 2 mm sieve to separate them into large and small samples. The MPs larger than 5 mm in size were visually classified as mesoplastic and were analyzed individually.

Extraction, Characterization, and Identification of MPs

To isolate MPs, the soil samples were passed through a 2 mm sieve. Approximately 50 g of the sieved soil was transferred into 500 mL glass beakers. The organic matters and plant fibers were digested by adding 20 mL of 30% hydrogen peroxide solution and an equivalent volume of 0.05 M Fe (II) Solution. Iron solution was prepared adding 7.5 g of FeSO₄.7H₂0 (= 278.02 g/mol) and 3 mL of concentrated H₂SO₄ in 500ml of water. The reaction was maintained below 40 °C with an ice bath if necessary. While monitoring closely, the deionized water was added for vigorous reactions. The beakers covered with aluminum foil were left to stand at room temperature for over one week (Masura et al., 2015). Density separation was performed with $ZnCl_2$ solution (1.6 g·mL⁻¹). Loder and Gerdts (2015) suggested ZnCl₂ as a good density separation medium due to its efficiency and costeffectiveness. Following this, agitation was done for 30 minutes. Then, the contents were allowed to settle for 24 h to allow the particles to float. The supernatant was then vacuumfiltered three times through 0.22-µm glass microfiber filter (GF/F) membranes. The filter membrane was air-dried in a glass petri dish covering the lid. After drying, MPs were stored for subsequent analysis.

For preparation meso-plastics for analysis, the larger fragments were isolated with tweezers while still moist. These meso-plastic samples were well-rinsed with distilled water for density separation with NaCl solution (1.2 g cm⁻³) (Masura et al, 2015). Vacuum filtration was performed using 0.45 µm filter paper (47 mm diameter). Particles measuring >5 mm were stored under normal temperature for further evaluation.

> MP Characterization and Identification

MPs were characterized and distinguished based on four criteria: abundance, size, color, and shape. According to the method of Zhao et al. (2018), a stereo-microscope (COIC) with a high-resolution digital camera having a magnification of 16-100 times was used to visually inspect and count MPs in both bulk soils and rhizosphere soils. After visual inspection of the color of MPs, the shape was analyzed according to Masura et al. (2015). Polymer identification constituted the final stage of MP characterization. Two or three randomly selected suspected MPs from sampling filters were analyzed using Fourier Transform Infrared Micro-spectroscopy (µ-FTIR). The obtained spectra were compared with standard databases such as HR Nicolet Sampler Library and Hummel Polymer Sample Library. To verify the reliability, only those spectra

ISSN No:-2456-2165

that matched over 70% with the standard database were acceptable. Additionally, a confirmatory test for 28 particles was performed and detected with μ -FTIR to check the consistency. About 80% of particles were correctly predicted by the pattern comparison. Moreover, the particle size was measured using ImageJ software while being categorized into ranges of <0.2 mm, 0.2–0.5 mm,0.5–1mm, and 1–5mm. All the identified MPs are classified by their shapes (pellets, foam, film, fiber, and fragment) along with various colors including white, blue, red, green, brown, yellow, black, and transparent.

Microbial Analysis: DNA Extraction, (PCR) Amplification, and Sequencing

The total genomic DNA of bulk soil, rhizosphere soil, and root endosphere was extracted using the TruSeqTM DNA Sample Prep Kit. Bacterial region was amplified using the 16S rRNA primers for soil 338F (5'-ACTCCTACGGGAGGCAGCAG-3') 806R (5'and GGACTACHVGGGTWTCTAAT-3') and plant endophyte primer obtained by endophytic bacteria 799F (5'-AACMGGATTAGATACCCKG-3') and 1193R (5'-ACGTCATCCCCACCTTCC-3'). Sequencing was performed using the Illumine MiSeq PE300 platform by Majorbio BioPharm Technology Co, Ltd. (Shanghai, China) with a paired-end format. QIIME 2 (Quantitative Insights into Microbial Ecology) (v2022.2) with DADA2 plugin was used to remove barcodes and sequence adapters, filter high-quality non-chimeric reads, cluster the reads in single amplicon sequence variants and perform quality control and feature table construction on raw sequences (Bolyen et al., 2019). High-quality sequences were clustered into amplicon sequence variants (ASVs) at highest similarity. The PCR productions were further purified using 2% agarose gel and AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, CA, USA). Alpha diversity was estimated upon the rarefaction of the datasets. Microbial species richness was determined by calculating the number of observed microbial species and using the Chao1 richness estimator.

Quality Control and Assurance

During sample collection, strict measures were taken to eliminate potential sources of contamination. During sample collection, all plastic-made equipment was directly avoided. In the case of any plastic lids, it was covered with aluminum foil to control contamination. The handling and processing of samples were scheduled at times of minimal activity within the laboratory to reduce airborne MPs or cloth-shredded fibers. To maintain an uncontaminated environment, 100% cotton-made lab coats were worn with masks and gloves. The cleaning protocol was strictly followed to minimize the risk of contamination. All scientific apparatuses were cleaned with an ultrasonic cleaner and oven-dried properly to eliminate any potential airborne contaminants. The apparatus was washed three times with deionized water. During sample processing, all the containers were consistently covered with aluminum foil. which has been reported to be able to minimize more than 90% of contamination (Wang et al., 2020). Blank assays were carried out to test external MP concentrations in air and water, as described by Chen et al. (2020). Through the detection of three air and three water samples, the average concentration of three procedure blanks was 1.8 items, and these results confirmed the non-significance of external factors. Continuous checking was done by keeping an open petri dish for suspecting airborne MPs. Control experiments were conducted in parallel with blank assays and it confirmed the absence of microplastic particles. While working, 70% ethanol alcohol was used to clean off the workbench.

https://doi.org/10.38124/ijisrt/IJISRT24MAR2137

D. Statistical Analysis

Statistical analyses were conducted with the STATA software (version 15) and Microsoft Excel (Microsoft Corporation) was used for data representation. One-way analysis of variance (ANOVA) was used to assess significant differences among different groups. Data were presented as means \pm SD, and statistical differences were assessed using the Chi-square test. The online analysis platform (Majorbio Cloud Platform) was used to draw the statistical charts (heatmap, bar plot, PCA, and Venn diagrams).

III. RESULT AND DISCUSSION

A. MP Abundance in different Farmlands

> Mesoplastic

Table 1 and Fig. 2 show the results of meso plastic analysis categorized by land use. In agricultural lands, packing string, fertilizer bags, and pieces of plastic mulching were found. Various garbage, plastics, and mesoplastics from wastes were found in factory areas, highway lands, and mulching areas, normal fields respectively. On factory and mulching lands, there were a lot of large plastic fragments and plastics such as PP, PE, PVC, and PS; with higher frequency. Their results confirmed that factory and mulching activities were a major factor affecting plastic contamination in these fields. Table 1 shows the results of meso plastic analysis found and categorized by type of fields.

Table 1: Number of (Meso) Plastics in Rice Soils (Particles/kg) According to Types of Fields.

Land use site	Field 1	Field 2	Field 3				
Factory area	2	6	7				
Highway	2	1	ND				
Plastic mulch	5	2	3				
Normal fields	ND	ND	1				

ND Not Detected



Fig 2: Meso Plastics Found in the Soil of Rice Fields

➢ Microplastic

A total of 24 soil samples of bulk and rhizosphere soils from 12 rice paddy fields were examined for MPs. Microplastics were present in all samples. A total of 70 plastic particles were counted and characterized from bulk soil and 15 particles were characterized from rhizosphere soils. Different concentrations of MPs were found in all 12 paddy fields. The paddies near the factory were significantly more polluted with MPs than others. The mean MP abundance in the rhizosphere soil near plastic mulching areas shows the highest abundance. The difference in MP abundances in rhizosphere soils between the near highway and near greenhouse is statistically significant. Also, the difference between near greenhouse and normal fields is statistically significant (p<0.02). Statistics show that the abundance of MP in bulk soils is not significant in different areas.



Fig 3: Abundances of MPs in Bulk Soil and Rhizospheric Soil by different Study Sites

There are several potential sources of MPs in bulk soils, including the application of soil amendment, plastic mulching practices in certain crop fields, atmospheric deposition and wind-driven transport, runoff from industrial areas, and attachment to root surfaces. According to our field experiment, in all field sites, bulk soil contained large particles of MPs. Interestingly, visual observation showed that MP abundances of particles between 1 and 5 mm and larger (meso plastics) were significantly highest in bulk soil of factory areas and second highest in greenhouse/ plastic mulching areas. As per the study of Tsering et al. (2021) the effluents from industries can diffuse into soils and result in

ISSN No:-2456-2165

https://doi.org/10.38124/ijisrt/IJISRT24MAR2137

larger MP deposition in soils nearby. Another study by Wang et al. (2022) fertilizer industry near the paddy fields increased the input of film-type MPs. The waste generated in factory areas comes into contact either by runoff, wind, or by direct littering. So, this might cause a high amount of MP in bulk soils near the factory areas. Paddy soils near the greenhouse/mulching ranks second among the four sites.

The presence and distribution of microplastics (MPs) in bulk soil versus rhizosphere soils can vary significantly due to a range of factors. According to Bouaicha et al., (2022), rhizosphere soil immediately surrounding plant roots, appears to be a hotspot for MP accumulation and interaction with the soil ecosystem. We found the highest proportion of MP in rhizosphere soil near the mulching/ greenhouse areas. According to our study, MP in rhizosphere soil has the highest abundance $(233.33 \pm 57.73$ particles/kg) than bulk soil. The rhizosphere seems to be more susceptible to MP accumulation and interactions compared to bulk soil, due to the direct interface between MPs and the plant-soil-microbe system (Bouaicha et al., 2022). This might be a cause of a higher percentage of MP

present in rhizosphere soils. There is very little information found about the difference between MP in bulk soil and rhizosphere soils. Dong et al. (2020) pointed out about strong electrostatic attraction forces of small plastic particles, which promote aggregation among particles. There is no information on how electrostatic attraction forces exist between plastic particles.

When microplastics are aggregated, they are easily moved by invertebrates, such as springtails (Maaß et al., 2017). Often aged microplastics are colonized by microorganisms as microplastics could be more palatable to soil invertebrates (Helmberger et al., 2020). More studies are needed to understand how these electrostatic forces and invertebrates affect the transport processes of MPs in the soil. Moreover, Plant roots can fragment larger pieces of plastic debris in the soil, contributing to the formation of MPs in the rhizosphere soil. Usually, the rhizosphere has a higher microbial activity compared to bulk soil. Some studies suggest that microbial degradation of plastics can occur, potentially influencing MP abundance and size distribution in the rhizosphere soil.



Fig 4: Bar Plots Showing the Variance Analysis of the Distribution Characteristics of MPs: Distribution of MPs' a) Colour b) Size c) Shape d) Polymer Composition in Bulk Soils.

Volume 9, Issue 3, March – 2024 ISSN No:-2456-2165



Fig 5: Bar Plots Showing the Variance Analysis of the Distribution Characteristics of MPs: Distribution of MP a) Colour b) Size c) Shape d) Polymer Composition in Rhizosphere Soils.

B. Shape and Color

Table 2: Number of Average Microplastics by Shape and Color in % of Soils [Others (Black, Red, Yellow, Pink)]

Color	Bulk soil %	Rhizospheric Soil %	
Blue	8.6	13.3	
Brown	12.9	13.3	
Green	10.0	13.3	
Transparent	11.4	20.0	
White	32.9	26.7	
Others(bryp)	24.3	13.3	
Shape			
Film	11.4	13.3	
Foam	12.9	13.3	
Fragment	37.1	33.3	
Pellet	24.3	20.0	
Fiber	14.3	20.0	

Table 2 shows a list of microplastics detected in the collected soil, divided into five shapes and seven colors. Regardless of land use, detected microplastics had a significantly higher proportion of fragments (37.1%) and white color. In the present study, the proportion of microplastics in bulk soil near the factory area, is 37.04% in pellet form, 33.33% in fragment form, and 14.81% in fiber form. The proportion in highway areas was 36.36% in fragment form, 18.18% in foam form, and 27.27% in fiber

form. The proportion near the greenhouse area, is 50% in fragment form, 25% in film form, and 12.5% in both foam and pellet form. The proportion of shape distribution in normal lands is (37.5%) fiber, and 25% pellet. The proportion of microplastics in rhizospheric soil near the factory area is 75% in fragment form and 25% in film form. The proportion in highway areas was 50% in fragment form and 50% in fiber form. The proportion near the greenhouse area is 42.86% in pellet form and 14.29% in fiber form. The proportion of shape distribution in normal lands is 50% fiber, and 50% fragment. Wang et al. (2021) reported that 54.05% of microplastics found in agricultural land soil were fragments, with the majority of microplastics found in rice paddies and orchards showing fiber and fragment forms. Furthermore, there are previous studies similar to this research result where fragment forms are mainly distributed, leading to the assumption that the occurrence forms of microplastics are similar (Han et al., 2019). However, Zhang and Liu (2018) found that 92% of MPs in the soil of forest areas were in fiber form, with only 8% in fragment and film type. The distribution by color in bulk soil near the factory was 26% white, 26% green, 14.81% blue, and 25% others. In greenhouse/ mulching areas, 45.83% white, 12.5% transparent, 20.83% brown, 16.67% others. In near highway areas, 27.27% brown, 18.18% both transparent and white, and 27% are others. In bulk soil other colors like black, yellow, pink, and red were found in small quantities. However, in other studies, black and green colors were most abundant (Yoon et al., 2024). The distribution by color in rhizosphere soil near the factory area is 50% transparent, 25% white, and brown. In mulching areas, 28.57% were white and green, and 14.29% for white, blue, and brown. In highway areas 50% of blue. In normal fields 50% of white and others were present. In rhizosphere soil, other colors like black, yellow, and pink were not found. Only red was denoted as others. However, in other studies, black and green colors were

https://doi.org/10.38124/ijisrt/IJISRT24MAR2137

most abundant (Yoon et al., 2024). The reason for the different distribution patterns according to land use is due to the different patterns of plastic use in each area. It is presumed that the use of green plastic and various farm machinery in nearby factory areas of agricultural lands resulted in higher numbers of these colors (Choi et al., 2021).

C. Size Analysis of Microplastic by Land Use

Figure 6 shows plastics detected in samples for each land use divided by size: 0.02-0.2 mm, 0.2 mm ~ 0.5 mm, 0.5 mm- 1mm, and 1-5 mm. The proportion of MPs measuring 1mm - 5mm is about 33% in bulk soils. 27.1% of particles were found ranging from 0.5- 1mm, 23% of particles in the 0.2mm – 0.5 mm range, and 17.1% of particles were lies in the 0.02 mm - 0.2 mm range. While MP size less than 0.2 mmwas the highest in rhizospheric soils, accounting for about 60%. 26.7% in 0.2mm- 0.5mm range, 13.3% particle in 0.5 mm- 1mm range. In rhizospheric soil, there is no particle detected in the 1 mm - 5 mm range. The proportion of microplastics larger than 1 mm was the highest in all sites of bulk samples. Although normal fields contain larger particles it can be suspected that whether there is any disturbance or not, MP can be available in all soils. Results of rhizospheric soil show that most of the MP of 1mm - 5mm can be found in normal fields and 0.5mm - 1mm particles in near highway fields.

It appears that microplastics generated by humans on the highway, and normal rice fields are broken down into smaller pieces due to physical force such as compaction and friction or influence of the environment in a short time. Also, highway waste can disintegrate into smaller particles and accumulate near the rhizosphere zone of plants. According to Yoon et al., (2024), forest soil areas are seldom visited by people, and plastics with sizes over 1 mm had a small amount because the waste input was small. Though small particles of plastic had a high percentage, they originated from plastic fallout or plastic that was already there and decomposed over a long period. Smaller particles of 0.02- 0.2mm can be found near factory and greenhouse/mulching areas suggesting that longterm disintegration of MP particles can occur near root zone and rhizosphere soil. This is a bit alarming for soil quality and agricultural purposes.





D. Polymer Analysis of Microplastic by Land Use



Fig 7: Photographs of Mps Observed Under the Stereomicroscope Illustrating Different Shapes: Foam (a), Film (b,c), and Fragment (d)

Table 3: Polymer Composition of MP Found in all Soil Samples
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Polymer Composition	Bulk Soil	Rhizospheric Soil	
EPDM	4.3	ND	
PB	10	ND	
PE	14.3	26.7	
PP	15.7	13.3	
PP/PE	24.3	33.3	
PS	21.4	13.3	
PU	5.7	ND	
others	4.3	13.3	

ND= Not Detected

FT-IR is called the fast-mapping method for qualitative analysis of microplastics. According to Yoon et al., (2024), agricultural land in a metropolitan area had an abundance of packing string, fertilizer bags, and pieces of plastic mulching components. According to our studies various garbage, plastics, and meso plastics from discarded waste such as plastic bags, packing material, used plastic sheets, foam, and rubber were found in fields near factory areas. Polymers in near factory fields were, 37.04% of PP/PE, 14.81% of PE, 14.81% of PS, 7.41% of PU, and 11.11% of EPDM etc. In rhizosphere soils near factories, PP, PS, and PP/PE polymers were detected. Interestingly, EPDM, a frequently used infill material of artificial turfs was found in one of the fields. According to a study by Rittelmann-Woods et al., (2023) EPDM granules had a clear negative effect on plant growth. EPDM is widely used in a variety of industrial and commercial applications due to its desirable properties in parts (seals, gaskets, hoses, weatherstripping). It can be anticipated that this came from plastic factory waste or construction. Fields near factories pose a great threat to rice plants and soil health. Pollutants also create stress on plant roots and this may indirectly affect plant growth and yields.



Fig 8: MP from Fields Near the Highway Fields (Black and Blue Color)

https://doi.org/10.38124/ijisrt/IJISRT24MAR2137

In rice fields near the highway, PB (36.36%), PP (18.18%), and PP/PE (18.18%) were detected. In the rhizosphere soil of this area, PE (25%) and PP/PE (25%) were detected. LDPE (denoted as others) and PS were also found in roadside soil. PP is also a material found in road maintenance materials and parking blocks. It is commonly encountered on roads. Though polybutadiene (PB) was detected in near-highway samples, more component of tire wear was not detected like in some previous studies. However, styrene-butadiene rubber (SBR) generated from automobiles was not identified in the soil because the road was well maintained, and the location had a good drainage system. Sommer et al. (2018) have reported that in urban areas such as roadside and residential areas, SBR is detected as a commonly used material in the tire industry. Their report also showed an increase in SBR around highways, roadside, or parking lots due to the use and wear of car tires.

Polymers present in bulk soil near greenhouse/ plastic mulch 37.5% of PS, 20.83% of PP, 16.67% of PE, and 12.5% of PP/PE. Also, in rhizospheric soils near greenhouse areas, most of the polymers detected were, 42.86% of PP/PE, 28.57% of PE, and 14.29% of PS. Usually, PE and PP are preferred materials widely used for mulching on agricultural land. However, due to their low density, they can be easily washed away along with soil erosion. PS, PE, PP, PVC, and PET have been detected in agricultural land soils in Shanxi province, China (Ding et al., 2020). Polystyrene (PS) also can be

sourced from package materials or mulching items. Choi et al. (2021) have reported that PE, PP, PS, and polyvinyl chloride (PVC) account for the majority of microplastics found in Yeoju soil.

It is suspected that the PP/PE copolymer came from slow-release fertilizers applied in the fields as most of the fields had distinct shapes of PP/PE pellets or fragments. Often some of the fertilizer coatings are called biodegradable but they seem to persist in the field and create micro and nanoparticles of PP/PE blend (Ghumman et al., 2023). In this study, we found PU in bulk soil near factories (7.41%) and greenhouse areas (8.33%) but not in rhizosphere soil. PU is used mostly as a synthetic leather material because it has higher elasticity and flexibility than hard PVC, making PU a preferred alternative. Therefore, PU detected in the soil was presumed to be fragments from worn-out shoe soles, insulation materials, cushioning materials, gloves, and artificial leather (Rusu et al., 2020). MPs released from tires by tire wear are considered to be one of the main sources of MPs in road dust (An et al., 2020). Though our study results got limited data to conclude about highway waste involving rice paddy soil it is undeniable that MP waste from highways might have some impact on paddy fields. Microplastic pollutants on roads can be from tire debris, road paint, asphalt, paints on roads and buildings, and materials used for traffic safety facilities (Magnusson et al., 2016, Sundt et al., 2014).



Fig 9: Transmittance of Polymers of PP/PE, PP, PS and EPDM Found in Rice Paddy Soils

ISSN No:-2456-2165

> Findings in Normal Fields

The abundance of MPs in normal paddy fields in this area was 66.66 ± 30.55 Particle/kg (Fig. 3), which is close to that reported in other agriculture fields (Chen et al. 2020). Four shapes, namely, fibers, pellets, fragments, and films were detected. The diameter of the detected MPs ranged from 0.02 mm to 0.2 mm (25%) and 1mm to 5 mm (50%). White and transparent were the dominant colors. Polyethene (PE; 25%), PP (25%), and PP/PE (25%) were the main polymer types in this area and the rest were polybutadiene (PB), polystyrene (PS). Regardless of land use, detected microplastics had a significantly higher proportion of fragments and white-colored particles. White fragments found in rice land were from mulching film and the use of farm machinery. From the results, it can be depicted that whether it is a normal field without other disturbance, MP particles can still be found ubiquitously. The results from Yoon et al., (2024) show that the occurrence of microplastic is greatly affected by human activities. These results confirmed that the frequency of farm activity and anthropogenic activities is a major factor affecting plastic contamination in rice fields.

E. Limitations

The initial soil and land properties may determine the subsequent response of microplastic properties. However, the lack of data prevented us from incorporating these indicators into the results part. Moreover, results might be different for larger datasets of soil. The response of soil microbial communities to plastic is still scarce. It is necessary to conduct subsequent laboratory studies based on the present results. However, in this study, only small amounts of samples were detected because of small number of sample soils. Judging from our analyses, the agricultural land soil of this study contained a lot of large organic matter. So much amount of time is needed to do pretreatment and

https://doi.org/10.38124/ijisrt/IJISRT24MAR2137

some steps have to be done over and over again. As organic matter and other particles might interfere with transmittance, we have to take only the suspected polymer particles for testing in micro-FT-IR. Due to the presence of large particles in high quantities, fine particles might be filtered thereby it might reduce the accuracy of the analysis.

F. Microbial Community Analysis: Composition and Diversity of Bacterial Communities in Rice Roots, Bulk Soil, and Rhizosphere Soil

▶ Bioinformatics analysis of 16S rRNA gene profiling

For microbial community analysis, the samples of rice roots, bulk soil, and rhizosphere soils were divided into four sites only. Sample numbers 1, 2, 3, and 4 are denoted as factory area, highway area, greenhouse/mulching area, and normal field area respectively. For all microbial analysis samples, the raw 16S rRNA gene sequencing reads were demultiplexed, Quality control was done using fastp (v0.19.6), merged with FLASH (v1.2.7), and Noise reduction with the DADA2 plugin.

• Alpha Diversity Analysis

Alpha diversity analysis mainly evaluates the information on the richness and diversity of microbial communities in all samples through multiple diversity indices and explores the difference in alpha diversity index between the control group and the sample group through inter-group difference testing. The following table depicts the genus-level data of root, bulk soil, and rhizosphere soils. MPs can have variable impacts on the overall microbial diversity in soil, as measured by diversity indices like Shannon and Chao1. In some cases, MP exposure led to a decrease in microbial diversity, while in others, the diversity remained relatively stable.

Sample/Estimators	Sobs	ACE	Chao	Shannon	Simpson		
Root_1	243	243.293	243	2.518121	0.243782		
Root_2	263	263	263	3.268038	0.075654		
Root_3	365	368.434	366.2162	3.768459	0.055891		
Root_4	441	446.165	443.625	4.168036	0.045386		
R_Soil_1	671	673.523	671.6667	4.948555	0.019709		
R_Soil_2	579	579	579	4.497071	0.033934		
R_Soil_3	634	639.786	635.6438	4.977367	0.015347		
R_Soil_4	592	596.315	593.3	4.751559	0.022955		
B_Soil_1	789	789.864	789.0366	5.513042	0.008478		
B_Soil_2	796	798.28	796.3784	5.430255	0.009049		
B_Soil_3	725	726.145	725.075	5.367639	0.008523		
B_Soil_4	687	690.975	687.8684	5.104962	0.013909		

Table 3: Alpha Diversity Indices of Different Samples

In this table, Sobs, Ace, and Chao indexes reflect the species richness or evaluate the total number of species in the community. The higher the value, the more OTUs in the community, and the greater the richness of the community. According to Sobs highest microbe community was found in highway bulk soil, it has the highest richness among other samples. Root samples specially from near factory lands have the lowest richness among the three samples, resulting that, factory waste can seriously affect microbial community of root. As per Ace and Chao index, overall findings are same. In bulk soil, microbial species richness is, highway>factory> mulching > normal fields. As for roots, The Shannon index and Simpson index were used to reflect community diversity, including species richness and species evenness. The higher the Simpson index, the lower the community diversity. As per Shannon and Simpson, it can be depicted that rice roots have

ISSN No:-2456-2165

more rich and diverse species than rhizosphere soils and bulk soil have the lowest diversity.

> Root Endophyte Microbes

This diagram suggests that there is a significant amount of variation in the endophyte genera found across the four root samples. However, there is also a substantial number of endophyte genera that are shared between two or more of the root samples. Each root zone has unique microbes not found in the others. The root samples from near the factory area have 24 unique species, the root from near highway land has 41, roots from near the plastic mulching have 57, and land of normal area with no disturbance rice roots have 111 unique species. Also, it can be indicated that 129 microbial species are common to all roots. Normal fields have more unique species and their range is Normal>greenhouse/mulch>highway>factory areas.

https://doi.org/10.38124/ijisrt/IJISRT24MAR2137

Moreover, the diagram can reflect the influence of different environmental conditions on microbial diversity. As our previous study states normal fields have less disturbance from MP particles microbial also have more unique species in normal fields while plastic mulching and factory areas have less unique species due to MP disturbance.



Fig 10: Venn Diagram Focuses on the Root Endophyte Genus Level for Microbes of Unique and Shared Species in Different Groups

The microbial community structure of rice roots of different sites is shown in Fig. 8. At the phylum level, the main bacterial communities included Proteobacteria, Actinobacteriota, Firmicutes, Bacteroidota, Myxococcota, Desulfobacterota, Verrucomicrobiota, Chloroflexi. Acidobacteriota, Patescibacteria, etc. Proteobacteria was the dominant phylum in all root samples from near factories, highways, greenhouse/mulching, and normal field areas. In root samples from the greenhouse/ mulching area, Proteobacteria has the highest abundance but in the factory area, Proteobacteria is found in lower abundance. While in highway and normal fields, the amount was quite similar for this phylum. For Actinobacteriota highway areas rose the most abundant phylum while greenhouse/ plastic mulching had the lowest abundance. Normal fields have no size effect on the abundance of Actinobacteriota but roots near the

Factory area show lower abundance than normal fields. Firmicutes have the highest abundance of rice roots in factory areas. For greenhouse/plastic mulching areas and normal land areas, it has the lowest abundance but is higher in highway areas. Similarly, Bacteroidota has a higher abundance in samples of normal fields, but quite similar and lowest abundance in factory and highway areas. Other phyla like Desulfobacterota, Myxococcota, Verrucomicrobiota. Chloroflexi, Acidobacteriota, Patescibacteria, etc have quite a similar abundance in a smaller amount. All the abundances show that the roots from near factory and highway areas have a higher abundance of the following top 5 phyla but less or no abundance of other phylum like Myxococcota, Desulfobacterota, Verrucomicrobiota, Chloroflexi, Acidobacteriota, Patescibacteria etc.



Fig 11: Relative Abundance of Microbial Community Analysis (Phylum Level) with Root Endospheric Samples where Root_1, Root_2, Root_3, and Root_4 Denoted as Root Samples from Factory Area, Highway Area, Greenhouse/Mulching Area, and Normal Field Area Respectively

 Relative Abundance of Microbial Community Analysis (Genus Level) With Root Endospheric Samples.

Root samples from near the factory have an abundance of the *Exiguobacterium* genus. The abundance of *Exiguobacterium* genus in sample sites can be ranged as, Factory>greenhouse/plastic mulch>Highway> Normal field area. *Chryseobacterium* can be found mostly in roots from normal fields and the range is Normal fields>greenhouse/mulch>Factory> Highway areas. For *Pseudomonas*, Highway > Factory > greenhouse/plastic mulch > Normal field area. But for *Arthrobacter*, the range of abundance is Highway > greenhouse/plastic mulch > Normal field > Factory area.



Fig 12: Relative Abundance of Microbial Community Analysis (Genus Level) with Root Endospheric Samples where Root_1, Root_2, Root_3, and Root_4 is Denoted as Root Samples from Factory Area, Highway Area, greenhouse/Mulching Area, and Normal Field Area Respectively

Bug Base Phenotype Prediction of Root Samples

The functional prediction analysis (Bugbase phenotypic prediction) can be used for the initial understanding of the functional characteristics and functions of the microbial community of the samples. BugBase phenotype prediction can determine the high-level phenotypes present in the control and treatment group samples and enable phenotypic prediction. The phenotypic types include Gram Positive, Gram Negative, Biofilm Forming, Pathogenic, Mobile Element Containing, and oxidative stress tolerance (Oxidative Stress Tolerant). In root samples, stress-tolerant bacteria can be found Highway> Normal field > Factory > greenhouse/plastic mulch area.



Fig 13: Relative Abundance of Root Microbial Variations in the Composition of Phenotypes (genus level) with Root Endosphere Samples where Root_1, Root 2, Root 3, and Root 4 are Denoted as Root Samples from Factory Area, Highway Area, Greenhouse/Mulching Area, And Normal Field Area Respectively

• Rhizosphere Soil and Bulk Soil Microbes

This diagram suggests that there is a significant amount of variation in the microbial genera found across the rhizosphere and bulk soil samples. However, there is also a substantial number of shared genes between two or more of the soil samples. The bulk soil samples from near the factory area have 50 unique species, from near the highway it has 61, from near the plastic mulching have 29, and land of normal area with no disturbance rice roots have 24 unique species. The rhizosphere soil samples from near the factory area have 32 unique species, from near the highway it has 29, from near the plastic mulching have 21, and land of normal area with no disturbance rice roots have 22 unique species.mAlso, it can be indicated that 303 microbial species are common to all soils. The bulk soil samples from fields near the highway have more unique species and their range is highway > factory> greenhouse/mulch> Normal areas. While for rhizosphere soil the range is factory> highway > Normal areas> greenhouse/mulch. Moreover, the diagram can reflect that for soil samples of bulk and rhizosphere area, more unique species can be found in highway and factory areas regardless of other areas.



Fig 14: Venn Diagram Focuses on the Rhizosphere Soil and Bulk Soil with Genus Level for Microbes of Unique and Shared Species in Different Groups

Relative Abundance of Microbial Community Analysis

The microbial community structure of rhizosphere soil and bulk soil of different sites is shown in Fig. 12. Thirteen phyla (communities included Chloroflexi, Actinobacteriota, Proteobacteria. Firmicutes. Acidobacteriota, Desulfobacterota. Myxococcota, Cyanobacteria, Bacteroidota, Nitrospirota, Gemmatimonadota, Methylomirabilota, Verrucomicrobiota, etc. as well as a few unspecified communities were identified. Such bacterial communities were present in both soils. Chloroflexi was the dominant phylum in all soil samples and ranks highest in rhizosphere soil of normal fields. Actinobacteriota has the highest abundance in rhizosphere soils of factory area while lowest in bulk soil of factory area. Actinobacteriota has more abundance in rhizosphere soil but less abundance in bulk soils. These findings also align with a study from Tian et al., (2023). In particular, factory areas and greenhouse/mulching areas have the highest amount of microplastic present in bulk and soil respectively. rhizosphere Microplastic addition significantly reduced the relative abundance of Proteobacteria in rhizosphere soils. Proteobacteria has the highest abundance in bulk especially in highway areas. Firmicutes have the highest abundance in rhizosphere soils of all areas, mostly in highway areas but the least abundance in bulk soil samples, and lowest in bulk soil of factory areas. In addition, in factory and highway areas, rhizosphere soil has significantly decreased relative abundance of Acidobacteria.

In greenhouse/plastic mulching areas and normal land areas, it has the lowest abundance but is higher in highway areas. Similarly, Bacteroidota has a higher abundance in samples of normal fields, but quite similar and lowest abundance in factory and highway areas. Other phyla like Nitrospirota, Gemmatimonadota, Methylomirabilota, and Verrucomicrobiota, etc have quite a similar abundance in a smaller amount. All the abundances show that the roots from near factory and highway areas have a higher percentage of the following phyla but less or no abundance of other phylum like Nitrospirota, Desulfobacterota, Verrucomicrobiota, Methylomirabilota, Gemmatimonadota, Patescibacteria etc.

At the genus level, norank f Anaerolineaceae, unclassified f Micrococcaceae, Bacillus. norank f norank o Vicinamibacterales, Fonticella etc can be found. Among these, norank f Anaerolineaceae has lower relative abundance in all other areas but is highest where no disturbance is found (Normal fields). While in rhizosphere soil from near factory and highway areas, unclassified f Micrococcaceae has the highest abundance rather than other areas, but the abundance is decreased in bulk soil of all areas. Bacillus has the highest abundance of rhizosphere soil in highway areas. Fonticella has a higher abundance in rhizosphere soil of all areas and ranks high in normal fields. For bulk soil, Fonticella abundance changed to the opposite. The diversity of the rhizosphere soil microorganisms decreased with the increase of microplastic quantity in areas like factories, and greenhouse/plastic mulching. Bulk soil also has a similar trend but the most decreased trend in greenhouse/plastic mulching areas. This indicates that different amounts and areas of microplastics can produce a certain dose effect on rhizosphere soil as well as bulk soils.



Fig 15: Relative Abundance of Microbial Community Analysis (Phylum Level) with Rhizosphere (R_soil_1, R_Soil_2, R_Soil_3, R_Soil_4) and Bulk Soil (B_Soil_1 B_Soil_2 B_Soil_3 B_Soil_4) Samples where 1, 2, 3, 4 as Soil Samples from Factory Area, Highway Area, Greenhouse/Mulching Area, and Normal Field Area Respectively



Fig 16: Relative Abundance of Microbial Community Analysis (Genus Level) With Rhizosphere (R soil 1, R Soil 2, R Soil 3, R_Soil_4) and Bulk Soil (B Soil 1 B Soil 2 B Soil 3 B Soil 4) Samples where 1, 2, 3, 4 as Soil Samples from Factory Area, Highway Area, Greenhouse/Mulching Area, And Normal Field Area Respectively

https://doi.org/10.38124/ijisrt/IJISRT24MAR2137

ISSN No:-2456-2165

Bug Base Phenotype Prediction of Soil Samples

BugBase phenotype prediction of soil samples shows MPs present have less effect or no change in the phenotype of bacteria. According to Buks and Kaupenjohann, (2020), The responses of soil microbial communities can vary based on the characteristics of the plastic, the microbial groups, and the soil qualities. Though our study had limited data and with field response, it is hard to identify the specific effect of MPs.



Fig 17: Relative Abundance of Variation in Composition of Phenotypes of Microbial Community with Rhizosphere (R soil 1, R_Soil_2, R_Soil 3, R Soil 4) and Bulk Soil (B Soil 1 B Soil 2 B Soil 3 B Soil 4) Samples were 1, 2, 3, 4 as Soil Samples From Factory Area, Highway Area, Greenhouse/Mulching Area, And Normal Field Area Respectively.

G. Impact of MPs on Bacterial Communities: Potential Effects of MP Exposure on the Structure and Diversity of the Microbial Communities

Usually, rice soil has a balanced community with various bacterial groups playing specific roles. They are responsible for breaking down organic matter and releasing nutrients for plants. The effects on diversity seem to depend on the specific MP type and the duration of exposure. The presence of MPs in soil can exert selective pressures on the microbial community, favoring the growth and proliferation of certain bacterial taxa over others. The potential effects of microplastics (MPs) on bacterial communities have a significant impact due to the ubiquitous presence of MPs in various environments. Research indicates that exposure to MPs may lead to changes in bacterial community structure and diversity, which can have profound implications on functions ecosystem and interactions between microorganisms and their environment. The plastic materials differ substantially, and these differences lead to distinct responses of microbial communities. According to some studies, PP had a noticeable promotion effect on microbial αdiversity and all ecological functions. This may be related to the chemical properties of PP, which has a methyl group sidebranched chain and is more prone to aging and degradation to produce low-molecular-weight degradation products. Usually, the degradation period is a fraction or even a tenth of that of

PVC and PE (Zhang et al., 2021). In some cases, MP exposure led to a decrease in microbial diversity, while in others, the diversity remained relatively stable. The presence of MPs, such as low-density polyethylene (LDPE), polystyrene (PS), and polyvinyl chloride (PVC), can significantly alter the relative abundances of different bacterial phyla in soil. (Palansooriya et al., 2023). Exposure to MPs can promote the growth of certain bacterial groups, such as Actinobacteria and Candidatus, Saccharibacteria, while decreasing the relative abundance of others, like Acidobacteria, Microgenomates, and Chloroflexi (Palansooriya et al., 2023). In our study, we also found an abundance of Actinobacteria, especially in rhizosphere soils. The specific effects on the microbial community structure depend on factors like MP type, concentration, and exposure duration. As our study was fieldbased, the significant effect of a specific type or concentration of MP could not be identified.

IV. CONCLUSION

In this study, MP pollution in typical paddy fields in Songjiang, Shanghai is investigated. Our results showed that factory production activities and mulching nearby have great impacts on the distribution of MPs in rice soils. Not only bulk soil is affected by this, but rhizosphere soil is also counting. Usually, plastic film mulching in the seedling process

ISSN No:-2456-2165

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significantly increases the abundance of MPs in paddy fields (Wang et al., 2022). The paddy fields near the factory have the highest abundance of MP while PP/PE copolymer and PS were the dominant type. Bulk soils had most fragments from soils of this area. Another study compared MP abundance in soils with different land uses. They found greenhouse soils (1880 particles/ kg) and soils near greenhouses (1302 particles/kg) to be most polluted with MPs. Additionally, they showed that rice fields (160 particles/kg) contained more MPs than other agricultural soils (81 particles/kg; (Kim et al., 2021). Irrigation water can be another significant plastic source in rice lands. Moreover, we suspect that rhizospheric soils can contain smaller particles from existing MP in soil, or accumulated MP near the root surface can cause MP abundance in rhizosphere soils. The use of PP/PE-coated slow-release fertilizers applied in the fields raises concerns about the environmental impact because they can contribute to microplastic pollution in rice fields (An et al., 2020; Alaswad et al., 2022). According to Wang et al (2021) paddy lands had significantly higher MP abundances than other croplands. If this situation continues it may cause dramatic changes to the nitrogen cycle (Feng et al. 2021) and carbon cycle (Romera-Castillo et al. 2018) in rice fields. Most studies on microplastics have been focused on aquatic ecosystems. The effects of MPs-generated nano plastics on terrestrial ecosystems, especially on farmlands are still unexplored (Duan et al. 2020).

This study examines the abundance and distribution of microplastics (MPs) in rice-growing soils near various areas (factories, highways and mulching). MPs pose a potential threat to soil microbiota (Huang et al. 2020; Zhang et al. 2020) and soil physiochemical properties. MPs in soils act as carriers of other pollutants (Yu et al. 2020) by increasing mobility and reducing the adsorption capacity of natural soils (Huffer et al. 2019). In factory waste, a variety of toxic substances and chemicals may be produced (Li et al. 2011). It has also been proven that MPs can act as vectors to increase various POPs and heavy metals in soil (Xu et al. 2021, Cheng et al. 2020). Therefore, plastic mulch and highway waste can also degrade soil health. The discharge of MPs into the rice fields, intentionally or unintentionally should be strictly controlled. The combined effects of MPs with other pollutants as well as their relationship with the microbial community of soils and crops need to be further studied. MPs can affect the functional potential and activities of soil microbial communities, potentially impacting key ecosystem processes like nutrient cycling and organic matter decomposition. The changes in microbial community structure and diversity driven by MP exposure may have effects on the overall soil ecosystem functions. This further confirmed that the existence of different microplastics aggravated the potential risk to the root zone, rhizosphere soil and bulk soil ecosystem.

Overall, this study has found some important data on MP distributing characteristics and explored some important MP pollution sources in rice-growing areas. Accordingly, these findings it is necessary to provide significant guidance for subsequent MP prevention and management in rice fields.

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