

Original Article

Application of floating aquatic macrophytes in treating wastewater from the recirculating aquaculture system of catfish, *Clarias macrocephalus*

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Abstract: Recirculating aquaculture systems (RAS) present a problem involving the accumulation of NO_3^- and PO_4^{3-} at the end of the culture. The study aimed to identify native aquatic plants of *Pistia stratiotes*, *Lemna minor*, *Salvinia molesta*, and *Eichhornia crassipes* with wastewater treatment capabilities in RAS. The catfish, *Clarias macrocephalus*, weighing 60 g each, were stocked at a density of 65 fish/100L and fed a diet of floating pellets containing 41% crude protein. *Pistia stratiotes* was the most effective macrophyte for treating wastewater from the catfish culture system during the first 10 days of the experiment. Following treatment with *P. stratiotes*, the concentrations of CO_2 , COD, TAN, N-NO_3^- , P-PO_4^{3-} , and TP in the wastewater decreased by 65.83, 34.28, 40.70, 46.70, 24.56, and 9.16%, respectively, while dissolved oxygen increased by 37.68% compared to the initial concentrations. Further research is required to thoroughly comprehend the efficacy and long-term effects of the *P. stratiotes* in RAS.

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Introduction

In Vietnam, where aquaculture plays a significant role in the economy and food security, addressing the environmental impacts of aquaculture wastewater is imperative. Adopting recirculating aquaculture systems (RAS) has gained traction in developing nursery models for shrimp and fishes like pangasius, snakehead, and yellow catfish (Nho et al., 2021; Sayanthan et al., 2024). The advantages of RAS encompass reduced water consumption, enabling large-scale fish farming with minimal water usage and negligible pollutant discharge (Sayanthan et al., 2024). Additionally, such systems facilitate efficient waste management and nutrient recycling, enhancing disease control measures and mitigating biological pollution risks (Sayanthan et al., 2024). However, by the end of the culture process, NO_3^- and PO_4^{3-} concentrations in RAS were often very high (Nho et al., 2021; Do et al., 2021; Sayanthan et al., 2024). For example, NO_3^- concentrations could exceed 30 mg/L in yellow catfish with seasonal water changes, and PO_4^{3-} concentrations could surpass 8 mg/L in pangasius nurseries without water changes and 5 mg/L

in yellow catfish with seasonal water changes (Nho et al., 2021). High levels of NO_3^- and PO_4^{3-} in the aquatic environment will stimulate algae overgrowth (algae bloom) in the pond and the process of algae decomposition will cause the aquatic environment to be polluted, lacking oxygen to provide respiratory activity in the water body; if wastewater containing high levels of NO_3^- and PO_4^{3-} is discharged directly into rivers that may pollute the environment and water sources used for daily life (Do et al., 2021, Nathanailides et al., 2023). Hence, treating wastewater generated from RAS is crucial for maintaining environmental sustainability and ecosystem health.

Among various wastewater treatment strategies, using aquatic plant systems in phytoremediation has gained attention for its potential to remove nitrogen and phosphorus compounds, such as nitrate (NO_3^-) and phosphate (PO_4^{3-}), from aquaculture effluents (Sayanthan et al., 2024). Recent studies have highlighted the importance of selecting appropriate floating aquatic plant species for efficient nutrient removal in aquaculture wastewater treatment systems. For example, Khanh et al. (2023) showed that the

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Table 1. Experimental design.

Treatment	Experimental description
T1 (control)	65 fish/100L only
T2	65 fish/100L with <i>Pistia stratiotes</i>
T3	65 fish/100L with <i>Lemna minor</i>
T4	65 fish/100L with <i>Salvinia molesta</i>
T5	65 fish/100L with <i>Eichhornia crassipes</i>

application of floating rafts of native aquatic plants (*Eichhornia crassipes*, *Ipomoea aquatica*, and *Commelina diffusa*) in treatment tanks improved aquaculture wastewater quality by reducing the SS (92.6%), COD (89.6%), BOD5 (93.9%), N-NH₄⁺ (93.4%), Total N (64.3%), and Total P (94.6%) in the first three months of study. Another study by Nho et al. (2021) demonstrated that *Pistia stratiotes* effectively treated wastewater from RAS that cultured yellow catfish (*Clarias macrocephalus*) at various growth stages. In addition to floating aquatic plants, duckweed (*Lemna minor*) and *Azolla microphylla* also showed a significant yield in phytoremediation of heavy metals, nitrogen, and phosphorous from wastewater effluent (Heitzman et al., 2024; Sayanthan et al., 2024). These studies indicate that floating native aquatic plants are promising in improving the quality of aquaculture wastewater.

Although extensive research exists on using aquatic plants in aquaculture wastewater treatment in Vietnam, their application in RAS remains limited. This study aimed to design a nature-based solutions model incorporating *Clarias macrocephalus* and common aquatic plants of *Pistia stratiotes*, *Lemna minor*, *Salvinia molesta*, and *Eichhornia crassipes* in Vietnam to evaluate their effectiveness in reducing NO₃⁻ and PO₄³⁻ in RAS. The findings will serve as a basis for designing and operating an integrated circulatory system for yellow catfish that combines culture and waste treatment.

Materials and Methods

Experimental setup of RAS: The experiments were conducted indoors at the Faculty of Biotechnology, Dai Nam University, Vietnam, in December 2023. The experiments were arranged in a RAS. The components of the RAS were adapted from (Nho et al., 2021), including culture tanks (100 L), settling

tanks (30 L), container tanks of 70 L, and biofilter tanks (70 L). The biofilter tank uses RK-Bioelements (with a specific surface area of 750 m²/m³) with a total substrate surface area of 30 m² (40 L of RK-Bioelements). The biofilter tank was continuously aerated with oxygen and the inlet maintained a constant flow rate of 2.3 L/min.

The plant planting system was constructed by connecting 3 plastic troughs (35x40x20 cm). The inlet water of the plant growing system is piped from the biological filter tank, and the outlet water is fed to the culture tank. The aquatic plants, including, *Pistia stratiotes*, *Lemna minor*, *Salvinia molesta*, and *Eichhornia crassipes* were collected from nature in the suburbs of Ha Noi City. Aquatic plants grow healthy, do not suffer from pests and diseases, are relatively uniform in height, and have the same growth period. The plants were left in an aquatic environment for 2 days to gradually get used to the conditions in small tanks. For each experiment, plants (eighty grams of fresh weight) were planted in the entire area of all 3 plant troughs. The experiment was equipped with LED lighting during the day to supplement light for plants to grow (the experimental system reached 2.000-4.000 lux in the morning, 9.000-11.000 lux at noon, and 20.000-25.000 lux in the afternoon; the fluctuation depends on weather conditions).

The catfish, *Clarias macrocephalus* (with an average weight of about 60 g/fish), was arranged into different experiments from T1 to T5 (Table 1) and fed twice daily with 41% protein commercial feed. The pH of the water was kept between 7.0 and 8.5 by using NaHCO₃. Each experiment was repeated three times and was 30 days long.

Fish aquaculture wastewater remediation: The experimental indicators such as temperature, pH, DO (Dissolve oxygen), CO₂, alkalinity, total suspended solids (TSS), COD (chemical oxygen demand), total

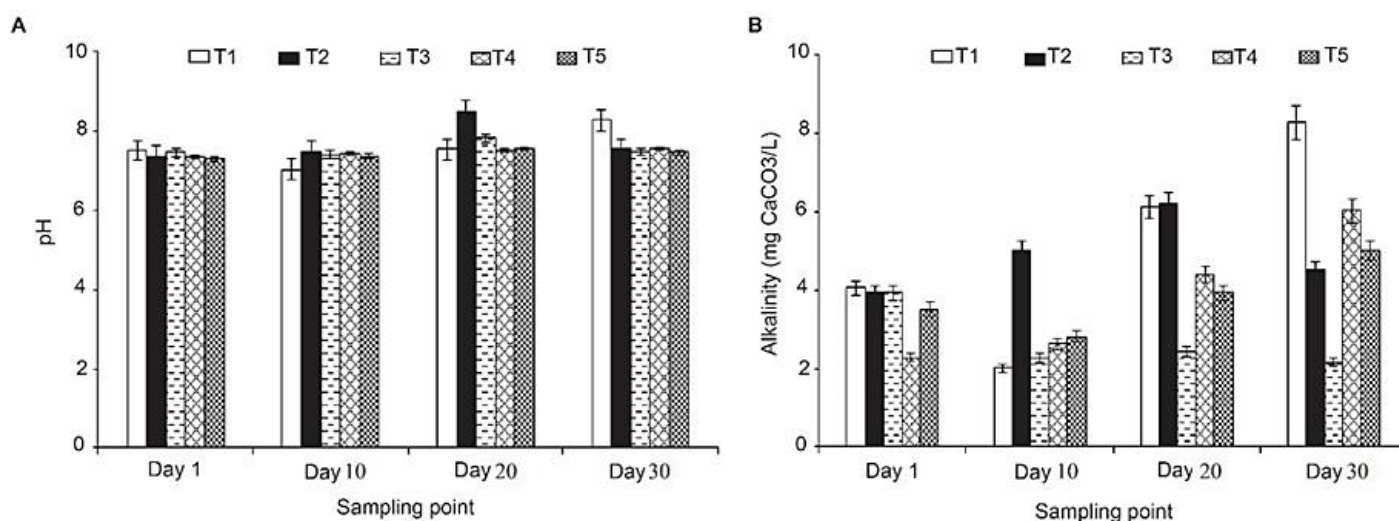


Figure 1. Fluctuation of pH (a) and alkalinity (b) in experimental systems. T1: 65 fish/100L only, T2: 65 fish/100L with *Pistia stratiotes*, T3: 65 fish/100L with *Lemna minor*, T4: 65 fish/100L with *Salvinia molesta*, T5: 65 fish/100L with *Eichhornia crassipes*.

ammonium-nitrogen (TAN), N-NO_2^- , N-NO_3^- , P-PO_4^{3-} , Total nitrogen (TN), and total phosphate (TP) were collected every 5 days at the output of the planting system. The parameters, including pH, TSS, and temperature, were measured using a Milwaukee Mi805 Portable pH/EC/TDS/Temperature Meter (Mi805 Milwaukee, CO, USA). Dissolve oxygen (DO) was monitored by a Milwaukee Dissolved Oxygen Meter (MW 600, CO, USA). The remaining water environmental indicators were collected and analyzed according to the American Public Health Association et al. (1995). During the experiment, the monitoring and removal of dead plants from the plant trough system was carried out regularly.

Data analysis: Data was analyzed using the SPSS software package version 17.0. The one-way analysis of variance (ANOVA) was applied to analyze the mean difference among treatments. Duncan's multiple range test ($P < 0.05$) was performed to determine the significance of the results.

Results

Water quality is categorized into physical parameters, organic contaminants, biological contaminants, and pathogens (Su et al., 2020). Physical parameters, influenced by climate and environment, are crucial in aquaculture, particularly in RAS, where water is continuously circulated. Key monitoring parameters include temperature, pH, dissolved oxygen (DO),

ammonia, nitrite, and others (Tziortzioti et al., 2019).

Temperature: During the experiment, the average temperature between the experiments fluctuated between $26.57 \pm 0.25^\circ\text{C}$.

pH and alkalinity: During the experiment, the pH in the T2 experiment was highest and lowest in the control (T1), with values ranging from 7.1 to 8.3 (Fig. 1A). The pH differences between sampling-day experiments were insignificant ($P > 0.05$). Both pH and alkalinity in experiments with aquatic plants (T2-T5) increased from day 1 to day 10, then decreased at the end of the experiments (Fig. 1). In contrast, pH and alkalinity in the control experiment (T1) decreased by day 5 before rising again. The results also showed that by day 15, pH and alkalinity in T2 and T3 experiments decreased. In contrast, pH and alkalinity in T4 and T5 increased (Fig. 1B).

Dissolved oxygen (DO), CO₂, COD, and TSS: The dissolved oxygen (DO) levels in the water tended to increase in experiments T2 to T5, while the DO levels in the control (T1) decreased throughout the experiment (Fig. 2A). On the 10th day, the DO in the T2 rose significantly more than in the other experiments, reaching 5.67 ± 0.23 mg/L, which is a 38.02% increase compared to the initial setup.

Figure 2B indicated that CO₂ concentrations were lowest in experiments using aquatic plants, T2 to T5, while CO₂ levels were highest in control (T1), with a significant difference ($P < 0.05$) observed on days 5,

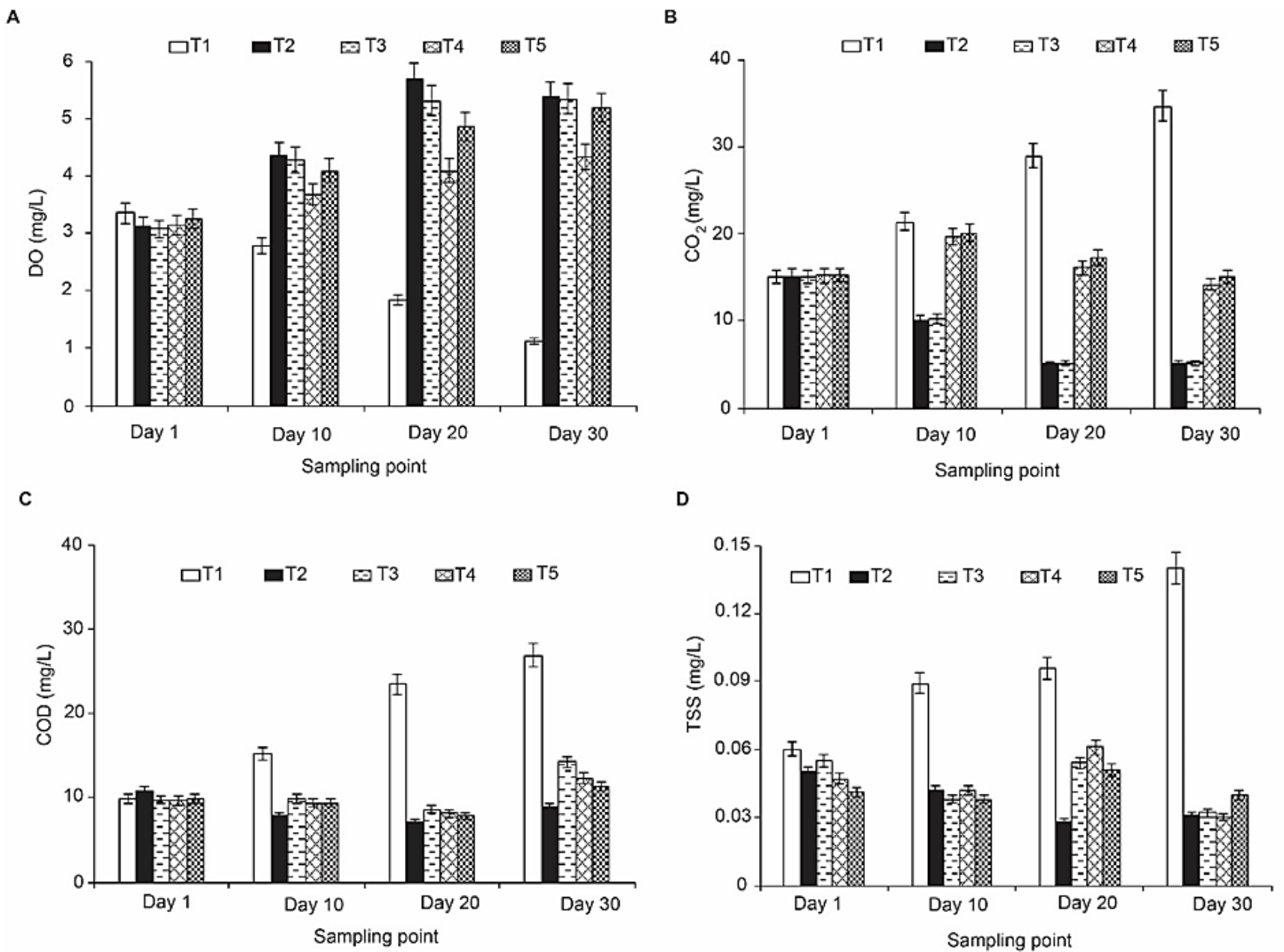


Figure 2. Fluctuation of DO (a), CO₂ (b), COD (c), and TSS (d) in experimental systems. T1: 65 fish/100L only, T2: 65 fish/100L with *Pistia stratiotes*, T3: 65 fish/100L with *Lemna minor*, T4: 65 fish/100L with *Salvinia molesta*, T5: 65 fish/100L with *Eichhornia crassipes*.

10, and 15. On day 10, the CO₂ content in the plant-treated experiments decreased sharply, especially in T2 and T3 experiments, which ranged from 5.51 ± 0.43 mg/L, marking a 66.17% reduction compared to the experiment's initial.

The COD in the experiments (T2 to T5) varied throughout the sampling sessions depending on aquatic plants' growth and organic matter deposition in the water. In the control (T1), COD levels tended to increase towards the end of the experiment. They were higher than in the other experiments (T2 to T5) (Fig. 2C). The highest COD treatment efficiency after 10 days was observed in the T2 experiment (35.31%), while the lowest was in the T3 experiment (10.37%).

Figure 2D showed that TSS levels gradually

increased over time and reached their highest values in the control experiment (T1) (0.115 ± 0.021 mg/L) but decreased from days 1 to 10 in the experiments with aquatic plants (T2 to T5).

Nitrogenous compounds (TAN, NO₂⁻, N-NO₃⁻ and TN): The total ammonia nitrogen (TAN) content in the experiments combined with plants (T2-T5) was lower than in the control (T1) after 10 days, with the lowest levels in the T2 experiment and the highest in the control (T1) (Fig. 3A), showing a significant difference ($P < 0.05$). In the T2 to T5, the TAN treatment efficiency after 10 days was highest in T2 (41.03%), followed by T3 (16.51%). No significant TAN treatment performance was observed in the T4 and T5 experiments after 10 and 15 days. In the

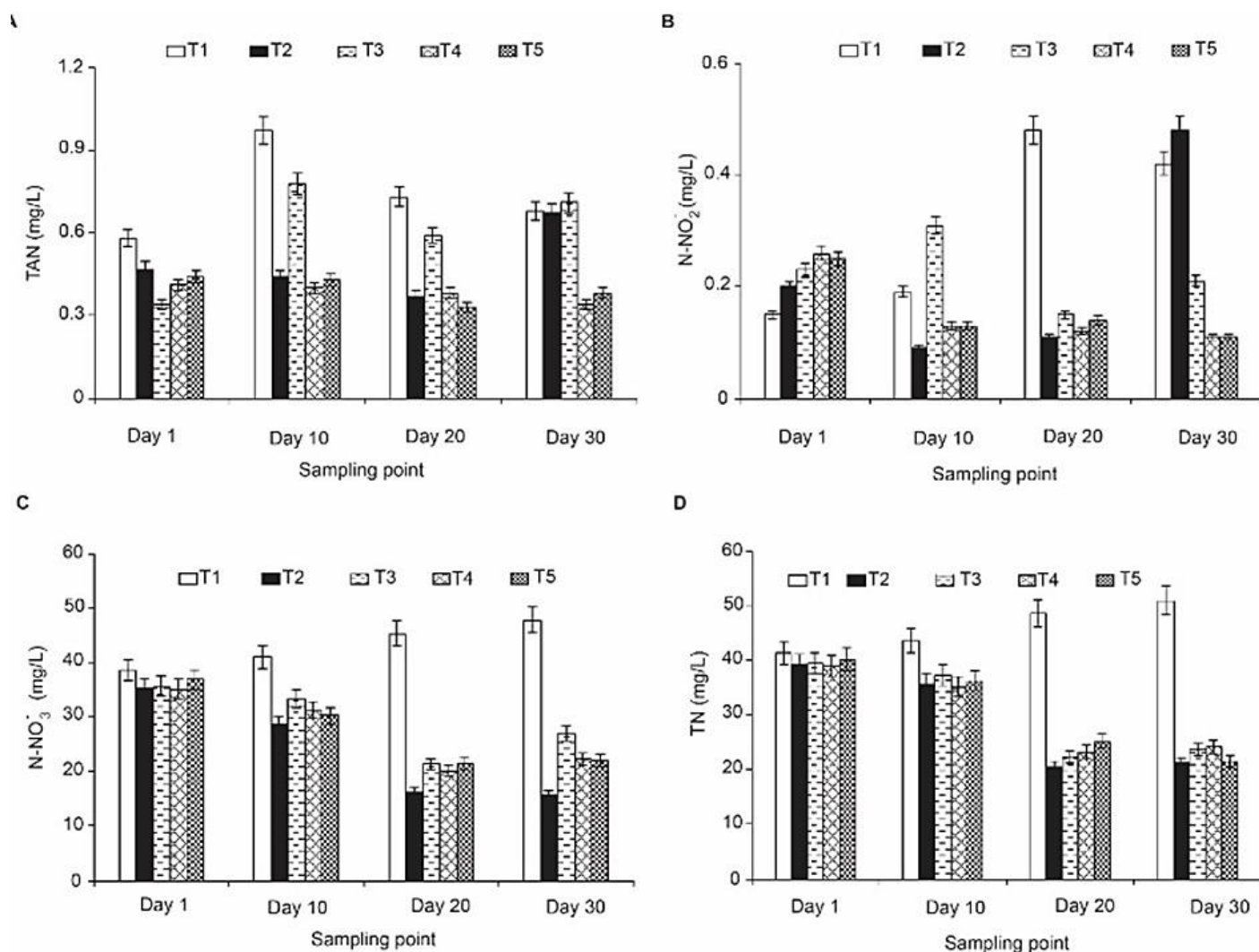


Figure 3. Fluctuation of TAN (a) and NO₂⁻ (b), N-NO₃⁻ (c), and TN (d) X in experimental systems. T1: 65 fish/100L only, T2: 65 fish/100L with *Pistia stratiotes*, T3: 65 fish/100L with *Lemna minor*, T4: 65 fish/100L with *Salvinia molesta*, T5: 65 fish/100L with *Eichhornia crassipes*. TAN: total ammonia nitrogen, TN: total nitrogen.

experiments with aquatic plants, N-NO₂⁻ levels decreased to their lowest point by the 10th day and then increased again by the 15th day (Fig. 3B). Moreover, N-NO₃⁻ levels tended to decrease from days 1 to 10 in the planted experiments (T2-T5) and increased in the control experiment (T1) (Fig. 3C). Figure 3D indicated that the total nitrogen (TN) content in T2 to T5 was lower than in the control one. The TN levels in experiments (T2 to T5) gradually decreased from day 1 to day 10, with the lowest level observed in T2 (22.47±1.37 mg/L).

P-PO₄³⁻ and TP: P-PO₄³⁻ and Total phosphorus (TP) concentrations in experiments T2 to T5 decreased by day 10, whereas in the control experiment (T1), these concentrations increased over time (Fig. 4A). After 10

days, the T2 experiment showed a 24.56% reduction in P-PO₄³⁻ and a 9.16% reduction in TP. The T4 experiment achieved a 13.80% reduction in P-PO₄³⁻ and a 15.08% reduction in TP (Fig. 4B), while the T3 and T5 experiments exhibited very low PO₄³⁻ absorption.

Discussions

Our results indicated that the temperature is well within the optimal range for the growth and health of catfish species commonly cultured in ponds (Nho et al., 2021; Jayadi et al., 2022). At optimal temperatures, fish exhibit rapid growth, efficient feed conversion, and increased disease resistance. While biofilter efficiency is also influenced by temperature,

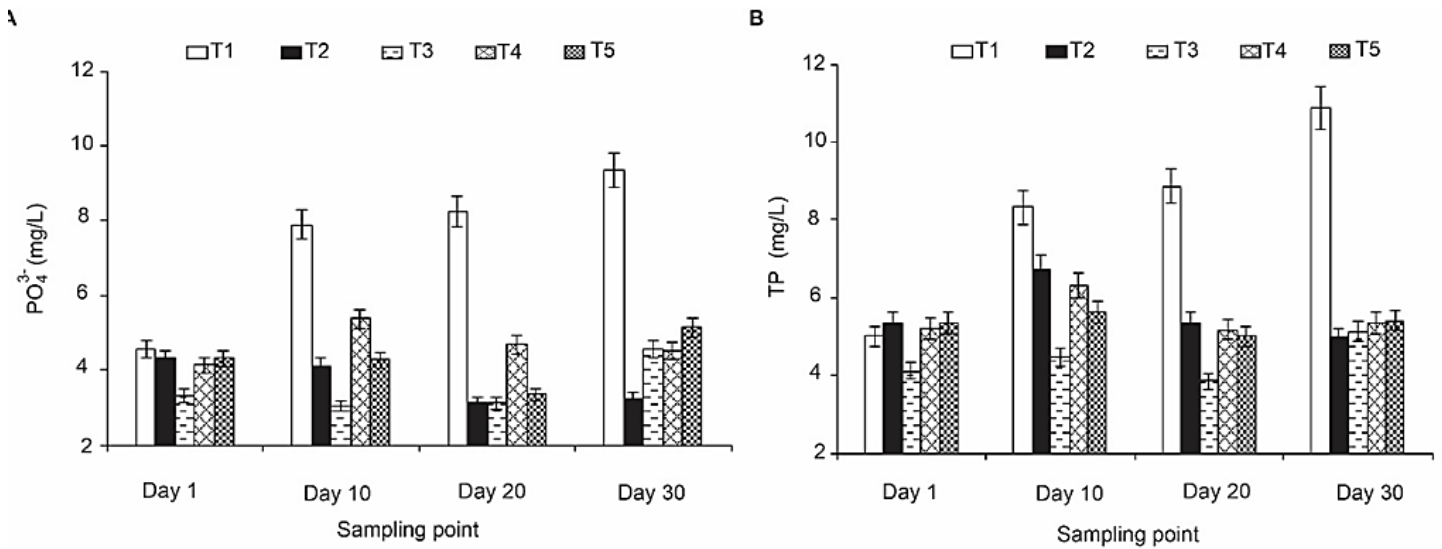


Figure 4. Fluctuations PO_4^{3-} (a) and TP (b) in the experimental system. T1: 65 fish/100L only, T2: 65 fish/100L with *Pistia stratiotes*, T3: 65 fish/100L with *Lemna minor*, T4: 65 fish/100L with *Salvinia molesta*, T5: 65 fish/100L with *Eichhornia crassipes*. TP: total phosphorus.

it typically remains effective in warm water systems (Nho et al., 2021). Suboptimal temperatures lower feed efficiency (Timmons et al., 2018) and can cause stress and disease.

The results indicated that when aquatic plants were combined (T2-T5), alkalinity and pH in the water increased during the first 10 days without $NaHCO_3$ supplementation. In the control experiment (T1), $NaHCO_3$ was applied to adjust pH. On the tenth day, the alkalinity in the T2 experiment and the control (T1) showed no significant difference. According to Summerfelt et al. (2015), the best circulatory systems have alkalinity levels ranging from 50 to 100 mg $CaCO_3/L$ or higher, whereas Boyd (2016) indicated that alkalinity levels below 10 mg $CaCO_3/L$ can negatively impact fish growth and development, with appropriate levels being above 20 mg $CaCO_3/L$. According to the surface water standards QCVN 08-MT:2015/BTNMT, pH values between 6.0 and 8.5 are deemed suitable for domestic use. Meanwhile, the wastewater standards QCVN 24:2009/BTNMT permit wastewater with a pH range of 5.5 to 9 to be discharged into the environment. Based on these standards, the quality of the treated water is adequate and does not pose a risk to the aquatic environment.

The increase in DO was significant compared to the other aquatic plant experiments (T3 to T5) and the control (T1). This rise in DO can be attributed to the

photosynthesis of the aquatic plants, which supplied oxygen to the system. Previous studies have shown that aquatic plants used in biofiltration systems in aquaculture can significantly boost oxygen levels (Do et al., 2021; Sayanthan et al., 2024). In contrast, the DO levels in the control (T1) were mainly affected by oxygen consumption from fish respiration, nitrifying bacteria, and organic matter decomposition, with oxygen supply relying solely on the aeration system. The DO levels in the control group on days 10 and 15 were significantly different from those in the other experiments. According to Timmons and Ebeling (2010), maintaining DO around 5 mg/L is crucial for the survival and growth of cultured species, and for the better efficacy of active biofiltration. The DO levels in this experiment remained within the appropriate range for farmed fish.

The decrease in CO_2 was due to the aquatic plants utilizing CO_2 during photosynthesis. Conversely, CO_2 levels in T1 rose sharply on day 10, reaching 28.75 ± 0.34 mg/L. The high CO_2 in the control group was attributed to the respiration of aquatic organisms (mainly fish) in the culture tank and the decomposition of organic matter.

The ability of aquatic plants to remove organic matter such as *Pistia stratiotes*, *Lemna minor*, *Salvinia molesta*, and *Eichhornia crassipes* has been published in several previous studies (Ijaz et al., 2015; Haidara

et al., 2018; Do et al., 2021). This is because, in the T2 to T5 experiments, aquatic plants obtained carbon dioxide and oxygen from the air. Under anaerobic conditions, many plants transport oxygen to their roots for metabolic purposes, providing excess oxygen to surrounding species (Ijaz et al., 2015). When plant roots are submerged in the water column, they serve as a living substrate for aerobic bacteria, which use the excess oxygen to break down water-soluble organic compounds, resulting in lower COD levels in the planted experiments. According to Yasin et al. (2021), higher aquatic plants can affect the oxidation status of sediments in wetlands by releasing oxygen from their roots and rhizomes into the root zone, enhancing aerobic decomposition. The highest COD content in the planted experiments was 19.03 ± 0.62 mg/L, which is still low compared to the industrial wastewater quality standard set by the Ministry of Natural Resources and Environment (QCVN 40: 2011/BTNMT - the maximum allowed COD content in industrial wastewater discharged into water sources used for domestic supply is 75 mg/L).

The decrease in TSS was due to the ability of aquatic plants to filter waste products through their root zones (Rhizofiltration), where pollutants are adsorbed on the root surface or precipitated by root secretions (Ijaz et al., 2015). Additionally, solid waste was eliminated by floating plants with extensive root systems (Do et al., 2021). Consequently, TSS levels decreased in the experiments with aquatic plants (T2 to T5), with the most significant reduction observed in the T2 experiment. According to the European Inland Fisheries Advisory Commission (1980), the total suspended matter should remain below 15 mg/L for the circulatory system to function properly, while Muir (1982) suggested an appropriate range of 20 to 40 mg/L. Thus, the results of this study were within the permissible range.

The reduction in TAN was due to the ability of aquatic plants to absorb nitrogen in inorganic forms such as N-NH_4^+ (Ijaz et al., 2015; Do et al., 2021). According to Ijaz et al. (2015), aquatic plants can remove dissolved waste in water through mechanisms such as (i) absorption by plants followed by harvesting

and removal from the system, (ii) ammonia evaporation, and (iii) nitrification and denitrification by microorganisms. According to Yasin et al. (2021), the plant root system is essential for nutrient absorption, which can absorb nutrients correlating with root surface area. The roots also provide a suitable habitat for microbial growth and development. The plant in the T2 experiment had a more developed root system and a much larger surface area than the other plants, leading to higher TAN processing efficiency.

The N-NO_2^- pattern occurred because the experimental plants grew well from day 1 to day 10, which increased alkalinity, pH, and oxygen in the system, thereby enhancing the activity of nitrifying bacteria (Mohd Nizam et al., 2020). However, between days 10 and 15, the duckweed (*L. minor*) in the T2 and T3 experiments began to die off, especially in T2, causing the N-NO_2^- content to rise sharply, reaching 0.49 ± 0.21 mg/L in the T2 experiment. According to Ajani et al. (2011), the N-NO_2^- content in a circulatory system should be below 0.5 mg/L, and Boyd (2016) stated that N-NO_2^- becomes toxic to shrimp and fish when levels exceed 2 mg/L. Therefore, the fluctuations observed in this experiment (less than 2 mg/L) are within an acceptable range and have minimal impact on fish growth and development.

The decrease in N-NO_3^- may be attributed to the growth of aquatic plants, which can absorb nitrogen in inorganic forms such as N-NH_4^+ and N-NO_3^- . Conversely, in the control experiment (T1), the metabolism of nitrifying bacteria in the biological filtration system caused N-NO_3^- concentrations to rise over the experimental period. On day 10, the NO_3^- concentration in T2 to T5 was not significantly different from each other but was significantly different from T1. The N-NO_3^- removal efficiency after 10 days was 47.12% in T2, 44.67% in T4, 43.78% in T3, and 31.26% in T5. These results were supported by the study of Li et al. (2015), in which aquatic plants primarily facilitate nitrate removal in RAS.

The decrease in TN is due to the plant's ability to

absorb inorganic nitrogen while not absorbing organic nitrogen, leading to a reduction in total TN primarily through the absorption of TAN and NO_3^- by aquatic plants. A study by Henry-Silva and Camargo (2006) in Brazil demonstrated that in a 4 m² experimental tank, *Pistia stratiotes* removed 0.15 mg/L TN, 62.4 mg/L TP, and 23.6 mg/L NO_3^- from tilapia pond wastewater over 14 weeks. Le et al. (2015) showed that *Hymenachne acutigluma* could significantly reduce N-NH_4^+ by 69.7-96.9%, N-NO_3^- by 99.3-99.9%, and TKN by 48.5-73.5%. Moreover, the reduction in phosphorus in aquatic plant treatment systems is attributed to absorption by plants or precipitation and adsorption into bottom sludge (Le et al., 2021).

The results of the current study showed that combining floating aquatic plants with a biofilter tank provides optimal phytoremediation efficiency for treating aquaculture wastewater. Integrating *P. stratiotes* yielded the best treatment effect during the first 10 days of the experiment, reducing NO_3^- by 46.70%, PO_3^- by 24.56%, and increased DO by 37.68%, CO_2 by 65.83%, COD by 34.28%, TAN by 40.70%, and TP by 9.16%. These data indicate that the aquatic plant *P. stratiotes* could be effectively integrated into the RAS model for fish culture.

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