



ORIGINAL RESEARCH PAPER

Microplastics contamination in two peripheral fish species harvested from a downstream river

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ABSTRACT

BACKGROUND AND OBJECTIVES: The occurrence of plastic waste pollution in waters has become a major issue globally. One of the waters which tend to be polluted with plastic waste such as bags, food wrappers, and unused fishing nets, is the Krueng Aceh River, which is located in the center of Banda Aceh city, Indonesia. Microplastics in the rivers potentially contaminate the fish through the food chains, and are then transferred to humans once consumed. The two species of fish that are frequently caught by fishermen in the Krueng Aceh River and consumed by the local people are mullet *Mugil cephalus* and bagok catfish *Hexanematichthys sagor*. Both have the potential of being contaminated with microplastics that enter the river. Therefore, this study aims to analyze the status of microplastic pollution in mullet *M. cephalus* and bagok catfish *H. sagor* harvested downstream of the Krueng Aceh River, Banda Aceh, Indonesia.

METHODS: The fish samples were caught in three locations, namely in the river estuary, residential, and agricultural areas. A total of 50 mullets and 46 bagok catfish were employed for analysis. Microplastics were analyzed in the digestive tract using a microscope, while waste in the carcass was detected using the fourier transform infrared analysis.

FINDINGS: In mullet, the highest number of microplastic particles were found in fish samples caught in river estuary (16 particles/fish on average), followed by the sample from residential areas (10 particles/fish on average). Meanwhile, the lowest abundance of microplastic was recorded in sample near agriculture areas (5 particles/gram body weight). In bagok catfish, microplastic abundance in samples from the river estuary and residential areas was almost the same, and it ranged from 7-8 particles/fish. The lowest particle number was in bagok catfish caught in the region near agricultural areas. This study indicated fiber as the most dominant microplastic in the two fish species at all sampling locations. It also had three colors in the alimentary tract of mullet and bagok catfish, namely red, blue, and black, which was predominant. The fourier transform infrared spectrum showed several wavenumber peaks signifying alkane compounds' presence, which are microplastic characteristics. Based on the peak values, the presence of two polymer types was suspected, namely polyethylene, and polypropylene.

CONCLUSION: Fiber and film microplastics were found in the digestive tract of mullet and bagok catfish, where the number of particles was most abundant in the mullet. The fourier transform infrared test was also detected the presence of microplastic pollutants in both species. This indicates that mullet and bagok catfish in Krueng Aceh River have been contaminated by microplastics and are not safe for consumption.

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INTRODUCTION

Water pollution is one of the major issues in the world, and it is often caused by waste from various sources including industries, oil spills, as well as anthropogenic waste, such as plastic, which is difficult to degrade in a short period (Rodrigues et al., 2020). Plastic waste is often obtained from lands in coastal areas, where the waste drifts off to open seas, and some sink to the sea bottom (Depledge et al., 2013; Hardesty et al., 2017; Law, 2017). Furthermore, Kubota et al. (2005) and Hardesty et al. (2017) stated that the distribution of plastic debris in the oceans is caused by currents, tides, and winds. Eriksen et al. (2014) reported that more than 250,000 tons of plastic waste have been floating in the ocean, while Haward (2018) estimated 4.8 – 12.7 million tons of waste, including plastic. According to Derraik (2002), approximately 6.5 million tons of plastic waste have been polluting the ocean since the 1990s. In the last 4 years, there has been a significant increase in the volume, which is 16-48 times higher than the previously recorded value. A similar phenomenon also occurred in Indonesia, and Purba et al. (2017) revealed that 68-80% of the waste found in the Indonesian sea contains plastics, it was higher than in China approximately 44.9% (Zhou et al. 2011). According to Hastuti et al. (2014), approximately 165,000 tons of plastic waste have been polluted the Indonesian water annually. Assuyuti et al. (2018) reported that its contamination in Pramuka, Panggang, and Air Islands in Jakarta causes low coral cover in these areas. Djaguna et al. (2019) stated that various sizes of plastic waste have been recorded in the coastal waters of Tongkaina and Talawaan Bajo, North Sulawesi. Furthermore, Joesidawati (2018) revealed that sediments in coastal areas near rivers in Tuban Regency have been contaminated with microplastics. Takarina et al. (2022) reported that the surface water and sediment in Jakarta Bay were contaminated by microplastic. Microplastics in the sea mostly come from the inland (Browne, 2015; Duhec et al., 2015; Nur and Obbard, 2014), and this microplastic contaminate marine biota, including plankton, benthos, and fish (Smith and Markic 2013; Wright et al., 2013), and also salt produced from contaminated seawater (Tahir et al., 2019). Wastes in the plankton are then transferred into other aquatic biotas through the food chains once the biotas feed on contaminated plankton (Mearns et al., 2014). For

instance, approximately 80% of *Sardinella lemuru* the planktivorous fish harvested from Northern Mindanao Philippines were contaminated by microplastics (Palermo et al., 2020). This indicates that it has the potential to cause damage to digestive organs, reduce growth rates, inhibit enzyme production, reduce steroid hormone levels, and inhibit the reproductive process (Wright et al., 2013). Therefore, microplastic pollution now threatens the health of aquatic wildlife and humans (Priya et al., 2022). Banda Aceh is an urban city located in a coastal area with rapid development. Furthermore, this development is followed by an increase in population density and activities, which also increase the production of waste, including plastics with the potential to pollute waters. One of the potentially polluted waters is the Krueng Aceh River, originating from Aceh Besar District and flowing into the city of Banda Aceh. This river crosses agricultural, residential, and urban areas that are densely populated and fond of discharging wastes into the Lampulo village where there is a fish landing and fish market. Sources of plastic waste that have the potential to pollute the Krueng Aceh River include plastic bags, food wrappers, and unused fishing nets. According to Evode et al. (2021), stated that approximately 40% of plastics were used for packaging materials in various industries. A previous report by Hadi et al. (2008) showed that the Krueng Aceh river has been contaminated with heavy metals, such as Lead (Pb), Cadmium (Cd), and Zinc (Zn) at a moderate level. A similar report was also published by Sarong et al. (2015) that oysters (*Crassoscrea* sp.) in the estuary of the Lamnyong River, one of the tributaries of the Krueng Aceh River were contaminated by these elements, which indicates the presence of plastics. However, no study has examined the status of plastic waste pollution in Krueng Aceh. In this study, microplastic contamination is determined in two fish species that are often caught downstream of Krueng Aceh, namely mullet (*Mugil cephalus*) and bagok catfish (*Hexanmatichthys sagor*). This study is crucial because the local community uses the bagok catfish and mullets commercially as a protein source. Yulianto et al. (2020) stated that mullets feed on algae and detritus at the bottom of the waters, while bagok is an omnivore. The two species have the potential to be polluted by microplastics due to their feeding habit. The current results obtained can be applied as scientific information to strategize

a better waste management policy, specifically for plastics waste disposal in urban city of Banda Aceh, and Indonesia in general. These tend to also become a theoretical basis for further studies. Therefore, this study aims to analyze the presence of microplastics in the digestive tract and carcass of mullet and bagok catfish harvested in the estuary of the Krueng Aceh River, Banda Aceh City, Indonesia in 2021. .

MATERIALS AND METHODS

Date and sampling

This study was carried out from January to February 2021 at the Laboratory of the Faculty of Marine and Fisheries, Universitas Syiah Kuala, Banda Aceh. The fish sample was collected at three stations in the estuary of Krueng Aceh River. Station 1 was located at the river mouth close to the fish market, jetty, and garbage processing station; Station 2 was situated approximately 7.46 Kilometers (km) from the river mouth, and was close to the residential area; Station 3 was located approximately 16.10 Kilometers (km) from river mouth close to agricultural plantations, as shown in Fig. 1. The fish sample was caught using two sets of gillnets with the mesh size of 15 millimeters (mm) and 20 mm with 25 meters (m) length and 1.2 m depth. The sampling was carried out at one-week intervals for one month. The samples were kept in an ice box at 4 degrees Celsius ($^{\circ}\text{C}$), and then transported

to the laboratory in Universitas Syiah Kuala Banda Aceh for further analysis.

Sample preparation

The fish samples were measured for their total length using digital calipers and weighed with a digital balance. The abdomen of the fish was dissected using scissors, after which the alimentary tract, namely the stomach and intestine was removed from the body cavity, and then preserved with 4% formaldehyde (Jantz *et al.*, 2013). Subsequently, the fish were weighed, dissected, and their contents were removed and placed on separate plates. The contents were then diluted using saturated NaCl three times the weight of the stomach and intestines.

Microplastics and data analysis

Approximately, 1 milliliter (mL) of the stomach and intestines content that was diluted with NaCl was taken and then placed on a sample plate. The sample was observed with the eye naked, where microplastic particles that are visible were separated from the rest. Meanwhile, those that are invisible were identified using stereo and binocular microscopes at 40x and 100x magnification. The microplastics obtained were then measured in size, followed by identification of their type and color. The abundance of microplastics was also calculated based on Boerger *et al.* (2010).

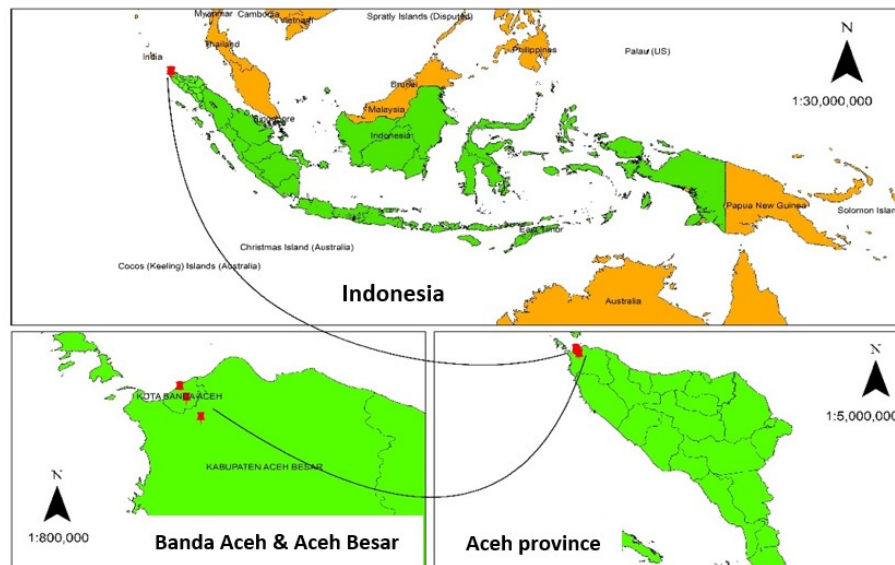


Fig. 1: Geographic location of the Indonesian archipelago showing the sampling location (red dots) from the river mouth to agricultural plantations

Microplastics contamination in mullet and bagok catfish

Table 1: The average abundance of microplastic at the alimentary tract of mullet *Mugil cephalus* and bagok catfish *Hexanematchthys sagor* according to location and type of microplastics

Species	Sampling site	N*	The average weight of fish (g)	Type of microplastic		Particles/ fish	Particles/ gram of fish
				Fiber	Film		
<i>M. cephalus</i>	River mouth	20	78	8	8	16	0.21
	Residential	15	106	5	5	10	0.10
	Agricultural	15	85	3	2	5	0.06
<i>H. sagor</i>	River mouth	16	118	4	4	8	0.07
	Residential	15	136	4	3	7	0.05
	Agricultural	15	129	2	1	3	0.02

*N= number of fish sample

Table 2: The average abundance of microplastic at the alimentary tract of mullet *Mugil cephalus* and bagok catfish *Hexanematchthys sagor* according to the location and colour of microplastics.

Species	Sampling site	Fish sample (N*)	Colour of microplastic (Particles/ fish)			
			Red	Blue	Black	Total
<i>M. cephalus</i>	River mouth	20	4	4	8	16
	Residential	15	2	3	5	10
	Agricultural	15	1	1	3	5
<i>H. sagor</i>	River mouth	16	1	3	4	8
	Residential	15	1	2	4	7
	Agricultural	15	1	1	1	3

*N= number of fish sample

FT-IR analysis

The type of microplastic polymer in fish carcasses was detected using Fourier Transform Infrared (FT-IR) spectroscopy with the KBr (Potassium Bromide) pellet method (Nor and Obbard, 2014). The wavelength spectrum of the polymeric produced by FT-IR was analyzed using a software to read the spectrum and then compared to the standard spectrum from the plastic polymer database using Euclidean Distance to determine the type of polymer in the sample (Lusher et al., 2013).

Data analysis

The data were presented in the tables and figures, followed by descriptive analysis using comparison with the previous reports and other related references.

RESULTS AND DISCUSSION

A total of 50 mullets and 46 bagok catfish were caught from three sampling sites. The results showed that in mullet, the highest number of microplastic particles was found in samples caught in the river mouth close to the fish market and garbage processing station,

namely 16 particles/fish on average, followed by the residential areas with 10 particles/fish on average. The lowest value was recorded at the sampling site close to agriculture areas, namely 5 particles/fish. In bagok catfish, the abundance of microplastics in samples from the river mouth and residential areas was almost the same, and it ranged from 7-8 particles/fish. The lowest abundance of microplastic (3 particles/fish) was in catfish caught in the region near agricultural areas, as shown in Table 1.

The fiber was the most dominant microplastic in the two species of fish at all sampling locations. It also had three colors in the alimentary tract of mullet and bagok catfish, namely red, blue, as well as black, which were predominant, as shown in Table 2.

The results showed that the size of microplastics in the mullet ranged from 20 micrometers (μm) to 120 μm , and from 20 μm to 80 μm in bagok catfish, where the dominant size was 20-40 μm in both species, as summarized in Table 3.

FT-IR analysis on the carcass of both fish species showed that the wavenumber of 3,570/cm – 3,200/cm, indicated that there was a peak of OH stretch. The range of 2,935/cm – 2,915/cm indicated the presence

Table 3: The size ranges of the microplastic found in the alimentary tract of the mullet *Mugil cephalus* and bagok catfish *Hexanematichthys sagor* according to location.

Fish species	Microplastic size	Sampling site (Particles/ fish)			Total
		River mouth	Residential	Agricultural	
<i>M. cephalus</i>	<20 μm	5	3	2	10
	20-40 μm	7	5	3	15
	40-60 μm	2	1	-	3
	60-80 μm	1	1	-	2
	80-120 μm	1	-	-	1
<i>H. sagor</i>	<20 μm	2	2	1	5
	20-40 μm	4	4	2	10
	40-60 μm	1	1	-	2
	60-80 μm	1	-	-	1
	80-120 μm	-	-	-	-

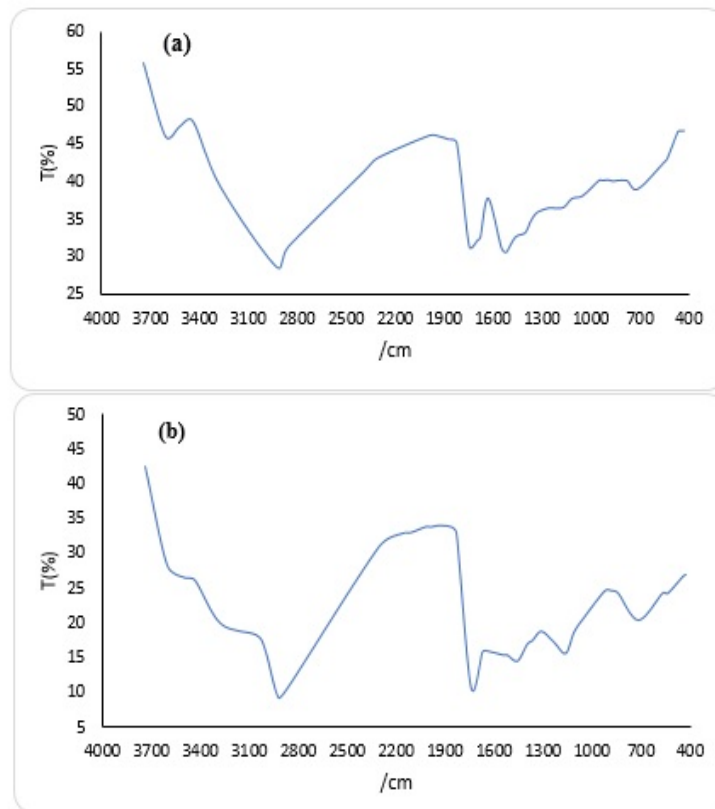


Fig. 2: The graph of the FT-IR analysis of carcass of (a) the mullet *Mugil cephalus*, and (b) bagok catfish *Hexanematichthys sagor*

of a peak of C-H stretch with strong intensity, while 1,375-1,450 showed CH_3 bending. A sharp and strong peak also occurred at 1,740/ cm due to C=O from acid, as shown in Fig. 2. Therefore, the study showed that mullet and bagok catfish harvested from Krueng Aceh River estuary were contaminated by microplastics,

and mullets contained more pollutants than the bagok catfish in all sampling sites.

The highest number of microplastic particles were found in fish samples caught in the river mouth area, followed by a site close to residential areas. The plastic waste in the river mouth mostly comes from

the garbage processing station and market near this site. The pollutant is blown by the wind into the river, and eaten by fish accidentally or through the food chain, for instance, plankton and other small fish that have been contaminated by microplastic. In sampling locations close to the residential area, the waste produced is often organic waste mixed with plastics. People who live on the banks of the river have a bad habit of throwing garbage directly into the water. Some of the waste are carried by the current to the river mouth, and this increases the plastic content of fish in this region, as recorded in this study. The study showed that the abundance of microplastics in the fish from Krueng Aceh river ranged from 10 -16 particles/fish. The stated values were higher than in fish from the Bengal Bay up to 2.2 particles per fish (Gosh et al., 2021). Conversely, they were lower compared to fish harvested from Great Lake, North America namely 35-59 particles/fish (Munno et al., 2021), and in cultured fish at the Pearl River estuary, South China, namely 35 particles/fish (Lam et al., 2022). In general, microplastic pollution in freshwaters such as rivers and lakes is greater than in the sea because freshwater is very intense and close to community activities, including industrial and residential areas (Rezania et al., 2018). The results showed that two types of microplastics were found, namely fiber, and film. These particles were most likely produced from used plastic bags, food wrappers, and gillnet that were dumped by fishermen into the river. This is because the first location (river mouth) was also close to the fish landing jetty. Napper et al. (2022) stated that fiber can be sourced from boat ropes and fishing gear discarded by fishermen. A'yun (2019) reported that it was dominantly found at the mouth of the Begawan Solo river, Gresik, Indonesia, which is a site for fishing boat mooring. Nor and Obbard (2014) also stated that fiber was produced from the degradation of various types of gillnet and boat ropes that broke down into the waters, and settled in the sediment. Pivokonsky et al. (2018) revealed that it is the most common morphotype of microplastics found in water samples. The abundance of microplastics in water depends on their density, where low-density particles, such as Polyethylene (PE) and Poly Propylene (PP) float in water, while others with high density, including High-Density Polyethylene (HDPE), tend to sink and be deposited in sediments (Sul and Costa, 2014). The PP and PE have densities of 900 kg/m³ and 857 kilogram

per cube meter (kg/m³), respectively, while the density of surface water ranges from 1001.50 - 1021.33 kg/m³. It was suspected that the microplastics found at the sampling sites mostly belonged to the PE and PP group, which came from used plastic bags, nylon gillnet, and fishing lines discarded by fishermen as mentioned above. Saleem et al. (2018) stated that these two categories are the most common plastic waste (Saleem et al., 2018), and these microplastics are frequently found in the waters in the forms of film and fiber (Liu et al., 2022; Zhang et al., 2019). Microplastic contamination in fish occurs through plastic waste that is eaten by fish accidentally. These particles cannot be digested and are difficult to discharge, hence, these wastes are left in the alimentary tract and absorbed into the tissue (Yona et al., 2020). This finding is in line with the FT-IR analysis, where microplastics were detected in the carcass (muscles) of the two species. The results showed that the abundance of these particles in the digestive tract of mullet was higher than that bagok catfish. This is caused by the feeding habit of mullets as detritophages (Jamabo and Maduako, 2015), and the mullet fed on detritus mixed with plastic waste at the bottom of the waters. Therefore, it was suspected that the sediment of the Krueng Aceh has also been polluted by microplastics. A series of studies are currently being carried out to examine this speculation. Black microplastics were the dominant particles in all fish samples due to their long duration in the waters or in the digestive tract of fish, and the color changed because of the discoloration process. Microplastics with dark colors probably come from polyethylene polymers, which have low density and are the main material for plastic bags and container production (GESAMP, 2015). Hidalgo-Ruz et al. (2012) stated that the dark coloration indicates the presence of many contaminants and other organic particles. The dark microplastics have a high ability to absorb pollutants (Basri et al., 2021; Shuo et al., 2021), including polycyclic aromatic hydrocarbons (PAHs), which are commonly found in aquatic ecosystems. Phenanthrene compounds (Phe) are one of the PAHs reported being toxic in fish and humans (Karami et al., 2016). Therefore, when these microplastics absorb the pollutants, their texture becomes coarse and dense (Hiwari, 2019). All types and colors of microplastics are harmful to health, but it was suspected that dark particles are more toxic because its contain higher pollutants. Sugandi et al. (2021), and Ghaffar et al.

(2022) reported that microplastics harm human health, including causing brain inflammation. This compound can also cause oxidative stress, which is a condition where the number of free radicals in the body exceeds the limits. Microplastics can also enter the intestines through food, and interfere with the digestive system (Hollman *et al.*, 2013). The microplastic in the digestive tract is then further absorbed, then enters the blood circulation to cause cancer, diabetes, skin irritation, cardiovascular disease, as well as respiratory and reproductive problems (Murray and Cowie, 2011; A'yun, 2019). The results showed that the size of microplastics varied in fish samples, where the dominant size was 20-40 μm . According to Ng and Obbard (2006) and Barnes *et al.* (2009) microplastic of sizes 1 μm to 500 μm are commonly found in seawater. It was influenced by several factors, one of which is the duration of time in the waters. The longer the time taken, the more it decomposes, which makes the size becomes smaller. Small microplastics are easier to enter the body because they are difficult to detect (Lim, 2021). The FT-IR analysis showed that the carcass of mullet and bagok catfish harvested from Krueng Aceh River have been contaminated by microplastics. The spectrum of the FT-IR shows several wavenumber peaks indicating the presence of alkane compounds, which are characteristic of microplastic. Based on peak values, it was suspected that there are two types of polymers present, namely polyethylene (PE) and polypropylene (PP). This was indicated by the peak wavenumber in the range of 2,935-2,915 cm^{-1} , which shows the presence of CH stretch bonds and 1,375-1,450 cm^{-1} for $-\text{CH}_3$ bending. The CH bond can be used as an indication of the presence of PE and PP (Syakti, 2017). The PE was produced from plastic bags, detergent packages, and shampoo bottles, while PP can be obtained from bottle caps, straws, buckets, and plastic toys (Barron and Spark, 2020; Hoseini and Bond, 2022).

CONCLUSIONS

Presently, the Krueng Aceh river in Banda Aceh city, Indonesia is potentially polluted by domestic wastes including microplastics. Similar to other urban cities in Indonesia, irresponsible people use rivers as a place for direct waste disposal without any processing it. Previous study has also reported the contamination of biotas such as the oyster *Crassostrea gigas* from this

river with heavy metals. The current study examined microplastic contents in the two dominant fish species found in the downstream of Krueang Aceh river, namely the mullet *M. cephalus*, and the bagok catfish *H. sagor*. The fish samples were collected from several locations in the downstream river of Krueng Aceh. The digestive tract of both fish was discovered to be contaminated with microplastics. The highest particle content was found in mullet and bagok catfish caught at the river estuary. There were two types of microplastics in this study, namely film and fiber. The dominant particle was black with a size range of 20 - 80 μm . The FT-IR analysis spectrum of the carcass mullet and bagok catfish samples showed several wavenumber peaks. This indicates Alkane compound's presence as a characteristic of microplastics with two polymer types, namely Polyethylene, and Polypropylene which mean the fish are not safe for consumption. Therefore, a better management strategy needs to be planned for the Krueng Aceh river by developing the laws and socializing with the people followed by consistent law enforcement. In Banda Aceh, even in Aceh province, a plastic waste recycling facility should be built immediately as an effort to reduce plastic waste disposed into the environment. The policy of selecting wastes based on their type and form also needs to be implemented to ensure plastic waste are separated and then recycled. In the short term, the obtained results have to be socialized to people in Banda Aceh that the two studied fish species and possibly other species living in this river are not safe for consumption because their microplastic content is harmful to health.

AUTHORS CONTRIBUTIONS

M.R. Maulana performed survey, collecting data, processing data, and prepared the first draft of the manuscript. S. Saiful conducted survey, analyzed and processed the FTIR data, and prepared the first draft. Z.A. Muchlisin developed the research concepts, data analysis, financial acquisition, and approval of the final draft of the manuscript

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest regarding the publication of this work. In addition, the ethical issues including plagiarism, informed consent, misconduct, data fabrication and, or falsification, double publication and, or submission, and redundancy have been completely witnessed by the authors.

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ABBREVIATIONS

μm	Micrometer
%	Percent
°C	Degrees Celsius
<i>Cd</i>	Cadmium
<i>CH</i>	Alkane
CH_3	Methyl
<i>C=O</i>	Carbonyl
<i>cm</i>	Centimeter
<i>FT-IR</i>	Fourier Transform Infrared
<i>g</i>	Gram
<i>HDPE</i>	High density polyethylene
<i>KBr</i>	Potassium bromide
<i>kg</i>	Kilogram

<i>km</i>	Kilometer
<i>m</i>	Meter
<i>mL</i>	Milliliter
<i>mm</i>	Millimeter
m^3	Cube meter
<i>N</i>	Number of fish samples
<i>OH</i>	Hydroxyl
<i>sp.</i>	Species
<i>PAHs</i>	Polycyclic Aromatic Hydrocarbons
<i>Pb</i>	Lead
<i>PE</i>	Polyethylene
<i>Phe</i>	Phenanthrene
<i>PP</i>	Poly Propylene
<i>Zn</i>	Zinc

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