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Microplastics in Sediment and Digestive Tract of Amazon Sailfin Catfish (Pterygoplichthys Spp.) in the middle segment of the Citarum River, Karawang, West Java, Indonesia

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Abstract: Microplastic pollution, primarily driven by industrial, residential, and agricultural activities, is a growing concern in the middle section of the Citarum River, Indonesia. Microplastics research was conducted three times in the central Citarum watershed from February to April 2022. This study investigates the types, abundance, and polymer composition of microplastics found in the sediment and digestive tract of the Amazon sailfin catfish (Pterygoplichthys spp.). Four types of microplastic-pellets, films, fibers, and fragments were identified, with an average abundance of 602.22 ± 563.87 particles/kg in sediment and 90.6 ± 40.7 particles/individual in catfish. The majority of particles were smaller than 0.3 mm. Identified polymers included Polyamide (PA), Polystyrene (PS), Polypropylene (PP), Cellulose Acetate (CA), Acrylonitrile Butadiene Styrene (ABS), Polyvinyl Chloride (PVC), Melamine, Ethylene Vinyl Acetate (EVA), Polyethylene (PE), Low-Density Polyethylene (LDPE), High-Density Polyethylene (HDPE), and Polyethylene Terephthalate (PET). Statistical analysis showed no significant difference in microplastic pollution among industrial, densely populated residential, and agricultural areas. These findings underscore the widespread distribution of microplastics in the Citarum River and highlight the need for comprehensive mitigation strategies.

Keywords: Citarum River, microplastics, pollution, polymers, sediment, Amazon sailfin catfish, Pterygoplichthys spp

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1. Introduction

The increasing production and use of plastics has certainly led to a growing amount of plastic waste (Mauludy *et al.*, 2020). As the number of people using plastics in their daily activities increases, the accumulation of plastic waste in the natural environment (Eriksen *et al.*, 2014; Kuncoro, 2018;) has become an environmental hazard (van Emmerik & Schwarz, 2020). An estimated 80% of plastic waste comes from land-based sources such as residential, commercial, public places, agriculture and industry (Ambarsari & Anggiani, 2022). Plastic waste has accumulated in the terrestrial environment, open ocean, coastlines, remote islands, and deep sea (Barnes *et al.*, 2009; Jakovljevic *et al.*, 2020). Plastic debris can carry toxic chemicals (Barnes *et al.*, 2009), including heavy metals (Hidalgo-Ruz *et al.*, 2012), which can adversely affect growth and reproduction (Cera *et al.*, 2020). Plastic is not biodegradable (Arisandi *et al.*, 2020; Mavuso & Singwane, 2020), and degradation is a slow process, so that plastic particles can persist in aquatic environments for long periods (Barnes *et al.*, 2009). When plastic degradation peaks, the abundance of microplastics and nanoparticles will increase and far outnumber plankton (Jovanović, 2017). Microplastics are pollutants commonly found in aquatic environments. (Nurhasanah *et al.*, 2021).

Microplastics are plastic particles that have a size of less than 5 millimeters, with the lower size limit of microplastics not determined (Masura *et al.*, 2015). According to its source, microplastics come from primary microplastics produced as resin pellets or as additives to personal care products (e.g., soaps and scrubs in cosmetics) and secondary microplastics are degradation products of larger plastic objects, which are broken down by biological degradation, UV radiation and physical abrasion into smaller fragments (Whitehead *et al.*, 2021). The presence of microplastics in water bodies, sediments, and aquatic organisms, including fish, has been studied extensively in various regions around the world. Aquatic organisms, including fish, can ingest microplastics containing toxic metals and become vectors for the spread of pathogens and microbes that tend to accumulate in the gills, liver, mantle and muscles, and digestive glands through various routes of exposure, such as drinking contaminated water, eating prey contaminated with microplastics, or ingesting microplastics through the gills. In fish, microplastics cause intestinal damage with rupture of the villus and rupture of enterocytes, leading to death, while in humans, microplastic hazards cause genomic instability and increased DNA damage, which can lead to infertility and cancer (Mandal & Mishra, 2023).

The Citarum River, a vital waterway in West Java, Indonesia, is among the most polluted rivers globally. Stretching 297 km (Hidayat *et al.*, 2022, it provides water for nearly 27 million people (MenPUPR, 2016). The river is divided into upstream, middle, and downstream sections, with its middle section, particularly in Karawang, West Java, characterized by agricultural, industrial, and densely populated residential areas. Despite studies focusing on the upstream and downstream sections, research on microplastics in the middle section remains scarce. It is one of the most polluted rivers in the world. The release of plastic waste as a secondary source of microplastics in the Citarum River is still quite high, reaching more than 1000 kg per hour (Hariyadi *et al.*, 2022). Polymer types found in previous studies in the Citarum River include Polyethylene Terephthalate, Polystyrene, High-Density Polyethylene, Low-Density Polyethylene, Polyvinyl Chloride, and Polypropylene (Aisyah *et al.*, 2022). Microplastics with a lower density than water float, while those with a higher density sink and settle in the sediment (Laksono *et al.*, 2021). The middle Citarum River has sediments from ancient volcanic activity, containing silt, clay, sand and gravel (Citarum, 2016), and the higher density of plastic compared to water causes microplastics to accumulate at the bottom of the river water (Azizah *et al.*, 2020). The slope of the river also accelerates the deposition of microplastic particles.

This study aims to assess the abundance and characteristics of microplastics present in the sediments of the middle section of the Citarum River in Karawang, West Java, and to identify the abundance and characteristics of microplastics found in the digestive tract of Amazon sailfin catfish inhabiting the same area. The results of this study are expected to provide important information on the current state of microplastic pollution in the Citarum River system and its potential impact on local aquatic life.

2. Material and Methods

2.1. Sampling Area

The sampling was collected in three repetitions from February to April 2022 in the heavily polluted middle Citarum River, Karawang district (see Table 1 and Figure 1). This area is a densely populated, industrialized and agricultural area. This leads to considerable pollution from plastics and other wastes (Hidayat *et al.*, 2022). Water levels were relatively stable during these three months, allowing for consistent sampling. This study aimed to assess the abundance and composition of microplastics in the sediment and digestive tract of Amazon sailfin catfish, a common invasive species found in the Citarum River.

Table 1. Sampling location.

Station	Location	Coordinates	Description
Station 1	Wadas, Teluk Jambe Raya	S 06°19'37.41", E 107°18'41.81"	Represents industrial areas with factories and warehouses.
Station 2	Kp. Pasir Panggang, East Teluk Jambe	S 06°18'39.71", E 107°16'34.83"	Densely populated residential area with hotels, restaurants, and showrooms.
Station 3	Sumedangan, Teluk Jambe Timur	S 06°16'47.63", E 107°16'27.83"	Agricultural area representing farming activities.

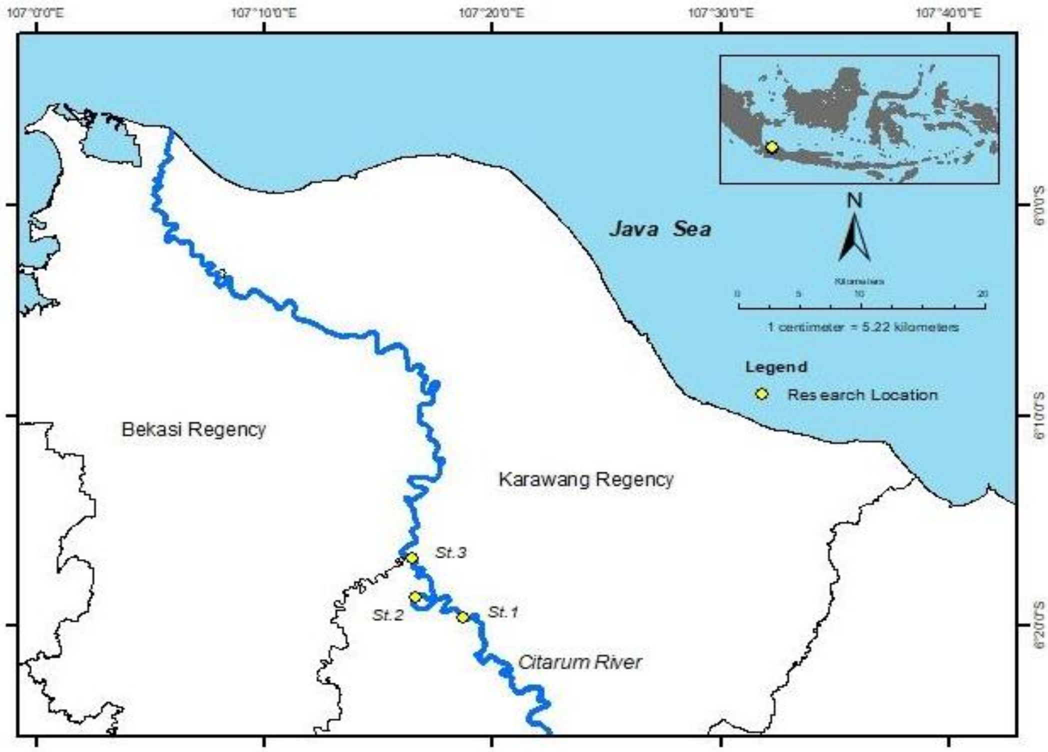

Figure 1. Map of sampling sites in the middle segment of the Citarum River, Karawang, West Java, Indonesia.

2.2. Sample collection

Sediment sampling was carried out by inserting a sediment core made of a transparent acrylic tube with a 2-inch diameter into a sediment column at a depth of 10 cm. The collected sediment sample was placed and labeled in an aluminum Ziplock container with 500 grams (wet weight).

Amazon sailfin catfish (Pterygoplichthys spp.) were collected from catches of local fishers specializing in Amazon sailfin catfish (Pterygoplichthys spp.) from study sites representing a range of community activities, including industrial areas, densely populated settlements, and agriculture. The number of samples was set at five fish to represent the number of fish caught each month (Julia *et al*., 2020), with February samples identified as samples 1 through 5, March samples as samples 6 through 10, and April samples as samples 11 through 15, respectively. Samples were then stored in a refrigerator before analysis.

2.3 Sample Preparations

a. Sediment Sample

A 300-gram wet sediment sample was dried in an oven at 50 oC for three days until completely dry (Rochman *et al*., 2015). The dry sediment was weighed, and 50 grams of it was placed into a beaker. Subsequently, 50 ml of saturated NaCl solution (prepared by dissolving 300 grams of NaCl in 1 liter of distilled water) was added and stirred until homogeneous. NaCl is used to separate the plastic waste from the sediment by density separation, where the plastic waste floats and separates from the sediment that settles due to the difference in density. The part separated from the sediment is called the supernatant. The mixture was covered with aluminum foil and left to settle for 24 hours to form a clear supernatant. The supernatant was filtered through a 5 mm sieve to remove particles larger than 5 mm and then filtered again through a 0.3 mm sieve to remove particles smaller than 0.3 mm. The filter results from the 0.3 mm filter were transferred to a beaker for further analysis.

The next step was adding 20 mL of 0.05 M Fe (II) solution and 20 mL of 30% hydrogen peroxide (H2O2) into the sample's beaker. The mixture was left at room temperature for 5 minutes, then heated on a hot plate to 70°C until gas bubbles appeared. The sample was removed and allowed to cool slightly. After that, it was reheated to 70°C for approximately 30 minutes (Bruge *et al*., 2020). If organic material is still present in the sample and visible with the naked eye, an additional 20 ml of 30% H2O2 could be added until the organic material is fully degraded (Mauludy *et al*., 2019). These steps were repeated as necessary to ensure complete decomposition of organic material. The sample was then filtered using a 0.3 mm filter to separate microplastics from degraded organic material, assisted by spraying with distilled water. Samples that are free from organic material are then refiltered using GFF filter paper and a vacuum pump. The ready-filter paper was further identified using a stereo microscope and FTIR microscopy.

b. Fish Sample

The microplastic content of the fish was analyzed through a two-step process. First, the fish samples were cleaned with distilled water to remove contamination from impurities and microplastics. The total length and weight of the fish were measured. The fish were dissected, and the digestive tract was weighed and transferred to a sterile beaker.

In the second step, 10% KOH solution was added to the fish gut samples in a beaker three times the sample volume and incubated overnight at 60°C to degrade the organic matter (Rochman *et al*. 2015). To further degrade the remaining fish intestines, 20 ml of 30% H2O2 solution was added. The sample was heated at 70°C until the salt dissolved and left overnight at room temperature. The sample was then filtered using Whatman GFF paper with the help of a vacuum pump, and the filtrate was further identified using a stereo microscope, the same as the sediment sample.

2.4 Microplastic analysis

Observations of microplastic particles in sediment and fish digestive tracts were carried out using a Nikon SMZ2B Stereo microscope with an additional 51-megapixel Koppace microscope camera, with a zigzag scanning pattern from left to right to ensure the entire sample area was thoroughly covered. This ensured all particles were detected and analyzed. During observations, microplastic particles were identified visually based on the following criteria: Resistance to torn with tweezers (particles have sufficient strength and durability, preventing them from being torn using tweezers); particles have a lack of organic structure (plastic particles do not have a structure associated with organic materials, such as wood fiber or other organic materials) (Peng *et al*., 2017). Microplastic particles identified and met those criteria were measured using a Nikon SMZ2B microscope, with a Koppache camera and software. In this study, only particles smaller than 5 mm were measured. Particles exceeding the size ranges were isolated and excluded from further analysis.

Fourier Transform Infrared Spectroscopy (FTIR) analysis was conducted at the Advanced Characterization Laboratory, National Research and Innovation Agency (BRIN) in Cibinong. The samples were analyzed using FTIR device employing the Attenuated Total Reflectance (ATR) method. The obtained spectrum was compared with the reference spectrum from the database, with matches accepted at a confidence level of 75% or higher.

2.5 Data analysis

The classification of microplastics is based on their types-pellets, films, fibers and fragments and their size, categorized as <0.3 mm, 0.3-<0.5 mm, 0.5-<1 mm and 1-5 mm (Liu *et al*. 2021). The abundance of microplastics in sediment was calculated based on the sample weight of 50 g, conserved to 1 kg (Hidalgo-Ruz *et al*. 2012). Microplastic concentration was expressed as the number of particles per kilogram of dry sediment (Claessens *et al*., 2011). For microplastics detected in the digestive tract of Amazon Sailfin Catfish (Pterygoplichthys spp.), the abundance was calculated in particles per individual (Gresi *et al*., 2021).

The statistical analysis method used was the Shapiro-Wilk normality test, followed by the Kruskal-Wallis test, to determine the differences in microplastic abundance across three stations in the middle segment of the Citarum River. Data was analyzed using Microsoft Excel 2019 and IBM SPSS Statistics 25.

3. Result and Discussion

3.1 Microplastics found in sediment

This study identified 21,680 microplastic particles from 9 sediment samples from industrial, residential and agricultural areas (see Table 2 and Figure 2). The average total abundance was 602.2 ± 563.9 particles/kg. The particles consisted of four types: pellets, films, fibers, and fragments. Fragments were the most common type in industrial areas, while fibers dominated in residential and agricultural areas. The particle sizes were predominantly less than 0.3 mm, followed by 0.3-0.5 mm and 0.5-1 mm. The highest concentrations were observed in March 2022 for industrial areas and in February 2022 for residential and agricultural areas.

At Station 1, the most common type of microplastics was fiber, with an average total abundance of 1533 ± 1307.3 particles per kilogram. At Station 2, fragments were the most common type, with an average total abundance of 686.7 ± 98.5 particles per kilogram. At Station 3, fiber was the predominant type, with an average total abundance of 1120 ± 429.7 particles per kilogram.

Microplastic particles were detected sediment samples in the middle section of the Citarum River. These particles found were in the form of pellets, films, fibers and fragments. The total abundance of plastic particles which dominantly found at Station 1 was fiber. The high abundance might be caused by the wastewater from washing process in garment factories in the area (Ali *et al*., 2021). Fiber particles can also derive from glass fibers (Sutanhaji *et al*., 2021).

At Station 2, which represents a densely populated residential area, microplastics enter the waters mainly through household waste and runoff from residential areas. The highest form of microplastic found at this station was the fragment. Fragment-shaped microplastics might come from items, such as drinking bottles, jars, buckets, mica folders, parallel pipes, containers/jerry cans, irrigation pipes, and plastic pots (Sutanhaji *et al*., 2021).

Table 2. Summary of microplastic data identified from sediments

		Station 1	Station 2	Station 3	Total
Average Number of MPs ± SD (Particles/kg)		658.3±828.5	446.7±264.7	701.7±431.5	602.2±563.9
Total number of MPs		7,900	5,360	8,420	21,680
Total number of MPs by type of debris	Pellet	400	1,020	1,660	3,080
	Film	1,000	300	660	1,960
	Fiber	4,600	1,980	3,360	9,940
	Fragment	1,900	2,060	2,740	6,700
Total number of MPs by size (mm)	<0.3	5,900	2,440	4,100	12,440
	0.3 - < 0.5	880	1,840	2,480	5,200
	0.5 - <1	1,020	1,040	1,440	3,500
	1-5	100	40	400	540

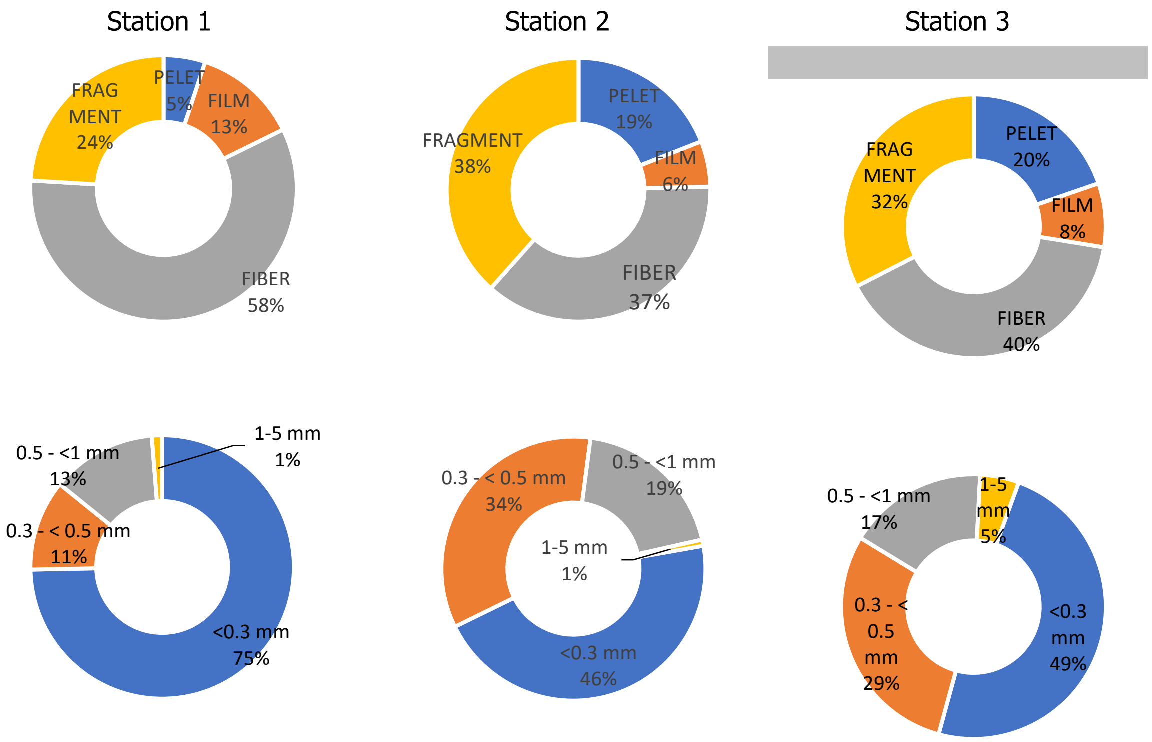

Figure 2. Distribution of microplastic particles by type, and size collected in sediments at three study sites Station.1 (Industrial Area); Station 2 (Densely Populated Residential Area); Station 3 (Agricultural Area)

At Station 3, an agricultural area, fiber was the highest form of microplastic particles. Similar to Station 1, fiber in these agricultural areas is likely derived from materials such as clothing, rope, cloth, mulch, plastic sacks, fiberglass raffia, and others (Sutanhaji *et al.*, 2021).

Comparing the sediment abundance average in the middle section of the Citarum River of 602.22 ± 563.87 particles/kg with several other rivers, microplastics in sediments in the middle section of the Citarum River are smaller than microplastics in sediments in the Opak River, Progo River of 1,799.33 ± 1,430.87 particles/kg and 645.33 ± 405.94 particles/kg, respectively. The average microplastic abundance in sediments in the middle section of the Citarum River is 602.22 ± 563.87 particles/kg. Compared to other rivers, this is lower than the concentration in the Opak River and Progo River, which are 1.799.33 ± 1.430.87 particles/kg and 645.33 ± 405.94 particles/kg, respectively (Utami *et al.*, 2022). However, it is higher than the levels in Muara Badak, Kutai Kartanegara Regency, which range from 69.3 to 90.12 particles/kg (Dewi *et al.*, 2015), and the Sei Kambing River at 32.3 particles/100g (Hasibuan *et al.*, 2020).

The particle size of microplastics in sediments from three sampling sites: industrial areas, densely populated settlements, and agricultural areas, is predominantly less than 0.3 mm, with the least represented size range being 1-5 mm. The size differences are influenced by the duration of the fragmentation process in the aquatic environment, where a longer process results in a smaller size of microplastics. Besides that, UV radiation also plays a role in breaking down larger plastics into smaller particles (Azizah *et al.*, 2020). Differences in microplastic concentrations among sampling sites are caused by variation in anthropogenic activities, sources of microplastic input, and the influence of natural factors such as water currents, wind, and river transport dynamics (Fitriyah *et al.*, 2022).

Based on the results of FTIR analysis on sediments (see Figure 3), MPs particles of polyamides, polystyrene, polypropylene, cellulose acetate, acrylonitrile butadiene styrene, and polyvinyl chloride were found. Microplastics in organisms can interfere with digestive enzymes that convert nutrients into forms that animals can use for energy. This can slow growth, delay maturation, and reduce reproduction (Trestrail *et al.*, 2021). Microplastics found in organisms may also transfer to humans, who risk exposure by consuming contaminated species. Whole organisms consumed pose a greater risk than those with digestive tracts removed. Potential human health impacts include skin, breathing, and digestive problems, as well as heart disease, reproductive issues, and cancer (Carbery *et al.*, 2018).

Based on statistical analysis using the Kruskal Wallis Test on sediment, the results show no significant difference in microplastics abundance among the sampling stations, with a significance value of 0.332 (p > 0.05).

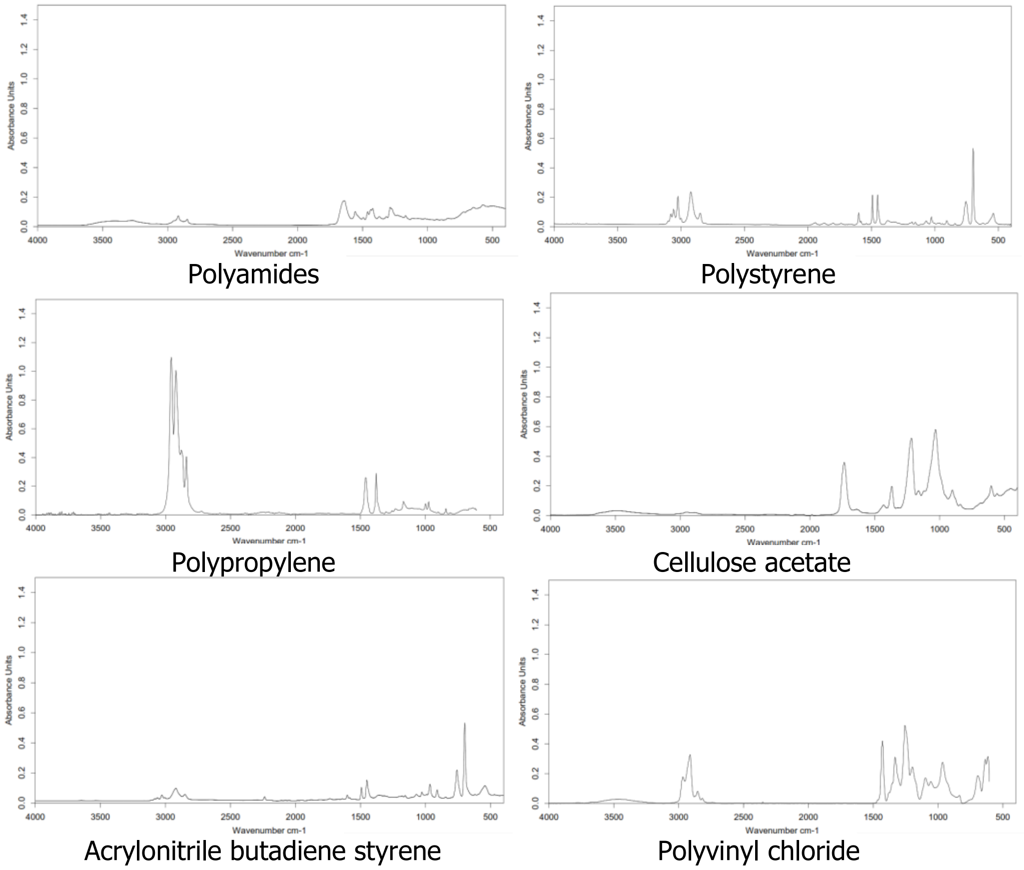

Figure 3. Results of analysis using FTIR on sediment samples

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Based on statistical analysis using the Kruskal Wallis Test on sediment, the results show no significant difference in microplastics abundance among the sampling stations, with a significance value of 0.332 (p > 0.05).

3.2. Microplastics found in Amazon Sailfin Catfish (Pterygoplichthys spp.)

A total of 15 digestive tract samples from Amazon Sailfin catfish (Pterygoplichthys spp.) contained 1359 microplastic particles, which were pellets, films, fibers and fragments (see Table 3 and Figure 4).

This study found a significant presence of microplastics in the digestive tract of Amazon Sailfin Catfish (Pterygoplichthys spp.) sampled from the middle section of the Citarum River in Karawang, West Java, Indonesia. Fiber was the most abundant type of microplastic found.

In February, an average of 79.4 ± 9.9 microplastic particles per individual was observed across all samples, with the highest concentration recorded in sample 1, suggesting a potential localized source of pollution. In March, the average increased to 107.2 ± 58.7 particles per individual, indicating a notable rise in microplastic abundance; the highest concentration during this period was detected in sample 10. By April, the average decreased slightly to 85.2 ± 36.4 particles per individual, with the peak concentration observed in sample 11. This fluctuation in monthly averages and varying spatial distribution across sampling points highlights the dynamic nature of microplastic contamination in the study area.

Table 3. Summary of data collected from Microplastics in the digestive tract of Amazon sailfin catfish (*Pterygoplichthys* spp.) in February-April 2022.

		Feb	**March**	**April**	**Total**
Average Number of MPs ± SD (Particles/kg)		79.4±9.9	85.2±36.4	107.2±58.7	90.6±40.7
Total number of MPs		397	426	536	1,359
Total number of MPs by type of debris	Pellet	60	60	152	272
	Film	14	23	17	54
	Fiber	196	259	187	642
	Fragment	127	84	180	391
Total number of MPs by size (mm)	<0.3	313	329	289	931
	0.3 - < 0.5	48	48	153	249
	0.5 - <1	32	37	88	157
	1-5	4	12	6	22

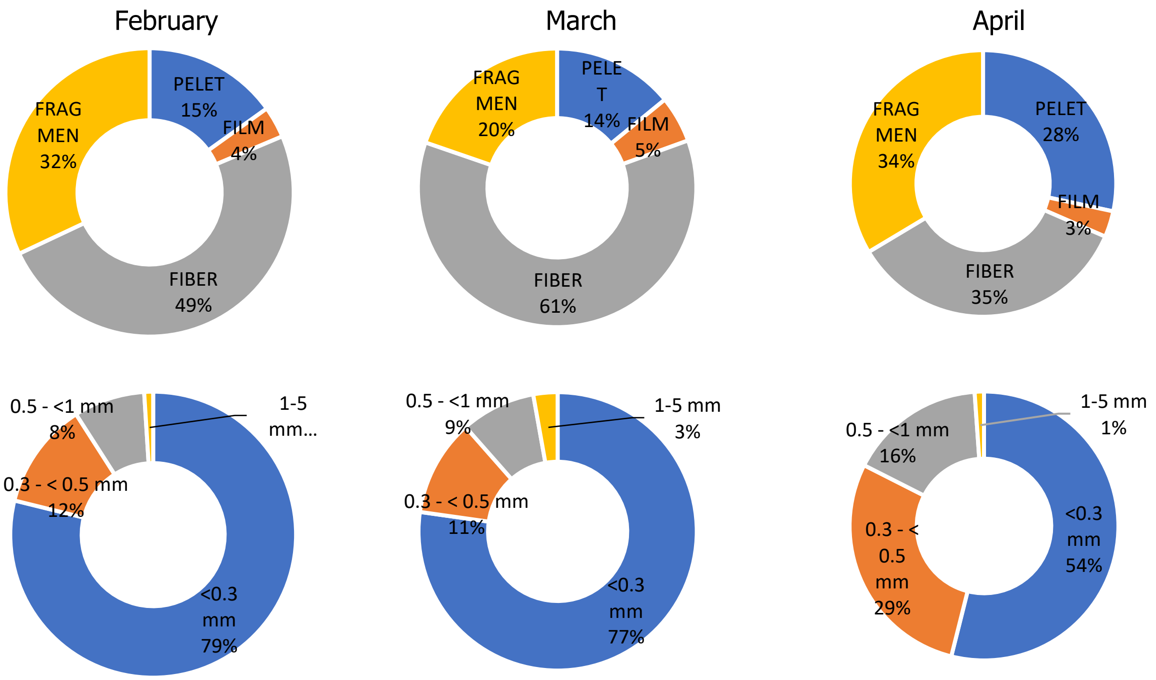

Figure 4. Distribution of microplastic particles by type and size collected from the digestive tract of Amazon Sailfin Catfish (*Pterygoplichthys* spp.) in the middle section of the Citarum River.

From a total of 15 Amazon Sailfin Catfish (Pterygoplichthys spp.) samples collected from February-April 2022, a total of 1359 microplastic particles were obtained, with an average total abundance of 90.6±40.7 particles/individual (Table 3). There are 4 types of particles obtained, namely pellets, films, fibers, and fragments, with the largest number of fibers at 47%, fragments at 29%, pellets at 20%, and films at 9%, respectively (Figure 4). In the overall microplastic particle size, sizes <0.3mm were obtained by 68%, sizes 0.3-<0.5mm by 18%, sizes 0.5-<1mm by 12%, and sizes 1-5mm by 2%, respectively.

The results of FTIR analysis show that the total types of polymers obtained from all research locations in the middle Citarum River are Polyamides, Low-density polyethylene, High-density polyethylene, Ethylene vinyl acetate, Polystyrene, Acrylonitrile butadiene styrene, Polypropylene, Polyvinyl chloride, and Polyethylene terephthalate (Figure 5).

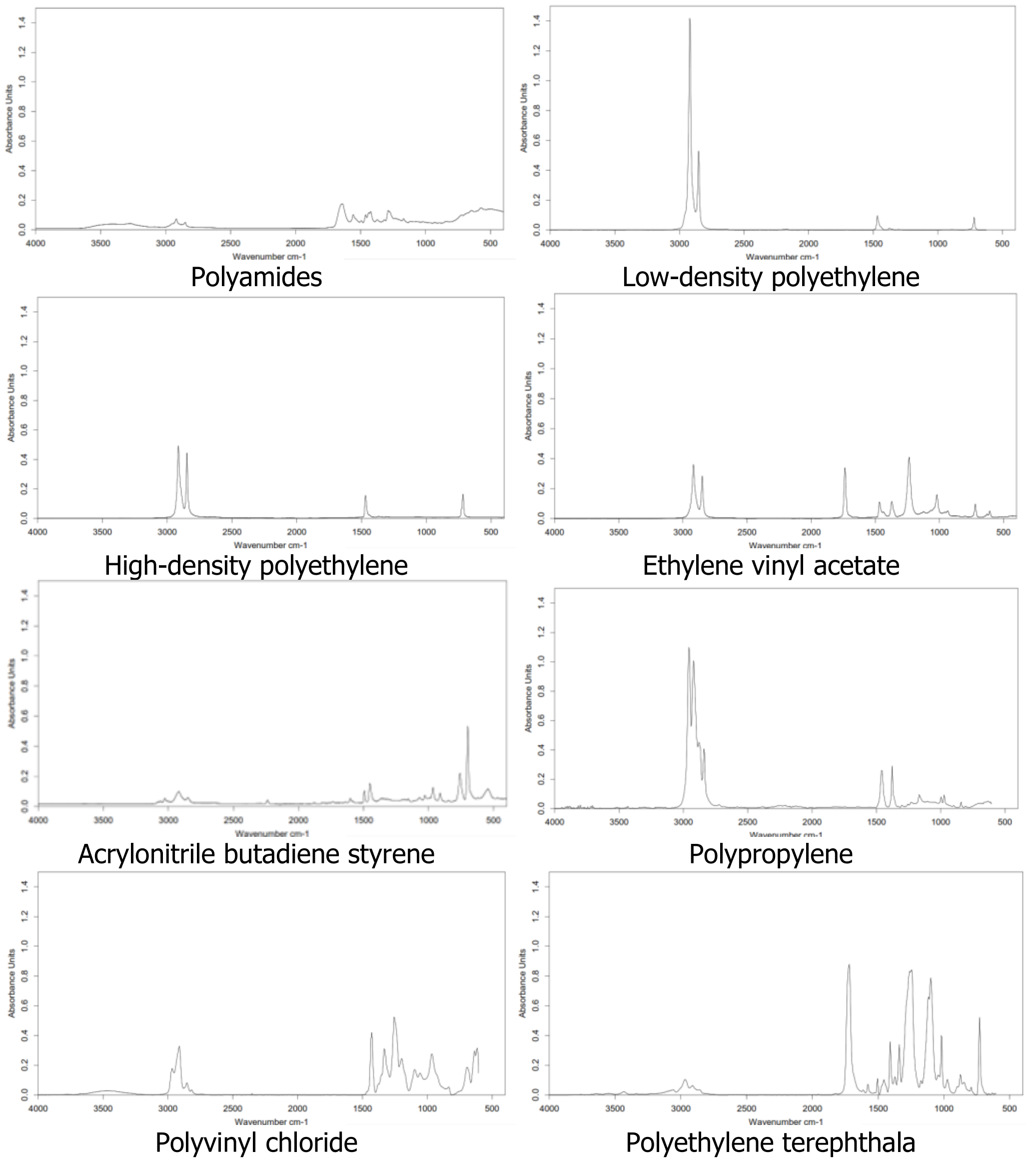

Figure 5. Results of analysis using FTIR on Amazon Sailfin Catfish digestive tract samples Amazon Sailfin Catfish

The Amazon Sailfin Catfish (Pterygoplichthys spp.) is one of the organisms found in the Citarum River. The Amazon Sailfin Catfish group is native to the South American Amazon River with the following classification; Class Actiopterygii; Nation Siluriformes; Tribe Loricariidae; Clan: Pterygoplichthys; Species: Pterygoplichthys ambrosettii, P. anisitsi, P.

disjunctivus, P. etentaculatus, P. gibbiceps, P. joselimaianus, P. lituratus, P. multiradiatus, P. pardalis, P. parnaibae, P. punctatus, P. scrophus, P. undecimalis, P. weberi, P. xinguensis and P. zuliaensis (Wahyudewantoro, 2018), is considered an invasive species (Qoyyimah *et al*., 2021). They have a remarkable adaptability, making them thrive in polluted waters. Their diet includes insect larvae, benthic algae, algae, detritus, and worms. Due to their small size and buoyancy, fish easily ingest microplastics. Fish ingest microplastics by mistaking the particles as natural prey or accidentally swallowing them. Microplastics can be unintentionally swallowed by fish or by mistake for natural prey (Jovanović, 2017). The presence of microplastics in aquatic organisms can damage the digestive tract, leading to internal injuries and, in severe cases, death. In addition, it may cause blockages in the digestive system, reducing the fish's ability to feed, which may result in starvation and energy depletion. Their accumulation in the digestive tract can also impair growth, lower steroid hormone levels, inhibit enzyme production, and negatively affect reproduction.

The microplastic abundance in the digestive tract of Amazon sailfin catfish in the middle Citarum River was 90.6 ± 40.7 particles per individual. This is relatively lower than the levels found in the digestive tracts of Amazon Sailfin Catfish from the Krukut River, which contain 486 particles per individual (Sandra & Radityaningrum, 2021), as well as the abundance from the Ciliwung River at MT. Haryono (5.344 particles/individual) and at the West Tanjung area (5,581 particles/individual) (Deriano *et al*., 2021).

5. Conclusions

This study investigated the presence and composition of microplastics in the sediment and digestive tract of Amazon catfish from the middle section of the Citarum River, West Java, Indonesia. The results indicate that microplastics were pervasive in the sediment and fish samples examined. Microplastics found in sediment samples were mainly fragments, fibres and films, ranging in size from <0.3mm to 5mm. Interestingly, raising concerns about the potential for trophic transfer and bioaccumulation of these contaminants within the aquatic food web, the majority of microplastics identified in fish digestive tracts were less than 1 mm in size. The results of this study highlight the urgent need for comprehensive strategies to address plastic pollution, particularly microplastics, in freshwater ecosystems to mitigate the adverse impacts on aquatic organisms and human health.

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Author Contributions

ISH contributed to research design, collecting, identification and analysis of data. **H**, **SH** and **T** contributed to the survey and evaluated the paper. **GPY** contributed to the paper evaluation and proofreading. **TS** contributed to the identification and paper writing. **E.T** contributed to the collection and preparation. All authors read and approved the final paper.

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