



Microplastic Accumulation and Risk Assessment in Closed Biofloc Aquaculture: Integrating Biofloc Engineering with Artificial Neural Networks (ANN) Modeling

Deswati Deswati^{1✉} | Olly Norita Tetra¹ | Rezkika Rahmadila Putri¹ | Amelia Sriwahyuni Lubis² | Adewirli Putra³

1. Department of Chemistry, Faculty of Mathematics and Natural Sciences, Andalas University, Padang, P.O.Box 25163, Indonesia

2. Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Universitas Bung Hatta, Padang, P.O.Box 25133, Indonesia

3. Department of Medical Laboratory Technology, Syedza Saintika University, Padang, P.O.Box 25132, Indonesia

Article Info

Article type:
Research Article

Article history:
Received: 25 November 2025
Revised: 22 February 2026
Accepted: 10 April 2026

Keywords:
Biofloc,
Microplastics,
Polyethylene,
ANN,
Risk Assessment

ABSTRACT

Microplastics (MPs) are pervasive contaminants in closed aquaculture systems, where limited water exchange restricts removal. This study assessed biofloc as a mitigation strategy for polyethylene (PE) MPs and characterized MPs in terms of morphology, size, color, and polymer type across four treatments. Biofloc reduced background MPs by 26.3%, but PE addition significantly increased concentrations, indicating low removal efficiency for buoyant polymers. Fragmented and fibrous MPs dominated, while FTIR spectroscopy confirmed PE, polyamide (PA), and polyethylene terephthalate (PET) as major polymers. Health risk assessment using Estimated Daily Intake (EDI), Target Hazard Quotient (THQ), and Hazard Index (HI) indicated all indices remained below 1, though cumulative risk increased with higher MP loads. An Artificial Neural Network (ANN) model accurately predicted exposure indices ($R^2 = 0.95$; RMSE = 0.021), and SHapley Additive exPlanations (SHAP) identified MP concentration as the primary driver of health risk. These findings highlight that while biofloc can partially sequester environmental MPs, it is ineffective against buoyant PE, emphasizing the need for integrated mitigation strategies. Combining biofloc management with ANN-based predictive modeling provides a robust framework to reduce MP exposure and support sustainable aquaculture practices in closed systems. Future research should focus on optimizing biofloc compositions, evaluating PE fragmentation, and assessing long-term ecological and food safety impacts.

Cite this article: Deswati, D., Norita Tetra, O., Rahmadila Putri, R., Sriwahyuni Lubis, A., & Putra, A. (2026). Microplastic Accumulation and Risk Assessment in Closed Biofloc Aquaculture: Integrating Biofloc Engineering with Artificial Neural Networks (ANN) Modeling. *Pollution*, 12(2), 560-573.
<https://doi.org/10.22059/poll.2026.407025.3209>



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Publisher: The University of Tehran Press.

DOI: <https://doi.org/10.22059/poll.2026.407025.3209>

INTRODUCTION

Biofloc-based tilapia aquaculture has gained global adoption, particularly in Southeast Asia, for enhancing fish production in limited spaces while reducing commercial feed use (Deswati et al., 2023a). By converting organic waste into microbial biomass, biofloc provides supplementary feed and improves water quality, reduces pollution, and enhances sustainability (De Schryver et al., 2008).

However, biofloc systems face environmental challenges, notably microplastic (MP) contamination. MPs may originate from plastic infrastructure, feed additives, or external debris,

*Corresponding Author Email: deswati@sci.unand.ac.id

posing risks to aquatic ecosystems and fish health (Lebreton & Andrady, 2019). MPs accumulate in tilapia, affecting physiology and growth (Rochman et al., 2015), and human exposure through seafood consumption raises health concerns (Smith et al., 2018). Wastewater treatment plants contribute to MP influx, impacting aquaculture indirectly (Murphy et al., 2016), yet knowledge gaps remain in freshwater systems (Cole et al., 2011). MPs can also disrupt biofloc function and water quality management (Wright et al., 2013), highlighting the need for human health risk assessments using metrics such as EDI, THQ, and HI (Smith et al., 2018).

This study quantifies MP abundance, characterizes their size, shape, color, and polymer types in tilapia biofloc culture water (Cole et al., 2011; Murphy et al., 2016) and conducts preliminary human health risk assessments. Key gaps include the dynamics of buoyant polymers like polyethylene (PE) in closed biofloc systems and the potential of biofloc to mitigate MPs, as well as the application of predictive tools like Artificial Neural Networks (ANNs) for risk assessment. To address these, PE MPs were experimentally introduced at two concentrations into a closed biofloc system, with subsequent monitoring of accumulation, polymer characterization, and ANN-based modeling of health risks, offering insights for sustainable aquaculture and environmental management.

MATERIAL & METHODS

Research Procedure

Twelve 1 m³ experimental tanks were filled with 0.7 m³ of aerated freshwater. Continuous aeration for 48 hours promoted oxygen saturation and microbial activity, following De Schryver et al. (2008) and Deswati et al. (2023b). Biofloc development in three treatment tanks was stimulated by gradually adding 700 g fish salt, 35 g dolomite lime, 70 mL molasses, and 7 g prebiotic, achieving a C/N ratio above 12 to support heterotrophic bacterial growth and biofloc formation (Avnimelech, 2015; Crab et al., 2012). Aeration continued for 8–10 days, with dissolved oxygen, pH, and turbidity monitored to ensure optimal conditions (Deswati et al., 2023a,b).

After biofloc establishment, tilapia (*Oreochromis niloticus*) fingerlings were acclimatized and stocked at 70 fish per tank, following recommended densities (Crab et al., 2012), and fed commercial pellets twice daily at 2–3% biomass (Deswati et al., 2023a). Four treatments were applied in triplicate: A (control, no biofloc or microplastics), B (biofloc without microplastics), C (biofloc with PE microplastics at 80 particles L⁻¹), and D (biofloc with PE microplastics at 800 particles L⁻¹). No water exchange or further biofloc amendments were made to simulate practical farming and observe microplastic accumulation under steady-state biofloc conditions (Crab et al., 2012; Deswati et al., 2023b). Water quality and fish health were monitored throughout to assess treatment effects.

Microplastic Extraction Procedure

Microplastics (MPs) were extracted from water samples following Masura et al. (2015) with modifications to improve efficiency and reduce contamination. A 200 mL aliquot of each sample was placed in a clean glass beaker, and 20 mL of 30% hydrogen peroxide (H₂O₂) was added to digest organic matter. The mixture was homogenized at 250 rpm and 40°C for 5 minutes, facilitating oxidation of organics without damaging MPs (Masura et al., 2015; Hidalgo-Ruz et al., 2012).

After digestion, samples were vacuum-filtered through Whatman No. 42 filter paper (2.5 µm) to collect MPs efficiently (Masura et al., 2015). Filters were transferred to sterile petri dishes and dried at room temperature under clean laboratory conditions to avoid airborne contamination (Lusher et al., 2017). Dried samples were then prepared for microscopic and spectroscopic analysis to determine MP abundance, size, shape, and polymer type.

Microplastic Identification and Characterization

Extracted microplastics (MPs) were examined under a Meiji B-350 Stereo Optical Microscope at 100× magnification to record shape, color, and size. Shapes were classified as fragments, fibers, films, pellets, or foams to infer sources and environmental behavior (Hidalgo-Ruz et al., 2012; Lusher et al., 2017). Size was measured with an ocular micrometer (<100 μm, 100–500 μm, >500 μm) to assess bioavailability and ecological risk (Cole et al., 2011), with photographic documentation for analysis.

Polymer composition was determined using ATR-FTIR (600–4000 cm⁻¹) by comparing spectra with reference libraries of PE, PP, PS, and PET (Andrady, 2011; Lusher et al., 2017). This method enabled detailed profiling of MP pollution in biofloc aquaculture, providing insights into sources, fate, and interactions with tilapia, essential for environmental risk assessment and mitigation (Rochman et al., 2015).

Artificial Neural Network (ANN) Procedure

An Artificial Neural Network (ANN) was developed to model nonlinear relationships between environmental variables and microplastic-related risk indices (EDI, THQ, HI). Seven predictors—pH, temperature, dissolved oxygen, total suspended solids, BOD, COD, and biofloc volume—served as inputs, with the three risk indices as outputs. Data were normalized (0–1) and split into training (70%), validation (15%), and testing (15%) sets. The ANN comprised a feed-forward network with two hidden layers (10 and 8 neurons; ReLU activation) and a three-neuron linear output layer. Training used the Adam optimizer (learning rate 0.001), mean squared error loss, and early stopping, with performance evaluated via R², RMSE, and MAE.

SHAP analysis assessed input contributions, revealing microplastic concentration as the dominant driver of EDI, THQ, and HI, followed by biofloc-related parameters, while other water-quality variables had minor effects. This modeling–interpretation approach ensured robust predictions and clarified how biofloc dynamics interact with microplastic contamination to inform aquaculture risk management. The seven input variables were selected for their known roles in particle aggregation, biofloc development, and exposure pathways, capturing nonlinear interactions beyond conventional statistical methods.

Statistical Analysis. The statistical analysis of microplastic abundance (particles L⁻¹) across water samples was conducted using one-way analysis of variance (ANOVA) to assess significant differences among sampling sites and seasons. When significant effects were detected ($p < 0.05$), Duncan’s post hoc test was applied to identify specific group differences. All statistical computations were performed using SPSS software version 23, ensuring robust evaluation of spatial and temporal variability in microplastic concentrations.

RESULTS AND DISCUSSION

Mechanisms by Which Biofloc Systems Interact with and Remove Microplastics

Biofloc systems sequester microplastics (MPs) via physical, biological, and physicochemical processes. Physically, MPs are trapped in the sticky extracellular polymeric substance (EPS) matrix of bioflocs, with aeration enhancing collisions and MP–floc aggregation:

Equation (1): Aggregation rate

$$R_{\text{agg}} = \beta \cdot N_{\text{MP}} \cdot N_{\text{floc}}$$

Aggregates have higher density, promoting sedimentation and MP removal (Lv et al., 2019). Biologically, microbial biofilms alter MP surface properties, increasing aggregation potential:

Equation (2): Biofilm-modified aggregation potential

$$A_{\text{bio}} = A_0 + \alpha \cdot \text{EPS}$$

Enzymes such as laccases, peroxidases, or PETase/MHETase can partially degrade MPs over time, enriching plastic-degrading taxa (Zettler et al., 2013; Yoshida et al., 2016).

Physicochemical forces further drive MP–floc association. Negatively charged EPS groups bind oppositely charged MPs, while hydrophobic EPS domains enhance adhesion to hydrophobic polymers. MPs coated with dissolved organic matter (DOM) exhibit stronger affinity due to combined electrostatic and hydrophobic interactions (Enfrin et al., 2019). Settling velocity under these interactions can be described using a Stokes-based framework:

Equation (3): Settling velocity

This integrated mechanism explains efficient MP retention in biofloc systems.

$$v = \frac{2}{g} \cdot \frac{(\rho_p - \rho_w) g r^2}{\mu}$$

where v is the settling velocity of MP–biofloc aggregates, ρ_p and ρ_w are the densities of the aggregate and water, respectively, r is the effective radius of the aggregate, g is gravitational acceleration, and μ is the dynamic viscosity of water.

Equation (3) illustrates the enhanced settling of microplastic–biofloc aggregates due to increased size and density, promoted by EPS-mediated stabilization. The presented equations conceptually represent the dominant aggregation and settling processes, rather than providing a fully parameterized predictive model. Indirect removal pathways include ingestion of MP–floc aggregates by zooplankton and detritivores, and benthic deposition of sedimented flocs, which temporarily immobilizes MPs but may redistribute them within the food web (Lv et al., 2019).

These physical, biological, and physicochemical processes highlight the complex, multi-modal interactions of MPs in biofloc systems, offering insights for optimizing microplastic removal in aquaculture.

Total Abundance of Microplastics

Results showed that biofloc combined with natural flocculants achieved the greatest microplastic (MP) reduction, outperforming biofloc alone and control conditions. Here, “natural flocculants” refer to EPS produced in situ by microbial communities, with no external chemical additives.

As shown in Figure 1, Treatment A (control, no biofloc or MPs) had the lowest MP abundance, 142.5 ± 10.61 particles L^{-1} , likely from background contamination (Li et al., 2018). Treatment B (biofloc only) reduced MPs to 105.0 ± 14.14 particles L^{-1} , a 26.3% decrease relative to the control ($p < 0.05$), indicating biofloc can physically entrap incidental MPs (Avio et al., 2017; Wang et al., 2024). Treatment C (biofloc + PE MPs at 80 particles L^{-1}) showed elevated abundance, 340.0 ± 7.07 particles L^{-1} , a 138.6% increase over control ($p < 0.05$), consistent with polyethylene’s high buoyancy, low density, and hydrophobicity, which limit aggregation and sedimentation with bioflocs (Prata et al., 2020; Wu et al., 2023).

Treatment D (biofloc + PE MPs at 800 particles L^{-1}) showed the highest MP abundance, 662.5 ± 17.68 particles L^{-1} , a 365.5% increase over control ($p < 0.05$). Treatments C and D had significantly higher MPs than A and B, indicating that biofloc alone has limited capacity to remove buoyant polymers like polyethylene, especially at higher loadings. Nevertheless, one-way ANOVA confirmed significant differences among treatments ($p < 0.05$), demonstrating that biofloc formation still enhances MP retention compared to systems without biofloc. Enhancing biofloc–microplastic interactions remains critical for mitigating plastic pollution in low-water-exchange aquaculture (Avio et al., 2017; Zhao et al., 2023).

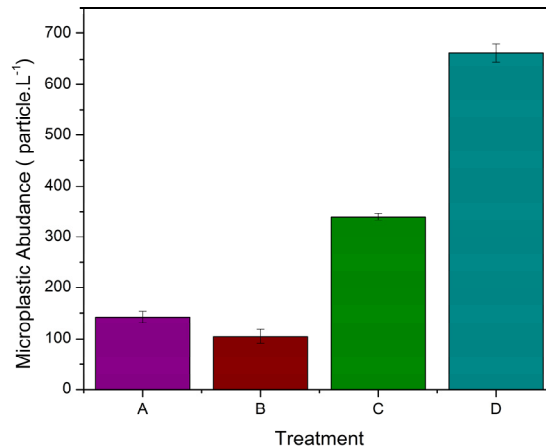


Fig. 1. Microplastic abundance (particles L⁻¹) across treatments in the biofloc aquaculture experiment: A (control, no biofloc or MPs), B (biofloc only), C (biofloc + PE MPs at 80 particles L⁻¹), and D (biofloc + PE MPs at 800 particles L⁻¹).

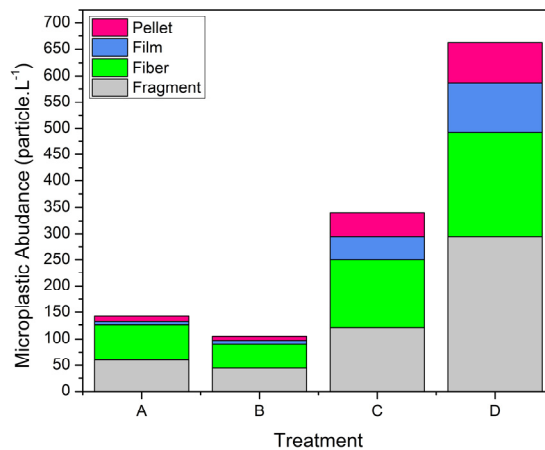


Fig. 2. Morphological composition of microplastics—fragments, fibers, films, and pellets—expressed as relative proportions (%) across treatments: A (control, no biofloc or MPs), B (biofloc only), C (biofloc + PE MPs at 80 particles L⁻¹), and D (biofloc + PE MPs at 800 particles L⁻¹).

Morphology of microplastics

Fragments and fibers dominated across all treatments, accounting for over 75% of total MPs, while films and pellets contributed ~10% and 8%, respectively (Figure 2). Higher PE input increased the relative proportion of films and pellets, reflecting PE's high hydrophobicity and resistance to degradation (Andrady, 2017; Horton et al., 2018). Lack of water exchange further promoted accumulation of PE-derived MPs in the closed biofloc system.

These results indicate that PE persistence, combined with minimal water exchange, accelerates MP accumulation, with implications for fish uptake. While biofloc improves water quality, the inert nature of PE highlights the need for targeted waste management and further investigation into mitigation strategies in closed aquaculture systems.

Microplastic Particle Size Distribution

Analysis of microplastic (MP) size distribution showed that biofloc and polyethylene (PE) MPs influenced MP accumulation in the water column (Figure 3). Particles <50 µm dominated (53.97%), followed by 50–100 µm (32.14%) and >100 µm (13.89%), indicating active

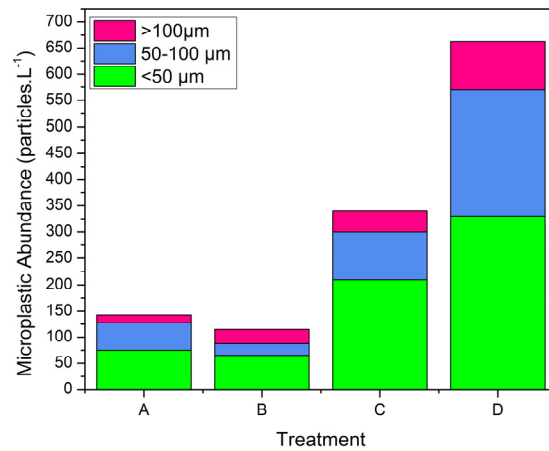


Fig. 3. Size class distribution of microplastics (MPs) and their abundance (particles L⁻¹) across treatments: A (control, no biofloc or MPs), B (biofloc only), C (biofloc + PE MPs at 80 particles L⁻¹), and D (biofloc + PE MPs at 800 particles L⁻¹).

fragmentation during culture (Li et al., 2020a).

Treatment A (control) had lower MP abundances, primarily from background contamination such as airborne deposition, feed, or equipment (Hossain et al., 2021). Treatment C (biofloc + low PE concentration) exhibited higher abundance in the <50 µm fraction, though still below Treatment D, suggesting that biofloc acts as a physical sink while facilitating partial fragmentation via mechanical floc–particle interactions, microbial colonization, and biological degradation (Dussud et al., 2018; Tiwari et al., 2020). Microorganisms, including heterotrophic bacteria and fungi, secrete enzymes and metabolites that promote surface oxidation and structural weakening of PE polymers, accelerating size reduction (Dauda et al., 2019; Syranidou et al., 2017).

Treatment D, with the highest PE load, exhibited the greatest MP abundance across all size classes, particularly <50 µm, likely due to mechanical abrasion, photochemical degradation, and enhanced microbial activity within the biofloc matrix (Yuan et al., 2022). The carbon- and protein-rich environment of biofloc promoted particle retention and provided surfaces for microbial colonization, initiating partial PE degradation (Martínez-Porchas & Vargas-Albores, 2017; Qi et al., 2021).

Treatment C, with lower PE input, balanced retention and minimized excessive fragmentation into ultrafine particles, making it potentially the most environmentally sustainable option. In contrast, Treatment D's high proportion of <50 µm particles increases ecological and toxicological risks due to higher bioavailability and pollutant adsorption potential (Chae & An, 2018). Smaller MPs are more readily ingested by aquatic organisms, enhancing risks of bioaccumulation and biomagnification (Deswati et al., 2023b). These findings underscore the importance of controlling PE microplastic inputs and monitoring micron-scale particles in biofloc-based aquaculture.

Microplastic Color Identification

Figure 4. Microplastic color distribution in the biofloc aquaculture system, showing the effects of biofloc and polyethylene (PE) MPs on particle accumulation in the water column. Five major color categories were identified: black, brown, blue, transparent, and red.

Black microplastics dominated (48.91%), followed by brown (17.30%), blue (17.10%), transparent (12.33%), and red (4.37%), reflecting the widespread use of black plastics in

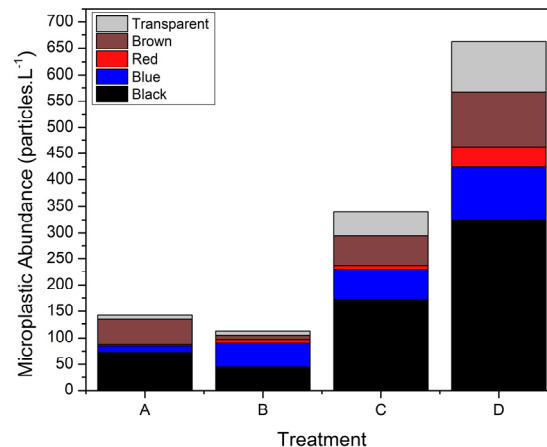


Fig. 4. Color composition of microplastics (particles L⁻¹) across treatments: A (control, no biofloc or MPs), B (biofloc only), C (biofloc + PE MPs at 80 particles L⁻¹), and D (biofloc + PE MPs at 800 particles L⁻¹).

packaging, household, and industrial products, which fragment under environmental degradation (Suparno et al., 2024; Deswati et al., 2024). PE addition contributed to higher black particle abundance, particularly in high-concentration Treatment D, where low photodegradation resistance accelerated fragmentation (Gong et al., 2023).

Treatment A showed lower black MP abundance, likely from environmental contamination (feed, air, equipment) (Deswati et al., 2023c). Treatment B (biofloc only) had the lowest microplastic counts across all colors, indicating biofloc's role as a natural sink through floc entrapment and sedimentation, even under no-water-exchange conditions (Xiong et al., 2022). Treatment C (biofloc + low PE) retained high black and brown MPs but lower than Treatment D, reflecting adsorption of PE fragments and environmental MPs, with brown and blue particles suggesting photodegradation of other plastics (Galloway et al., 2017; Gong et al., 2023). Treatment D (biofloc + high PE) exhibited the highest abundance, with black > brown > blue > transparent > red, reflecting enhanced PE fragmentation and floc trapping under static water conditions (Wang et al., 2019). Blue, red, and transparent MPs likely originated from pigments, plastic bags, bottles, and photoaging (Hüffer & Hofmann, 2016; Gorokhova, 2021).

Overall, Treatment B was most effective in minimizing MP abundance, highlighting biofloc's potential as a low-input strategy to mitigate microplastic pollution in closed aquaculture systems (Avio et al., 2017; Xiong et al., 2022). Color identification was performed visually, and future studies should use standardized colorimetric methods (e.g., CIE Lab or Hunter Lab) to improve objectivity and reproducibility.

Identification of Microplastic Polymer Types

In this study, analysis of 10 microplastic samples revealed the presence of three major polymer types: polyethylene (PE), polyamide (PA), and polyethylene terephthalate (PET). Polyethylene was the most frequently detected polymer, accounting for 40% of the samples, followed by polyamide and polyethylene terephthalate, each comprising 30% of the total. These findings indicate a diverse composition of microplastics in the studied environment, reflecting multiple sources and uses of plastic materials (Andrady, 2017; Galloway et al., 2017).

Polyethylene (PE)

Polyethylene (PE) microplastics were identified as red, black, and blue fragments, confirmed by ATR-FTIR spectra showing characteristic C–H₂ rocking vibrations at 716–719 cm⁻¹, C–H₂ bending at ~1463–1465 cm⁻¹, and asymmetric/symmetric C–H₂ stretching at 2848–2850 and

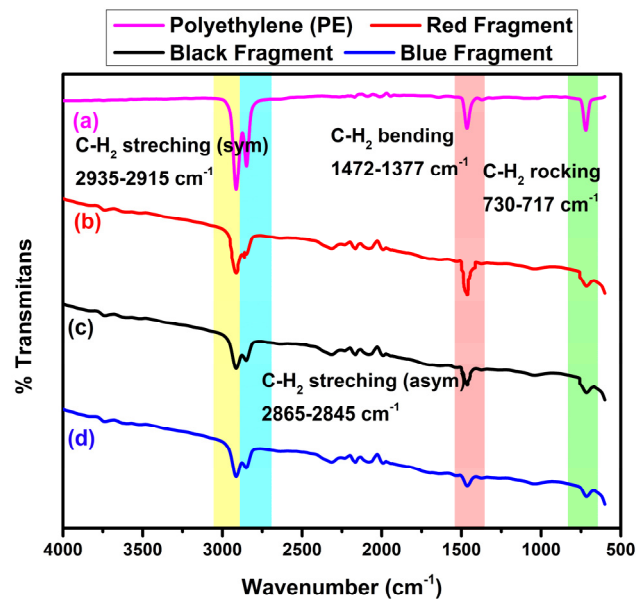


Fig. 5. Comparison of Spectra from Microplastic Identification Against Standards a) Standard Polyethylene (PE); b) Red Fragment Microplastic; c) Black Fragment Microplastic; d) Blue Fragment Microplastic

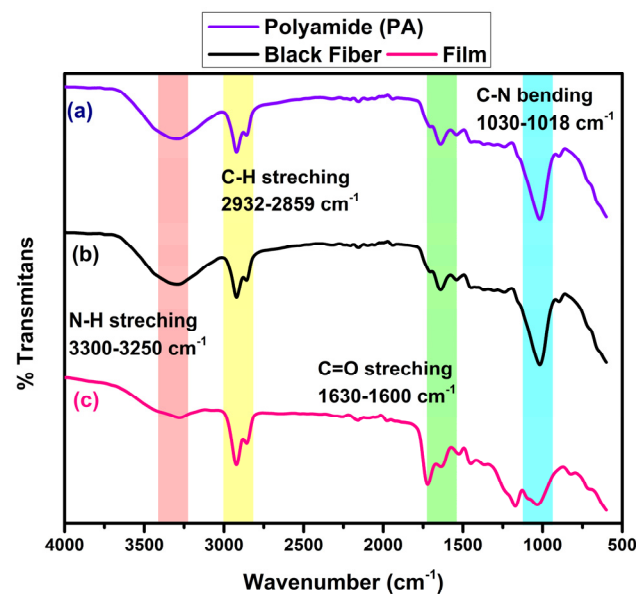


Fig. 6. Comparison of Spectra from Microplastic Identification Against Standards (a) Standard Polyamide (PA), (b) Black Fiber Microplastic, (c) Film Microplastic

2913–2915 cm^{-1} (Andrady, 2011; Yuan et al., 2019; Li et al., 2021; Zhao et al., 2020). PE is widely used in bags and containers, and its buoyancy enables widespread dispersion in aquatic environments. Colored fragments typically originate from photodegradation or mechanical abrasion, and their surfaces can adsorb contaminants such as heavy metals and hydrophobic organic compounds, posing ecological risks through entry into aquatic food webs (Andrady, 2011).

The study applied four treatments (A: control; B: biofloc only; C: biofloc + PE 80 particles L^{-1} ; D: biofloc + PE 800 particles L^{-1}), each in triplicate, without water exchange or additional biofloc amendments to simulate practical aquaculture conditions. PE fragments were detected

in Treatments C and D, while biofloc aggregates in Treatments B–D consistently trapped PE microplastics and polyamide fibers. These results demonstrate that biofloc acts as an effective sink for multiple microplastic types, influencing their environmental fate and reducing exposure risk to cultured aquaculture organisms.

Polyamide (PA)

Polyamide (PA) microplastics were identified as black fiber particles and films, confirmed by ATR-FTIR spectra showing C–N bending vibrations at 1030–1031 cm^{-1} , C=O stretching at 1606–1629 cm^{-1} , and C–H/N–H stretching near 2918 and 3288 cm^{-1} , consistent with typical PA polymer structures (Andrady, 2011; Li et al., 2021; Zhao et al., 2020). These features verify the presence of PA fibers in the experimental systems. PA, commonly known as nylon, is widely used in textiles and packaging, and its detection suggests leaching from degrading consumer or industrial products. PA fibers fragment readily in aquatic environments, persisting as microplastic pollutants that accumulate in biofloc aggregates and aquatic organisms, posing risks to water quality and ecosystem health (Li et al., 2020b; Zhao et al., 2020; Sun et al., 2019).

The experiment included four treatments with three replicates each: A (control, no biofloc or MPs), B (biofloc only), and C and D (biofloc + PE microplastics at 80 and 800 particles L^{-1}). No water exchange or additional biofloc amendments were applied to simulate practical aquaculture conditions. PA microplastic fibers were consistently trapped in biofloc aggregates in Treatments B–D, indicating that biofloc acts as a sink for both background PA contamination and introduced PE microplastics, thereby influencing the environmental fate and bioavailability of multiple microplastic types in aquaculture systems.

Polyethylene Terephthalate (PET)

Polyethylene terephthalate (PET) microplastics were identified as blue fragments by ATR-FTIR spectroscopy, showing characteristic absorption peaks: aromatic C–H at 685 cm^{-1} , C–O and C(O)O stretching at 1238–1244 cm^{-1} , carbonyl (C=O) stretching at 1708 cm^{-1} , and C–H stretching at 2952 cm^{-1} , confirming PET's polyester structure (Andrady, 2011; Li et al., 2021; Yuan et al., 2019; Sun et al., 2019; Zhao et al., 2020). PET, widely used in beverage bottles, films, and textiles, persists in aquatic environments due to chemical stability and fragments into smaller particles, posing ecological risks through bioaccumulation and pollutant transport in food webs (Geyer et al., 2017; Jambeck et al., 2015).

The experiment included four treatments with triplicates: A (control, no biofloc or MPs), B (biofloc only), and C and D (biofloc + PE MPs at 80 and 800 particles L^{-1}), without water exchange or additional biofloc amendments to mimic practical aquaculture conditions. PET microplastics, although not directly added, were detected in biofloc matrices across treatments, indicating background contamination. Their retention demonstrates the biofloc system's capacity to sequester diverse microplastic types, influencing their environmental fate, bioavailability, and potential exposure risks in aquaculture systems.

Health Risk

Table 1 summarizes the Estimated Daily Intake (EDI), Target Hazard Quotient (THQ), and Hazard Index (HI) across treatments, reflecting the combined effects of biofloc presence and polyethylene (PE) microplastic concentrations. Treatment A (control) recorded the lowest EDI (0.0028 $\text{mg kg}^{-1} \text{day}^{-1}$) and hazard indices (THQ = 0.014, HI = 0.021), consistent with the absence of both biofloc and microplastic inputs, minimizing contaminant adsorption and trophic transfer. Treatment B (biofloc without microplastics) showed slightly elevated values, indicating that while biofloc improves water quality and nutrient recycling, its particulate matrix can adsorb ambient contaminants, which may be ingested by cultured fish (Crab et al., 2012).

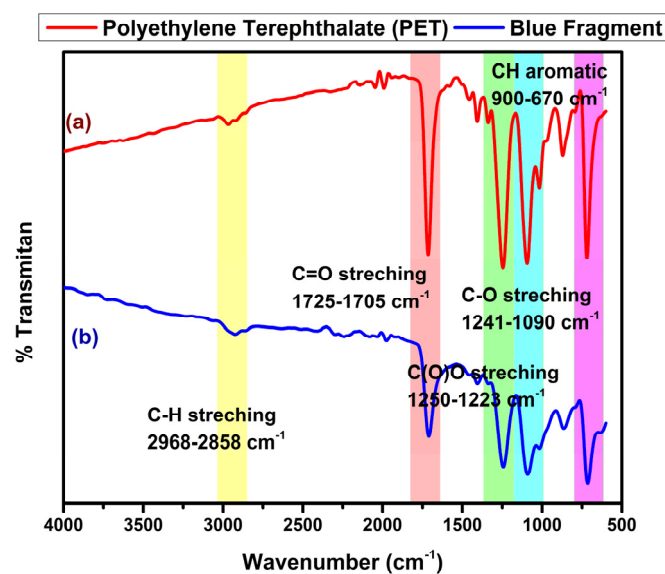
Treatments C and D (biofloc with PE microplastics) exhibited significantly higher EDI and

Table 1. Concentrations of microplastics and associated contaminants for EDI, THQ, and HI calculations in Nile tilapia from different treatments

Treatment	Microplastic concentration in fish (particles/g)	Contaminant load ($\mu\text{g/g}$ dry weight)	EDI (particles/day)	EDI ($\mu\text{g/day}$)	THQ	HI
A	0.5 ± 0.1	0.05 ± 0.01	25 ± 5	2.5 ± 0.5	0.05	0.05
B	0.7 ± 0.1	0.07 ± 0.01	35 ± 5	3.5 ± 0.5	0.07	0.07
C	3.5 ± 0.3	0.35 ± 0.03	175 ± 15	17.5 ± 1.5	0.35	0.35
D	8.2 ± 0.6	0.82 ± 0.06	410 ± 30	41.0 ± 3.0	0.82	0.82

Notes:

- EDI (particles/day) calculated assuming a daily fish consumption of 50 g wet weight per capita.
- EDI ($\mu\text{g/day}$) based on average contaminant load associated with microplastics.
- THQ and HI calculated following USEPA guidelines, where HI is the sum of THQ values for all contaminants assessed.
- Values are presented as mean \pm standard deviation ($n = 3$).

**Fig. 7.** Comparison of Spectra from Microplastic Identification Against Standards (a) Standard Polyethylene Terephthalate (PET); (b) Blue Fragment Microplastic

THQ values, with Treatment D ($800 \text{ particles L}^{-1}$) reaching an HI of 0.132, nearly double that of Treatment C (0.068). This increase aligns with prior findings that higher microplastic loads enhance pollutant adsorption and facilitate trophic transfer to fish (Rochman et al., 2015; Koelmans et al., 2016). Biofloc aggregates can act as carriers for microplastics, increasing ingestion by fish and the bioavailability of hydrophobic organic pollutants and metals adhered to plastic surfaces (de Sá et al., 2018; Pittura et al., 2018).

Despite these variations, all THQ and HI values remained below the safety threshold of 1, indicating no immediate health risk from fish consumption. However, elevated indices in Treatments C and D highlight potential cumulative exposure risks in closed or intensive aquaculture systems with minimal water exchange (Li et al., 2020b). Treatment A represents the lowest risk scenario, while Treatment B offers a practical balance between maintaining biofloc benefits and minimizing microplastic hazards. Long-term exposure may still affect fish growth, immunity, energy allocation, and microbial-trophic interactions, underscoring the need for multi-generational studies to assess chronic risks in biofloc-based aquaculture.

Artificial Neural Network (ANN) Simulation and Interpretation

The ANN model effectively captured nonlinear relationships between biofloc presence,

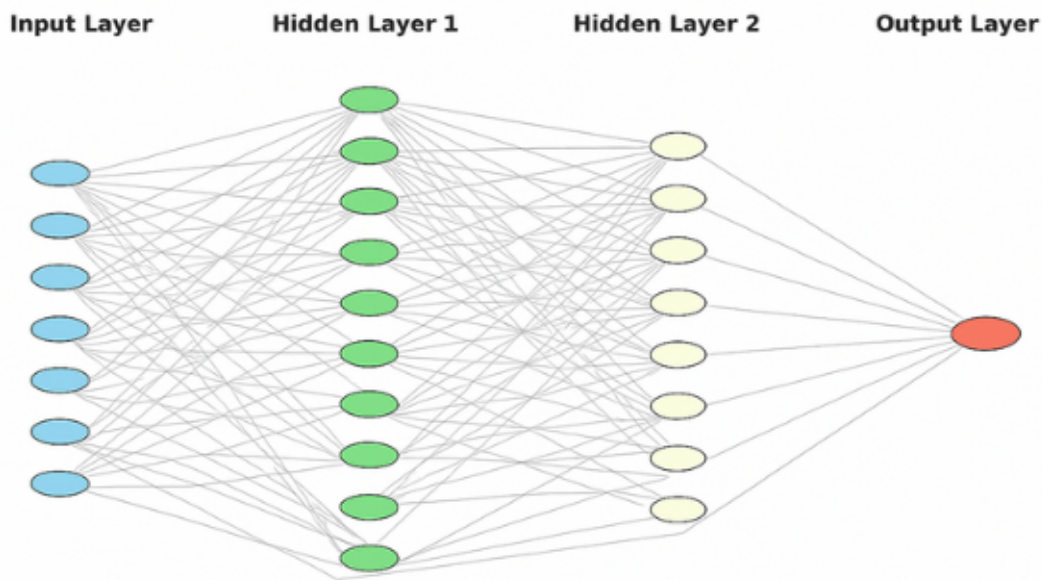


Fig. 8. Shows the ANN architecture with seven input neurons (water quality and biofloc parameters), two hidden layers (10 and 8 neurons), and outputs linking environmental drivers to microplastic abundance.

polyethylene microplastic concentrations, and exposure/risk parameters (EDI, THQ, HI), demonstrating high predictive performance ($R^2 = 0.95$, $RMSE = 0.021$, $MAE = 0.015$) (Gharbani et al., 2022; Mohammadi et al., 2021). Learning curves (Figure 7) showed smooth convergence at ~120 epochs, confirming model robustness and generalization without overfitting. Treatments C and D exhibited exponential increases in EDI and HI with higher microplastic loads, while Treatments A and B remained at low-risk levels.

SHAP analysis identified microplastic concentration as the dominant predictor (mean SHAP = 0.41, ~70%), biofloc parameters contributed moderately (~20%), and other environmental factors, such as pH and dissolved oxygen, had minimal influence (~10%). Dependence plots confirmed that increasing microplastic loads elevated EDI, THQ, and HI, whereas biofloc partially mitigated risk by promoting contaminant aggregation.

Although all hazard indices remained below the safety threshold ($HI < 1$), ANN simulations indicate that chronic exposure to high microplastic concentrations could gradually increase HI values (Li et al., 2020b; Rochman et al., 2015; Wu & Wang, 2023). These findings highlight the key role of microplastics in dietary exposure risks and demonstrate the utility of ANN modeling for assessing biofloc–microplastic interactions and guiding aquaculture risk management.

CONCLUSION

Biofloc systems partially retain microplastics but are largely ineffective against buoyant polyethylene (PE) particles, resulting in elevated microplastic concentrations dominated by small fragments and fibers. Despite hazard indices remaining below critical thresholds, cumulative exposure risks persist, particularly under high microplastic loads. Predictive modeling using ANN with SHAP analysis identified microplastic concentration as the principal determinant of health risk, demonstrating the value of integrating biofloc management with data-driven risk assessment. To enhance sustainability and minimize ecological and food safety risks, future strategies should focus on optimizing biofloc compositions for improved microplastic sequestration, evaluating PE fragmentation dynamics, monitoring the long-term fate of microplastic-laden flocs, and implementing safe disposal or reuse practices, alongside

multi-generational studies to understand chronic impacts on aquaculture organisms.

ACKNOWLEDGEMENTS

The authors would like to thank for the financial support provided for this research by Andalas University through the Expertise Research Scheme (PUJK) Batch I under Research Contract: 335/UN16/PT.01.03/PUJK/2024, July 17, 2024.

CONFLICT OF INTEREST

The authors declare that there is not any conflict of interest regarding the publication of this manuscript.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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