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Article

The Impact of Biofloc on Fish Growth Indicators and Health Risks Assesment from Polyethylene Terephthalate Microplastic Contamination

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Abstract. This study aims to evaluate the effects of biofloc technology on the growth performance of Nile tilapia (*Oreochromis niloticus*) and to assess potential health risks associated with polyethylene terephthalate (PET) microplastic contamination in aquaculture systems. Three treatments were applied: aquaculture without biofloc and microplastics, aquaculture with biofloc but without microplastics, and aquaculture with biofloc combined with PET microplastics. The novelty of this research lies in the integration of biofloc technology with a quantitative health risk assessment approach aspect that has received limited attention in previous studies. Furthermore, this study specifically utilizes PET microplastics, which differ in physicochemical properties and toxicological potential from the commonly studied polyethylene (PE) microplastics. The results revealed that the best performance was observed in the treatment with biofloc but without microplastics, showing an average body weight (ABW) of 5.478 g/fish, an average daily gain (ADG) of 2.343 g/fish/day, and a specific growth rate (SGR) of 4.208%. In terms of health risk, this treatment also demonstrated low to moderate risk levels, with a Polymer Load Index (PLI) of 2.53, a Potential Health Index (PHI) of 11, and a Potential Ecological Risk Index (PERI) of 10. These findings indicate that biofloc technology is not only effective in enhancing fish growth performance but also contributes to mitigating the adverse impacts of microplastic contamination on fish health and food safety in aquaculture systems.

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1. Introduction

Intensive farming of Nile tilapia (*Oreochromis niloticus*) has rapidly expanded in Indonesia as an effort to meet the population's growing demand for animal protein [1]. However, conventional aquaculture systems often face challenges such as high feed consumption, organic waste accumulation, and declining water quality [2]. Biofloc Technology (BFT) has been introduced as an innovative solution to address these issues. BFT utilizes heterotrophic microorganisms to convert nitrogenous waste into microbial biomass that can be consumed by fish, thereby improving feed efficiency and water quality [3]. Moreover, BFT has been shown to enhance fish immunity and growth performance [4]. In Nile tilapia, the application of BFT has proven effective in increasing growth rates, feed efficiency, and survival, while significantly reducing the need for antibiotics [5].

Biofloc also plays a significant role in reducing the abundance of microplastics (MPs) in aquaculture environments [6-7]. The floc mass formed in this system contains organic matter and microorganisms capable of trapping microplastic particles such as polyethylene (PE) and polyethylene terephthalate (PET) [8]. Physical and biological interactions within the flocs facilitate the reduction of microplastic concentrations in the water column through sedimentation or initial degradation by specific microorganisms [9]. Consequently, biofloc indirectly helps reduce microplastic exposure in fish while improving the overall quality of the aquaculture ecosystem [10].

However, the increasing contamination of microplastics (MPs), particularly polyethylene terephthalate (PET), in aquaculture environments raises concerns regarding fish health and food safety [11]. Microplastics are known to accumulate in fish tissues, particularly the liver and intestines, and can negatively impact the nutritional composition of fish muscle [12]. In the context of biofloc systems, high concentrations of PET microplastics can interfere with floc formation, alter microbial communities, and reduce the efficiency of nitrogen transformation [13-14]. The degradation of floc quality due to microplastics may hinder the bioconversion of waste into microbial biomass, consequently affecting water quality and fish growth performance [15,16]. Beyond their direct impact on fish, microplastics also pose risks to human health through the food chain, particularly through the consumption of farmed fish exposed to microplastics and adsorbed heavy metals [17]. Therefore, it is essential to conduct health risk assessments using indices such as the Pollution Load Index (PLI), Potential Ecological Risk Index (PERI), and Potential Hazard Index (PHI) to evaluate the extent of ecological and consumer risk associated with microplastic contamination [18-19].

This study aims to evaluate the effects of biofloc technology (BFT) on the growth and production of Nile tilapia (Oreochromis niloticus), as well as to assess health risks associated with high-dose polyethylene (PE) microplastic contamination. The parameters observed include average daily gain (ADG), average body weight (ABW), specific growth rate (SGR), survival rate (SR), floc density, and health risk indicators based on the Pollution Load Index (PLI), Potential Hazard Index (PHI), and Potential Ecological Risk Index (PERI) [16],[20]. The main contribution of this research lies in the integration of biofloc technology with a quantitative health risk assessment approach, which is still rarely applied, alongside the use of polyethylene terephthalate (PET) microplastics unlike most previous studies that focused on PE microplastics thereby providing new insights into the specific impacts of different types of microplastics within aquaculture systems [21].

2. Research Methodology

2.1. Research Procedure

Nile tilapia (*Oreochromis niloticus*) were cultured in glass aquaria measuring 75 × 50 × 50 cm^3, with a water volume of 150 liters. The aquaria were thoroughly cleaned prior to use, and each was then filled with 150 liters of water. An aeration system was installed using PVC pipes, with four air stones placed in each treatment tank. The initial stage of biofloc culture began with the addition of 150 g of non-iodized salt (specifically formulated for aquaculture) to each aquarium. After 30 minutes, 7.5 g of dolomite lime was added. Aeration was activated and maintained for two days until the water became clear, followed by pH measurement. When the water pH reached 8, 15 ml of molasses was added as a carbon source. After another 30 minutes, 1.5 g of biolakto (a probiotic to promote floc formation) was introduced. The culture was then left undisturbed for eight days.

This study used 25 juvenile Nile tilapia with an average length of 8–10 cm and an average weight of 16 g. Prior to the experiment, the fish were kept in a ventilated container for two days to reduce stress. Acclimatization was carried out by floating plastic bags containing the fish in the aquarium water and gradually adding tank water until the fish began to swim freely, minimizing stress. Each aquarium was stocked with 25 fish and fed commercial pellets twice daily at 7:00 AM and 5:00 PM, at a feeding rate of 2–3% of the total fish biomass.

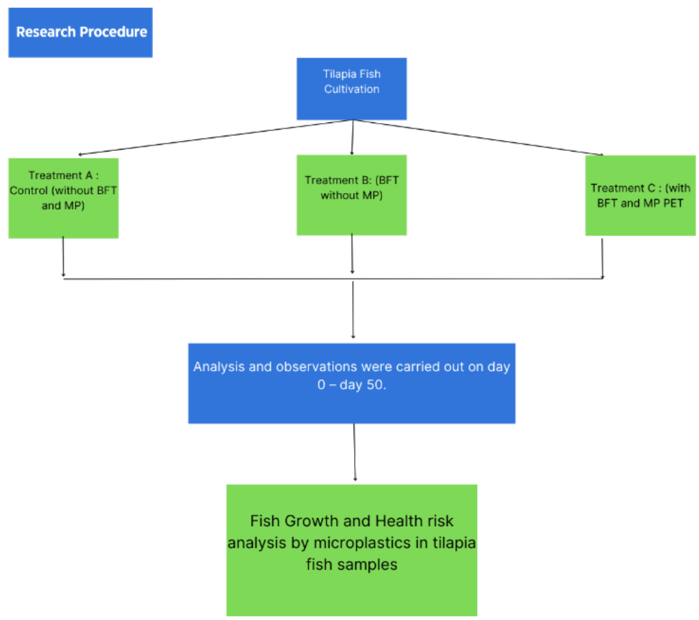

Figure 1. Research Procedure of Biofloc-based

2.2. Microplastics in Biofloc Systems

This study aims to observe the effects of polyethylene terephthalate (PET) microplastics in a biofloc based aquaculture system. The experiment was conducted using three different treatments, each with an identical aeration system. Observations were carried out over a period of 0 to 50 days.Treatment A: Without biofloc and without microplastics. Treatment B: With biofloc and without microplastics Treatment C: With biofloc and with PET microplastics (800 particles/L). The microplastic concentration used in Treatment C was based on the previous study by [20], in which 800 particles per liter corresponded to a measured weight of 0.045 grams.

2.3. The Parameters Measured Include

The observed parameters included Average Daily Growth (ADG), Average Body Weight (ABW), Specific Growth Rate (SGR), Survival Rate (SR), floc density, and health risk indicators based on the Pollution Load Index (PLI), Pollution Hazard Index (PHI), and Potential Ecological Risk Index (PERI). All analyses were conducted in triplicate to ensure accuracy and the reliability of the results.

2.4 Data Processing Design

The results of the water quality analysis for each treatment were calculated as mean ± standard deviation and presented in tables and graphs. To determine the significance of the treatments during the sampling process, statistical tests were done using one-way Analysis of Variance (ANOVA) at a 95% confidence level (α=0.05). A further test was conducted using the Duncan method if the p-value was considered significant ($p<0.05$). The IBM SPSS Statistics 23 software was utilized for all statistical analyses.

3. Result And Discussion

3.1. Fish Growth

Growth parameters such as Average Daily Gain (ADG), Average Body Weight (ABW), and Specific Growth Rate (SGR) are crucial in fish farming as they reflect the effectiveness of the rearing system and feed quality. These indicators help measure the overall growth rate and health of the fish. Monitoring these parameters allows farmers to evaluate the success of the applied farming technology. Additionally, this data is useful for optimizing production and ensuring fish quality. The following graphs present a comparison of ADG, ABW, and SGR values across different treatments, illustrating the impact of biofloc and microplastics on Nile tilapia growth.

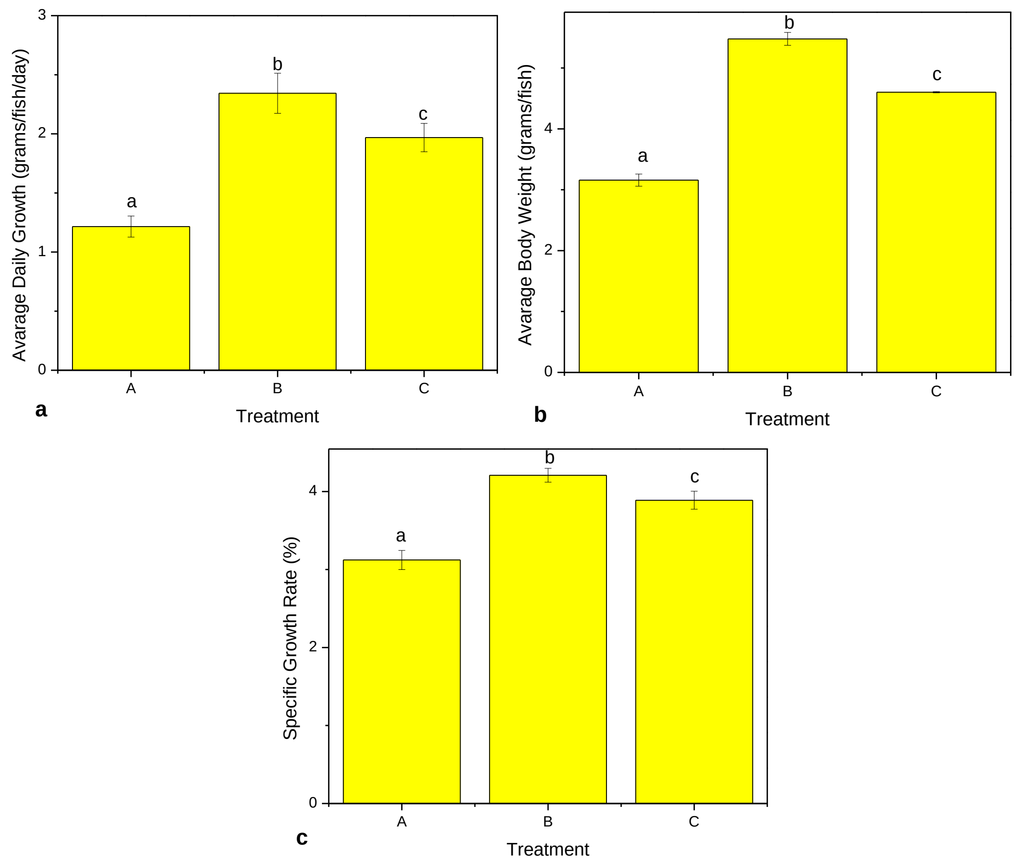

Figure 2. (a). Avarage Daily Growth (grams/fish/day), (b). Avarage Body Weight (grams/fish), (c). Specific Growth Rate (%)

In The study results indicate that the application of biofloc technology significantly enhances the growth of Nile tilapia (Oreochromis niloticus), as reflected by the Average Daily Gain (ADG), Average Body Weight (ABW), and Specific Growth Rate (SGR) [16,22,23]. In Treatment A (control, without biofloc and microplastics), fish exhibited the lowest growth performance with an ADG of 1.215 grams/fish/day, ABW of 3.158 grams/fish, and SGR of 3.122% per day. This was attributed to the absence of biofloc as an additional nutrient source and suboptimal water quality, which limited growth efficiency [20],[24].

Treatment B (with biofloc and no microplastics) produced the highest growth results, with an ADG of 2.343 grams/fish/day, ABW of 5.478 grams/fish, and SGR of 4.208% per day. Biofloc provided supplementary nutrients such as microbial protein, fatty acids, vitamins, and digestive enzymes that improved metabolism and feed conversion efficiency [25-26]. Additionally, biofloc helped break down organic waste, enhanced water quality, and created a stable farming environment, all of which supported optimal fish growth [16],[22].

Treatment C (biofloc with polyethylene terephthalate microplastics) showed reduced growth compared to Treatment B, with an ADG of 1.968 grams/fish/day, ABW of 4.602 grams/fish, and SGR of 3.888% per day. This decline suggests that the presence of PET microplastics interferes with nutrient absorption, induces oxidative stress, damages the digestive tract, and lowers the quality and density of the biofloc. Accumulated microplastics within the biofloc reduce the nutritional value available to the fish, thereby inhibiting growth efficiency [5],[17],[20],[24],[27].

Statistical analysis using ANOVA followed by Duncan's test confirmed that the differences in ADG, ABW, and SGR among treatments were significant. Each treatment was classified into different groups (a, b, and c), indicating that the observed variations in growth were due to the treatments rather than chance [23]. Overall, these findings reinforce that biofloc systems can improve the growth efficiency of Nile tilapia compared to conventional methods, but the presence of microplastics in biofloc may hinder achieving optimal growth.

This study demonstrates that biofloc technology, which utilizes microbial activity, effectively enhances the growth of Nile tilapia (Oreochromis niloticus) by decomposing organic waste and reducing pollutants such as ammonia and microplastics. The microorganisms in biofloc not only improve water quality but also produce nutrient-rich biomass that serves as supplemental feed for the fish. In treatments without biofloc, fish growth was hindered due to limited nutrients and poor water quality. Conversely, biofloc without microplastics significantly improved growth by providing microbial nutrients. However, the presence of PET microplastics disrupted microbial biochemical activity and fish health, resulting in reduced growth efficiency [28-29].

3.1.1. Survival Rate (SR)

Survival Rate (SR) is a crucial indicator for assessing the success of fish farming, representing the percentage of fish that survive until the end of the rearing period. SR is strongly influenced by factors such as water quality, population density, environmental stress, dissolved oxygen availability, disease infection, and feed quality [30].

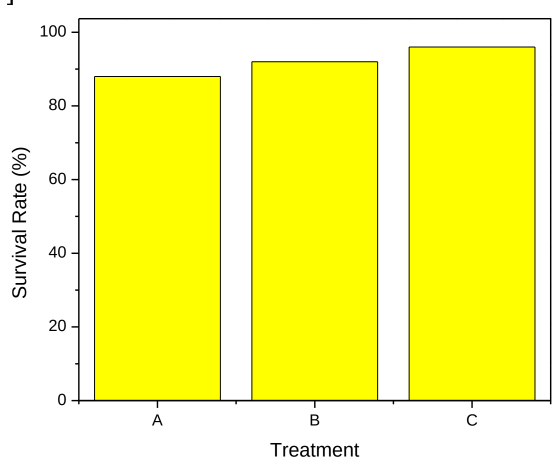

Figure 3. Survival Rate (%)

The results of this study show that the highest SR was observed in Treatment B (with biofloc and without microplastics), reaching 96%, followed by Treatment C (with biofloc and polyethylene terephthalate microplastics) at 92%, and the lowest SR in Treatment A (without biofloc and without microplastics) at 88%.

The high survival rate in Treatment B indicates that the biofloc system can create a more stable and healthy aquatic environment. Biofloc is known to maintain water quality through nitrogen cycling, reducing ammonia and nitrite concentrations, and supporting the growth of probiotic microorganisms that suppress pathogens [16],[25]. This condition creates a more optimal environment for the cultivation of Nile tilapia (Oreochromis niloticus), thereby reducing mortality risks. In Treatment C, despite using the same biofloc system, a slight decrease in SR compared to Treatment B was observed. This decline is strongly associated with exposure to polyethylene terephthalate microplastics (PET), which can trigger physiological stress, digestive disturbances, and morphological changes in organs such as the intestine and liver of the fish [20],[27]. Additionally, microplastic accumulation can impair the immune system and cause chronic inflammation, negatively impacting fish survival [18]. The reduced SR confirms that the presence of microplastic contaminants poses a significant challenge to the overall effectiveness of the biofloc system.

Meanwhile, the lowest SR in Treatment A indicates that systems without biofloc are more vulnerable to environmental stress and fluctuations in water quality. Uncontrolled parameters such as ammonia, dissolved oxygen, and pH result in unstable conditions for fish, leading to higher mortality rates.

3.2. Floc Density

Measuring floc density is essential to assess the extent of biofloc development during the cultivation process. This measurement helps determine the effectiveness of biofloc in promoting fish growth and mitigating health risks.

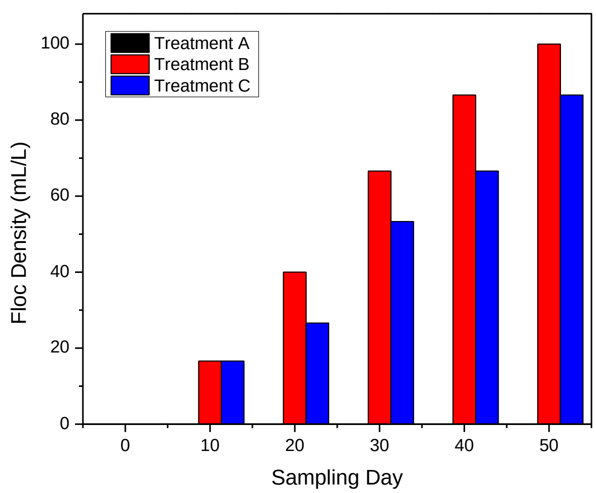

Figure 4. Floc Density (mL/L) with Sampling Day

The results of this study showed that the volume of biofloc formed varied among treatments. Treatment B exhibited biofloc volumes ranging from 16.6 to 100 mL/L, while Treatment C showed volumes between 16.6 and 86.6 mL/L. The higher biofloc volume in Treatment B is attributed to the accumulation of organic compounds derived from uneaten feed and fish metabolism, which were converted into biofloc. Since Treatment B was free from microplastic contamination, the biofloc volume increased more than in Treatment C. Treatment A, serving as the control, showed no change in biofloc density throughout the sampling period, remaining constant at 0 mL/L from day 0 to day 50. In contrast, Treatment B exhibited a significant increase in biofloc density starting from day 10 through day 50. Density rose from 16.6 mL/L on day 10 to 100 mL/L by day 50. This increase is due to the presence of biofloc, which facilitates the formation of suspended particles and aggregates that enhance water density. Active biofloc accumulates both organic and inorganic particles, leading to an overall increase in water density. Treatment C also demonstrated an increase in biofloc density, but at a slower rate compared to Treatment B. The density rose from 16.6 mL/L on day 10 to 86.6 mL/L on day 50. Although this increase reflects biofloc activity, the presence of microplastics appears to reduce the rate of density growth. This may be due to microplastics interfering with biofloc colonization, thereby slowing the increase in biofloc density [14].

3.3. Health Risk Assesment

3.3.1. Identification of Polymer Type

The analysis of microplastic polymer types found in fish and water samples in this study was conducted using ATR-FTIR. FTIR spectroscopy is a technique based on the ability of a sample to absorb infrared (IR) radiation, allowing for the identification of molecular composition through the infrared spectrum produced. By identifying the type of polymer, it becomes possible to calculate health risk analysis values (PLI, PHI, and PERI) associated with microplastic contamination in aquaculture systems [18-19]. The results of the analysis identified the white-colored fragments and pellets as polyethylene terephthalate (PET) polymers.

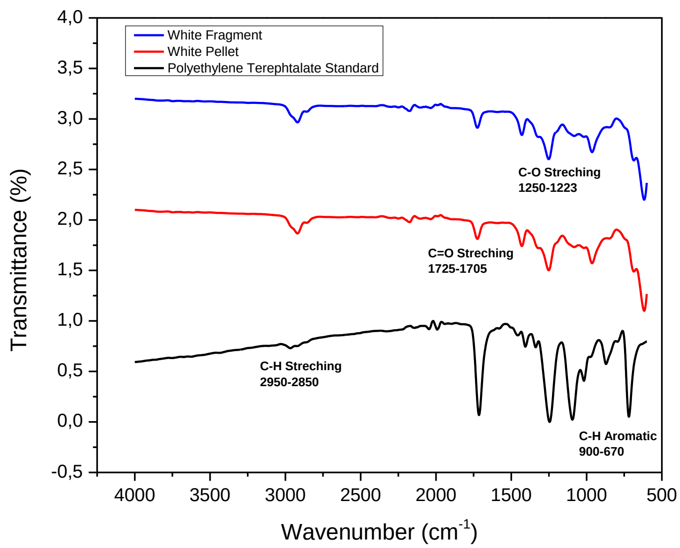

Figure 5. FTIR Spectrum of White Fragments and White Pellets (Polyethylene Terephthalate)

Polyethylene terephthalate (PET) was the predominant type of microplastic found in the water samples from Treatment C after the cultivation period, appearing in the form of white fragments. This type of plastic polymer was identified by characteristic absorption bands in the FTIR spectrum: C–H stretching vibrations at 2950–2850 cm^{-1}, C=O stretching at 1725–1705 cm^{-1}, C–O stretching at 1250–1233 cm^{-1}, and aromatic C–H vibrations at 900–670 cm^{-1}. PET is a commonly found plastic polymer in aquatic environments. It is known for its strength and lightweight properties, and is widely used in food and beverage packaging, electronic insulation, and various other applications [31].

PET can undergo hydrolysis and oxidation reactions in the presence of water and oxygen, which may lead to changes in its chemical structure. These changes can alter existing functional groups, leading to the formation of degradation products such as terephthalic acid and ethylene glycol. Over time, these processes can significantly affect the physical and chemical properties of PET fragments.

As ester bonds break down, the original functional groups within the polymer may no longer be easily identified [29].

3.3.2. Health Risk

Based on Figure 5, the values of Pollution Load Index (PLI), Pollution Hazard Index (PHI), and Potential Ecological Risk Index (PERI) provide a comprehensive overview of the microplastic pollution level and its potential ecological risks. These indices are crucial in assessing health risks associated with microplastic exposure in aquaculture environments.

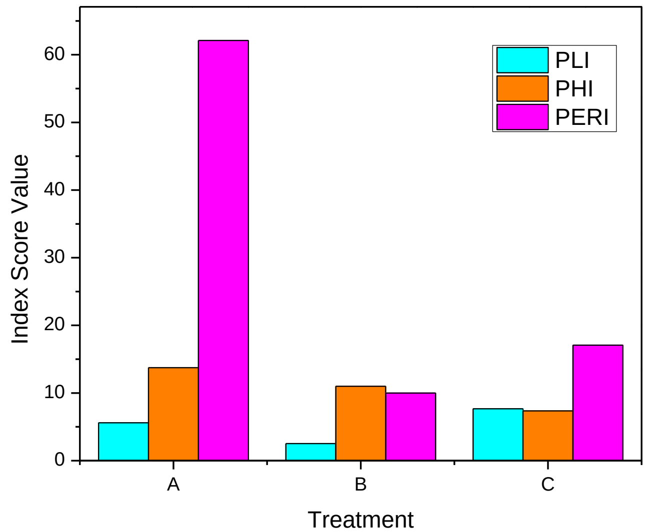

Figure 6. Health Risk Assesment by Microplastics

The PLI measures the burden of microplastic polymers accumulated in fish tissues and reflects the capacity of the biofloc system to retain or accumulate these particles. The results indicate that the highest PLI value was observed in Treatment C (7.67), followed by Treatment A (5.6), and the lowest in Treatment B (2.53). This suggests that biofloc without microplastic exposure (Treatment B) significantly reduces polymer accumulation. Conversely, polyethylene terephthalate (PET) microplastics in Treatment C contribute substantially to the high PLI, reinforcing evidence that PET contamination plays a major role in increasing polymer loads within fish bodies [18-19].

The PHI reflects the public health risk posed by consuming contaminated fish. The highest PHI was found in Treatment A (13.75), followed by Treatment B (11), and the lowest in Treatment C (7.36). Although PLI was highest in Treatment C, PHI was lower, indicating that factors beyond microplastic contamination, such as heavy metals or other toxicological parameters, also influence health risks. It is likely that Treatment C's biofloc system retains some filtration or biological interventions that mitigate overall toxicity effects on human health [17],[23]. The reduction in PHI may thus demonstrate the adaptive capacity of the biofloc system against certain toxic threats.

PERI evaluates the potential ecological risk posed by contaminants in the aquaculture environment. The results showed the highest PERI in Treatment A (62.115), followed by Treatment C (17.075), and Treatment B (10). The elevated PERI in Treatment A indicates that conventional systems without biofloc are highly vulnerable to toxic substance accumulation, adversely affecting not only fish but the entire aquatic ecosystem [32]. Treatment B's lowest PERI value again underscores the effectiveness of biofloc in mitigating ecological impacts and improving aquatic environmental quality.

4. Conclusion

This study demonstrates that the application of biofloc technology in Nile tilapia aquaculture significantly enhances growth performance and reduces health risks associated with microplastic exposure. Treatment B (biofloc without microplastics) proved to be the most effective, achieving the highest average body weight (ABW of 5.408 g), specific growth rate (SGR of 4.242% per day), and survival rate (SR of 96%) compared to other treatments. This highlights the critical role of biofloc in creating a stable culture environment, improving water quality, and providing additional nutrition through biofloc particles.

On the other hand, health risk analyses based on Pollution Load Index (PLI), Pollution Hazard Index (PHI), and Potential Ecological Risk Index (PERI) revealed that Treatment A (without biofloc and with microplastics) presented the highest risk values (PERI = 62.115), indicating significant ecological and health hazards. Conversely, Treatment B recorded the lowest risk index (PERI = 10), reflecting biofloc's capability to mitigate microplastic and heavy metal accumulation in fish tissues. Therefore, the optimal implementation of biofloc technology without microplastic contamination can substantially improve Nile tilapia culture performance while lowering environmental and health risks. These findings support the adoption of biofloc as a sustainable approach in modern aquaculture to ensure food safety and aquatic ecosystem conservation.

5. Acknowledgements

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