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Ecological Risk Assessment of Microplastics in Sediment and Bioaccumulation in *Melanoides tuberculata* Tissues in the Citarum River Downstream Area, Indonesia

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ABSTRAK

Pencemaran mikroplastik (MP) telah menjadi isu global di lingkungan perairan, khususnya sungai sebagai jalur utama transportasi menuju laut. MP tersusun atas berbagai jenis polimer dengan tingkat toksisitas berbeda sehingga penilaian risiko ekologi diperlukan untuk memahami potensi ancamannya. Penelitian ini mengevaluasi risiko ekologi MP pada sedimen dasar sungai serta akumulasinya pada gastropoda air tawar *Melanoides tuberculata* di hilir Sungai Citarum, lokasi yang rentan menjadi area akumulasi akibat rendahnya kecepatan arus, tingginya sedimentasi, dan meningkatnya masukan polutan dari hulu. Sampel sedimen dianalisis untuk menentukan kelimpahan dan karakteristik MP, sedangkan jenis polimer diidentifikasi menggunakan spektroskopi FTIR sebagai dasar perhitungan *Polymer Hazard Index* (PHI), *Pollution Load Index* (PLI), dan *Potential Ecological Risk Index* (PERI). Hasil penelitian menunjukkan kontaminasi MP pada sedimen tergolong tinggi dengan kelimpahan rata-rata 63,37 ± 33,06 partikel/100 g berat kering, sedangkan konsentrasi dan risiko tertinggi ditemukan di lokasi L3 (Tunggakjati). Polimer yang teridentifikasi meliputi polipropilena, polietilena, dan poliester. Nilai PHI menunjukkan kategori risiko tinggi hingga sangat tinggi, PLI mengindikasikan beban pencemaran signifikan, dan PERI menunjukkan risiko ekologi tinggi hingga sangat berbahaya. Pada jaringan *M. tuberculata*, rata-rata konsentrasi MP mencapai 3,47 ± 1,75 partikel/g bobot basah atau 0,738 ± 0,14 partikel per individu. Meskipun berada di hilir bendungan, kawasan ini tetap menjadi hotspot MP akibat pertemuan sungai yang mengonsentrasikan polutan dari hulu dan mempercepat deposisi MP pada sedimen maupun biota. Penelitian ini merupakan penilaian risiko ekologi terintegrasi pertama yang mengombinasikan data kontaminasi sedimen dan bioakumulasi gastropoda air tawar di hilir Sungai Citarum sebagai dasar strategi pengelolaan pencemaran.

Kata kunci: Citarum, *Melanoides tuberculata*, Mikroplastik, Penilaian Risiko Ekologi, Sedimen

ABSTRACT

Microplastic (MP) pollution has become a global concern in aquatic environments, particularly in rivers that serve as major pathways transporting pollutants to the ocean. As MPs consist of various polymer types with different toxicity levels, ecological risk assessment is essential to evaluate their potential impacts. This study assessed the ecological risk of MPs in riverbed sediments and their accumulation in the freshwater gastropod *Melanoides tuberculata* in the downstream Citarum River, an area prone to MP accumulation due to low flow velocity, high sedimentation rates, and increasing pollutant inputs from upstream. Sediment samples were analyzed to determine MP abundance and characteristics, while polymer types were identified using Fourier Transform Infrared (FTIR) spectroscopy. Ecological risk was evaluated using the Polymer Hazard Index (PHI), Pollution Load Index (PLI), and Potential Ecological Risk Index (PERI). The results showed high MP contamination in sediments, with a mean abundance of 63.37 ± 33.06 particles/100 g dry weight. The highest MP concentration and ecological risk were observed at site L3 (Tunggakjati). Identified polymers included polypropylene, polyethylene, and polyester. PHI indicated high to very high ecological risk, PLI revealed significant pollution loads, and PERI classified the sites as having high to very dangerous ecological risk. In *M. tuberculata*, the mean MP concentration was 3.47 ± 1.75 particles/g wet weight or 0.738 ± 0.14 particles per individual. Despite being located downstream of a dam, this section of the Citarum River remains an MP hotspot due to river confluences that concentrate upstream pollutants and enhance MP deposition in sediments and aquatic organisms. This study provides the first integrated ecological risk assessment combining sediment contamination and gastropod bioaccumulation in the downstream Citarum River, offering a scientific basis for microplastic pollution management.

Keywords: Citarum, Ecological Risk Assessment, *Melanoides tuberculata*, Microplastics, Sediment

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1. INTRODUCTION

The United Nations Environment Assembly (UNEA) has adopted a resolution to establish a legally binding international treaty aimed at eliminating plastic pollution, with implementation scheduled for 2025 (Arora et al., 2024). According to the United Nations Environment Programme (UNEP), Indonesia ranks as the second-largest producer of marine plastic debris, following China, with an estimated 0.48 to 1.29 million tons annually (Jambeck et al., 2015). A significant portion of this plastic waste originates from inland activities and is transported through river systems before reaching the marine environment. Rivers play a crucial role as major pathways for microplastics (MPs) from land-based sources to the oceans while also acting as reservoirs (Akdogan et al., 2023; Bratovcic et al., 2022; Rohais et al., 2024; Siegfried, 2017). The Citarum River, the largest river in West Java, provides water resources for 25 million people in the DKI Jakarta region (Kardhana et al., 2024; Prawirakusuma, 2023) and serves as a critical water source for hydroelectric power in the Java-Bali region (Sembiring et al., 2020). Moreover, the river also serves a population of 18.64 million people and is home to 2,822 industries that discharge wastewater, significantly contributing to its pollution (Sembiring et al., 2020). Several studies have reported that the Citarum River releases both MPs and macroplastics into the sea, discharging over 1,000 kg of plastic waste per hour (Cordova and Nurhati, 2019; Hariyadi et al., 2022).

MPs are easily transported by river currents and tend to accumulate in downstream regions, which often act as repositories for various contaminants (Ghinassi et al., 2023; Kumar et al., 2021). Studies on the Cisadane (Sulistyowati et al., 2022) and Ciliwung (Mahendra et al., 2023) rivers have shown that MP abundance increases as the flow moves downstream. Given that MPs in sediments exhibit varying levels of toxicity to aquatic ecosystems (Lithner et al., 2011), assessing their ecological risks becomes essential (Pan et al., 2021; Peng et al., 2018; Ranjani et al., 2021). Despite the significant pollution levels, ecological risk assessments of MPs in the downstream Citarum River remain limited. Therefore, this study represents the first attempt to investigate MP contamination and assess its potential ecological risks in sediment ecosystems of the downstream Citarum River.

MPs exhibit high bioavailability due to their small size, which allows them to enter organisms across various trophic levels (Carbery et al., 2018; Nelms et al., 2018), including zooplankton (Zheng et al., 2020), polychaetes, mollusks, and crustaceans (F. Wang et al., 2021), gastropods (Curren et al., 2024), insect larvae (Malafaia et al., 2020), and fish (Sembiring et al., 2020). Studies on MP occurrence in organisms have primarily focused on species occupying higher trophic levels, such as fish (Firdousie et al., 2021). However, research on MP contamination in organisms at lower trophic levels remains limited. Studies by Jitkaew et al. (2023) and Curren et al. (2024) demonstrated that gastropods can serve as effective bioindicators of anthropogenic pollution. As deposit feeders that consume fine particulate organic matter (FPOM), gastropods can accumulate contaminants such as MPs (Harahap et al., 2022). Understanding MP uptake by freshwater gastropods is essential for evaluating MP transfer through trophic levels in aquatic ecosystems. *M. tuberculata*, a dominant gastropod species in the Citarum River, is widely distributed and highly resilient to environmental stressors (Ho et al., 2019; Schweizer et al., 2019).

This study evaluates the ecological risks of MP contamination in the downstream area of the Citarum River and its bioaccumulation in *M. tuberculata*, a key bioindicator of aquatic pollution. Special attention is given to river confluence areas, where altered flow dynamics promote pollutant accumulation (Haque et al., 2024; C. Li et al., 2023). As the first comprehensive investigation integrating MP contamination in river sediments and its accumulation in biota within this area, this research provides critical information regarding the ecological risks posed by MPs. The findings will provide valuable guidance for policymakers in developing effective strategies to improve plastic waste management. We hypothesize that MP abundance and ecological risks are highest at river confluence sites due to combined pollutant loading from upstream industrial tributaries, and that M. tuberculata effectively bioaccumulates MPs in proportion to sediment contamination levels.

2. METHODOLOGY

2.1. Study Area and Sampling Time

The Citarum River, the longest and largest river in West Java, Indonesia, covers an area of 6,614 km^2 and serves a population of 18.64 million along its course (Sembiring et al., 2020). The study area spans a 51 km stretch, divided into four sampling locations (**Figure 1**), with site coordinates and characteristics outlined in **Table 1**. Focusing on the Citarum River downstream area, a lowland region heavily influenced by industrial, domestic (Laili and Sofyan, 2017), and agricultural activities (Santikayasa et al., 2014), which contribute to pollution through waste generation. Locations 1 and 2 (L1 and L2), located in Karawang Regency, are affected mainly by residential and agricultural activities. L1 is located downstream of the Curug Klari Dam outlet, whereas L2 is located downstream of the Walahar Dam outlet. L3 and L4 are located downstream of the confluence with the Cibeet River, where contamination may occur due to agricultural and industrial pollutants transported from nearby industrial zones through the Cibeet River. The final location (L4) is approximately 40 km upstream of the Citarum River estuary and beyond the reach of tidal influences. Sampling was conducted in May to September of 2023 with three replicates collected at each sampling station.

2.2. Sample Collection and MPs Extraction

Sediment samples were collected from riverbanks and midstream areas, with 1 kg of sediment obtained from each location. The samples were stored in aluminum containers and sealed to prevent contamination. MP analysis followed the methodologies described by Babel et al. (2022) and Li et al. (2021), which included drying, weighing, density separation, sieving, digestion, and microscopic observation. A total of 12 sediment samples were collected (4 locations × 3 replicates), representing a 51 km stretch of the downstream Citarum River to ensure spatial representativeness. Sediment samples were dried at 100 °C for 48 hours, and 100 g of each sample was subjected to density separation using NaCl solution (1.2 g/cm^3). After settling for 2 hours, samples were sieved into different size fractions (0.05–0.3 mm, 0.3–0.5 mm, 0.5–1.0 mm, and 1.0–5.0 mm). Organic matter in the <0.5 mm fraction was digested using 30% hydrogen peroxide for 24 hours, followed by filtration and microscopic observation for MP identification. *Melanoides tuberculata* samples were collected using a Surber net (25 × 40 cm^2) with four replicates per location and preserved in 70% ethanol. MP analysis in gastropod tissues involved sample preparation, organic matter digestion using Wet Peroxide Oxidation (WPO), density separation, and microscopic observation. Tissue samples were separated, dried at 105 °C for 48 hours, ground, and digested with 0.05 M Fe(II) solution and H_2O_2 at 75 °C. The digested samples were subsequently filtered and observed microscopically for MPs. The minimum detectable MP size was 0.05 mm, corresponding to the smallest sieve mesh size used in this study. Procedural blanks were processed alongside all samples, and particles detected in the blanks were subtracted from the final sample counts.

2.3. Polymer Identification

The polymer types of MPs in sediment samples were identified using Fourier Transform Infrared (FTIR) spectroscopy, which enables the identification of functional chemical groups within MPs. Polymer composition analysis was performed using Attenuated Total Reflectance (ATR)-FTIR spectroscopy within a wavenumber range of 4,000 to 500 cm^{-1} (Peng et al., 2018). These polymer identification results provided the basis for assessing the potential ecological risks associated with MP contamination.

2.4. Quality Control and Assurance

Strict contamination prevention protocols were implemented to ensure data reliability, following the guidelines of Bogdanowicz et al. (2021) and Prata et al. (2021). Non-plastic tools were used during sampling, with sediment samples collected using shovels and stored in aluminum-lined containers to prevent contamination. Glass-based equipment was used in the laboratory, with all instruments thoroughly cleaned before use. Containers were covered to prevent airborne contamination, and workspaces were sterilized to minimize external contamination.

Table 1. Sampling Location Descriptions. Characteristics of Sampling Locations along the Lower Citarum River, Including Coordinates, Depth, Width, Current Flow, Substrate Composition, and Notable Surrounding Environmental Features

Location	Coordinates	Characteristics				
		Depth (m)	Width (m)	Current Flow (m/s)	Substrate	Other
Location 1 (Curug)	6°26'21.98"S 107°22'42.14"E	6	68.4	0.33	Clay (58%), mud (41%)	• Rural area with residential houses near the river • Approximately 0.85 km from the outlet of Curug Klari Dam • Agricultural along the riverbanks • Dense aquatic vegetation along the riverbanks
Location 2 (Walahar)	6°21'29.22"S 107°19'58.33"E	3	53.7	0.25	Mud (40%), clay (27.5%), sand (22.5%)	• Rural area • Approximately 6.5 km from the outlet of Walahar Dam • Scattered riparian vegetation • Agricultural along the riverbanks
Location 3 (Tunggak-jati)	6°14'47.11"S 107°16'31.01"E	4.5	43.1	0.38	Mud (54.8%), clay (34.3%)	• Rural area with sparse settlements • Approximately 5 km from the confluence with Cibeet River • Agricultural areas and rice fields along the riverbanks • Sparse riparian vegetation • River crossing activities using small traditional wooden boats
Location 4 (Pebayuran)	6°13'3.04"S 107°17'21.59"E	6	39.9	0.27	Clay (47%), mud (46%)	• Rural area with sparse settlements • Approximately 12.6 km from the confluence with Cibeet River • Agricultural areas and rice fields along the riverbanks • Sparse riparian vegetation • River crossing activities using small traditional wooden boats

During microscopic analysis, samples were exposed only when necessary, and pre-filtered solutions were stored in sealed containers to prevent contamination. To further reduce contamination risks, non-white materials were used for laboratory coats and containers (Baalkhuyur et al., 2018). Procedural blanks and distilled water blanks were processed to detect potential contamination, and any identified contaminants were subtracted from the final MP counts. Particles resembling those found in control samples (primarily white cotton fibers) were excluded from the analysis.

2.5. Risk Assessment

To evaluate the potential ecological risks of MPs in sediments, both concentration and chemical composition were considered critical factors (Lithner et al., 2011; Xu et al., 2018). Polymer toxicity assessment was conducted for different MP polymer types using the Polymer Hazard Index (PHI) method proposed by Lithner et al. (2011). This assessment incorporated the relative abundance of each MP polymer in the total sample (Pn) and the corresponding polymer hazard score (Sn). The overall hazard of MP polymers was calculated using the following equation:

$$PHI = \sum_{n=1}^{n} P_n \times S_n \quad (1)$$

The Pollution Load Index (PLI) was used to assess MP pollution levels in sediments. The PLI calculation formula was introduced by Tomlinson et al. (1980).

$$CF_i = \frac{C_i}{C_{oi}} \quad (2)$$

$$PLI = \sqrt{CF_i} \quad (3)$$

The PLI value for each location was associated with the MP concentration factor (CFi) (Ranjani et al., 2021), where CFi represents the ratio between MP concentration at each site (Ci) and the background concentration (Coi).

The Potential Ecological Risk Index (PERI) was applied to evaluate the potential environmental impacts associated with MP contamination in sediments. This index considers both MP contamination levels and polymer toxicity. PERI was calculated using the formula proposed by Hakanson (1980), as follows:

$$C_f^i = \frac{C^i}{C_n^i} \quad (4)$$

$$T_r^i = \sum_{n=1}^{n} \frac{P_n}{C^i} \times S_n \quad (5)$$

$$E_r^i = T_r^i \times C_f^i \quad (6)$$

CFi represents the ratio between MP concentration at each location (Ci) and the background concentration (Cin). The toxicity coefficient (Tir) indicates polymer toxicity and biological sensitivity (Ranjani et al., 2021). The Tir value was calculated by multiplying the relative abundance of MP polymers (Pn/Ci) by the corresponding polymer hazard score (Sn). The hazard scores were derived from the polymer hazard classification proposed by Lithner et al. (2011), as presented in **Table 2**. Cn denotes background concentration.

Table 2. Hazard Score of Polymers (Lithner et al., 2011)

Polymer	Abbreviations	Shape	Score
Polyethylene	PE	Fragment, Pellet	11
Polyamide	PA	Fiber	47
Polyethelene terephthalate	PET	Fragment	4
Polyester	PES	Fiber	4
Polypropylene	PP	Film, Fragment	15
Polystyrene	PS	Foam	30

These three indices are interrelated and complementary in assessing the ecological risks of MP contamination in sediments. The Polymer Hazard Index (PHI) evaluates polymer-specific toxicity based on chemical composition, whereas the Pollution Load Index (PLI) provides a quantitative assessment of MP contamination levels relative to background concentrations. Meanwhile, the Potential Ecological Risk Index (PERI) integrates polymer toxicity and contamination levels to provide a comprehensive evaluation of ecological risks associated with MPs in sediments. Together, these indices provide a holistic assessment of MP pollution by considering pollutant load, toxicity, and potential ecological impacts (Ranjani et al., 2021).

The background concentration used in PLI and PERI calculations served as a reference value representing baseline MP contamination levels (Veerasingam et al., 2021). In this study, the background concentration was set at 0.57 MP particles per 100 g, as reported by Zhou et al. (2021) from the Fuhe River estuary, China. This reference was selected due to the absence of standardized background concentrations for Indonesian freshwater systems and has been previously applied in regional MP risk assessment studies (Ilmi et al., 2023; Pan et al., 2021). Nevertheless, future studies are recommended to establish Indonesia-specific background concentrations to improve the accuracy of ecological risk assessments in freshwater environments.

The results from PHI, PLI, and PERI calculations were categorized according to MP contamination criteria defined by Ranjani et al. (2021)(**Table 3**).

2.6. Data Analysis

Statistical analyses were conducted using PAST version 4.03 software. Normality (Shapiro–Wilk) and homogeneity tests were performed prior to statistical analyses. Differences in MP abundance among sampling locations were evaluated using one-way ANOVA followed by Tukey's HSD post hoc test for pairwise comparisons (Wakhid et al., 2020).

Hierarchical Cluster Analysis (HCA) was performed using the UPGMA method based on the Bray–Curtis similarity index to evaluate similarities in MP characteristics among sampling sites (Bray and Curtis, 1957).

Table 3. Ecological Risk Criteria based on PHI, PLI, and PERI Values According to (Ranjani et al., 2021)

PHI	Hazard Category	PLI	Hazard Category	PERI	Risk Category
0-1	I	<10	I	<150	Minor
1-10	II	-	-	150-300	Medium
10-100	III	10-20	II	300-600	High
100-1000	IV	20-30	III	600-1220	Danger
>1000	V	>30	IV	>1200	Extreme Danger

3. RESULTS AND DISCUSSION

3.1. Spatial Distribution of Microplastics in Sediment Samples

The analysis of MPs in sediment samples revealed an average concentration of 63.37 ± 33.06 particles per 100 g of dry sediment. Significant differences in MP abundance were observed among sampling locations ($p < 0.05$). Site L3 exhibited the highest concentration, with an average of 104 ± 7.3 particles/100 g sediment, followed by L4 (76.5 ± 10.3 particles/100 g), L2 (39 ± 5.7 particles/100 g), and L1 (34 ± 7 particles/100 g) (**Figure 1**). These concentrations were relatively high compared to those reported in other areas of the Citarum River. The Upper Citarum contained 34 ± 5.3 particles/100 g (Adrian & Roosmini, 2024), the Midstream Citarum contained 22.92 ± 19.94 particles/100 g (Ilmi et al., 2023), and the Citarum River Estuary recorded 16.66 ± 0.57 particles/100 g (Sembiring et al., 2020). These findings are consistent with previous studies (Gabriel & Bacosa, 2024; Ismiyati et al., 2023; Rakib et al., 2022; Sulistyowati et al., 2022), which reported increased MP abundance in downstream regions.

A post-hoc Tukey HSD test showed significant differences ($p < 0.05$) between L1–L2 and L3–L4, separating the sampling locations into two distinct groups. These spatial variations were mainly influenced by differences in land use and additional pollution inputs from river confluences. L1 and L2 were primarily influenced by domestic and agricultural activities, whereas L3 and L4 received additional input from agricultural and industrial pollutants transported through the Cibeet River. River confluences, where complex hydrodynamic interactions occur, can act as hotspots for MP accumulation because changes in flow conditions may enhance particle retention and sediment deposition, a process further influenced by hyporheic exchange (Drummond et al., 2022; Haque et al., 2024; C. Li et al., 2023). Several studies (Dahms et al., 2020; Dikareva & Simon, 2019; Nel et al., 2018) have also confirmed the important role of tributaries in MP transport and distribution within larger river systems. However, Sembiring et al. (2020) reported a decrease in MP concentration in the estuarine area of the Citarum River. Although river transport generally facilitates downstream MP accumulation (Kumar et al., 2021), local pollution sources and environmental conditions must also be considered. The downstream-estuary region of the Citarum River is predominantly rural, consisting mainly of agricultural areas and plantations, in contrast to the more urbanized upstream and midstream regions. Therefore, fewer local MP inputs in these areas may contribute to the reduction in MP concentrations toward the river mouth.

The relationship among substrate characteristics, hydrological conditions, and MP concentration further explains the observed spatial distribution. Substrate composition data (**Table 1**) indicate that sites with higher mud content, particularly L3 (54.8%) and L4 (46%), exhibited elevated MP concentrations, consistent with the tendency of fine-grained sediments to retain MP particles through physical entrapment and particle interactions (Drummond et al., 2022; Kumar et al., 2021). In contrast, L1 and L2 showed lower MP concentrations, which may reflect differences in hydrodynamic conditions downstream of dam outlets and sediment characteristics. Regarding hydrological parameters, river width decreased progressively from L1 (68.4 m) to L4 (39.9 m), potentially affecting flow patterns and promoting particle retention in localized depositional zones. Notably, L3 exhibited the highest current velocity (0.38 m/s) despite having the highest MP concentration, suggesting that MP accumulation at this location was primarily controlled by continuous pollutant inputs from the Cibeet River confluence rather than flow conditions alone. The combination of tributary inputs, localized hydrodynamic conditions, and fine muddy substrates creates a favorable depositional environment for MP accumulation at L3, supporting observations from other confluence-dominated MP hotspots (Haque et al., 2024; C. Li et al., 2023).

3.2. Sizes, Shapes and Colors of Microplastics in Sediment Samples

During transport in the river, MPs undergo natural processes such as aggregation, degradation, flocculation, and biofouling (Kumar et al., 2021; G. Wang et al., 2021), which gradually reduce their size as they move downstream (Siegel et al., 2021). MP observations in sediments across all study locations revealed a predominance of smaller particles, particularly within the 0.05–0.3 mm size range, accounting for 57% of the total MPs. In contrast, particles within the 0.3–0.5 mm, 0.5–1 mm, and 1–5 mm size ranges accounted for approximately 20%, 11%, and 12%, respectively. Studies by Firdaus et al. (2020), Haque et al. (2024), and Wicaksono et al. (2021) have also reported the dominance of smaller

MPs in downstream areas, primarily driven by physical and chemical degradation during transport. Smaller MPs may settle more readily due to biofouling under eutrophic conditions, which alters their buoyancy (Long et al., 2015; Z. Wang et al., 2018). Furthermore, as MPs size decreases, their bioavailability increases (Carbery et al., 2018), making them more easily ingested by aquatic organisms and facilitating their transfer through food webs, thereby posing greater ecological risks (Cole et al., 2013; Triebskorn et al., 2019).

MP observations in the sediment of the downstream Citarum River revealed various types, including fibers (70%), fragments (17%), films (12%), and pellets (1%) **(Figure 3).** The dominance of fibers is consistent with previous studies reporting fibers as the most prevalent MP type in both upstream and downstream areas of the Citarum River (Alam et al., 2019; Sembiring et al., 2020). Fibers primarily originate from the laundering of synthetic textiles and textile industry effluents (Bui et al., 2020; Dris et al., 2015; Napper & Thompson, 2016; Yin et al., 2019). Their prevalence was likely influenced by residential settlements near the river, as observed at L1 (Curug) and L2 (Walahar). Fragments result from the degradation of larger plastic items, such as bottles and packaging materials (Horton et al., 2017; Susanti et al., 2020), and may have been introduced through household waste or agricultural packaging, which are common sources across locations surrounded by agricultural areas.

Films are formed from the breakdown of thin plastic sheets commonly used in packaging (Ta & Babel, 2020). Their occurrence was likely associated with agricultural activities and rice fields, particularly at L2, L3, and L4. Pellets, or nurdles, are primary MPs produced as raw materials in plastic manufacturing (Susanti et al., 2020). Although rarely detected in this study, pellets may originate from upstream transport or incidental releases associated with human activities around the river.

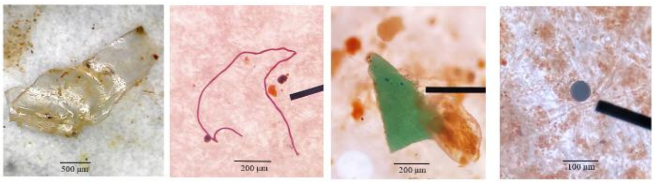

Figure 3. Examples of MPs Shapes Collected in the Midstream and Downstream of Citarum River (from left to right: film, fiber, fragment and pellet)

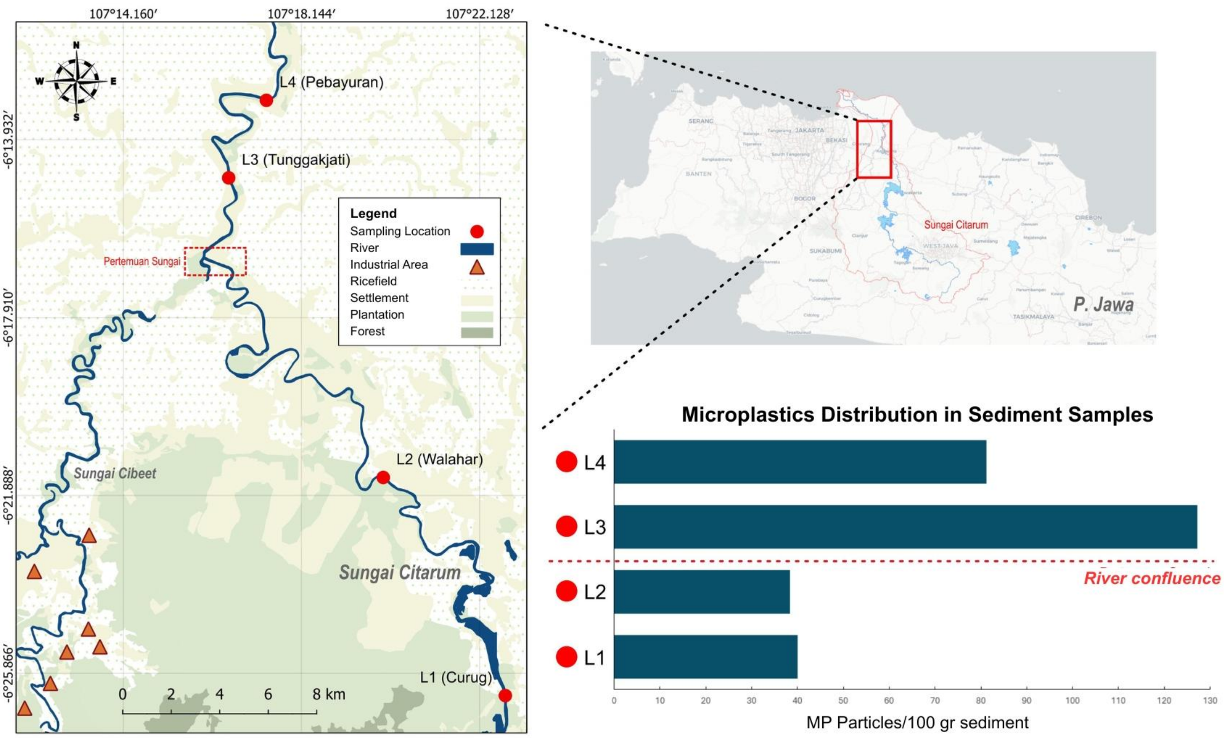

Figure 1. Occurrence of MPs Detected in the Sampling Locations in The Downstream of Citarum River, West Java, Indonesia

Note: Map on the left shows the sampling locations, while the graph displays the abundance of MP particles (items/100 g) found in sediments at each station.

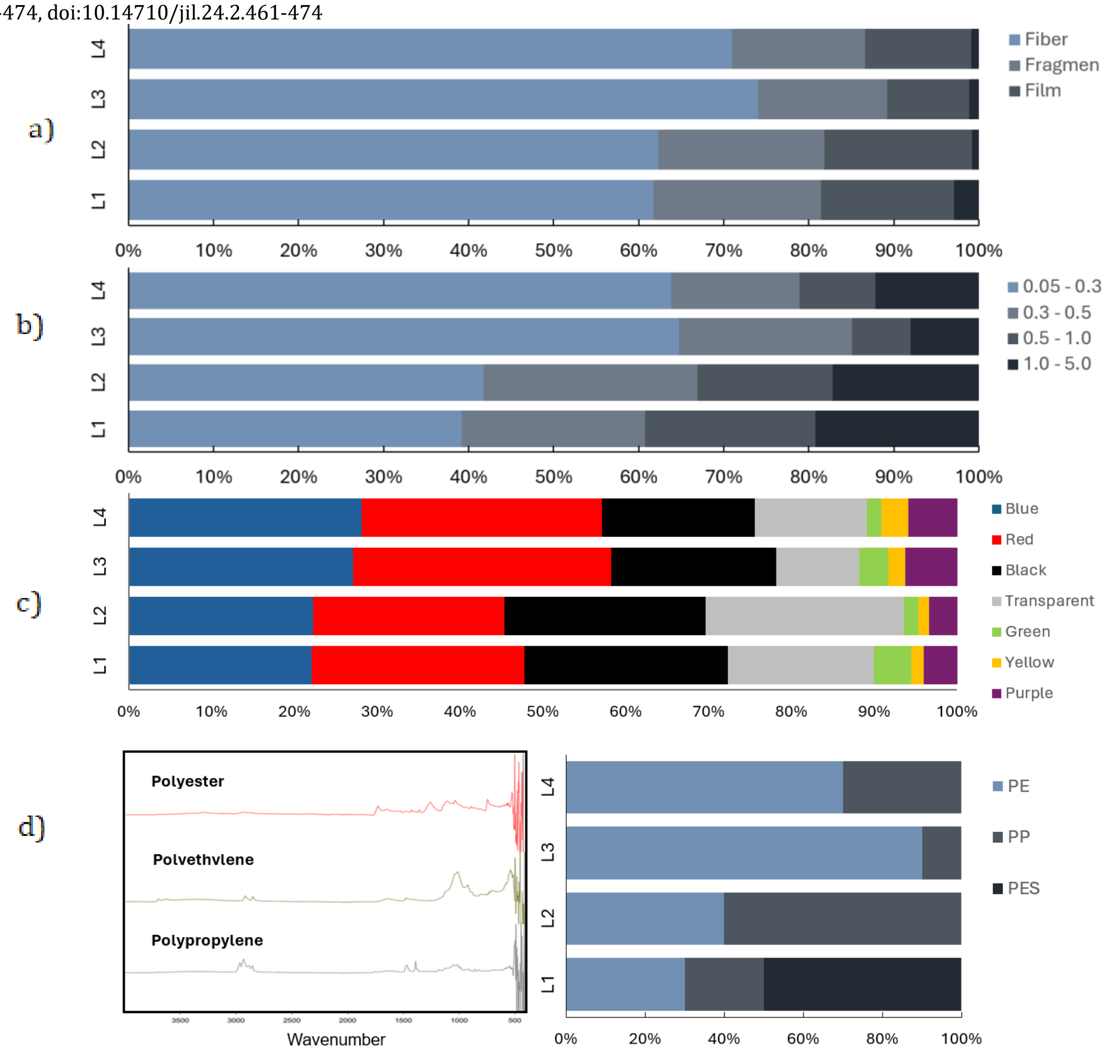

Figure 2. Distribution of MP Characteristics Detected at Each Sampling Locations, Showing Similarity using Hierarchical Cluster Analysis (HCA) with Unweighted Pair Group Method with Arithmetic Mean (UPGMA) for (a) Shapes, (b) Sizes, (c) Colors, and (d) Polymers

Seven distinct MP color groups were identified in this study: red, blue, yellow, green, purple, black, and transparent (**Figure 2**). Among these colors, red (30%) and blue (27%) MPs were dominant in sediment samples from the downstream Citarum River. These findings are consistent with previous studies. Drabinski et al. (2023) reported the dominance of blue and black MPs, while Yang et al. (2021) observed blue, red, and black MPs in rivers on the Northern Slope of the Himalayas, China. The observed colors of MPs in this study may not represent their original colors, as physical and chemical degradation processes can alter their appearance (Laksono et al., 2021). Understanding the color distribution of MPs is crucial for assessing their interactions with aquatic organisms (Wright et al., 2013). Visual characteristics such as color can influence ingestion because aquatic organisms may mistake brightly colored MPs for food particles. Previous studies have shown that exposure to MPs with different physical characteristics, including color and polymer type, may induce physiological and behavioral responses. For example, *Oryzias latipes* and *O. javanicus* exhibited bioaccumulation of MPs in tissues and alterations in gene expression related to cell adhesion, xenobiotic metabolism, and brain development (Assas et al., 2020). Similarly, *Sparus aurata* exposed to low-density polyethylene (LDPE) MPs showed changes in antioxidant enzyme activity, increased lipid peroxidation, and behavioral alterations, including impaired feeding and social interactions (Rios-Fuster et al., 2021). These findings highlight the importance of considering MP color and physical characteristics when evaluating ecological risks.

3.3. Polymers Identification

FTIR spectroscopy analysis of forty suspected MP particles confirmed their polymeric composition and identified several polymer types in downstream Citarum River sediments, including polypropylene (PP), polyethylene (PE), and polyester (PES) (**Figure 4**). Among these polymers, PE and PP were the most prevalent types across all sampling locations (Figure 2). These findings are consistent with previous studies reporting PE and PP as dominant polymers in riverine environments (Chen et al., 2022; Ilmi et al., 2023; Koelmans et al., 2019; Lestari et al., 2020; Ni'am et al., 2022; Pan et al., 2020; Wicaksono et al., 2021). As the most widely produced polymers globally, PP and PE are more likely to enter aquatic environments through various pathways (Wicaksono et al., 2021). PP is commonly used in products such as trays, funnels, and bottles (Maddah, 2016), while PE is typically utilized for packaging, including shopping bags, plastic bottles, and films (Romani et al., 2020). PES, detected only at site 1, aligns with findings near textile industries, as seen in Ciwalengke river (Alam et al., 2019) and Saigon River (Lahens et al., 2018). Different polymers exhibit varying levels of toxicity, with additives such as those for flexibility, UV protection, and flame retardancy, can leach and cause toxic effects (Lithner et al., 2011). Understanding polymer composition is crucial for effective environmental risk assessment, which is further detailed in the following section.

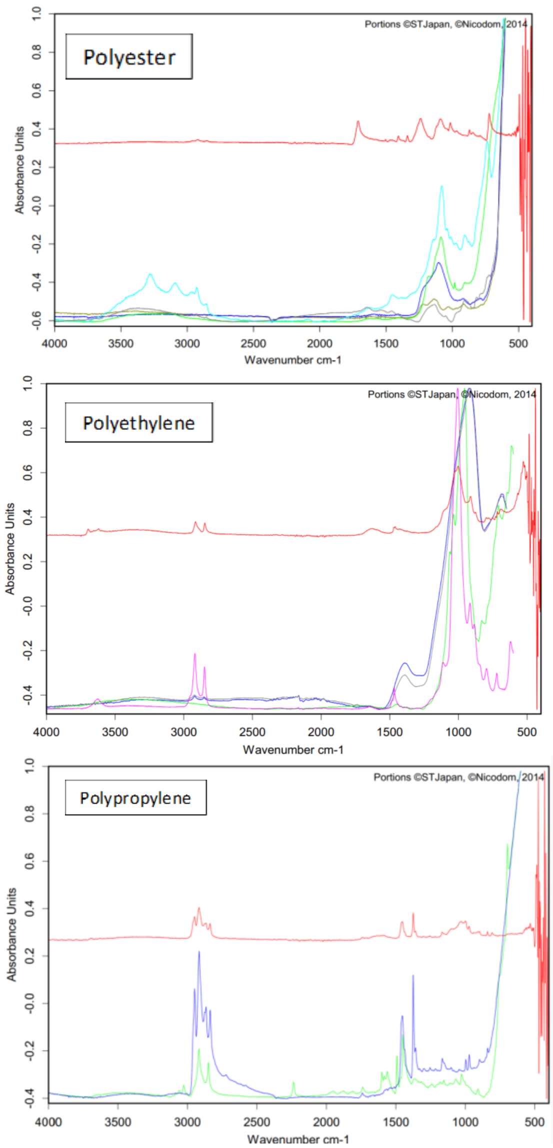

Figure 4. Types of MP Polymers Found in the Downstream Area of Citarum River, Identified using FTIR spectra. The red Spectra Represent the FTIR Readings from the Analyzed MP Samples, While Other Colors Correspond to Reference Spectra Obtained from Spectral Libraries for Comparison

3.4. Microplastics in Freshwater Gastropod *M. tuberculata* Tissues

MPs can be ingested by freshwater invertebrates, including gastropods, potentially causing toxicological effects (Pan et al., 2021; Weber et al., 2021). MP analysis in M. tuberculata tissues revealed an average concentration of 3.47 ± 1.75 particles/g wet weight or 0.738 ± 0.14 particles/individual. These findings align with Akindele et al. (2020), which reported MP concentrations of 4.57 ± 1.07 particles/g and 1.7 ± 0.42 particles/individual in *M. tuberculata* tissues. The results of this study are higher than those reported by Zaki et al. (2021), which ranged from 0.5 to 1.75 particles/g (wet weight) or 0.25 to 0.88 particles/individual, but lower than those observed by Xu et al. (2020), which ranged from 0.025 to 9.684 particles/g or 0.08 to 18.4 particles/individual. The elevated MP concentration in *M. tuberculata* tissue observed in this study is strongly influenced by the presence of a dam. The dam reduces water flow, creating a depositional environment that facilitates microplastic accumulation in sediments, increases exposure to benthic organisms, and the feeding behavior of *M. tuberculata* as a sediment-ingesting species. These environmental and biological factors potentially increase MP ingestion and retention in tissue.

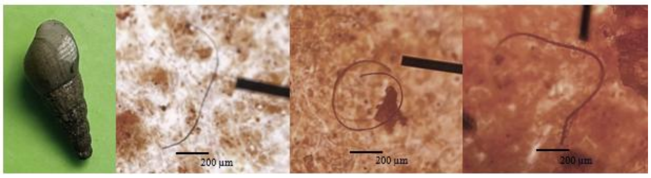

Figure 5. Detection of MPs Within the Tissue of the Freshwater Gastropod *M. tuberculata* Samples

The MP forms found in all *M. tuberculata* samples were predominantly fibers, accounting for 55 particles (96.5%) identified (**Figure 5**). The dominance of fibers is consistent with findings from previous studies on gastropods (Curren et al., 2024; Doyle et al., 2020; Jitkaew et al., 2023; Zaki et al., 2021). Thin, elongated fibers are more easily ingested by gastropods through their radula (Curren et al., 2024), suggesting they have higher bioavailability compared to other forms. Consequently, MP accumulation can interfere with feeding processes and potentially affect energy intake. Ehlers et al. (2019) found that MP fibers (50 mg/L) significantly reduced feeding capacity and led to high mortality in *Planorbella campanulata*. Additionally, MPs can adsorb toxic chemicals like heavy metals and persistent organic pollutants (POPs) (e.g., PCBs and dioxins), amplifying environmental and biological risks (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2016; Engler, 2012; Enyoh et al., 2020; Franzellitti et al., 2019; Holmes et al., 2012; Naji et al., 2018).

3.5. Risk Assessment

The ecological risk assessment of MP contamination in sediments was conducted using the Pollution Load Index (PLI), Polymer Hazard Index (PHI), and Potential Ecological Risk Index (PERI). The average PLI in this study was 10.3, higher than the midstream Citarum of 6.31 (Ilmi et al., 2023) but lower than other regions, such as the Changjiang Estuary in China (18.4) (P. Xu et al., 2018) and Dongshan Bay (14.2) (Pan et al., 2021). The elevated MP abundance likely reflects the impact of urbanization, agriculture, and industrial activities along the downstream Citarum River, as human activity often drives local MP accumulation (Lechner et al., 2014).

Variations in polymer composition do not directly influence PLI values because this index is primarily based on MP abundance relative to background concentrations. Ecological risk assessment, however, considers both abundance and toxicity, with toxicity evaluated using the PHI. PHI was determined using hazard scores for different MP types (**Table 3**), classified according to their potential risks to the environment and human health (Lithner et al., 2011). PHI categorizes ecological risk based on proportions of MP polymers found along the sampling stations. PHI emphasizes that certain polymers, despite lower concentrations, may have high hazard scores (Gao et al., 2024). PHI values in this study ranged from 258 to 1,144, indicating heavy contamination (categories IV–V) (**Figure 6**). L1, characterized by a high proportion of PES (50%), which has a relatively low hazard score (4), exhibited the lowest PHI value, while other sites had higher PHI values due to the presence of PE and PP, particularly PP, with a hazard score of 15.

PERI categorization revealed ecological risk levels ranging from category II (high) to category IV (very dangerous), with an increasing trend downstream. L3 recorded the highest PERI value (6,362, extreme danger), while L1 had the lowest (453, high) (**Figure 6**). PERI values reflect MP contamination levels based on both concentration and polymer toxicity (Pan et al., 2021; Peng et al., 2018; Ranjani et al., 2021). The elevated findings in this study are likely due to the combined effects of increased pollution load (PLI) and high polymer hazard levels (PHI), both contributing to a higher potential ecological risk index (PERI) (Ranjani *et al.*, 2021). High contamination levels (PLI) and polymer-associated hazards (PHI) at L3 resulted in a high PERI score, whereas lower concentration and toxicity levels at site 1 corresponded to the lowest PERI score. The average PERI value in this study (2,887.75) was significantly higher than previous Citarum River studies, such as at the outlets of the Saguling, Cirata, and Jatiluhur dams (39.2) (Ilmi et al., 2023). Comparatively, this result was also higher than studies from the Bay of Bengal, Bangladesh (Low) (Al Nahian et al., 2022), Japan (Low-High) (Kabir et al., 2022), the Changjiang Estuary, China (Medium-High) (P. Xu et al., 2018), and Dongshan Bay, India (Ranjani et al., 2021).

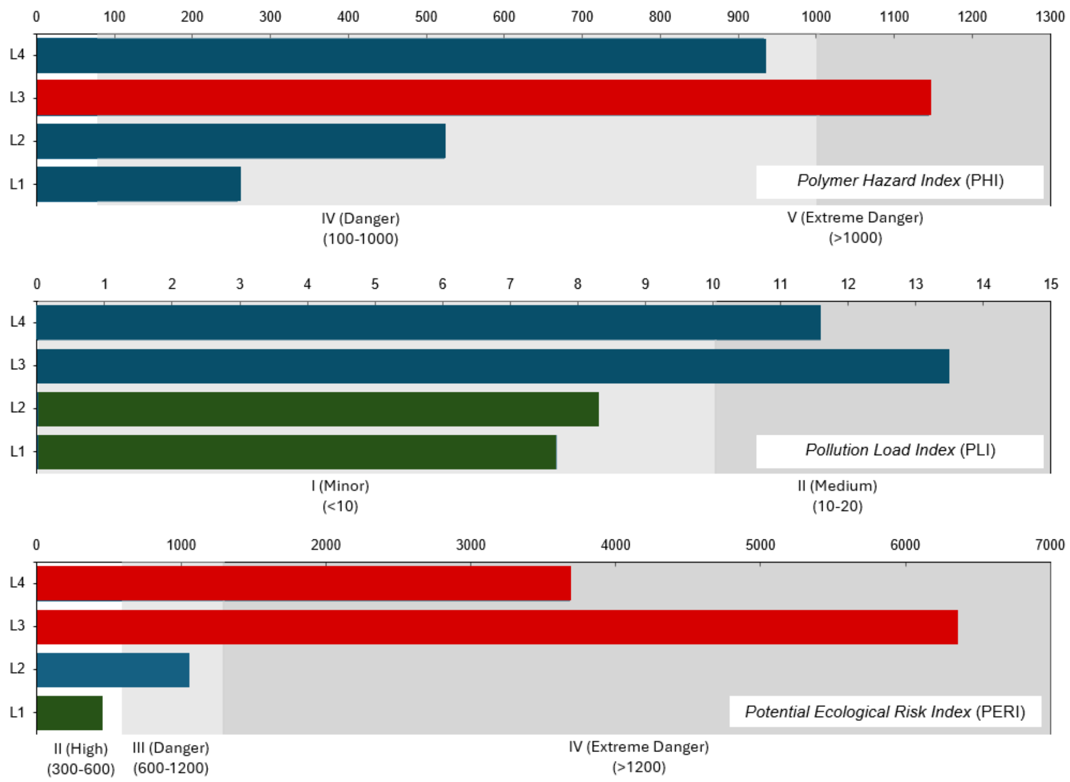

Figure 6. PHI, PLI, and PERI Values in the Downstream Area of Citarum River. PHI Indicates Polymer Toxicity (Red: Extreme Danger, Blue: Danger), PLI Reflects Sediment Pollution Levels (Green: Minor, Blue: Medium), and PERI Assesses Ecological Risk (Green: High, Blue: Danger, Red: Extreme Danger)

High-risk levels indicate significant threats to the ecosystem, as MPs can act as vectors for hazardous contaminants, posing serious risks to aquatic organism's health (Franzellitti et al., 2019). Furthermore, MPs not only affect organismal health but can also transfer through the food chain and undergo bioaccumulation and potential trophic transfer (Au et al., 2017; Saeedi, 2023; Sari et al., 2021), presenting potential risks to both aquatic organisms and human health. Addressing the ecological risks of MP contamination in the Citarum River requires increased attention, as both previous studies and this research emphasize the high ecological risk posed by MPs in the river.

It is important to note that PERI is an index designed to evaluate ecological risk to aquatic organisms and benthic ecosystems, specifically macroinvertebrates, deposit feeders, and the broader sediment-associated community, rather than a direct as-sessment of human health risk. Risks to human health arising from MP contamination are indirect, occurring through the bioaccumulation of MPs in consumed aquatic organisms such as M. tuberculata and fish, followed by trophic transfer and potential biomagnification through the food web (Au et al., 2017; Sari et al., 2021).

4. CONCLUSION

This study provides a comprehensive ecological risk assessment of MP contamination in sediments and bioaccumulation in *M. tuberculata* tissues in the downstream Citarum River. MP concentrations in this area were notably higher compared to other sections of the river. Variations in MP abundance across sampling locations indicate that land use, human activities, and inputs from tributaries significantly influence MP distribution. Bioaccumulation analysis revealed substantial MP occurrence in M. tuberculata tissues, predominantly in the form of fibers, consistent with sediment characteristics and indicating sediment ingestion as a potential uptake pathway. Risk indices (PLI category II, PHI categories IV–V, and PERI categories II–IV) collectively indicate that the downstream Citarum River represents an area of considerable ecological concern, with river confluences, particularly the Cibeet River confluence, identified as important zones for MP accumulation. These findings provide critical baseline data for policymakers to target plastic input reduction, sediment monitoring, and rural waste management interventions. Future research should prioritize: (1) in vivo toxicity studies examining sub-lethal effects of the dominant polymers (PP and PE) on *M. tuberculata* at the concentrations observed in this study; (2) trophic transfer experiments tracking MP movement from gastropods to higher trophic levels (e.g., fish and waterfowl); (3) temporal monitoring studies to assess seasonal variation in MP abundance relative to flow regime and land use changes; and (4) establishment of Indonesia-specific background MP con-centration values to improve the accuracy of future PLI and PERI calculations in Indonesian river systems.

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