

Study on the dynamics of microplastics in the biofloc system for Nile tilapia (*Oreochromis niloticus*) aquaculture

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ABSTRACT

Microplastics accumulated in bioflocs can potentially jeopardize fish health and threaten the sustainability of aquaculture. Although biofloc technology (BFT) improves feed efficiency and water quality, the interaction between microplastics and bioflocs and their impact on fish remains an under-researched issue. This study explored the dynamics of microplastics in biofloc systems used for tilapia (*Oreochromis niloticus*) aquaculture to understand the accumulation of microplastics in bioflocs and fish tissues and their impact on fish health and ecological risks. Four treatments were applied: control without bioflocs and microplastics (A), bioflocs without microplastics (B), bioflocs with polyethylene (PE) microplastics of 80 particles·L⁻¹ concentration (C), and bioflocs with PE of 800 particles·L⁻¹ concentration (D). Microplastics were extracted from fish tissue using the NaCl solution density method and characterized using ATR-FTIR to identify polymer types. Results showed that in the treatments without microplastics (A and B), the accumulation of microplastics in fish tissues was shallow, with 0 particles·g⁻¹ in muscle and an average of 1.667 particles·g⁻¹ in the intestine. However, in treatments with microplastics (C and D), the accumulation increased significantly to 0.25 particles·g⁻¹ in muscle and 9 particles·g⁻¹ in the intestine for treatment D. The polymer types identified included polyethylene (PE), polyamide (PA), and polyethylene terephthalate (PET). The ecological risk index showed that the PLI ranged from 1.386–2.038, while the PHI reached a value of 122.966–212.665, indicating a high hazard level. This study confirmed that bioflocs without microplastics significantly reduced the risk of contamination in fish, while bioflocs with microplastics increased exposure and toxicity. Management of microplastic waste is essential to support aquaculture sustainability and food safety.

Keywords: aquaculture sustainability, biofloc, contamination, food safety, microplastics.

INTRODUCTION

Microplastics, defined as plastic particles less than 5 mm in size, have been identified as one of the most significant threats to global aquatic ecosystems. The primary sources of microplastics include domestic waste, industrial activities, and the natural degradation of plastics in the environment (Lebreton et al., 2017). Beyond acting as physical pollutants, microplastics also serve as vectors for persistent organic pollutants (POPs), heavy metals, and chemical additives used in plastic manufacturing (Vo and Pham, 2021). The presence of microplastics has been shown to have detrimental

impacts on aquatic organisms, with risks extending throughout the food chain, ultimately affecting human health as the final consumer (Muhib and Rahman, 2023). Moreover, studies have demonstrated that the prevalence of microplastics in aquatic environments has increased exponentially over the last decade, particularly in inland waters frequently utilized for aquaculture (Vivekanand et al., 2021; Wang et al., 2021).

One of the most common types of microplastics found in aquatic environments is polyethylene (PE), which is also detected in salt products in the form of microplastics (Deswati et al., 2023; Deswati et al., 2024; Suparno et al., 2024;

Syamsu et al., 2024). PE's physical and chemical properties that make it lightweight, resistant to biological degradation, and low-density allow it to float and become widespread in various aquatic ecosystems (Koelmans et al., 2019). A study by Duis and Coors (2016) showed that PE was frequently identified in seawater, freshwater, and beach and river sediments, confirming that this polymer is one of the main contributors to microplastic pollution in aquatic environments (Duis and Coors, 2016). One of the main reasons for selecting PE in this study is its high prevalence in aquatic environments compared to other polymers such as polypropylene (PP) or polystyrene (PS) (Horton et al., 2017).

On the other hand, BFT has emerged as a pivotal innovation in modern aquaculture, enhancing productivity and sustainability. This system leverages microbial consortia to recycle organic waste, improve water quality, and provide additional feed resources for fish through biofloc biomass (Ahmed Alkhamis et al., 2023; Liu et al., 2019). By reducing water exchange requirements, this technology offers an environmentally friendly solution for aquaculture. However, the infiltration of microplastics into biofloc systems presents a novel challenge that may endanger cultured organisms, particularly fish.

Recent research has indicated that in biofloc systems, microplastics can become entrapped within biofloc aggregates, which are rich in microorganisms and organic materials, potentially increasing the bioavailability of microplastics to fish (Meng et al., 2023; Wang et al., 2020). Further studies by Hossain et al. (2023) revealed that interactions between microplastics and bioflocs could alter the dynamics of biofloc aggregates, including the microbial composition and the quality of bioflocs as feed. These studies suggest that exposure to microplastics may affect feed conversion efficiency and negatively impact fish physiology, including gastrointestinal damage and oxidative stress.

Fish exposed to microplastics have been reported to experience a range of disturbances, such as digestive tissue damage, metabolic alterations, and the accumulation of harmful chemical compounds in their tissues (Kadac-Czapska et al., 2024; Lu et al., 2016). A recent study by Abbasi et al. (2018) also demonstrated that microplastics can carry additional pollutants, such as heavy metals, increasing the toxicity risks to aquaculture fish. This not only reduces the quality of fish as a food

commodity but also poses health risks to human consumers (Rajmohan et al., 2019). Furthermore, the long-term effects of microplastics on biofloc systems, particularly on microbial ecosystem dynamics, remain poorly understood, as highlighted by Hu et al. (2023).

Research on the impacts of microplastics in aquaculture, particularly within biofloc systems, remains limited. Most existing studies focus on the accumulation of microplastics in marine environments, whereas studies on the interactions between microplastics and biofloc technology in inland aquaculture are scarce (Deswati et al., 2023; Deswati et al., 2023). To address this gap, the present study aims to explore the dynamics of microplastics in biofloc systems used for cultivating Nile tilapia (*Oreochromis niloticus*). This research will analyze the distribution of microplastics within bioflocs and fish tissues, identify the types of polymers present, and evaluate the associated ecological risks using indices such as the pollution load index (PLI) and potential hazard index (PHI).

The findings of this study are expected to provide deeper insights into the interactions between microplastics and bioflocs and their implications for fish health. Furthermore, this research has the potential to generate practical recommendations for mitigating the risks of microplastics in aquaculture and supporting more sustainable management practices. As such, this study is not only relevant to the development of the aquaculture sector but also holds significant implications for global food security and the protection of aquatic ecosystems (Posthuma et al., 2019; Siddique et al., 2023).

MATERIALS AND METHODS

Research procedure

Fish ponds measuring $1 \times 1 \times 1 \text{ m}^3$ were prepared according to the established treatments, filled with 0.7 m^3 of water, and aerated for two days. After aeration, 700 g of fish salt was evenly distributed across the pond, except for the control pond. After 30 minutes, 35 g of dissolved dolomite lime was added, excluding the control pond. When the water became clear, 70 mL of molasses was mixed in, except the control pond. Subsequently, 7 g of dissolved prebiotic was added to all ponds except for pond A (Deswati, Zein,

Suparno, et al., 2023). The carbon-to-nitrogen (C/N) ratio was monitored and adjusted to > 12 (Dauda et al., 2018; Khanjani et al., 2023). Aeration continued, and biofloc growth was observed over 8–10 days.

Once the biofloc was adequately formed, acclimatized tilapia (*Oreochromis niloticus*) fry was transferred to each pond at a density of 70 fish per pond to ensure proper growth and health. The fish were fed twice daily at 07:00 and 17:00 WIB using pellets amounting to 2–3% of the total body weight of the fish per pond. This feeding rate was essential to support fish growth and maintain water quality in the pond.

To monitor biofloc growth regularly, a 1 L water sample was collected in the morning using an Imhoff cone. After collection, the sample was allowed to settle for 30 minutes. This sedimentation process helped separate biofloc particles from the water. After the sedimentation period, the flocculated solids adhering to the side of the cone were counted to determine the biofloc formation efficiency. The volume of the floc produced was calculated using the formula proposed by Deswati et al. (2023b), enabling the evaluation of biofloc dynamics in this aquaculture system (Deswati et al., 2023).

Microplastic dynamics in the biofloc system

The objective of this study is to examine the behavior and dynamics of microplastics in a biofloc system and to determine their impact on the health of cultured tilapia (*Oreochromis niloticus*). Four treatments were investigated, each with three replicates. The four treatments were designated as follows: A (no bioflocs and microplastics), B (bioflocs without added microplastics), and C (bioflocs added polyethylene (PE), at a concentration of $80 \text{ particles} \cdot \text{L}^{-1}$), and D (bioflocs added PE, at a concentration of $800 \text{ particles} \cdot \text{L}^{-1}$). During the course of the study, no alterations were made to the water or to the biofloc technology, including the introduction of salt, dolomite lime, molasses, or prebiotics. The parameters measured included the extraction of microplastics from fish, microscopic identification based on shape, size, and color, quantification of microplastic abundance, characterization using ATR-FTIR, and an analysis of the potential health risks associated with microplastic exposure (Syamsu et al., 2024; Vasudeva et al., 2025).

Microplastic extraction

The microplastic extraction procedure from tilapia follows the modified method of Karami et al. (2017). The weight and length of the fish were recorded, and all equipment was cleaned with distilled water. The samples were washed with deionized water, weighed for wet weight, dried at $60 \text{ }^\circ\text{C}$, and then weighed again for dry weight. The dry sample was placed in a 500 mL beaker, 20 mL of 30% H_2O_2 was added, and the beaker was heated at $40 \text{ }^\circ\text{C}$ with stirring at 250 rpm, followed by a 30-minute incubation in an oven. After degradation, 400 mL of saturated NaCl solution (5 M) was added, homogenized for 15 minutes, and allowed to stand for 24 hours. After separation, the sample was filtered using Whatman No. 42 paper ($2.5 \text{ } \mu\text{m}$) with a vacuum pump. The microplastic filter paper was dried in a sterile petri dish (Karami et al., 2017).

Microscopic examination

The extracted and dried microplastics were identified based on their shape, color, and size using a Meiji B-350 stereo optical microscope. The microscope was equipped with a camera connected to a laptop and controlled with Motic Image Plus 3.0 software to ensure accuracy. Observations were conducted at 100x magnification, allowing clear visualization of microplastic particles. The microplastics were manually counted by counting the visible particles on the laptop screen to ensure accuracy and classification according to their characteristics (Suparno et al., 2024).

Characterization with ATR-FTIR

Polymer identification of microplastics in this study was performed using Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR). The technique was operated in single reflection mode with a resolution of 8 cm^{-1} and a spectral range of $600\text{--}4000 \text{ cm}^{-1}$, allowing in-depth analysis of the functional groups of each microplastic particle in the sample. Each detected spectral peak represents a specific functional group, which was then compared with library spectra to identify the polymer type accurately (Deswati, Zein, Suparno, et al., 2023).

Microplastic abundance calculation

Microplastic abundance was calculated using the method proposed by Wang and Wang (2018), which allows for estimating the number of microplastic particles based on the volume or mass of the sample being tested (Wang and Wang, 2018). By comparing the number of particles detected in the sample against the total volume or mass of the sample, this method provides an accurate quantitative estimate of microplastic abundance. This technique is crucial for obtaining consistent data that can be compared across studies regarding microplastic distribution in different environments.

They are assessing pollution levels and ecological risk

Assessing pollution levels and environmental risks is essential for maintaining ecosystem balance. The measurement of the PLI, PHI, and potential ecological risk index (PERI) plays a crucial role in evaluating the degree of pollution and potential ecological risks within an environment. PLI provides an overview of pollution levels in a particular area by comparing current pollutant concentrations to baseline values, facilitating the identification of highly polluted sites (Niu et al., 2021). PHI identifies specific hazards posed by various types of microplastic polymers, aiding in understanding microplastic pollution's impact on ecosystems (Chaukura et al., 2021). Meanwhile, PERI estimates the long-term ecological risk of various pollutants and hefty metals based on toxicity and bioaccumulation potential, providing a foundation for implementing more sustainable environmental management policies (Looi et al., 2019).

Data processing design

The results of the water content analysis were calculated as the mean \pm standard deviation and presented in tables and graphs for more straightforward interpretation. Statistical analysis was conducted using one-way ANOVA at a 95% confidence level ($\alpha = 0.05$) to assess significant differences between treatments. A p-value < 0.05 was considered important, and if differences were found, a post hoc Duncan test was performed to identify significant differences. The analysis was conducted using “IBM SPSS Statistics 23” to ensure the accuracy of the results.

RESULTS AND DISCUSSION

Total microplastic abundance in fish samples

The results of the study on microplastic abundance indicate that the use of biofloc and microplastics in aquaculture systems has a significant impact on the accumulation of microplastics in fish tissues, as evidenced by the data presented in Figures 1a and 1b.

This experiment (Figs. 1–4) involved four different treatments: A (no bioflocs and microplastics), B (bioflocs without added microplastics), and C (bioflocs added PE, at a concentration of $80 \text{ particles} \cdot \text{L}^{-1}$), and D (bioflocs added PE, at a concentration of $800 \text{ particles} \cdot \text{L}^{-1}$).

Treatment A, as the control, showed that without biofloc intervention or additional microplastics, the microplastic abundance in fish tissue remained low, with $0 \text{ particles} \cdot \text{g}^{-1}$ in the muscle and $2.167 \text{ particles} \cdot \text{g}^{-1}$ in the intestine. This emphasizes the importance of keeping

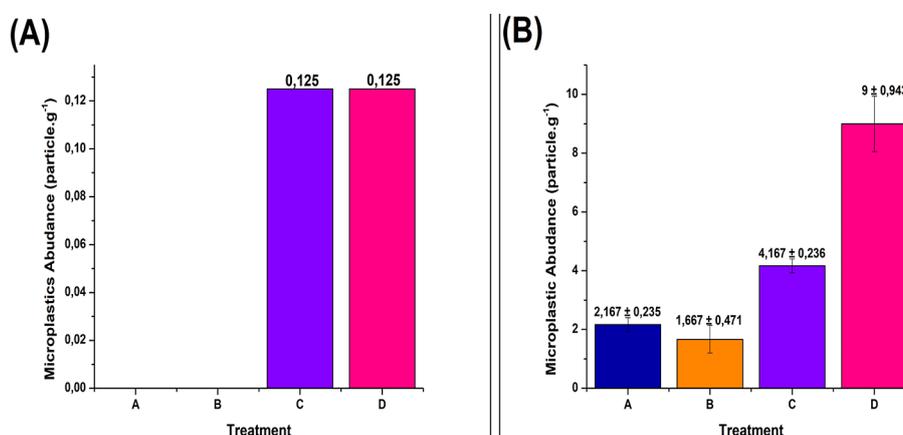


Figure 1. Total microplastic abundance (a) fish muscle; (b) fish intestine

aquaculture contamination-free for safe harvests (Hamelink et al., 2024).

In Treatment B, biofloc was applied without adding microplastics to improve water quality through the breakdown of organic waste. The results showed no microplastics in the muscle and only 1.667 particles·g⁻¹ in the intestine, indicating that biofloc can help reduce the risk of microplastic accumulation in fish tissues (Ding et al., 2018; Reis et al., 2019). Treatment C involved mixing biofloc with PE microplastics at 80 particles·L⁻¹ concentrations. This led to a slight increase in microplastic abundance, with 0.25 particles·g⁻¹ in the muscle and 4.167 particles·g⁻¹ in the intestine. At the same time, biofloc can reduce the direct exposure of fish to microplastics, but accumulation in tissues still occurs due to consuming contaminated biofloc (Issac and Kandasubramanian, 2021; Zhou et al., 2021).

In Treatment D, with biofloc and a high microplastic concentration of 800 particles·L⁻¹, the microplastic accumulation increased to 0.25 particles·g⁻¹ in the muscle and 9 particles·g⁻¹ in the intestine. This condition demonstrates the health risks for fish, as exposure to high levels of pollutants can affect metabolism and organ function (Turan et al., 2021; Vázquez-Rowe et al., 2021).

Microplastic shape abundance in fish samples

The study of microplastic shape abundance indicates that using biofloc and microplastics in aquaculture systems significantly impacts the accumulation of microplastics in water and fish tissues, both in muscle (Fig. 2a) and intestine (Fig. 2b).

Treatment A serves as a baseline for evaluating the impact of microplastics on fish in biofloc

systems. Previously conducted studies have demonstrated that microplastics can accumulate in the fish digestive tract, with results indicating an abundance of 0.125 particles·g⁻¹ in fish muscle and 1 particle·g⁻¹ in the fish intestine. This reflects the risk of potential harm to fish health and provides insight into the broader ecosystem impacts (McGoran et al., 2017; Parker et al., 2021). In treatment B, the biofloc system was operated without the addition of external microplastics. The mean abundance in fish muscle remained at 0.125 particles·g⁻¹, while the intestine increased to 1.667 particles·g⁻¹. This finding indicates that fish in biofloc systems, despite the partial protection afforded to them, remain susceptible to microplastic exposure, which has the potential to affect their metabolic processes and overall health (Guzzetti et al., 2018; Zhou et al., 2021).

Treatment C, adding 80 microplastic particles·L⁻¹, resulted in microplastic fragments detected in the fish muscle with an abundance of 0.25 particles·g⁻¹. In the intestine, it increased to 2.167 particles·g⁻¹. Previous research by Huang et al. (2020) indicated that microplastic accumulation in fish tissues could be influenced by higher concentrations of microplastics in the water, which may disrupt biological functions and the overall health of the fish (Huang et al., 2020).

Treatment D showed the most significant results by adding 800 microplastic particles·L⁻¹. In this condition, the fish muscle contained 0.25 particles·g⁻¹, while the intestine showed three particles per gram. The high concentration further strengthens the finding that adding microplastics to the biofloc system contributes to the widespread distribution of microplastics in fish. This presents potential risks for fish health

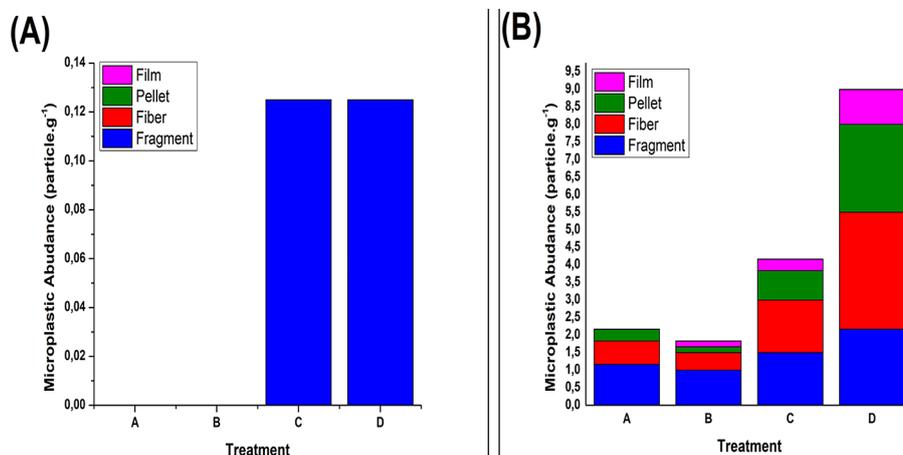


Figure 2. Microplastic shape abundance: (a) fish muscle; (b) fish intestine

and food safety, especially if these microplastics contain harmful chemicals that could lead to toxic effects (Auta et al., 2017; Barceló and Picó, 2019). Research by Elizalde-Velázquez and Gómez-Oliván (2021) also confirmed that high microplastic exposure could lead to inflammation, hormonal disruption, and even death in certain fish species (Elizalde-Velázquez and Gómez-Oliván, 2021).

Based on these findings, it can be concluded that microplastics play a significant role in the accumulation of particles within the fish’s body. While biofloc can enhance water quality, the introduction of microplastics still poses a risk of contamination in fish, especially in the digestive system. This highlights the growing concerns regarding waste management and underscores the necessity for further research on the impact of microplastics on fish health and food safety.

Microplastic size abundance in fish samples

The study of microplastic size abundance indicates that using biofloc and microplastics in aquaculture systems significantly impacts the accumulation of microplastics in fish tissues, both in muscle (Fig. 3a) and intestine (Fig. 3b).

In Treatment A, both the fish muscle and intestine show deficient levels of microplastic accumulation, with fewer than 1 microplastic particles.g⁻¹ in both fish body parts. Although no microplastics were added to the biofloc system, microplastic exposure can still occur through internal sources, such as waste from uneaten fish feed released into the water. Previous studies have shown that microplastics can persist in the environment and enter aquatic organisms’ bodies

by consuming these particles directly or through the food chain (Vital et al., 2021).

In Treatment B, while using biofloc helps reduce microplastic concentrations in the water, fish still show microplastic accumulation in their tissues. The muscle tissue contained 1–2 microplastic particles.g⁻¹, while the intestine showed similar levels. This suggests that although biofloc acts as a filter, absorbing microplastic particles from the water (Schuhen and Sturm, 2021), fish are still exposed to microplastics that can accumulate in their tissues. This process indicates that water management using biofloc does not eliminate the risk of microplastic accumulation in fish.

In treatment C, with the addition of microplastics to the system, there was a significant increase in the concentration of microplastics in the fish’s body. Fish muscle contained 3–4 microplastic particles per gram, and the intestine had around 2–3 microplastic particles.g⁻¹. Research by Garrido Gamarro et al. (2020) shows that microplastic exposure in aquatic systems can lead to the direct accumulation of microplastics in marine organisms’ bodies (Gamarro et al., 2020). This significant addition of microplastics may indicate potential health risks for both fish and humans consuming microplastic-contaminated fish, especially since microplastics can contain harmful chemicals that may be released into the body.

In Treatment D, with higher microplastic concentrations in the biofloc system, fish muscle contained 5–6 microplastic particles.g⁻¹, while the intestine contained 4–5 microplastic particles.g⁻¹. These results suggest that higher microplastic exposure in the fish farming environment directly increases microplastic

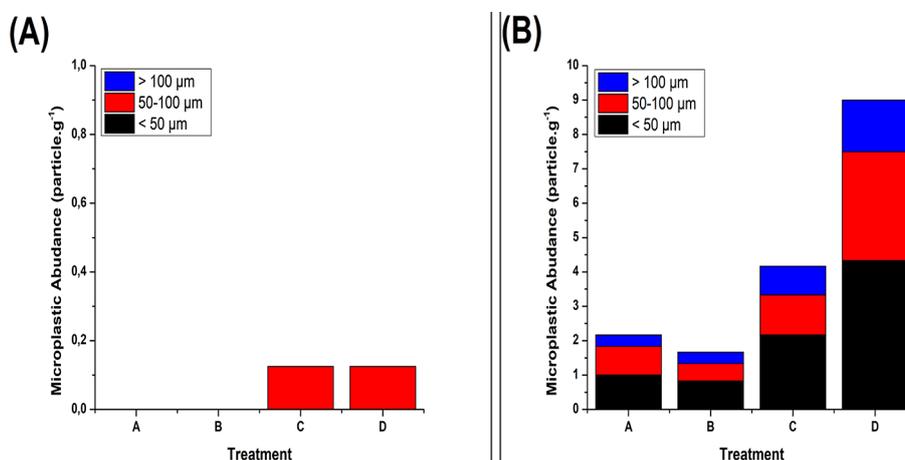


Figure 3. Microplastic size abundance: (a) fish muscle; (b) fish intestine

accumulation in fish bodies. Research by Guerrero et al. (2021) states that high exposure to microplastics can affect fish health and increase health risks for human consumers, as microplastics may contain harmful chemicals and potentially cause hormonal disruptions, cancer, or other health issues (Guerrero et al., 2021).

Biofloc systems in aquaculture have an important role in improving water quality and providing an additional source of nutrients for fish through the formation of aggregates of microorganisms called bioflocs (Deswati et al., 2023; Deswati et al., 2023; Deswati et al., 2025; Lusher et al., 2017). However, although bioflocs can capture small particles, including microplastics, this does not completely prevent the accumulation of microplastics in the fish body (Cole et al., 2013). Some of the main mechanisms that lead to the accumulation of microplastics in fish in biofloc systems include direct ingestion, consumption of contaminated bioflocs and trophic transfer within the biofloc system (Cole et al., 2013; Lusher et al., 2017; Setälä et al., 2014).

Based on the results from the four treatments, it is clear that biofloc can reduce microplastic pollution levels in the water. Still, it cannot eliminate microplastic accumulation in fish tissues. Treatments A and B show that good management can minimize the risk of microplastic accumulation in fish. At the same time, treatments C and D demonstrate that adding microplastics can significantly increase contamination in fish bodies. This underscores the importance of controlling and monitoring microplastic pollution in fish farming systems to ensure food safety and protect aquatic ecosystem health.

Microplastic color abundance in fish samples

The study of microplastic color abundance indicates that using biofloc and microplastics in aquaculture systems significantly affects the accumulation of microplastics in fish bodies, both in muscle (Fig. 4a) and intestine (Fig. 4b).

In treatment A, no microplastics were detected in the fish muscle, suggesting that fish raised in an environment free from microplastic contamination are safe for consumption. This is important in ensuring aquaculture products do not contain harmful substances that could affect human health throughout the food chain (Guerrero et al., 2021). Additionally, no microplastics were found in the fish intestine, reinforcing that no contaminants accumulate in the fish's digestive system. This is crucial to prevent potential health impacts caused by microplastics that could affect fish digestion and metabolism (Gola et al., 2021).

Although biofloc successfully maintained environmental quality in treatment B, the fish muscle remained free from microplastics. Using biofloc as a medium helps reduce microplastic exposure, creating a healthier environment for the fish and decreasing the potential for contamination in aquaculture products (Yu et al., 2023). The fish intestine also showed no presence of microplastics, indicating that biofloc can act as a binding agent for other particles, thus protecting the health of the fish's digestive system.

In treatment C, microplastics were detected in the fish muscle with an abundance of $0.125 \text{ particles} \cdot \text{g}^{-1}$, although in low quantities. Studies have shown that microplastics can enter fish bodies through the consumption of contaminated food, potentially leading to the accumulation of

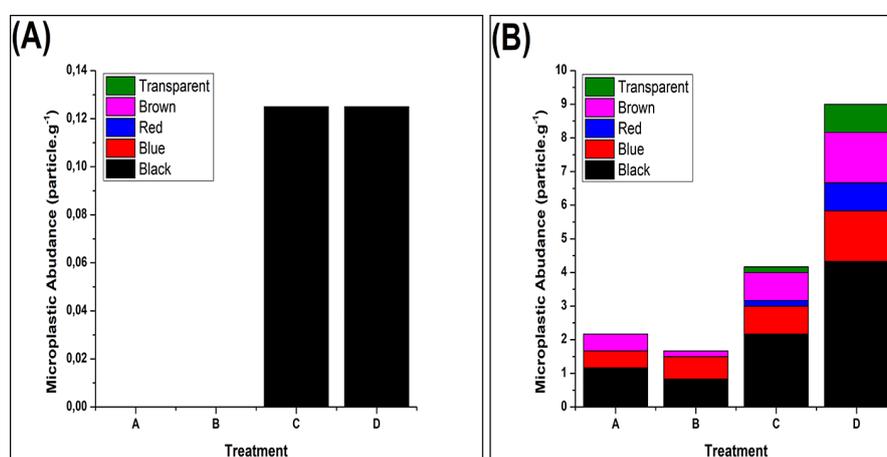


Figure 4. Microplastic color abundance: (a) fish muscle; (b) fish intestine

contaminants in the fish's tissues (Lusher et al., 2017). The abundance of microplastics ranged from 0.833 to 4.333 particles·g⁻¹ in the fish intestine, suggesting that black-colored microplastics resemble natural food sources, making fish more likely to ingest them. This indicates the need for stricter monitoring of microplastic contamination in aquatic environments.

In treatment D, a significant increase in microplastics was detected in the fish muscle, reflecting higher exposure to contaminants. Fish living in environments with higher microplastic concentrations are more susceptible to absorbing these harmful particles, which could affect the quality of aquaculture products and fish health (Guo and Wang, 2019). The fish intestine in this treatment showed a higher abundance of microplastics, ranging from 1 to 4.333 particles·g⁻¹. Previous research suggests that exposure to microplastics in the fish digestive system can affect metabolism and digestive health, potentially leading to negative impacts on the overall health of the fish (Ma et al., 2020).

Identification of microplastic polymer types

Identification of functional groups was carried out manually by comparing the peak spectra of each analyzed particle with reference data specific to each polymer. The analysis revealed several dominant polymer types present in both the water and fish samples, such as PA, PE, and PET (Vahur et al., 2016). Identifying polymer

types from black fibers, brown fragments, and red fragments in the samples showed that these particles are PA type microplastics, as seen in Figure 5.

Polyamide (PA) polymers like nylon can be analyzed using FTIR spectroscopy. This technique allows identification based on infrared absorption at specific wavenumbers associated with molecular bonds (Shan et al., 2019). The primary characteristic of PA is observed at an absorption around 1017.56 cm⁻¹, indicating C–N bending vibrations—a distinctive feature of the polyamide structure (Fan et al., 2021). Additionally, absorption at 1640.39 cm⁻¹ signifies C=O stretching vibrations, often found in amide and carboxyl bonds, further confirming the presence of PA in the sample (Fan et al., 2021; Rodrigues et al., 2019). At a wavenumber of 2923.62 cm⁻¹, this absorption is associated with C–H stretching vibrations, indicating the presence of carbon chains in the PA polymer structure (Shan et al., 2019). A strong absorption at this wavenumber indicates hydrocarbon components in the polyamide network. Finally, the absorption at 3292.84 cm⁻¹, indicating N–H stretching vibrations, is also a unique characteristic of PA, which contains nitrogen atoms in its polymer structure (Fan et al., 2021).

The presence of polyamide in aquatic ecosystems, particularly in Nile tilapia (*Oreochromis niloticus*), poses serious environmental risks. PA, which is difficult to degrade, can accumulate in organisms through ingestion or direct

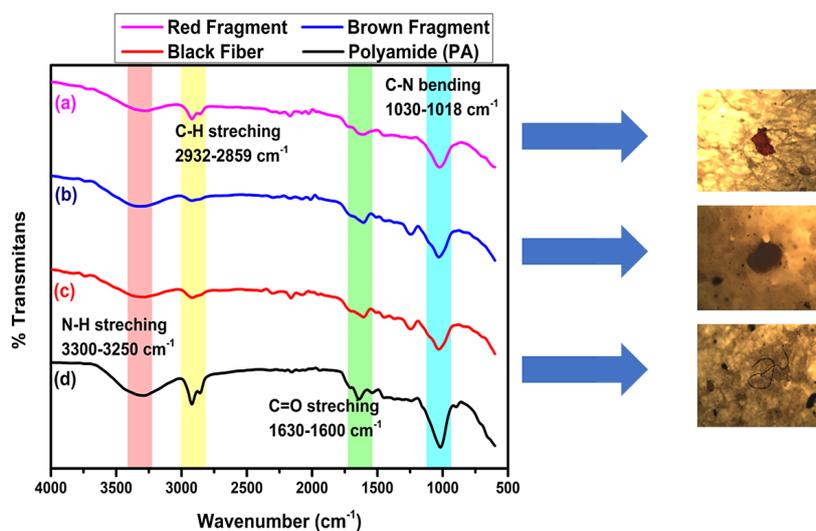


Figure 5. Comparison of microplastic identification spectra with standards: (a) red fragment microplastic; (b) brown fragment microplastic; (c) black fiber microplastic; (d) polyamide (PA) standard

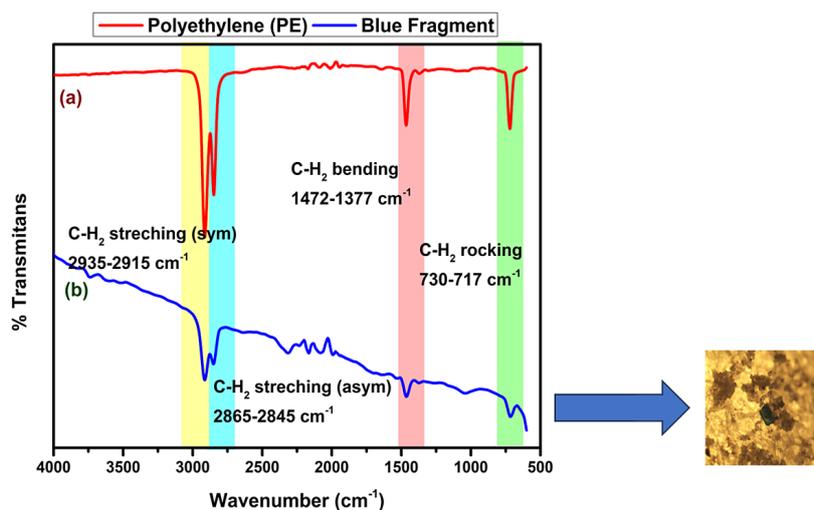


Figure 6. Spectrum comparison of microplastic identification results against standards: (a) polyethylene (PE) standard; (b) blue fragment microplastic

contamination from the aquatic environment (Rajmohan et al., 2019). Consumed PA microplastics can disrupt the digestive system, reduce appetite, and interfere with metabolic processes (Vo and Pham, 2021). Additionally, there is potential for the leaching of harmful chemicals from PA into fish tissues, leading to hormonal disruptions or tissue damage (Alimba and Faggio, 2019). Other negative impacts include growth and reproductive disturbances in fish, with bioaccumulation risks that may affect higher trophic organisms in the food chain, including humans (Gola et al., 2021). The identification results of the plastic polymer type from the blue fragment in the

sample indicate that the particle is polyethylene (PE) microplastic, as shown in Figure 6.

PE is a type of plastic polymer that can be identified using FTIR spectroscopy through its characteristic absorption patterns. Absorption peaks in the wave number range of 2936–2915 cm^{-1} correspond to symmetric CH_2 stretching vibrations, while the peaks at 2865–2845 cm^{-1} indicate asymmetric CH_2 stretching. Additionally, CH_2 bending vibrations are detected in the range of 1472–1377 cm^{-1} , and CH_2 rocking appears at 730–717 cm^{-1} , confirming the presence of PE structure in the sample (Morgado et al., 2021). These characteristics are crucial for identifying

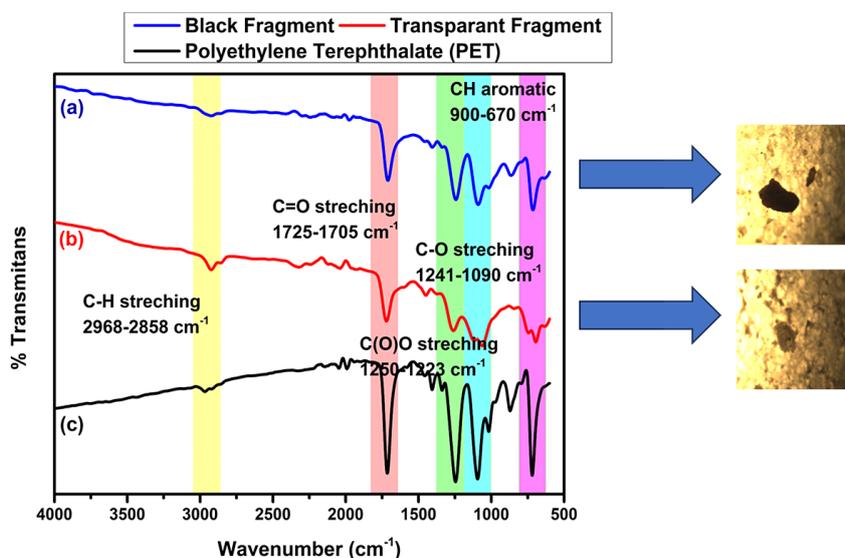


Figure 7. Comparison of spectra for microplastic identification against standards: (a) black fragment microplastic; (b) transparent fragment microplastic; (c) PET standard

PE in environmental analyses, mainly to understand its impact on organisms such as fish.

PE in aquatic environments can have detrimental effects if ingested by Nile tilapia (*Oreochromis niloticus*). Accumulation of PE polymer in the fish’s digestive tract can disrupt the digestive system and hinder nutrient absorption, potentially compromising the overall health of the fish (Dehaut et al., 2016). Studies have shown that microplastics in fish bodies cause physiological stress, leading to weight loss, stunted growth, and developmental issues (Vo and Pham, 2021). In the long term, microplastics in fish can result in bioaccumulation and negative health impacts, including for human consumers who eat the fish (Gola et al., 2021). Additionally, PE is a non-biodegradable material and may release harmful chemicals that could damage the fish’s internal organs (Hahladakis et al., 2018). The identification results of plastic polymer types from the black and transparent fragments in the samples indicate that these particles are microplastics of the polyethylene terephthalate (PET) type, as shown in Figure 7.

PET is a type of plastic polymer that can be identified using FTIR spectroscopy based on its characteristic absorption patterns at specific wave numbers. Absorption in 2968–2858 cm^{-1} indicates CH stretching vibrations, while absorption at 1725–1705 cm^{-1} indicates C=O stretching vibrations, characteristic of the carbonyl group in PET’s ester structure. C(O)O stretching vibrations are detected in the 1250–1223 cm^{-1} range, and C-O stretching appears at 1241–1090 cm^{-1} . Additionally, aromatic CH vibrations in the range of 900–670 cm^{-1} indicate the presence of aromatic groups in PET (Bailey and Winey, 2020).

The presence of PET in aquatic environments, particularly when ingested by fish such as Nile tilapia (*Oreochromis niloticus*), can pose serious health risks. Accumulated PET microplastics in the digestive tract of fish can hinder digestion and nutrient absorption and damage the fish’s digestive

tissues (Alimba and Faggio, 2019). Furthermore, PET may contain harmful chemicals, both from additives used during plastic production and from PET degradation in the environment. These chemicals can leach into the fish’s body and disrupt the endocrine system, affecting fish growth and reproduction (Karapanagioti and Werner, 2019). Long-term consumption of PET can also reduce fish resistance to diseases, hinder reproduction, and harm overall health, ultimately affecting fish populations and the aquatic food chain (Vo and Pham, 2021).

Assessing pollution levels and ecological risk

Based on Table 1, the values of the PLI, PHI, and PERI provide a comprehensive overview of the level of microplastic pollution and its potential ecological risks. The PLI values, ranging from 1.386 to 2.038, indicate that the study area experiences pollution levels exceeding safe thresholds, potentially leading to long-term effects on the aquatic environment and the organisms within it. A PLI value above 1 signifies moderate to high pollution, suggesting an accumulation of pollutants, particularly microplastics, which may originate from various sources, including plastic waste from human activities around the area. According to Lebreton et al. (2017), river flow and coastal activities can increase plastic emissions into waters, ultimately impacting water quality and the health of aquatic biota (Lebreton et al., 2017).

The PHI index, with a value range of 122.966 to 212.665, indicates a significant potential hazard from microplastics to aquatic organisms. The PHI assesses potential hazards based on the toxic properties of microplastics and their effects on ecosystems. This value falls within the risk category II, indicating moderate intellectual and ecological health risks. Research has shown that microplastics can cause toxic effects in fish through accumulation in body tissues, potentially affecting their biological functions (Karapanagioti and

Table 1. Health risk index values for fish due to the presence of microplastics

Risk index	PHI	PLI	PERI	Category	Levels of risk
Category	0–1	<10	< 150	I	Low
	1–10	–	150–300	II	Medium
	10–100	1020	300–600	III	High
	100–1000	20–30	600–1200	IV	Danger
Station	> 1000	> 30	> 1200	V	Extreme danger
Current study	122.966–212.665	1.386–2.038	56.751–98.152	I-IV	Low to danger

Werner, 2019). Additionally, microplastics can act as vectors for other organic pollutants, amplifying toxicity risks that can impact the food chain and, ultimately, humans who consume fish from the area (Siddique et al., 2023).

The PERI index, ranging from 56.751 to 98.152, indicates a high potential ecological risk to aquatic organisms, including Nile tilapia, the subject of this study. The PERI assesses risks to the marine ecosystem by considering the possible negative impacts of microplastics on the health and survival of biota. High PERI values indicate that microplastics affect fish and other organisms within the ecosystem, creating imbalances in the food web. This impact could seriously affect biodiversity, as some species may experience population declines or developmental disruptions due to microplastic exposure (Pan et al., 2021). Additionally, microplastic particles entering fish bodies can induce oxidative stress, affecting fish health and reproduction (Kadac-Czapska et al., 2024).

Overall, the PLI, PHI, and PERI values indicate that microplastics in the waters pose a significant threat to the health of Nile tilapia and the aquatic ecosystem. This threat can affect fish's quality of life, reproduction, growth, and ecosystem stability. Appropriate mitigation efforts are needed to reduce this risk, including strengthening regulations on plastic waste and raising public awareness of the importance of maintaining clean aquatic environments. An integrated approach involving waste management, environmental monitoring, and public education can help curb the accumulation of microplastics in waters and mitigate their impacts on ecosystems and the people who rely on these resources (Knoblauch and Mederake, 2021; Lebreton et al., 2017; Muhib and Rahman, 2023).

CONCLUSIONS

This study reveals the dynamics of microplastics in biofloc systems used for Nile tilapia farming, showing that microplastics can accumulate in biofloc and potentially affect fish health. Adding microplastics to biofloc increases accumulation in fish tissues, which may influence the digestive system and fish growth. Based on the PLI, PHI, and PERI, microplastics in the water pose a significant threat to fish health and the overall aquatic ecosystem. Waste management and controlling microplastic contamination in biofloc systems are critical to maintaining fish health, food safety, and the sustainability

of aquaculture ecosystems. These findings provide valuable insights for the aquaculture industry in mitigating microplastic contamination risks and ensuring the sustainability of fish farming.

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