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Microplastic Contamination in Fish, Water and Sediment from Milkfish Ponds: Environmental Insights from Kasemen District, Banten Province, Indonesia

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Abstract: Plastic waste discarded into the environment can easily enter water bodies. One as such for an example is the milkfish farming ponds in Kasemen Subdistrict, which have become a dumping ground for household waste. This has led to the entry of plastic waste into the pond waters, which can be degraded into microplastics. This study aims to identify the content and abundance of microplastics in fish and other fish parts such as: flesh, intestines, stomach, gills, water, and sediment, as well as to measure the water quality in the milkfish farming ponds in Kasemen Subdistrict, Serang City, Banten Province. A total of 12 milkfish were collected from 6 stations, with 2 fish taken from each station using the random sampling method across 6 hectares of the farming ponds. The quantity and types of microplastics identified in the samples were analyzed using descriptive statistical analysis with software brand Meiji Techno. The results showed that the milkfish farming ponds in Kasemen Subdistrict were contaminated with microplastics, found in the fish's flesh, gills, intestines, stomach, water, and sediment. The microplastics identified were fiber, film, and fragment types. FTIR test results indicated that the microplastics found were made of polypropylene (PP) polymer.

Keywords: milkfish (Chanos chanos), microplastics, polypropylene, pond

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1. Introduction

Waste has become a problem for humans and the environment. Waste comes in various types, one of which is plastic waste (Suprijanto *et al.*, 2021). Plastic waste takes a relatively long time to decompose. Due to this extended period, plastic in aquatic environments will undergo a degradation process, breaking down into smaller particles known as microplastics (Tuhumury and Ritonga, 2020). Microplastics are plastic particles smaller than 5 mm. Microplastics have been studied for differences in molecules, structure, size, shape, color, and source, making them diverse contaminants or pollutants (Rochman *et al.*, 2019). Water, sediments, plants and aquatic animals can be contaminated by microplastics at varying concentrations (Cole *et al.*, 2011).

The milkfish (Chnos chanos) farming ponds in Kasemen Subdistrict have become a dumping ground for household waste. The surrounding community disposes of household waste, especially plastic waste, around the edges of the ponds. This contributes to the entry of plastic waste into the ponds, where it can be degraded into microplastics, allowing

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the Kasemen ponds to accumulate microplastics, leading to the contamination of aquatic organisms. Furthermore, these farming ponds are located close to the sea, where plastic waste is present, allowing water flow to carry plastic waste into the aquaculture environment.

The entry of microplastics into water can occur through two categories: 1) primary microplastics, which are small plastic particles that enter marine environments directly, and 2) secondary microplastics, larger particles resulting from the fragmentation of plastic pieces (Ambarsari and Anggiani, 2022). Fish can ingest microplastics accidentally during feeding because their shape is similar to the fish's food, or because the prey itself has been contaminated by microplastics (Yona *et al.*, 2020). Monitoring efforts of microplastics in water, sediment, and organisms (both qualitatively and quantitatively), field sampling techniques, and laboratory tests (identification and quantification of particles) have been extensively conducted to enhance understanding of the negative impacts of microplastics on the environment (Wang and Wang, 2018).

Milkfish is a leading brackish water fish commodity, providing good nutritional value at an affordable price ranging from Rp. 36.000 to Rp. 40.000 for 1 kg, and is popular among the Indonesian population (Akhmadi *et al.*, 2019). The production of milkfish in Banten Province in 2020 reached 12,585 tons (Eris *et al.*, 2020). The location of the milkfish farming ponds, which are close to both the sea and residential areas, makes it possible to find microplastic content accumulated in the body organs of milkfish.

The objective of this research is to identify the content and abundance of microplastics in fish, sediment, and water, as well as to measure water quality in the milkfish farming ponds. Since research on microplastic accumulation in milkfish farming ponds is still limited, the result of this research is expected to provide important information for relevant parties regarding the presence of microplastic accumulation in fish, sediment, and water in the milkfish farming ponds in Kasemen Subdistrict, Serang City.

2. Materials and Method

2.1. Time and Location of Sampling

The research was conducted from March to April 2023. Samples of fish, sediment, and water were collected, and water quality measurements were taken at the milkfish farming ponds in Kasemen Subdistrict, Serang City, Banten Province. The analysis of microplastic content in milkfish samples was carried out at the Aquaculture Laboratory, Sultan Ageng Tirtayasa University. Additionally, Fourier Transform Infrared (FTIR) ATR (Attenuated Total Reflectance) tests were conducted at the Integrated Laboratory and Research Center (ILRC), University of Indonesia.

2.2. Sample Collection

Fish sampling was carried out in the milkfish farming ponds, located in Kasemen Subdistrict, Serang City. A total of 12 milkfish were collected from 6 stations, with two fishes taken from each station using the random sampling method across 6 hectares of the farming ponds. This method refers to the research conducted by Sawalman *et al.* (2021). Simple random sampling, also is a method of selecting samples from the population randomly and straightforwardly, ensuring each population member has an equal chance of being chosen (Harahap *et al.*, 2018).

Sampling was conducted weekly: week 1 (station 1), week 2 (station 2), week 3 (station 3), week 4 (station 4), week 5 (station 5), and week 6 (station 6). The samples taken from these 6 points include the inlet, middle, and outlets areas. Stations 1 and 2 represent the inlet area, stations 3 and 4 represent the middle area, and stations 5 and 6 represent the outlet area.

Sediment samples were collected from each station using a 2-inch PVC pipe. Sediment sampling was conducted at a depth of 16 cm from the surface. The sediment samples were labeled with identification tags. Meanwhile, water samples were taken from each station using a plankton net at the water surface and stored in sample bottles. Water quality parameters (pH, temperature, and salinity) were measured at each station. According to Humaerah and Rasyid (2024), pH, temperature, and salinity are significant factors affecting microplastics.

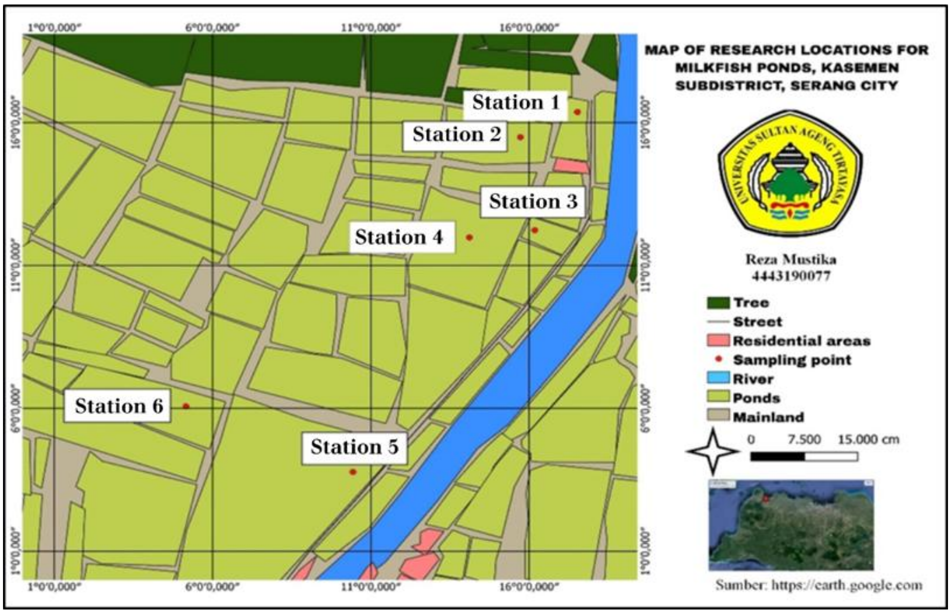


Figure 1. Map of the research location for sampling in the milkfish aquaculture pond

2.3. Microplastic Sample Analysis

2.3.1 Milkfish Sample Analysis

The total length of the milkfish was measured using a 30 cm ruler, and the weight was recorded using a digital scale. The fish were dissected from the anus to the dorsal area, following the lateral line to the head and downward to expose the stomach and internal organs. The flesh, gills, and digestive organs (intestines and stomach) were removed. Each organ was weighed and placed in labeled sample bottles 100 ml.

The sample extraction method followed Purnama *et al*. (2021), the samples were placed in sample bottles, then a 10% v/w KOH solution was added at a 1:3 sample volume ratio. The bottles were sealed and incubated at room temperature for 24 hours. Once the organs had dissolved, the samples were filtered using Whatman No. 42 filter paper and dried in an oven at 75°C for three hours. The dried samples were observed using a stereo microscope, and documentation was done with a camera. Identified microplastics were placed into a tube with a sample weight of 1 gram for FTIR ATR testing.

After collecting sample data, the abundance of microplastics was calculated. According to Purnama *et al*. (2021), microplastic abundance can be calculated using the following formula:

$$K = \frac{\mathrm{Ni}}{\mathrm{N}} \quad \text{.... Eq.1}$$

where:

K = microplastic abundance (particles/individual)

Ni = number of microplastic particles found (particles)

N = number of fish (individuals)

2.3.2 Sediment Sample Analysis

Sediment samples were dried in an oven at 70°C for 48 hours. Once dried, the sediment was ground and sieved using Whatman No. 42 filter paper, and 25 grams of the sample were placed in a sample bottle (Layn *et al*., 2020). The filtered samples were mixed with NaCl solution at was added at a 1:3 sample volume ratio and left for 24 hours. Afterward, the samples were observed using a stereo microscope. The formula to calculate microplastic abundance in sediment is as follows (Putro, 2021):

$$Microplastic\ abundance = \frac{\text{The number of microplastics in sediment (particles)}}{\text{Sediment weight (grams)}} \quad \text{.... Eq.2}$$

2.3.3 Water Sample Analysis

The water samples, stored in 150 ml sample bottles, were mixed with NaCl solution at a ratio of 1 part water to 3 parts NaCl solution and left for 24 hours to separate any remaining plastic contaminants. After 24 hours, 1 ml of the water sample was taken using a pipette and observed under a stereo microscope (Hasibuan *et al*., 2021). Chemical substances like NaCl can separate polymers with lower density since NaCl has a high density (Sutanhaji *et al*., 2021). The formula to calculate microplastic abundance in water is as follows (Syachbudi, 2020):

$$C = \frac{n}{v} \quad \text{.... Eq.2}$$

where:

C = microplastic abundance (particles/ind)
n = number of microplastic particles in water
v = filtered water volume (mL)

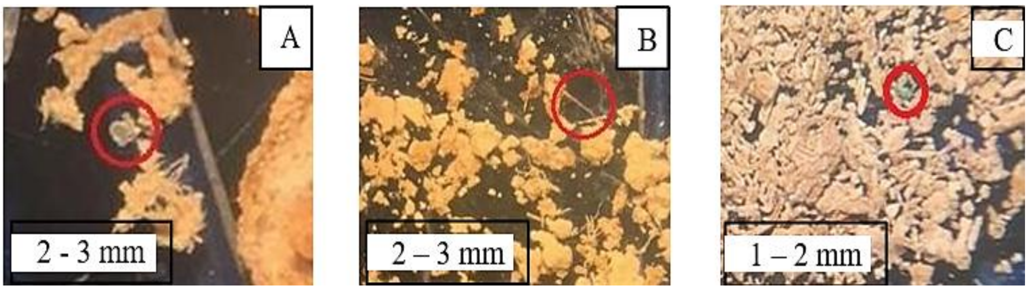
2.3.4 Fourier Transform Infrared (FTIR) Test

The Fourier Transform Infrared (FTIR) test aims to determine the type of plastic polymer contained in the microplastics found in the digestive organs of milkfish. The identified microplastics were placed in tubes for classification based on type (film, fiber, and fragment), texture (hard, soft, thick, and thin), and color of the microplastics found in the milkfish samples.

3. Results and Discussion

3.1. Abundance of Microplastic Types

The identification of microplastics using a stereo microscope on milkfish, sediment, and water in aquaculture ponds revealed the presence of film, fiber, and fragment types (Figure 2). The presence of microplastics in an environment is influenced by oceanographic factors such as currents and waves, allowing them to travel vast distances from their pollution sources (Yona *et al*. 2020). This milkfish aquaculture pond is located close to the sea and residential areas, which affects the abundance of microplastics found in this research. Visually, during data collection in the field, various types of plastic waste could be seen in the area. According to Ayuningtyas *et al*. (2019), the entry of microplastics from the sea into the water column is caused by current. Microplastics carried by currents will accumulate in the aquaculture pond in Kasemen Distric.



a) Film b) Fiber c) Fragment

Figure 2. Types of microplastics found in the milkfish pond

In figure 2, film-type microplastics are found in the flesh organ, fiber-type microplastics are found in the gill organ, and fragment-type microplastics are found in the intestine organ. Film-type microplastics have characteristics resembling sheets or plastic fragments and are transparent in color. They are commonly used in the production of plastic bags or packaging. Amin *et al*., (2020) stated that film-type microplastics have a lower density compared to other types of microplastics, allowing them to float in the water column. Consequently, when fish feed, microplastics may unintentionally enter their mouths. Fiber-type microplastics are typically characterized by fibers or shapes resembling fishing nets, often colored red or black. Fragment-type microplastics appear as shards from bottle waste, jars, mica, or small pieces from PVC pipes, and they are typically blue, green, or yellow (Ambarsari and Anggiani 2022). Fragment-type microplastics are frequently found in fish intestines due to their widespread dispersion in the water column (Amin *et al*., 2020). Microplastics enter aquatic environments from the sea, carried by currents (Ayuningtyas *et al*., 2019).

3.2. Microplastic Abundance in Milkfish

Microplastic abundance in milkfish was identified in the gills, flesh, stomach, and intestines. At stations 1, 4, and 5, microplastic abundance was dominated in the gills, with 22 particles/ind, 6 particles/ind, and 11 particles/ind, respectively. Meanwhile, at stations 2, 3, and 6, the flesh had the highest abundance, with 8 particles/ind, 6 particles/ind, and 6 particles/ind, respectively. This may be due to the milkfish consuming microplastics either intentionally or unintentionally.

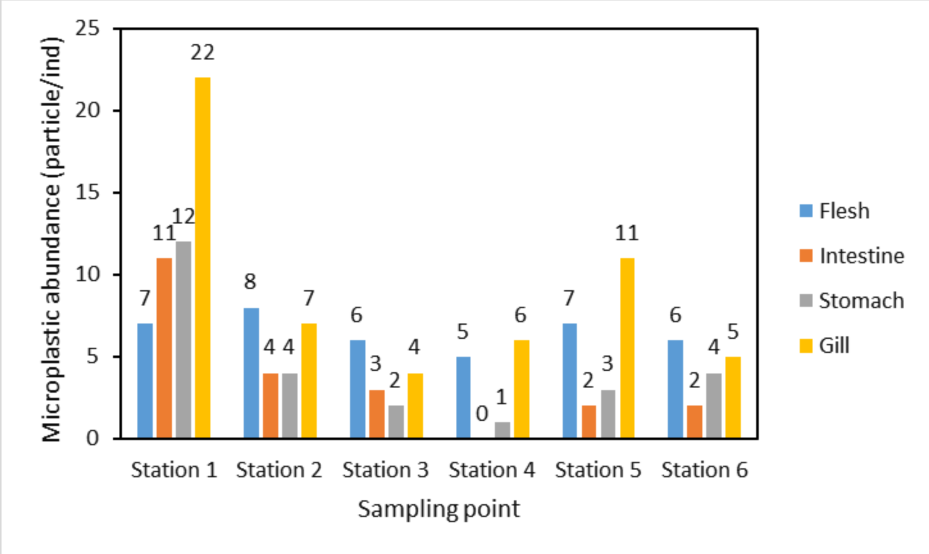


Figure 3. Microplastic abundance in milkfish (flesh, intestines, stomach, and gills)

Microplastics can accumulate in various fish organs, including flesh, gills, and the digestive system (intestines and stomach). The digestive system is where microplastics accumulate as part of the fish's feeding process. Research shows that flesh and gills contain higher microplastic levels than the digestive system (Figure 3). This aligns with Yona *et al.* (2020), who found that microplastics accumulate more in the gills than in the digestive system. Utomo *et al.* (2022) explained that microplastics consumed by fish enter the gastrointestinal tract. If not excreted through urine or feces, microplastics can pass through the gastrointestinal walls and spread to other tissues or organs via the bloodstream. The flesh is one tissue where microplastics can accumulate. Utomo *et al.* (2022) stated microplastics consumed by fish then enter the gastrointestinal tract. If microplastics are not excreted from the body through urine and feces, they can pass through the gastrointestinal tract walls and spread to body tissues or other organs via the bloodstream. Annas (2023) added that aquatic organisms may stop feeding due to a false sensation of fullness caused by indigestible microplastics in the stomach, potentially leading to death from starvation. Yona *et al.* (2020) stated that microplastics entering fish gills come directly from the water as part of the fish's respiration process.

3.3. Correlation Between Milkfish Weight and Microplastic Abundance

The relationship between milkfish weight and microplastic abundance was studied to determine whether fish weight affects microplastic abundance. The relationship between fish weight and microplastic abundance is presented in table 1.

Table 1. Relationship between milkfish weight and microplastic abundance

Model Summary		**Anova**			**Coefisien**	
R	R Square	df	F calculated	Significance	t calculated	Significance
0.255	0.065	1 22 23	1.525	0.230	1.235	0.230

The correlation coefficient (R) is 0.255, and the coefficient of determination (R square) is 0.065. With a significance value of 0.230 > 0.05, this indicates that fish weight has no significant effect on the number of microplastics. Fachrudin (2022) concluded that fish weight does not influence the amount of microplastics. The amount of microplastics entering fish bodies is more influenced by feeding habits. Microplastics have a size of less than 5 mm. Hermawan *et al*. (2022) stated that fish may unintentionally consume microplastic particles while foraging. Margaretha *et al*. (2022) found that the presence of microplastics in fish is related to the species studied, habitat, feeding habits, plastic particle density, and the presence of microplastics in the aquatic environment.

3.4. Microplastic Abundance in Aquaculture Pond Water

The study results show that microplastic abundance in aquaculture pond water is dominated by the film type. Station 3 had the highest number of microplastics, with 6 film-type particles/mL, while station 1 had the fewest, with 1 film-type particle/mL. Fiber-type microplastics were the least common in pond water.

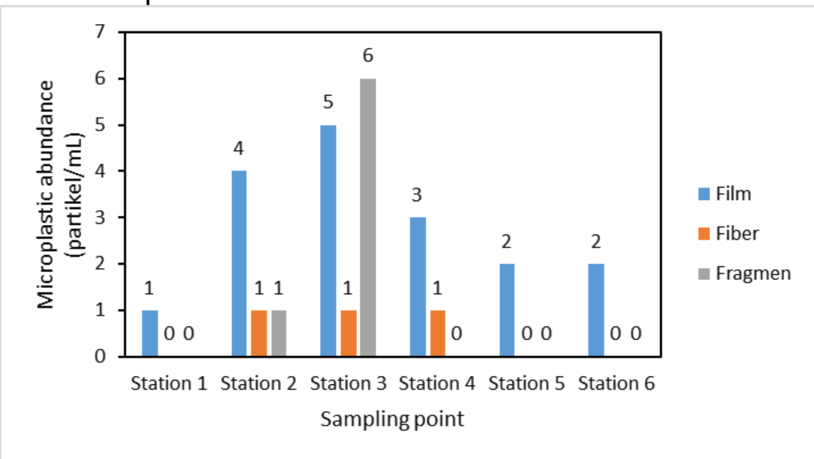


Figure 4. Microplastic abundance in aquaculture pond water

The abundance of film microplastics in aquaculture ponds suggests the presence of macro debris, such as plastic bags, bottles, plastic wrap, and other waste. The dominance of film-type microplastics in pond water is due to human activity, such as disposing of food packaging near ponds or coastal areas close to the ponds. These wastes are carried into the ponds by currents or waves. At stations 2 and 3, fragment-type microplastics were found. This corresponds to the condition of stations 2 and 3, where a significant amount of plastic bottle and mica waste was carried by currents from the sea. Amin *et al*. (2020) stated that film-type microplastics have a lower density than other types, allowing them to float in the water column. Ayuningtyas *et al*. (2019) noted that film-type microplastics consist of thin sheets from packaging, are transparent, and have a lower density, making them easily transportable. This accounts for the dominance of film-type microplastics in aquaculture pond water. Putri (2021) stated that microorganisms and other particles can cause film microplastics to sink, accumulating at the bottom of the water and potentially being ingested by fish.

3.5. Microplastic Abundance in Aquaculture Pond Sediment

Microplastic abundance in pond sediment was dominated by fragment-type microplastics at all stations. Station 1 had the highest number, with 14 fragment-type particles/g, while station 4 had the fewest, with 1 fragment-type particle/g (Figure 5). Station 1 is the station located near the sea, which may result in a large accumulation of plastic waste carried by currents from the sea to station 1. This leads to a very high abundance of fragment-type microplastics at station 1.

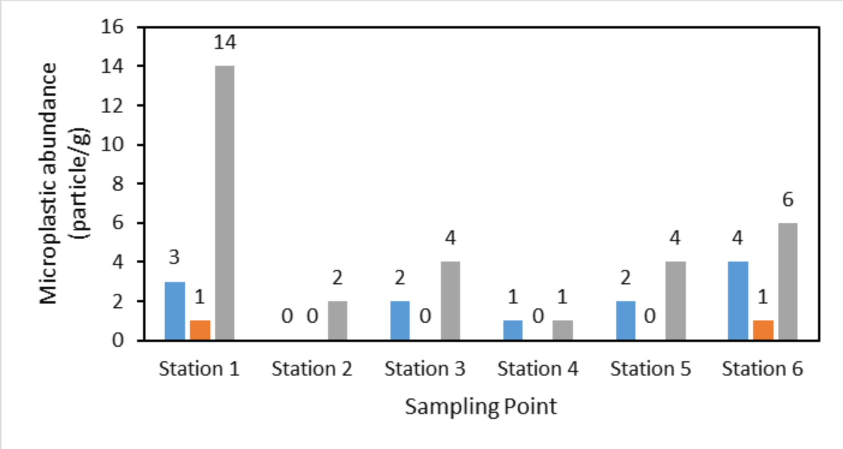


Figure 5. Microplastic abundance in aquaculture pond sediment

Azizah *et al.* (2020) explained that microplastics entering water bodies eventually settle in sediment. The presence of microplastics in sediment poses ecological risks. Fragment-type microplastics dominate sediment due to human activities, particularly the disposal of plastic bottles and mica-based waste near aquaculture ponds. Layn *et al.* (2020) stated that the high abundance of fragment-type microplastics results from the dominant plastic bottle waste along water bodies. Sarasita *et al.* (2020) noted that fragment-type microplastics have higher density, making them more likely to sink to deeper waters or sediment. Yona *et al.* (2019) found high levels of fragment-type microplastics in aquatic sediment. Another factor that can influence the presence of microplastics in fish ponds is the local residents disposing of waste in the pond area, as well as the currents from the sea flowing into the pond. As a result, the fish in the pond can accumulate microplastics.

3.6. Aquaculture Pond Water Quality Measurement

Water quality measurements in aquaculture ponds were conducted at each research station, assessing parameters such as temperature, pH, and salinity. Water quality parameters were measured at the same time as fish and sediment sampling. The results are shown in Figure 6.

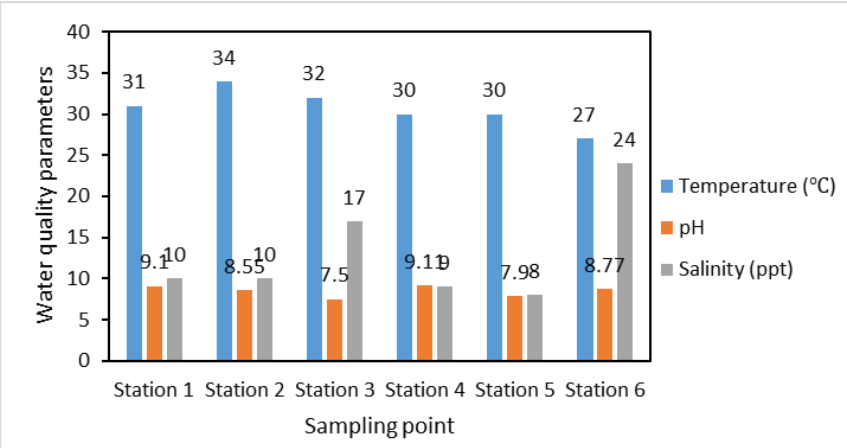


Figure 6. Aquaculture pond water quality

Milkfish can survive in temperatures of 27–35°C. A pH range of 6–9 is ideal for milkfish farming, and the fish can live in salinity levels of 0–35 ppt (Wahyuni *et al.*, 2020). This shows that the water quality in aquaculture ponds remains within normal limits for milkfish farming. Water quality affects the survival, growth, and development of aquatic organisms. If water quality is good, it supports the survival of aquatic life such as fish.

Water temperature in ponds fluctuates more than in coastal waters due to the smaller water volume and larger surface area, making pond water heat and cool more quickly (Jefri *et al.*, 2020). pH fluctuations occur because of rainwater input, which lowers pH levels. The pH may rise when measured during the day due to photosynthesis, which reduces CO2 levels and increases pH (Faizin, 2018). Yunarty *et al.*, (2022) noted that salinity fluctuations are due to evaporation during the day.

Table 2. Interpretation of FTIR Test on the Digestive Organs of Milkfish

Sample Name	**Peak Result (cm^{-1})**	**Functional Groups**	**Polymer Characterization**
Station 1 and 2	3374.91	N - H stretch	*polypropylene* (PP)
	2918.95	C - H stretch	
	2585.02	C - H stretch	
	1628.9	C = O stretch	
	1371.82	CH_2 bend	
	997.4	CH_3 rock, CH_3 bend, CH bend	
	909.88	Aromatic ring stretch or CH bend	
	830.16	Aromatic CH out-ofplane bend	
	792.96	Aromatic CH out-ofplane bend	
	752.69	CH2 rock, C = O bend	
Station 3 and 4	3361.3	N - H stretch	*polypropylene* (PP)
	2921.55	C - H stretch	
	2620.72	C - H stretch	
	2582.78	C - H stretch	
	1620.77	C = O stretch	
	1391.66	CH_2 bend	
	829.36	Aromatic CH outof-plane bend	
	699.96	Aromatic CH out-ofplane bend	

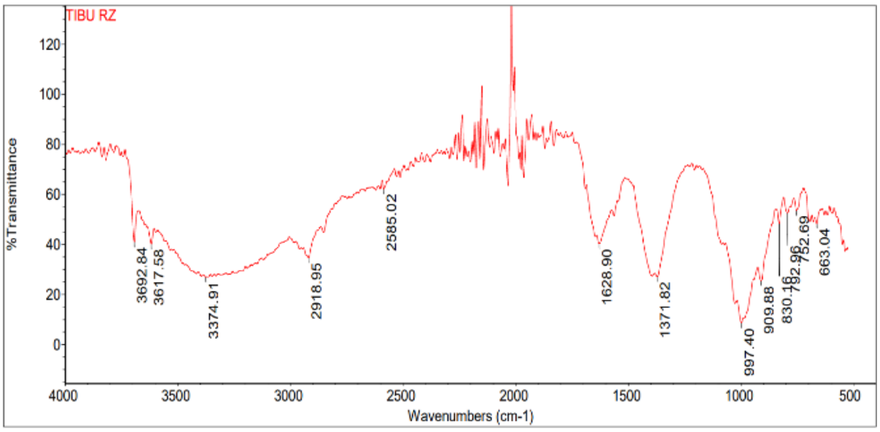


Figure 7. FTIR Test Results of Intestinal Samples from Stations 1 and 2

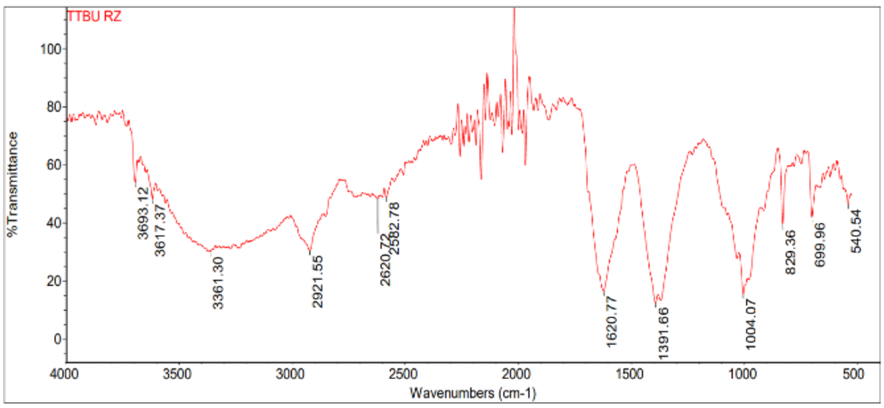


Figure 8. FTIR Test Results of Intestinal Samples from Stations 3 and 4

3.7. FTIR Test on Milkfish Digestive Organs

FTIR (Fourier Transform Infrared) tests were conducted to identify the type of polymer in the samples. The polymer identification used FTIR ATR. Seftianingrum *et al*., (2023) stated that FTIR ATR spectra are obtained from an electro-nexus spectrophotometer equipped with a Diamond Smart Orbit. Murtadho (2023) added that ATR (Attenuated Total Reflectance) is suitable for analyzing thick or highly absorbent solid materials.

The estimation of microplastic polymers can be determined based on wavelength analysis, the estimation can be compared based on the research by Jung *et al*., (2018). The results of the FTIR test on the digestive organs of milkfish indicated the estimation of the polymer type as polypropylene (PP).

The type of microplastic found in the FTIR test of the digestive organs of milkfish is PP. Plastic is a synthetic polymer made from petroleum, characterized by its flexibility and difficulty in biodegradation. The polymer most commonly used in plastic production is PP (Murtadho, 2023). PP has strong characteristics, low vapor permeability, excellent resistance to fats, durability at high temperatures, and lightweight properties. Additionally, this type of polymer is resistant to corrosion and has a high melting point. Due to these properties, PP is widely available in the market. Polypropylene is commonly used in household plastic products such as funnels, glass bottles, buckets, plastic bags, milk bottles, detergent packaging, margarine packaging, water pipes, trash bins, and various types of reusable containers (Faqih 2022). The results of the FTIR analysis are consistent with the conditions of the aquaculture pond in Kasemen District, which is very close to human activities and the sea, where the surrounding population disposes of used waste near the pond.

4. Conclusion

The types of microplastics found in milkfish, water, and sediment include film, fiber, and fragments. Based on the data from the research conducted, it can be concluded that the abundance of microplastics in the organs of milkfish from Kasemen pond was dominated by film-type microplastics. The abundance of microplastics in the sediment of the aquaculture pond in Kasemen District is dominated by fragment-type microplastics. The types of microplastics found in milkfish, water, and sediment include film, fiber, and fragments. The FTIR test results on the digestive organs of milkfish indicated the dominant polymer type as PP. The quality of the water, including temperature, pH, and salinity in the aquaculture pond of Kasemen District, remains within normal limits for milkfish farming.

Data availability statement

Data used in this study can be requested from the author.

Conflict of interest

The authors declare there is no conflict of interest.

Authors contribution

RM, **DH**, **DA**, and **MBS**: idea concept, data collection, conceptualization, data analysis, and writing the original draft. **RK**: review and editing.

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