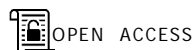


Microplastic Occurrence in Different Fish Organs from Two Coastal Waters in Java Sea, Indonesia

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Abstract

Plastic debris and microplastic (MP) have been associated with marine pollution. The present study aimed to assess the MP accumulation in different organs (gut and gill) of 6 economically important fish species from two essential coastal areas, Jakarta Bay and Cirebon Bay. MP was isolated with NaCl, followed by the identification of MP profiles with digital microscope and Fourier Transform Infrared Spectroscopy (ATR-FTIR), respectively. The result showed that MP was found in all samples. Generally, MP concentration in fish from Cirebon Bay was slightly higher than that from Jakarta Bay, with benthic species more concentrated than pelagic fish in both sites. Microplastic was more concentrated on the gill of fish from Jakarta Bay, in contrast with Cirebon Bay. The predominantly MP in Jakarta Bay fish was fiber, while in Cirebon Bay was fragmented with minor granule and film in both locations. Polyethylene (PE), polyvinyl chloride (PVC), and polypropylene (PP) were the most dominant MP in the fish samples from both study areas. Microplastic size showed that the majority (65%) was the small size (<1 mm), compared to the bigger one (1-5 mm), while blue, black, and red are the predominantly MP colors. This research supports the need to minimize plastic pollution in aquatic ecosystems to tackle the detrimental impact of MP accumulation to fish and human health. It is suggested to compare MP profiles on fish samples and in water or sediment compartments and identify the chemical constituents of MP.

Keywords: microplastic, fish gut, fish gill, Jakarta Bay, Cirebon Bay

Introduction

The population growth and economic development have led to an increasing solid and liquid waste emission in Indonesia, especially in big cities such as Jakarta and Surabaya (Cordova & Nurhati, 2019; Dwiyitno et al., 2016; Yona et al., 2019). Importantly, plastic pollution has been recognized as harmful and reported to expose a wide range of wild organisms, including seafood, marine mammals, birds, and other products related to marine sources such as sea salt (Devriese et al., 2017; Kim et al., 2018; Rochman et al., 2015). Other studies reported that Indonesian waters are contaminated by plastic debris (Cordova & Nurhati, 2019; Fitri & Patria, 2019; Jambeck et al., 2015; OECD, 2020; Yona et al., 2019).

Through the bio-magnification pathway via the aquatic food web, MP contaminants potentially accumulate in aquatic organisms, including seafood. Furthermore, MP has shown possible ecotoxicological

effects on marine organisms, such as weight loss, reduced feeding rate, increased phagocytic activity, transferable to the lysosomal system, bioaccumulation, and inhibition of acetylcholinesterase activity (Avio et al., 2017; Ribeiro et al., 2017, 2021). Additionally, MP can adsorb organic contaminants from the surrounding environment, such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) and may produce a more harmful effect (Yeo et al., 2020).

Comprehensive studies on MP contamination in fish species from the Indonesian coast are relatively limited. A study on MP contamination in Indonesian fish was started by Rochman et al. (2015), revealing the exposure in 11 fish species collected from the fish market in Makassar. However, there are limited studies that investigate various essential parameters of MP in coastal fish, i.e. in the digestive tract of tuna, bream, and croacker (Suwartiningsih et al., 2020), the digestive tract of threadfin, mackerel, threadfin bream, and hairtail (Hidayati et al., 2023), and stomach, intestines,

and gills of spotted scat fish (Marchellina et al., 2024). Additionally, five other studies only identified partial parameters and did not analyze either the MP concentration or the polymer type (Amin et al., 2020; Hastuti et al., 2019; Indriyasaki et al., 2023; Ismail et al., 2019; Rochman et al., 2015; Sarasita et al., 2020). These studies were conducted in economically important fish of both pelagic and demersal species from different regions in Indonesia.

The present study aimed to assess the profile of MP contamination in fish species from Jakarta Bay, known as the most polluted coast in Indonesia (Cordova & Nurhati, 2019; Dwiwitno et al., 2024; Kompas, 2017; Nabillah, 2022) with a population of 10.67 million, and compared to those from Cirebon Bay, known as a newly developing region with 2.78 million inhabitants that potentially increase the pollution loads (BPS-Statistics Indonesia, 2023; Cirebon Regency, 2021; Setiawan & Chalil, 2023). Both regions produced significant capture fishery as 146,000 ton fish/year was landed in Jakarta while in Cirebon was 2,700 tons/year (Statistic Indonesia - Cirebon City, 2024; Statistic Indonesia - DKI Jakarta Province, 2020).

Most studies in Indonesian fish investigated MP in the digestive tract or gut. For that reason, besides in the gastrointestinal tracts, the present study also identified the MP concentration in the fish gill, in which both are important to understand the uptake pathway of MP in fish species (Su et al., 2019; Yin et al., 2022).

Materials and Methods

Sample Collection

The present study was conducted in two coastal regions related to the Java Sea, i.e. Jakarta Bay and Cirebon Bay. Jakarta Bay is conducted near Jakarta Capital City ($6^{\circ}00'00.0''\text{S}$; $106^{\circ}51'32.4''\text{E}$), the most populated region in Indonesia, with more than 10 million inhabitants. Furthermore, Cirebon Bay ($6^{\circ}38'20''\text{S}$; $108^{\circ}41'13''\text{E}$) is situated 200 km eastern part of Jakarta Bay (Figure 1), a relatively small region with approximately 3 million residences, which is currently known as a newly developing region with potential economic and urbanization growth.

In order to evaluate the MP contamination in fish species, at least ten individuals of each fish species were provided directly from fishermen in the study area. Five fish species were collected from Jakarta Bay, i.e., rabbitfish (*Siganus javus*), croaker (*Agyrosomus amoyensis*), sea catfish (*Netuma thalassina*), bigeye (*Priacanthus* sp), and fringescale sardine (*Sardinella fimbriata*). These species are known among economically important fish species in Jakarta Bay. Additionally, three economically important fish species were provided from Cirebon Bay, i.e., rabbitfish (*Siganus javus*), croaker (*Agyrosomus amoyensis*), and anchovy (*Stolephorus comersonii*). A single sampling campaign was conducted in the morning time (09.00-10.00 am) in March (Cirebon Bay) and



Figure 1. Location of the sample collection.

April (Jakarta Bay) of 2019. Most of the fish samples are benthic/demersal species, except *S. fimbriata* and *S. comersonii*, which are classified as pelagic species. All fish samples were conserved at chilling temperature (0-4°C) and transported to the laboratory in Jakarta.

Microplastic Analysis

Identification of MP profiles included MP concentration and distribution in gill and digestive/gut organs, type of MP polymer, shape, color, and size. A number of 3 individual fish samples were randomly selected from each fish species, i.e. 5 species from Jakarta Bay and 3 species from Cirebon Bay. These species were found as the most dominant species captured by the local fishermen during the sampling campaign. All species are classified as economically important fish in Indonesia (White et al., 2013). Fish samples were dissected and treated in a clean-air room to reduce environmental contamination. All glassware and equipment were pre-cleaned with *Milli-Q* water, and a blank protocol was performed as a quality control procedure.

Microplastic was isolated and identified following the method of Avio et al. (2015). The fish MP was isolated from oven-dried organs (gastrointestinal tract/gut and gill). Microplastic in each organ was separated from organic materials with 50 ml of concentrated H₂O₂ 30% (Merck) at 60°C for 24 hours. Density separation was performed with 150 ml concentrated NaCl (Merck) and filtered with a glass microfiber filter of 2.7 µm pore size and 4.7 cm diameter (*Whatman GF/D*) under vacuum conditions.

Density separation and filtration were repeated 2 times to ensure all MP had been extracted. The visualization and quantification of MP objects were observed by a binocular microscope (*Olympus BX-*

53) equipped with a *DP21* camera at 40x magnification. The composition of the MP polymer was identified using a universal ATR-FTIR spectrometer (*PerkinElmer-Spectrum One*) by comparing the spectra to that of commercial standard or reference materials at 400-4000 cm⁻¹ and co-adding of 16 scans at a resolution of 4 cm⁻¹.

Quality Control

Microplastic analysis was performed under quality control procedures. Non-plastic materials were used for all laboratory applications and pre-cleaned glassware with prefiltered distilled water (pore size 2.7 µm cellulose nitrate *Whatman* membrane). MP isolation was conducted in a laminar flow cabinet to avoid contamination. Prior to analysis, fish samples were washed with deionized water. All suspected MPs in the samples were counted and presented as identified MPs. Untreated prefiltered distilled water was used as a blank protocol, with no MP contamination found.

Results and Discussion

Microplastic Abundance in Fish Samples

In the present study, MP was found in all fish species, either from Jakarta Bay or Cirebon Bay. However, minor samples (3/22 or 13.64% of the individual samples) showed no MP contamination, namely in one sample of *S. fimbriata*, *S. javus*, and *A. amoyensis*, respectively. Additionally, some fish samples showed that MP was only identified in the gut or gill organ. In general, MP concentration in fish from Cirebon Bay was slightly higher than that from Jakarta Bay, with *S. javus* known as the most contaminated species in both locations at an average concentration of 11.33 and 15.00 particles/fish (Figure 2A). Similar to *S. javus*,

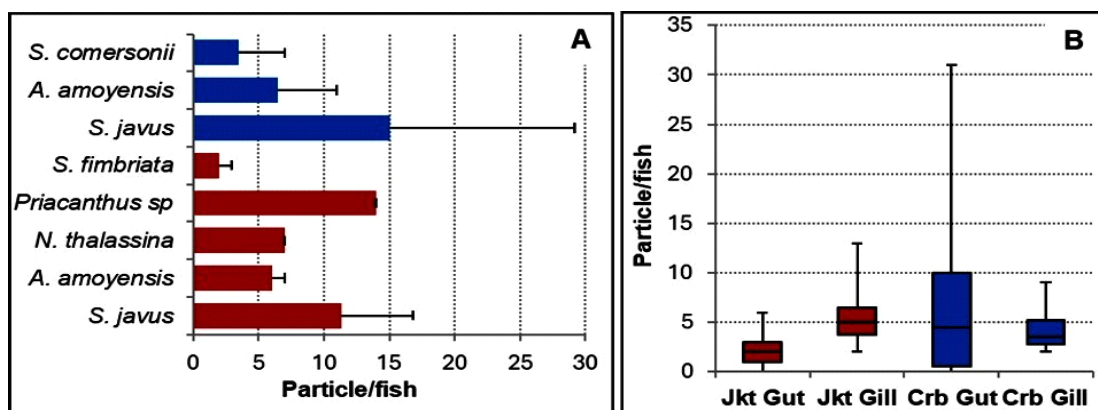


Figure 2. Microplastic concentration in different fish species from Jakarta Bay/Jkt (red bars) and Cirebon Bay/Crb (blue bars) (A: concentration based on species; B: average concentration in gut and gill organs)

Priacanthus sp from Jakarta Bay was also known to be relatively contaminated by MP at an average concentration of 14 particles/fish, whereas this species was not provided from Cirebon Bay. A pelagic species of *S. fimbriata* collected from Jakarta Bay was known as the least contaminated species by MP, i.e. , 2 particles/fish on average. Microplastic contamination was more dominant on the gill organ of fish samples from Jakarta Bay, in contrast with that from Cirebon Bay (Figure 2B). This difference could be associated with the fact that MP profiles (shape, polymer, and size) in both locations are relatively varied.

Gill and gut are common organs to accumulate MP in fish species (Su et al., 2019; Yin et al., 2022). In the present study, MP concentration was identified at relatively comparable concentrations in fish guts and

gills from both locations. However, a relatively high concentration of MP in fish guts and gills was investigated in Cirebon Bay. This could be related to the different sizes and shapes of fish MP from Jakarta Bay and Cirebon Bay, reflecting the different sources of plastic waste emission in both regions. Since the smaller size of MP (0.1-1.0 mm) was more dominant in fish from Cirebon Bay (Figure 5A), this size is relatively accumulated more in the gill rather than in the gut, as also reported by Su et al. (2019). The most dominant MP in fish gills from Jakarta Bay could be associated with the predominant fiber MP in Jakarta Bay, in contrast to that in Cirebon Bay (Figure 3A). Another reason is that MP accumulation in the gill is a non-selective process as the gill can capture MP passively by filtration. In contrast, MP exposure in the gut could be accidentally ingested from the water

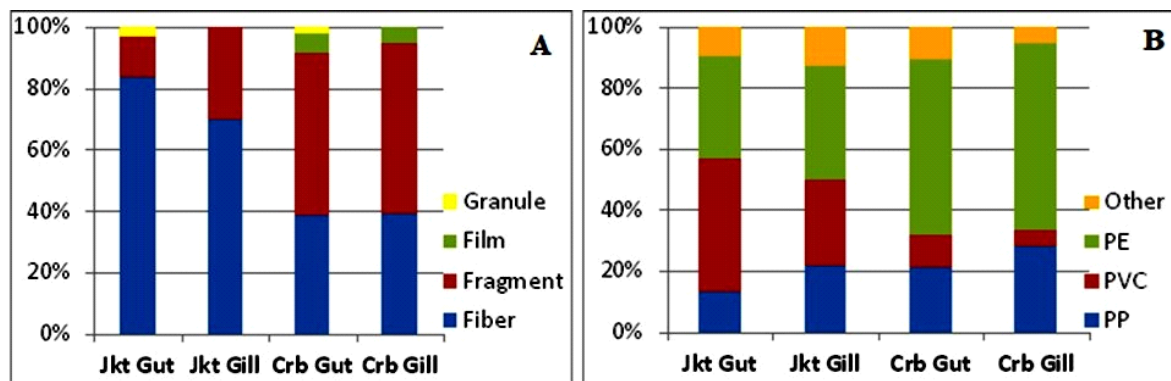


Figure 3. Microplastic profile in the gut and gill organs of fish from Jakarta Bay (Jkt) and Cirebon Bay (Crb) based on microplastic shape (A) and plastic polymer (B).

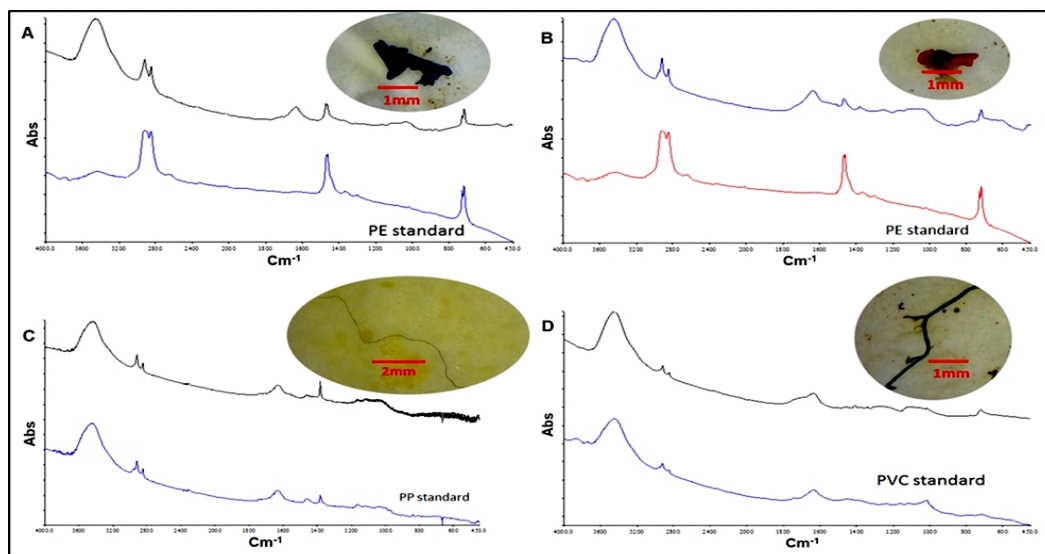


Figure 4. Visualization of microplastic samples and the FT-IR spectra(A-B: PE fragments; C-D: PP and PVC fibers)

column and seabed or from the adhered MP into natural feed (Neves et al., 2015).

Type, Size, and Color of Microplastic

As presented in Figure 3A, the predominantly MP contamination in Jakarta Bay was fiber, while in Cirebon Bay was fragmented. Microplastics in the form of granules were identified in the minor fish gut (2% of total samples) in both locations. In contrast, film MP was only detected in 4% of fish samples, especially from Cirebon Bay, both in the gut and gill organs. Identification of the polymer with FTIR showed that polyethylene (PE), polyvinyl chloride (PVC), and polypropylene (PP) were the most dominant MP in the fish samples from Jakarta Bay and Cirebon Bay (Figure 4B). In Cirebon Bay, PE was significantly more dominant than the other polymers, while in Jakarta Bay, PE and PVC were presented relatively similarly in fish samples. The examples of identified MP and their FTIR spectra are presented in Figure 4.

Based on the MP size, the majority of the identified plastics (65%) were the small size of less than 1 mm, while the bigger MP (1-5mm) accounted for 31%. Additionally, there were minor MP (4 % of the total samples) with a size of more than 5 mm found in the fish gut from Jakarta Bay (Figure 5A). Due to the instrumentation limit, the MP less than 0.1 mm could not be identified in the present study. Further identification of MP color revealed that the most dominant MP in fish from both locations were blue, black, and red with slightly different compositions between in gut and gill organs (Figure 5B). Other colors that are inconsistently present only in the gut organ are green, white, and transparent.

In aquatic ecosystems, MP potentially absorbs environmental pollutants that can disrupt the food web and cause harm to the health of aquatic organisms.

Accumulation of MP in fish organs could be vulnerable due to the possible acute toxicity to fish, such as neurotoxicity, oxidative stress, energy-related damages, intestinal blockage, reproductive toxicity, and immune response changes (Amelia et al., 2021; Barboza et al., 2018; Gao et al., 2023). Additionally, microplastics can also pose a threat to human health, as they can enter the food chain and potentially negatively impact to the consumer of contaminated seafood. More attention has been paid to MP due to its invisible size and the potential to endanger human health. Less than 20 μm MP may be transported to the human internal organ, while the smaller one (<10 μm) could penetrate the cell membrane and transport through the vascular organs and might affect the immune system and intestinal disorders (Bouwmeester et al., 2015; Lusher et al., 2017; Visalli et al., 2021). Recent studies also reported that exposure to MP has been detected in the human organs of placenta (Braun et al., 2021), testis (Zhao et al., 2023), and gallstones (Zhang et al., 2024).

The different composition of MP shapes in fish from both locations could indicate the different profiles of MP pollution in Jakarta Bay and Cirebon Bay. The result in Jakarta Bay is in line with the earlier study in PIK mangrove, Baron Bay, Bali Strait, and Papua, which showed fiber, as the most dominant MP in fish samples (Hastuti et al., 2019; Sarasita et al., 2020; Suwartiningsih et al., 2020; Yona et al., 2020). In contrast, the fragment was reported as the most dominant MP in fish from Makassar and Pangandar Bay (Ismail et al., 2019; Rochman et al., 2015). The different shapes of MP could be attributed to the different types of polymers that affect the accumulation pattern in fish, as they have different degradation rates, sizes, and densities.

The composition of plastic polymer in fish from Jakarta Bay and Cirebon Bay supports the different profiles of MP pollution in both locations. PE is also

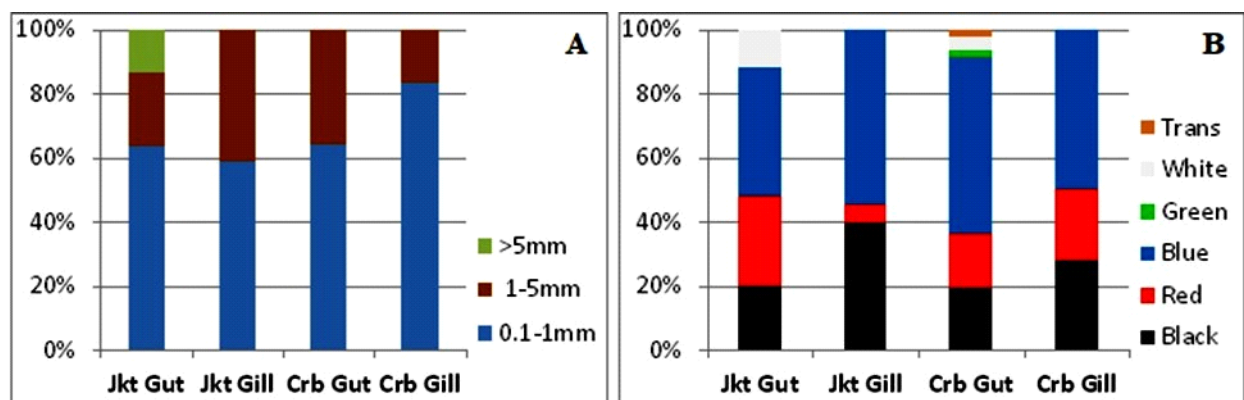


Figure 5. Distribution of microplastic in different organs of fish from Jakarta Bay (Jkt) and Cirebon Bay (Crb) based on microplastic size (A) and microplastic color (B)

Table 1. Profile of microplastic in fish from Indonesian coasts and some Asian waters

| Location | Target organ* | Nr. of species | Microplastic (particle/fish) | Dominant microplastic | | | | Ref. |
|-----------------------|--------------------------|----------------|------------------------------|-----------------------|-------------|-----------|------------|------------|
| | | | | Shape | Color | Size (mm) | Polymer*** | |
| Jakarta Bay, INA | Gut, Gill | 5 | 0-19 | Fiber | Blue, Black | 0.1-1.0 | PE, PVC | This study |
| Cirebon Bay, INA | Gut, Gill | 3 | 0-34 | Fragment | Blue, Black | 0.1-1.0 | PE, PP | |
| Baron Bay, INA | Gut | 4 | 7-97 | Fiber | Black | 0.05-0.1 | PA | [1] |
| Bengkalis, INA | Gut | 3 | 56-72 | Film | White | 0.1-0.5 | NA | [2] |
| Makassar, INA | Gut | 10 | 0-21 | Fragment | NA | +3.5 | NA | [3] |
| Bali Strait, INA | Gut | 4 | 4-7 | Fiber, Film | NA | NA | Varied | [4] |
| PIK Mangrove, INA | Gut | 9 | 0-52 | Fiber | Transparent | 0.06-0.08 | NA | [5] |
| Pangandaran, INA | Gut | 2 | 2-28 | Fragment | Varied | Varied | NA | [6] |
| Papua, INA | Gut, Gill | 8 | 1.6-16.5** | Fiber | NA | >1.0 | NA | [7] |
| East Java, INA | Gut, Gill, Stomach | 1 | 4-10 | Pellet | Black | <0.1 | NA | [8] |
| Cilacap, INA | Gut | 4 | 12-28 | Film, Fiber | Transparent | NA | Varied | [9] |
| East Java, INA | Gut, Gill, Stomach | 1 | 5-99 | Fiber | Black | 1-5 | Varied | [10] |
| Tokyo, Japan | Gut | 1 | 2-3 | Fragment | NA | <1.0 | PE, PP | [11] |
| Yangtze, China | Gut | 13 | 1-7 | Fiber | Transparent | 2.0-5.0 | CE | [12] |
| Malaysia | Gut, Gill | 11 | 0-10 | Fragment | NA | 0.1-0.5 | PP | [13] |
| Atlantic coast, Spain | GIT, Gill, Liver, Muscle | 3 | 0-19 | Fiber | Black | 0.15-1.5 | Ry, PES | [14] |
| Caspian Sea, Iran | GIT | 4 | 3-9 | Fiber | Black | 0.5-2.0 | PP | [15] |

INA: Indonesia; NA: not applicable; * GIT: gastrointestinal tract, **: (particle/g);

***PE: Polyethylene, PVC: Polyvinylchloride, PP: Polypropylene, PA: Polyamide, CA: Celophane, Ry: Rayon, PE: Polyester

[1] (Suwartiningsih et al., 2020), [2] (Amin et al., 2020), [3] (Rochman et al., 2015), [4] (Sarasita et al., 2020), [5] (Hastuti et al., 2019), [6] (Ismail et al., 2019), [7] (Yona et al., 2020), [8] (Indriyasaki et al., 2023), [9] (Hidayati et al., 2023), [10] (Marchellina et al., 2024), [11] (Tanaka & Takada, 2016), [12] (Jabeen et al., 2017), [13] (Karbalaei et al., 2019), [14] (Guilhermino et al., 2021), [15] (Gholizadeh et al., 2024)

known as the most reported MP in fish globally, followed by polystyrene (PS) and PP (de Sá et al., 2018). The result in Jakarta Bay is in line with the investigation that reported PE and PP are the most dominant meso and macroplastic pollution in the surface water of Jakarta Bay (Dwiyitno et al., 2020). A similar result was reported from the study in fish, from Bali Strait, which showed PE and PVC are the most dominant MP (Sarasita et al., 2020). Nevertheless, no available study on plastic pollution in Cirebon Bay, either in water or sediment samples.

A relatively similar result was revealed in MP color in fish from Jakarta Bay and Cirebon Bay. Blue, black, and red are the most dominant colors, with minor additional colors of green, white, and transparent in the gut organ. Black MP was also reported as the most dominant in fish from Baron Bay (Suwartiningsih et al., 2020), while white and transparent were reported as the most dominant MP from fish in Bengkalis and PIC mangrove, respectively (Amin et al., 2020; Hastuti et al., 2019). Since the majority of MP is a secondary product from macroplastic, MP color could reflect the origin of macroplastic pollution in the study area. An earlier study on meso and macroplastic in Jakarta Bay revealed that the most dominant plastic litter comes from consumer products, packaging, and fishing gear (Dwiyitno et al., 2020).

Comparison to Other Studies

Compared to the earlier studies, the concentration of MP in fish samples from Jakarta Bay and Cirebon Bay in the present study is relatively higher than that reported from Bali Strait (Sarasita et al., 2020) but comparable to that reported from Makassar (Rochman et al., 2015), Pangandaran Bay (Ismail et al., 2019), PIK mangrove (Hastuti et al., 2019), and Cilacap (Hidayati et al., 2023). A more concentrated MP was reported from Bengkalis (63 particles/fish), Baron Bay (46 particles/fish), and East Java Coast as reported by Amin et al. (2020), Suwartiningsih et al. (2020), and Marchellina et al. (2024).

Table 1 summarizes the MP contamination in fish samples from different Indonesian and some global waters as a comparison. However, the result is difficult to compare due to the different methods, sampling locations, and fish species. Compared to the other Asian waters (Malaysia, China, and Japan), the MP concentration in fish from Jakarta Bay and Cirebon Bay was relatively higher. However, the MP profiles were relatively similar to those from Malaysia, Japan, Atlantic coast, and Caspian Sea, especially in shape, size, and polymer type.

Referring to the fish species, we found the MP in benthic species (*S. javus* and *Priacanthus* sp) is more

concentrated than that in pelagic fish (*S. fimbriata* and *S. comersonii*). This result is in accordance with the earlier study conducted in PIK mangrove reported by Hastuti et al. (2019) that the same genus of *Siganus* (*S. canaliculatus*) was the most concentrated species by MP (4-52 particles/fish). Similar finding was also reported by Hidayati et al. (2023) on fish samples from Cilacap and Gholizadeh et al. (2024) on commercial fish from Caspian Sea. The different concentrations of MP between pelagic and benthic species could be associated with the feeding and migratory behaviors. Benthic species tend to ingest more MP, as most of them sink to the seabed depending on the density (Bellas et al., 2016; Gholizadeh et al., 2024). In contrast, pelagic fish accumulate MP in the water column. Therefore, it is essential to compare MP profiles in fish samples and water or sediment compartments as a suggestion for future study.

With regard to the potential risk of MP accumulation to both fish and human health when consumed via the food chain, it is important to authoritative bodies and related stakeholders to tackle and minimize plastic pollution in aquatic ecosystems. This is necessary to reduce the detrimental impacts of MP and the consequences of absorbed chemicals and pathogens for preventing potential risks to aquatic organisms and the environment. Furthermore, identification and analysis of the chemical constituents of MP is crucial due to their potential impact on fishery products and humans.

Conclusion

Microplastics were found in all fish species, either from Jakarta Bay or Cirebon Bay. In general, MP concentration in fish from Cirebon Bay was slightly higher than that from Jakarta Bay, with benthic species are more concentrated than that in pelagic fish. Microplastic contamination was more concentrated on the gill organ of fish samples from Jakarta Bay, in contrast with that from Cirebon Bay. The predominantly MP contamination in Jakarta Bay was fiber, while in Cirebon Bay was fragment with minor granules and film in both locations. PE, PVC, and PP were the most dominant MP in the fish samples from both study areas. Microplastic size showed that the majority (65%) was the small size of less than 1 mm, compared to the bigger one (1-5 mm), while blue, black, and red are the predominantly MP color in both locations. To reduce the detrimental impact of MP accumulation to fish and human health, efforts to minimize plastic pollution in aquatic ecosystems are important. Furthermore, identification and analysis of the chemical constituents

of MP is crucial due to their potential impact on fishery products and human health.

Acknowledgments

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Supplementary Materials

Supplementary materials is not available for this article

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