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The impact of microplastics on water quality, heavy metals, and health risks in bioflocbased tilapia farming systems

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Abstract

Along with microplastics, pollution of heavy metals, including iron (Fe), zinc (Zn), and copper (Cu), in freshwater ecosystems poses a serious environmental threat that can adversely affect human health. This study investigates the use of biofloc technology to reduce microplastic and heavy metal contamination while improving water quality. By utilizing microbial aggregates that capture microplastic and heavy metal particles through flocculation and biosorption processes, four experimental treatments were applied, i.e.: A (without biofloc and microplastics); B (with biofloc, without microplastics); C (with biofloc and low-density polyethylene microplastics); and D (with biofloc and high-density polyethylene microplastics). The results indicate that fish in Treatment B maintain a stable condition, with reduced biochemical oxygen demand (BOD) value and controlled heavy metal accumulation. Furthermore, the Target Hazard Quotient (THQ) values suggest that consumption of fish from all treatments does not incur a significant health risk, despite the bioaccumulation of Cu, Fe, and Zn at varying concentrations. The application of biofloc technology demonstrates effectiveness in mitigating pollution and enhancing food safety.

Keywords

Heavy metal pollution; biofloc technology; microplastics; Water Quality; health risk

RESEARCH PAPER

The Impact of Microplastics on Water Quality, Heavy Metals, and Health Risks in Biofloc-based Tilapia Farming Systems

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Abstract

Along with microplastics, pollution of heavy metals, including iron (Fe), zinc (Zn), and copper (Cu), in freshwater ecosystems poses a serious environmental threat that can adversely affect human health. This study investigates the use of biofloc technology to reduce microplastic and heavy metal contamination while improving water quality. By utilizing microbial aggregates that capture microplastic and heavy metal particles through flocculation and biosorption processes, four experimental treatments were applied, i.e.: A (without biofloc and microplastics); B (with biofloc, without microplastics); C (with biofloc and low-density polyethylene microplastics); and D (with biofloc and high-density polyethylene microplastics). The results indicate that fish in Treatment B maintain a stable condition, with reduced biochemical oxygen demand (BOD) value and controlled heavy metal accumulation. Furthermore, the Target Hazard Quotient (THQ) values suggest that consumption of fish from all treatments does not incur a significant health risk, despite the bioaccumulation of Cu, Fe, and Zn at varying concentrations. The application of biofloc technology demonstrates effectiveness in mitigating pollution and enhancing food safety.

Keywords: Heavy metal pollution, Biofloc technology, Microplastics, Water quality, Health risk

1. Introduction

The notable rise in the level of microplastics (MPs) and heavy metals in water brought on by increased industrial activities, plastic production, and plastic consumption has posed a serious risk to the environment, particularly impairing water quality and harming the health of water organisms [1]. Most MPs are among the fastest-growing sources of pollution due to their ability to absorb heavy metals through their pores and Van der Waals forces [2]. The manufacture of plastics and the sponge-like PDMS cover may produce pollutants and contaminants and release greenhouse gases into the atmosphere, greatly affecting the transmission and bioavailability of these pollutants [3]. MPs enable heavy metals, such as mercury, cadmium, and lead, to cling to and profligate through water, making marine life and humans incredibly susceptible to them.

Heavy metals that accumulate in the tissues of aquatic organisms through ingested MPs can enter the food chain, harming ecosystems and posing health risks to humans [1,4]. Emissions and contaminations of MPs and heavy metals can indicate broader environmental pollution, thus requiring comprehensive environmental management measures to manage them [5].

In lakes, the most prevalent plastic polymer is polyethylene (PE), which accounts for 12 % of the world's plastic production [6]. It accumulates in the environment, both on land and in water, due to its high resistance to degradation processes [7,8]. Therefore, resolving pollution issues, particularly in Lake Maninjau, requires better waste management and greater public awareness. This study

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investigates the application of biofloc technology to improve water quality by minimizing the accumulation of microplastics and heavy metals, particularly in lakes that are highly susceptible to pollution. The experiments used Nile tilapia (*Oreochromis niloticus*) as the model organism since it can function as a bioindicator of contaminant bioaccumulation, thereby serving a crucial role in aquaculture.

Biofloc technology utilizes microorganisms that can bind and precipitate pollutants, offering a viable, eco-friendly solution for dealing with microplastics and heavy metals. This study aims to reduce the contamination of microplastics and heavy metals to minimize their impact on biota and human health and improve water quality in freshwater ecosystems. Biofloc technology can remove these contaminants by forming microbial aggregates that capture their particles through flocculation and biosorption processes [9]. After treatment, the formed biofloc can be separated from the water, allowing contaminants to be removed from the ecosystem. This makes biofloc an effective strategy for simultaneously addressing pollution and improving environmental quality. This study develops a novel approach to the utilization of biofloc technology in mitigating microplastics and heavy metal contamination in lake ecosystems and promoting food safety and sustainable aquaculture.

2. Materials and methods

2.1. Research procedure

This study examines the application of biofloc technology in tilapia (*Oreochromis niloticus*) farming in 75 × 50 × 50 cm aquariums. The fish used in the experiment were purchased from a certified fish farmer to ensure their quality and health in the Lubuk Buaya area. The general procedure of this study can be seen in Fig. 1.

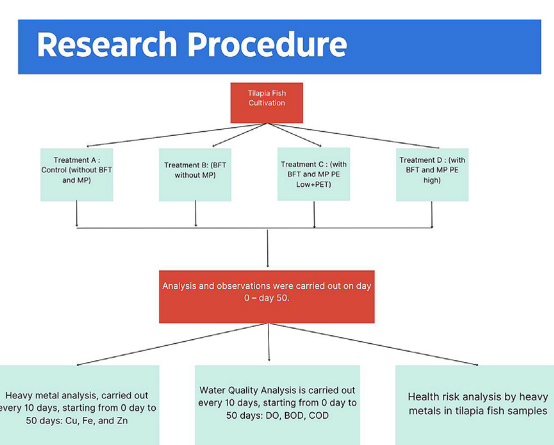

Fig. 1. Research procedures of biofloc-based tilapia cultivation.

A ½-inch PVC air lift aeration system, including an aerator to circulate air through four hoses connected to an air stone at the bottom, was installed in the aquarium. Then, 15 ml of molasses and 1.5 g of Biolacto probiotics were added to the water after the pH reached 8. It took eight days for the biofloc formation process. Water quality was monitored, and floc density was measured using an Imhoff cone, following the formula developed in previous research [9].

This study examined 100 tilapia fingerlings (*Oreochromis niloticus*), measuring 8–10 cm in length and weighing an average of 16 g. Each aquarium housed 25 fish. To ensure their quality and health, the fish used in this study were purchased from a certified fish farmer in Lubuk Buaya, Padang, Indonesia. Before the experiment, the fish were aerated for two days to reduce stress from transportation and environmental changes. To allow the fish to gradually adapt to the new environment, acclimatization was performed using a floating method. Pellets made up 2–3 % of the total biomass were fed twice daily at 07:00 and 17:00 Western Indonesian Time (WIB).

2.2. The influence of microplastics and heavy metals

This study assesses the impacts of microplastics on water quality by applying four treatments with three replications. Each treatment involved an excess of microplastics in glass aquariums as well as various tests for water quality (DO, BOD, and COD) and heavy metal concentration (Cu, Fe, and Zn). Furthermore, health risk analyses of 3 fish samples were performed using Bioconcentration Factor (BCF), Target Hazard Quotient (THQ), and Hazard Index (HI) [10] to monitor microplastics with abundance, shape, size, color, and ATR-FTIR specific parameters [11].

As shown in Fig. 1, the four treatments applied in this study are: A – without biofloc and microplastics; B – with biofloc, without microplastics; C – with biofloc and low concentration polyethylene (PE) microplastics (80 items L^{-1} or 30 μg L^{-1}); and D – with biofloc and poly-concentrated polyethylene terephthalate (PET) microplastics (800 items L^{-1} or 300 μg L^{-1}) [2,9]. Floc incorporation was carried out at intervals of 10 days, and both microplastics and heavy metals were monitored for 50 days.

2.3. 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 ($\alpha = 0.05$). 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. Results and discussion

3.1. Mechanism of heavy metal adsorption by microplastics

There are three main stages in the adsorption of heavy metals onto microplastics: external diffusion, intraparticle diffusion, and adsorption (Fig. 2). In the first stage, pollutants spread to the water layer on the surface of the hydrated microplastics within one to 3 h, demonstrating rapid adsorption. They begin to reach the interior of the microplastics during the intraparticle diffusion stage, where a decrease in the number of available adsorption sites causes the adsorption rate to drop, The adsorption rate continues to decline in the final stage until it completely stops due to the diminishing number of available adsorption sites and the decrease in pollutant concentration in the system [2,12].

Physical adsorption occurs through the simple diffusion of water molecules into microplastics without the formation of new substances. Electrostatic interactions are essential in this process, especially in microplastics with simple structures and certain types of polymers. In weak acid solutions, where free ions can stick to the surface of microplastics or get trapped inside their pores, electrostatic attraction frequently acts as a primary mechanism influencing the interaction between cationic pollutants and microplastics. Depending on the type of polymer and contaminant, the toxicity of microplastics increases with the absorption of pollutants from the environment [1,13].

3.2. Floc density

Fig. 3 displays the changes in floc density (mL L^{-1}) observed in the four treatments at different sampling times. No floc formation was observed in Treatment A, throughout the experiment, as evidenced by a consistent floc density of 0 mL L^{-1} at all sampling points. This is possible since Treatment A is the control group, without biofloc and MP

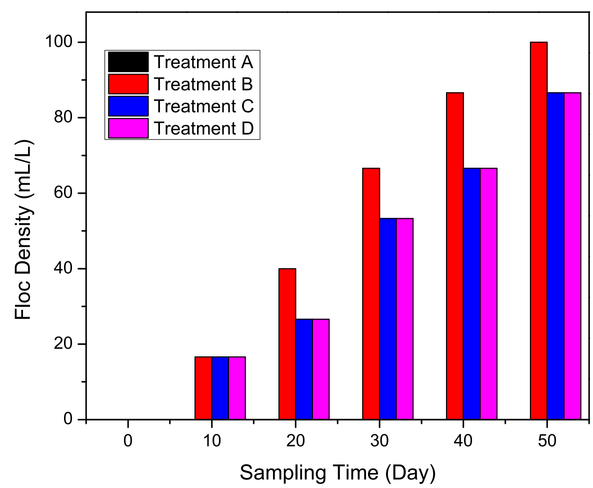

Fig. 3. Floc density (ml L^{-1}) at different sampling times (days).

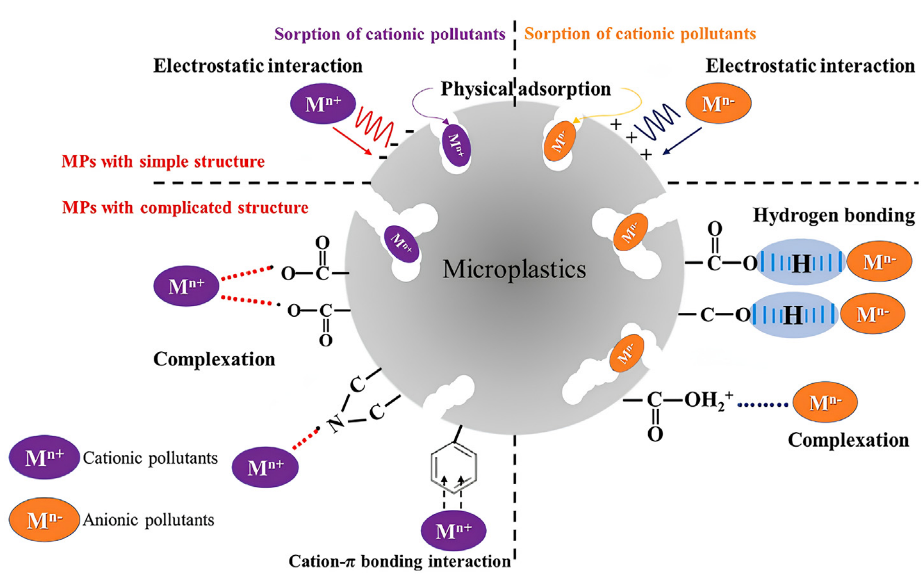

Fig. 2. Mechanism of heavy metal interaction with microplastics.

contamination. In Treatment B, on the other hand, floc density increased from 16.6 mL L^{-1} on Day 10–100 mL L^{-1} on Day 50. This notable rise in floc density indicates an effective floc formation with the use of biofloc technology, as noted in the quality of aeration and the availability of carbon in the water. Compared to Treatment B, Treatments C and D showed a gradual increase in floc density, albeit more slowly. In both treatments (C and D), the floc density increased from 16.6 mL L^{-1} on Day 10–86.6 mL L^{-1} on Day 50. The presence of MP contamination, which inhibits floc growth, explains this slower development [14]. Overall, these findings imply that biofloc technology positively affects water quality and floc density in aquaculture systems, with variations in response depending on aeration treatment and nutrient availability.

3.3. Dynamics of water quality (DO, BOD, COD)

As seen in Fig. 4, biofloc in the Nile tilapia cultivation system significantly affects the dynamics of water quality, which includes DO, BOD, and COD concentrations. Biofloc plays a crucial role in maintaining water quality through the nitrogen cycle, which converts toxic nitrogen compounds into forms that aquatic organisms can benefit from. Water quality declined over time in Treatment A which did not contain biofloc. This is due to the absence of mechanisms that support the degradation of organic compounds.

In contrast, biofloc density in Treatment B increased from 16.6 mL L^{-1} to 100 mL L^{-1} by Day 50. Water quality improved as a result of biofloc's assistance in lowering nitrogen compounds and other organic materials, and feed efficiency was enhanced through the reuse of the produced biomass [15]. Additionally, biofloc stimulates the growth of beneficial microorganisms, accelerates nutrient cycling, and promotes the stability of the aquatic ecosystem.

Compared to Treatment B, the growth of biofloc was slower in Treatments C and D. The increase in biofloc density was inhibited particularly in Treatment D which had a higher concentration of microplastics. Through physical and chemical interactions, as well as the absorption of other pollutants, microplastics—such as polyethylene—can stress aquatic organisms and disrupt the efficiency of biofloc in processing organic materials [16]. As a result, the water quality in Treatment D was not as optimal as that in Treatment B, indicating the negative effect of microplastics in the biofloc system on the dynamics of water quality and the growth of the biofloc.

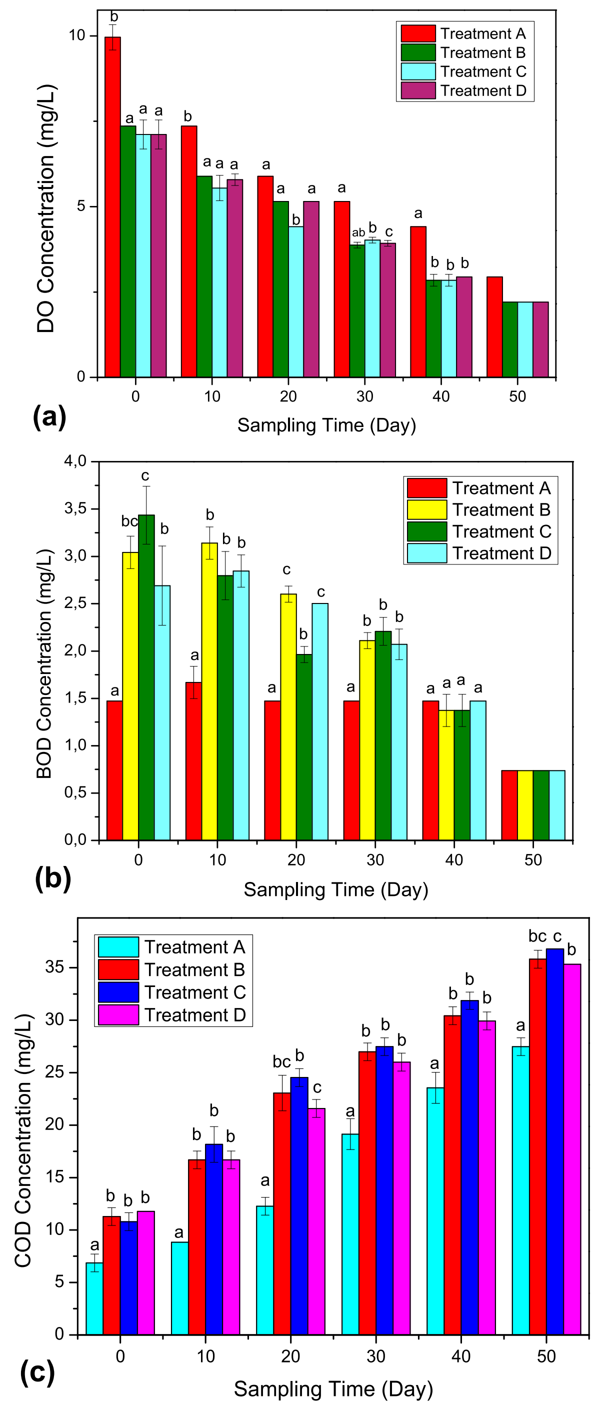

Fig. 4. (a) Concentration of DO (mg L^{-1}), (b) Concentration of BOD (mg L^{-1}), and (c) Concentration of COD at different sampling times (days). †Mean ± SD (n = 3) with different letters (a, b, c, d) for each sampling indicate significant differences (p < 0.05) between treatments at the same sampling time. Treatments A (without biofloc and microplastics), B (with biofloc, without microplastics), C (with biofloc and PET + PE microplastics), and D (with biofloc and PE microplastics).

3.3.1. Dissolved Oxygen (DO)

Fig. 4a presents the average DO values of 2.29–9.96 mg L^{-1}, 2.21–7.36 mg L^{-1}, 2.21–7.11 mg L^{-1}, and 2.21–7.11 mg L^{-1} for

Treatments A, B, C, and D, respectively. It is critical to maintain DO levels since low DO can stress fish and reduce biofloc quality, which may affect feed consumption [17]. Although the DO levels in all treatments were initially within the acceptable limit for tilapia growth (>3 mg L^{-1}), with concentrations of 9–10 mg L^{-1} on the first day, significant changes were observed over time. Different from other treatments, Treatment A showed the sharpest decline from 10 mg L^{-1} to 6 mg L^{-1} between Days 10 and 20. Meanwhile, Treatments B and C had a slower decline to 7–8 mg L^{-1}. The presence of biofloc contributes to the maintenance of oxygen levels, particularly in Treatment C, where microplastic degradation began.

Differences in DO levels became more pronounced on Day 30. The DO level in Treatment A dropped to 4 mg L^{-1}, approaching the minimum threshold of 3 mg L^{-1}. This finding differs significantly from the other treatments. As biofloc reduced organic matter and controlled the nitrogen cycle, the DO in Treatment B, which contained biofloc, remained constant at 6 mg L^{-1}. The fact that the results in Treatment B were significantly different ($p < 0.05$) from those in Treatment A but not from those in Treatment C signifies biofloc's effectiveness in maintaining DO. Because microplastic degradation requires oxygen, Treatment C—which contained a mixture of microplastics (PE and PET)—experienced a quicker decline to 5 mg L^{-1}. In contrast to Treatments B and C, Treatment D (which had a higher polyethylene content) had a sharper drop to 4 mg L^{-1} by Day 40.

From Days 50–60, the DO level in Treatment A failed to meet the standards as it dropped below 3 mg L^{-1} by Day 60. Conversely, the DO in Treatment B remained above the standard, at 5 mg L^{-1}. Significantly different from those in Treatments A and B, the DO in Treatment C dropped to 4 mg L^{-1}. This is most likely due to the adsorption of heavy metals by microplastics, thereby accelerating the decline in the DO level [18]. With its high polyethylene content, Treatment D showed a sharp drop in the DO level to 3 mg L^{-1} (significantly different ($p < 0.05$) from Treatments B and C), indicating that microplastics accelerate oxygen depletion.

3.3.2. Biochemical Oxygen Demand (BOD)

In Treatment A, BOD levels ranged from 0.7360 to 1.4720 mg L^{-1} (Fig. 4b). This finding is consistent with another prior study that reported BOD levels below 2 mg L^{-1} in catfish farming without biofloc. Treatments with biofloc exhibited BOD levels between 0.7360 and 3.4350 mg L^{-1}, similar to the findings of a previous study conducted by author, where BOD in biofloc systems ranged from 0.450 to 2.598 mg L^{-1}. All of these values are within the acceptable limit of <3 mg L^{-1} [19].

At the beginning of the experiment, the BOD level in Treatment B exceeded 3.5 mg L^{-1}, while those in Treatments C and D (with microplastics) were around 3.3 mg L^{-1}. Meanwhile, the BOD level in Treatment A was close to 1.5 mg L^{-1}. A significant difference ($p < 0.05$) in BOD levels between Treatment A and the other treatments highlighted biofloc's role in boosting oxygen consumption through the rapid decomposition of organic matter. The degradation was slightly hindered by microplastics in Treatments B, C, and D, whose BOD levels exceeded 3 mg L^{-1}.

By Day 20, the BOD remained high in Treatments B and D, while that in Treatment C showed a slight decrease. Treatment A continued to differ significantly ($p < 0.05$) from the other treatments, particularly from Treatments B and D, whose BOD levels remained above 3 mg L^{-1}. In Treatment C, the BOD level approached the standard, indicating that PET microplastics started to slow down the degradation of organic matter. The BOD levels declined significantly for all treatments on Day 30. With the BOD value dropping below 3 mg L^{-1}, there was biofloc stabilization in Treatment B, while Treatments C and D declined more slowly to around 2.5 mg L^{-1}. There were significant differences ($p < 0.05$) across Treatments C, D, and B, indicating that the type and quantity of microplastics influenced the degradation rate. The BOD in all treatments continued to decrease by Day 40, with the BOD in Treatment B being around 1 mg L^{-1} and those in Treatments C and D approaching 2 mg L^{-1}. By Day 50, all treatments showed further declines in BOD, with that in Treatment B falling below 1 mg L^{-1} and those in Treatments C and D nearing 1.5 mg L^{-1}.

Despite initial delays in Treatments C and D, all treatments achieved BOD levels below 1 mg L^{-1} at the end of the experiment. Both Treatment C and Treatment D ultimately reached values comparable to that of Treatment B, indicating that the microplastic degradation process was complete and that heavy metal adsorption was no longer preventing BOD reduction. No significant differences ($p > 0.05$) were observed among treatments at the end of the experiment, with Treatment B reaching the quality standard (<3 mg L^{-1}) faster [20].

3.3.3. Chemical Oxygen Demand (COD)

As shown in Fig. 4c, the concentration of COD increases in all treatments, indicating an accumulation of organic compounds from fish waste, uneaten feed, and dead microorganisms. In Treatment A, COD ranged from 6.8693 to 27.4773 mg L^{-1}. This finding supports a prior study on catfish farming without biofloc. COD levels varied between 11.2853 and 36.800 mg L^{-1} in Treatments B, C, and D. Meanwhile, those in treatment without biofloc ranged from 5.861 to 11.307 mg L^{-1}. All values remained below the permissible limit of 40 mg L^{-1} [21,22].

On Day 0, COD concentrations were low across treatments (6.8693−11.7760 mg L^{-1}), with no significant differences ($p > 0.05$). By Day 10, however, Treatment C showed an increase in COD to 18.1546 mg L^{-1}, which is most likely linked to microplastic degradation. On Day 20, the COD level in Treatment C reached 24.5333 mg L^{-1}, showing a significant difference ($p < 0.05$) from other treatments. This finding is in line with other studies that noted an increased organic load from degraded microplastics, along with heavy metal absorption by biofloc [15,22].

The COD values reached 27.4773 mg L^{-1} in Treatment C and 26.0053 mg L^{-1} in Treatment D on Day 30, with no significant differences from Treatments A and B ($p > 0.05$). This increase is associated with microplastic degradation and heavy metal absorption [1]. Similarly, the COD values continued to rise to 31.8600 mg L^{-1} in Treatment C and 35.3280 mg L^{-1} in Treatment D by Day 40, with no significant differences ($p > 0.05$). This finding is consistent with a previous study [23], which found that microplastic degradation escalates the decomposition of organic matter. On Day 50, the COD value peaked at 36.8000 mg L^{-1} in Treatment C and at 35.3280 mg L^{-1} in Treatment D, with significant differences ($p < 0.05$). In this regard, the release of organic compounds from microplastic degradation was accelerated, as reported in another prior study [23]. Polyethylene (PE) microplastics in Treatment D may adsorb organic material, thus reducing the availability of easily degradable compounds. In Treatment B, on the other hand, all organic matters are available for microbial degradation, potentially enhancing COD. The degradation of microplastics in Treatments C and D significantly contributed to the increase in their COD values, eventually accelerating oxidation through dissolved organic compounds and heavy metal absorption [24].

3.4. Metal concentrations in water (Cu, Fe, Zn, and Cd)

In aquaculture, metals are essential elements for both physiological and enzymatic processes in the fish; insufficient levels of metals can disrupt bodily functions, while excessive metal concentrations may lead to toxicity [21]. Contaminants in biofloc systems, which include heavy metals, can come from uneaten feed and unmanaged water. Since heavy metals can enter fish through their gills, monitoring becomes crucial to preventing harm to the ecosystem [25]. The sources of metal contamination include feed, salt, dolomite lime, and molasses. A prior study has found that the composition of fish meal contains potassium (0.94−0.97 %), iron (103.1295 mg kg^{-1}), zinc (40.6344 mg kg^{-1}), and copper (12.9651 mg kg^{-1}) [26].

Iodized salt used in aquaculture is sodium chloride (85−98 %) which also contains magnesium chloride (0.5−1.5 %), calcium chloride (0.1−0.5 %), potassium chloride (0.2−1 %), and added iron, copper, and zinc [27,28]. Dolomite lime consists of magnesium oxide and calcium oxide (more than 18 %), as well as alumina and iron oxide (less than 3 %). Meanwhile, molasses is made up of sucrose and fructose as well as K, Fe (100−300 mg kg^{-1}), Cu (2−10 mg kg^{-1}), and Zn (10−50 mg kg^{-1}). Data on metal concentrations in water (Cu, Fe, and Zn) were taken on Days 0, 10, 20, 30, 40, and 50, as displayed in Fig. 5.

3.4.1. Copper (Cu)

In all treatments, Cu concentration was initially found to be below 0.01 mg L^{-1} (0.0006−0.0109 mg L^{-1}) on Day 0, indicating low interactions between water, biofloc, and microplastics at the beginning of the experiment. After that, all treatments recorded an increase in copper concentration. By Day 10, the Cu concentrations were 0.0058 mg L^{-1}, 0.0121 mg L^{-1}, 0.008 mg L^{-1}, and 0.0124 mg L^{-1} for Treatments A, B, C, and D, respectively. This can be attributed to the Cu adsorption of microplastics and biofloc [25,29]. Each treatment (A, B, C and D) had a different weight result.

Day 20, the concentration of Cu was found at 0.011 mg L^{-1} in Treatment A and 0.021 mg L^{-1} in Treatment D. Greater differences were observed in Treatment D due to the active maintenance of elevated Cu ions within treatment composites brought on by the high concentration of polyethylene. Those dissimilarities were also present on Day 30, in which Cu concentrations were 0.0453, 0.0454, and 0.0451 mg L^{-1} in Treatments A, B and C. This established further differences ($p < 0.05$) on Day 40. Owing to the active interaction of microplastics, biofloc, and ionic particles, Cu concentrations in-creased to 0,065 mg L^{-1} in Treatment B and 0.061−0,059 mg L^{-1} in Treatment C and D. This indicates the occurrence of microplastic degradation in Treatments C and D.

By Day 50, Cu concentrations reached 0.1532 mg L^{-1}, 0.1880 mg L^{-1}, 0.1664 mg L^{-1}, and

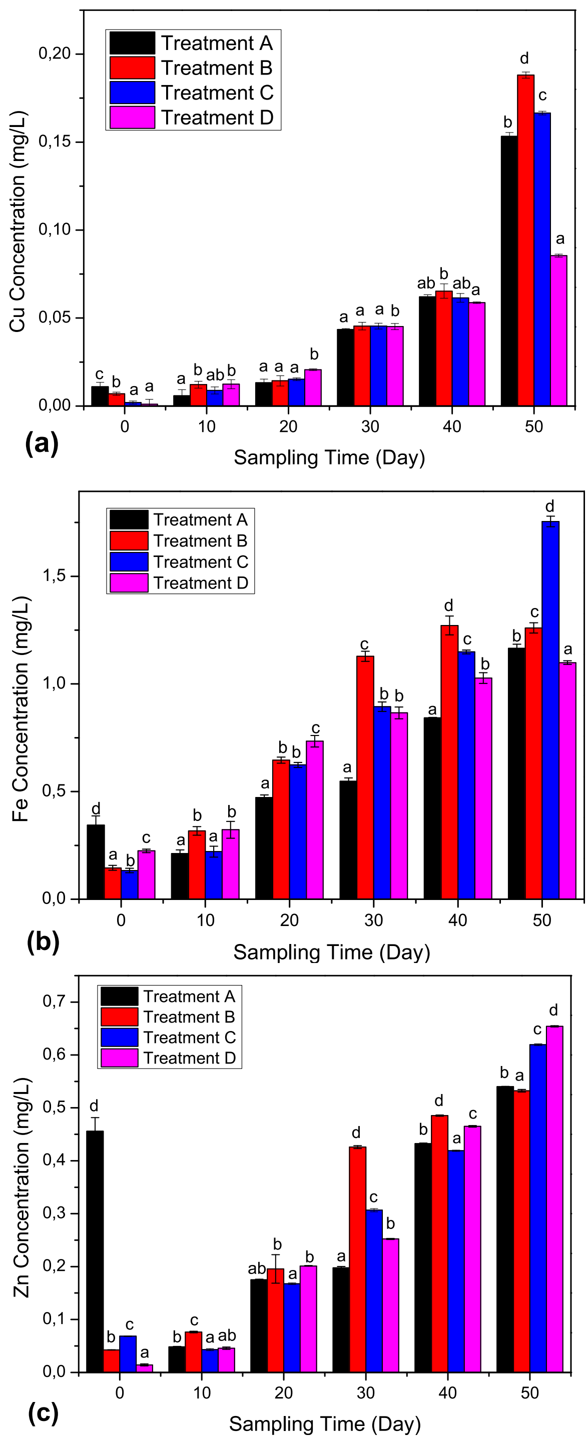

Fig. 5. (a) Concentration of Cu (mg/L), (b) Concentration of Fe (mg/L), and (c) Concentration of Zn (mg/L) at different sampling times (days). ‡Mean ± SD (n = 3) with different letters (a, b, c, d) for each sampling indicate significant differences (p < 0.05) between treatments at the same sampling time. Treatments A (without biofloc and microplastics), B (with biofloc, without microplastics), C (with biofloc and PET + PE microplastics), and D (with biofloc and PE microplastics).

0.0854 mg L^{-1} in Treatments A, B, C, and D, respectively. The increase in Cu concentration in Treatments C and D can be linked to microplastic degradation and biofloc absorption of heavy metals.

3.4.2. Iron (Fe)

Fig. 5b shows fluctuations in iron (Fe) concentration across all treatments. In Treatment A, Fe concentrations ranged from 0.2120 to 1.1652 mg L^{-1}. This is in line with a prior study on catfish farming which reported Fe concentrations between 0.185 and 1.594 mg L^{-1}. According to another study, fish is reported to use its gills, fins, and skin to absorb iron, which is present in a more soluble form Fe^{2+} [27].

Between Days 0 and 10, the Fe concentration in Treatment A was 0.3439 mg L^{-1}, whereas those in Treatments B, C, and D were between 0.1330 and 0.2246 mg L^{-1}. In Treatment B, a further decrease in Fe concentration was noted (0.3171–1.2716 mg L^{-1}). This ultimately motivated the use of iron-enriched feed and the adoption of natural processes in organic matter between Days 10 and 40. Higher Fe concentrations of 0.2211–1.1488 mg L^{-1} were noted in Treatments B and C. Meanwhile, the Fe concentration in Treatment D ranged from 0.3226 to 1.0990 mg L^{-1}.

The degradation of biofloc contributes to the release of iron which supports fish growth [15,21]. On Day 60, the Fe concentration in Treatment D reached 1.0990 mg L^{-1}, while that in Treatment C peaked at 1.7547 mg L^{-1}. Microplastics, such as polyethylene (PE) and polyethylene terephthalate (PET), function as adsorbents for iron due to their porous, electrostatically charged surfaces [30]. Environmental factors, including pH, temperature, and redox, potentially influence this interaction, leading to the desorption of Fe as conditions change. This explains the increased Fe levels in Treatments C and D. Nonetheless, the highest Fe concentration found in this study (1.7547 mg L^{-1}) is still below the safe limit of 2 mg L^{-1} for aquaculture.

3.4.3. Zinc (Zn) metals

As seen in Fig. 5c, the average Zn concentration in Treatment A ranged from 0.1751 to 0.5401 mg L^{-1}. These values are higher than those in a prior study, which reported Zn levels between 0.0280 and 0.0896 mg L^{-1} in catfish aquaculture without biofloc. Treatments B, C, and D had Zn levels ranging from 0.0429 to 0.6542 mg L^{-1}, comparable to the Zn concentrations found in another study in biofloc systems (0.328–3.85 mg L^{-1}). Tilapia may consider biofloc a food source, increasing the risks of heavy metal accumulation, which could explain the higher Zn concentration in treatments with biofloc [31]. Nevertheless, zinc is essential for fish growth.

On Day 1, Zn levels were low in all treatments (0.0142−0.0684 mg L^{-1}), suggesting minimal accumulation of zinc. Treatment A had slightly higher Zn levels than other treatments, presumably from the initial water used. In addition, all treatments showed statistically significant differences ($p < 0.05$). During this time, Zn concentrations increased, especially in treatments with biofloc. The highest value was found in Treatment D at 0.6542 mg L^{-1}, which is still lower than the acceptable limit of 1.5 mg L^{-1} set by the WHO. As a vital co-factor for an important class of enzymes [32], zinc is derived from feed, salt, dolomite, and molasses. There are significant differences across all treatments in this study ($p < 0.05$). According to previous studies, Zn deficiencies and excesses can affect fish health [27,33]. If consumed beyond their safety limits, heavy metals such as Fe, Zn, and Cu from fish grown in aquaculture can have an adverse effect on human health [16]. Due to its accuracy, spectrophotometry is often used to detect these metals in fish [26]. Hence, regular monitoring is essential for food safety [34].

As shown in Table 1, Fe, Zn, and Cu were detected in all fish samples, with Fe and Zn concentrations being higher than Cu concentrations, reflecting their roles in fish metabolism. Although remaining within safe limits, Zn and Fe deficiencies may increase the risk of anemia and chronic illnesses. According to a prior study, fish feeding behavior and environmental metal levels affect the bioaccumulation of heavy metals [21].

Table 2 shows the bioaccumulation of Cu, Fe, and Zn in fish samples observed from Day 0, which is likely due to hatchery contamination. By Day 50, Treatment D and Treatment B had the highest and the lowest copper accumulation, respectively. This suggests that the biofloc system reduces metal accumulation. Treatment B showed the highest iron levels. Meanwhile, Treatment C had the lowest iron levels, presumably because of microplastics suppressing iron accumulation. Even while metal concentrations remain below permissible limits, the consumption of contaminated fish still poses serious health risks [20,35] The Target Hazard Quotient (THQ) evaluation (Fig. 6) underscores the need for monitoring heavy metal exposure through fish consumption.

As seen in Fig. 6, the Target Hazard Quotient (THQ) values for tilapia from all treatments are <1. This indicates no serious health risks associated with the consumption of this fish. The THQ values in all treatments, except Treatment C, are in the following order: Zn < Cu < Fe. Treatment C,

Table 2. Bioaccumulation of metals in fish samples.

No.	Samples	Bioaccumulation of metals in fish (mg Kg^{-1})		
		Cu	Fe	Zn
1	Initial treatment	163.432	108.0403	85.5093
2	Treatment A	22.0245	10.0187	41.3193
3	Treatment B	22.9286	11.3062	41.3612
4	Treatment C	33.149	4.711	45.7235
5	Treatment D	52.0432	8.0422	31.5906

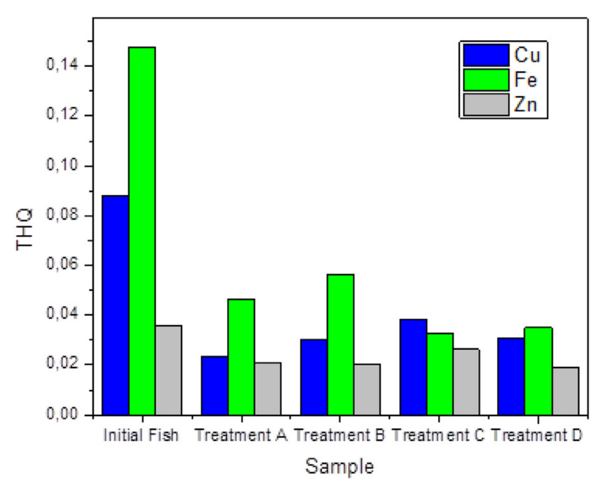

Fig. 6. THQ values in fish samples.

Table 1. Metal concentrations in fish.

No.	Samples	Average metal concentration in fish (mg kg^{-1})		
		Cu	Fe	Zn
1	Initial treatment	12.6814	37.155	38.996
2	Treatment A	3.3741	11.6738	22.3166
3	Treatment B	4.3105	14.248	22.0016
4	Treatment C	5.516	8.2664	28.3166
5	Treatment D	4.4444	8.8384	20.6666
		<20 (mg kg^{-1})[a]	<50 (mg kg^{-1})[b]	<100 (mg kg^{-1})[b]

[a] WHO.
[b] KEP-POM- No.03725/BSK/VII/89.

however, has a different order (Zn < Fe < Cu), with Fe showing the highest potential health risk. According to the Hazard Index (HI), which is determined by adding the THQ for each metal, the initial treatment poses higher health risks than other treatments with biofloc (B, C, and D). This implies that the use of biofloc technology in Tilapia farming produces fish that are safer for consumption [35].

Despite its numerous advantages in tilapia farming including increased feed efficiency, reduced waste, and improved water quality, the application of biofloc technology, particularly in commercial-scale settings, still has several limitations and potential negative impacts to evaluate. If the water source or feed is contaminated, improper management of biofloc can lead to the accumulation of toxic compounds, such as ammonia, nitrite, and heavy metals. Long-term exposure to these compounds can cause stress in fish, which may result in reduced growth rates or even mass mortality [10].

4. Conclusion

The application of biofloc technology has shown enormous potential in improving freshwater quality by reducing contamination from microplastics and heavy metals. When compared to systems without biofloc, treatments with biofloc consistently exhibited better water quality, as reflected by more stable dissolved oxygen levels and reduced biochemical oxygen demand (BOD). Although chemical oxygen demand (COD) slightly increases, its value in treatments with biofloc are within acceptable environmental limits. The effectiveness of biofloc systems in limiting toxic substances in aquaculture environments is further demonstrated by the lower accumulation of heavy metals, particularly copper. These results highlight the potential of biofloc technology as a sustainable strategy for improving food safety and environmental health in fish farming. Nevertheless, the potential risks associated with bacterial metabolites in biofloc systems must be carefully managed to protect consumer health. Further research is needed to evaluate the effects of environmental factors—such as temperature and dissolved oxygen levels—on biofloc performance, as well as the economic feasibility and long-term ecological impact of biofloc technology to ensure its sustainable application on a commercial scale.

Ethics information

This study involved non-invasive observations and standard aquaculture practices with Oreochromis niloticus. No procedures causing harm or distress were conducted. All handling of fish followed internationally accepted guidelines for the ethical treatment of aquatic animals.

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Conflicts of interest

The authors declare that they have no competing interests.

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