

Utilizing catfish waste-derived liquid organic fertilizers for sustainable microalgae cultivation

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ABSTRACT

Peatlands are important ecosystems in Sumatra and Kalimantan in Indonesia that support the livelihoods of local people especially fisheries. The study investigated the effect of the addition of *A. microphylla* on water quality parameters in the rearing media of Siamese catfish in peat water to identify the optimal rate of *A. microphylla* that can enhance water quality and ultimately improve the growth and survival of Siamese catfish in aquaculture systems. The research was a randomized design with 4 treatment levels of *A. microphylla* with 3 replications, namely AM₀ (Control), AM₁ (20 g/m²), AM₂ (40 g/m²), and AM₃ (60 g/m²). The results showed that the best treatment was the AM₃. It was able to reduce TSS by 68.5%, TDS by 37.65%, turbidity of 70.5%, and the brightness by 15.58 cm. The treatment improved the pH value of water (4.70 to 6.63), increased the dissolved oxygen content of water (3.30 mg/L to 7.66 mg/L), decreased water-free carbon dioxide (8.00 mg/L to 7.00 mg/L), increased nitrate content in water (0.43 ppm to 16.86 ppm), increased water orthophosphate content (0.64 ppm to 3.52 ppm) and produced an average abundance of plankton of 8461 ind. per L. The favourable condition was able to support the rearing of catfish for 60 days by increasing weight gain (2.39 g per fish), absolute length (2.50 cm per fish), feed efficiency (88.01%) and a high survival rate (93%). The results contribute to the development of efficient and eco-friendly aquaculture practices for fish.

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1. INTRODUCTION

Peatlands occur in large areas in Sumatra and Kalimantan in Indonesia and support the livelihoods of people living in the area. Peatlands provide ecosystem services and support fisheries, cropping, plantations, and forestry [1, 2]. To encourage conservation and reduce the degradation of peatlands, peatlands can be utilized for fisheries by implementing various strategies that take advantage of the unique characteristics of the peat swamp ecosystem [3, 4]. The water in peatlands is brown, rich in dissolved organic matter and acidic, which can be challenging for aquaculture and fish to thrive in [5]. However, this water can be processed and enriched to create a suitable habitat for fish rearing.

One approach is to cultivate fish in ponds or cages in the peat swamp areas, using peat water that has been treated to enhance its quality. This can involve adding fertilizers or other nutrients to increase the water's productivity and create a more favorable environment for fish growth. Another strategy is to incorporate the cultivation of aquatic plants, such as *Azolla microphylla*, which can help to improve water quality and create a more diverse ecosystem. These plants can serve as a food source for fish, and their growth can also help to remove excess nutrients from the water [6].

Azolla is an aquatic fern native to Asia, Africa, and America that is abundant in nature, grows as a floating mat on the surface of the water and can have many beneficial uses. It can serve as feed, green manure, and a biofilter to enhance water quality in fish-raising environments. This plant is typically found in still waters, including lakes, ponds, rivers, and rice fields [7]. Azolla microphylla can act as a phytoremediation agent by absorbing excess nutrients from fish and food waste, thereby improving water quality and promoting fish biomass growth. Additionally, this plant is capable of fixing atmospheric nitrogen and establishing a symbiotic relationship with *Anabaena azollae* [8]. Research by Candra et al. (2019) shows that Azolla added as a supplement could sustain Nile tilapia growth by serving not only as feed ingredient but as a nitrogen source, thus improving water productivity [9].

In aquatic ecosystems, water quality is a critical determinant of the success of fish-raising efforts. The use of *A. microphylla* in aquaculture can serve as a cost-effective and eco-friendly solution to improve peat water quality. The plant's ability to utilize dissolved organic matter from peat water, excess nutrients from fish waste can reduce the need for chemical treatments to control water quality, which can adversely affect aquatic organisms and the environment. In addition, *A. microphylla* can support the growth of beneficial microbes that contribute to nutrient cycling, further improving water quality in fish-raising environments.

The Siamese catfish (*Pangasianodon hypophthalmus*) is one of the main fishery commodities that can be cultivated using peat water in areas such as Sumatra and Kalimantan. This species is a mainstay commodity in Riau Province, Indonesia due to high market demand, which necessitates aquaculture businesses to adopt intensive cultivation systems to achieve optimal production results [10]. Research conducted by Tahapari et al. (2015) demonstrated that growing Siamese catfish on peatlands led to good cultivation diversity, with a daily weight gain (PBH) of 3.33 – 3.83 g per day, feed conversion ratio (FCR) of 0.85 – 1.23, and survival rate (SR) of 95% – 97.92% [11]. These findings suggest that the use of peat water for the aquaculture of Siamese catfish can be a viable and productive strategy, providing an opportunity for sustainable and profitable fisheries practices.

The study seeks to address the need for effective and sustainable solutions to improve water quality in peat water based aquaculture, which can lead to increased production and profitability for local livelihoods. Thus, this study aims to investigate the effect of Azolla microphylla biomass on water quality parameters in the rearing media of Siamese catfish (*Pangasianodon hypophthalmus*) cultivated in peat water. The objective is to identify the optimal rate of *A. microphylla* that can enhance water quality and ultimately improve the growth and survival of Siamese catfish in aquaculture systems. Furthermore, by determining the optimal rate of *A. microphylla* for improving water quality parameters in the rearing media, the study can contribute to the development of efficient and eco-friendly aquaculture practices for Siamese catfish cultivation in peat water based systems.

2. RESEARCH METHOD

2.1. Research Set Up

The study was conducted in the Experimental Pond Area at the Faculty of Fisheries and Marine Sciences, University of Riau in Pekanbaru, Indonesia. A complete randomized design was conducted with four treatment levels, each replicated three times. The biomass dosage utilized in the study was based on the outcomes of a preliminary test that identified the best treatment within the 20-60 g m⁻² range. The research treatments included AM0 (no *A. microphylla* biomass), AM1 (20 g m⁻² *A. microphylla* biomass), AM2 (40 g m⁻²), and AM3 (60 g m⁻²).

Peat water was collected from a river in the village of Kuala Nenas, Kampar Regency in the province of Riau, Indonesia. Jerry cans with a 30-liter volume were used to obtain the water directly from the river banks, and transport it to the research location. To prepare the research containers, the containers were cleaned with water and 10% KMnO₄. Then, 12 units were randomly arranged, labeled according to treatment, and filled with peat water. The *A. microphylla* was cultured in a 100-liter bucket before being transferred to the research container. The Azolla was weighed every four days to ensure its biomass remained aligned with the initial dose for each treatment. If there was an increase in biomass, it was reduced to ensure consistency with the prescribed dose for each treatment.

Siamese catfish of 5-7 cm in size and weighing approximately 1 gram were selected from the same parent to ensure genetic uniformity. The fishlings were introduced into the research containers at a stocking density of one fish per litre of water, resulting in 60 fish per container, or a total of 720 fishes. The fish were acclimatized for three days in the peat water before being placed in the research containers to prevent stress. The maintenance of Siamese catfish continued for 28 days to 60 days, during which they were fed three times a day with commercial pellets trademarked as PF-800, at a feeding rate of 5% of the fish population's weight. The feeding times were at 07.00, 12.00, and 17.00 hour. The Siamese catfish's weight and length were measured on the 0th, 7th, 14th, 21st, and 28th until 60 days. A sub-sampling technique was used to obtain 30% of the sample fish from each treatment, approximately 20 individuals, for measurements.

2.2. Water Quality Parameters

The quality of peat water was evaluated through physical, chemical, and biological measurements. The physical and chemical parameters of the peat water were measured five times throughout the study, on day 1, day 7, day 14, day 21, and day 28 until 60 day. On the other hand, the biological parameters of the peat water were measured every two days throughout the study.

2.2.1. The Peat Water Physical Parameters

The measurement includes:

- Total Suspended Solid (TSS) was measured by passing the water sample through a pre-weighed filter and calculated from:

$$TSS (g) = \frac{A-B}{V} \times 1000 \quad (1)$$

where, TSS = Total Suspended Solid (g), A = Dry weight of filter paper after filtering (g), B = Dry weight of filter paper after washing (g), and V = Volume of filtered water (L).

- Total Dissolved Solids (TDS), using the electrometric measurement method with the TDS meter (digital HM).
- Turbidity, using the nephelometer measurement method with a turbidity meter (Lutron TU 2016).
- Brightness level of peat water, using a Secchi disk tool.
- Water temperature using a thermometer.

2.2.2. The Peat Water Chemical Parameters

The measurement includes:

- pH with portable pH Meter Hanna HI 9124 with an accuracy level of 0.1.
- Dissolved Oxygen (DO) using the DO meter with a YSI Pro20i,
- Free CO₂ in peat water, using the titrimetric method, where Na₂CO₃ was titrated to form NaHCO₃ and calculated with the following formula:

$$CO_2 (mg/L) = \frac{mL \text{ titrant} \times N \text{ titrant} \times 44/2 \times 1000}{\text{Sample Volume}} \quad (2)$$

where, CO₂ = Concentration of free carbon dioxide (mg/L), mL titrant = Volume of titrant read (mL), N titrant: Normality Na₂CO₃, and 1000 = Correction factor.

- Nitrate concentration using the Naphthyl method.
- Orthophosphate concentration using the Stannous Chloride method as recommended by Boyd and Tucker (1998) [12].

2.2.3. The Peat Water Biological Parameters

The measurement includes:

- The abundance of plankton in this study, calculated using the formula [13]:

$$K = \frac{N \times C}{V_0 \times V_1} \quad (3)$$

where, K = Abundance of plankton, N = Number of cells, C = Volume of water in the sample bottle, Vo = Volume of filtered water, and V1 = Volume of dropper pipette (0.1 ml).

- Species diversity index (H') of plankton, calculated according to Shannon-Weiner formula:

$$H' = \sum_{i=1}^S P_i \log_2 P_i \quad (4)$$

where, Pi = proportion of plankton species u.

- Diversity index (H') of plankton, calculated according to Shannon-Wiener formula:

$$C = \sum_{i=1}^S \left(\frac{n_i}{N} \right)^2 \quad (5)$$

where, C = Species dominance index, ni : Number of individuals of type i, N = Total individuals of all species, and S = total number of plankton types

2.2.4. Siamese Catfish Growth Parameters

The measurement includes:

- Absolute Fish Weight gain.
- Absolute Length of Fish growth.
- Larval survival rate.
- Feed Conversion Ratio (FCR), calculated from: $FCR = F / ((W_t + W_d) - W_o)$, where Wo: Weight of the fish biomass at the start of the experiment (g), Wt: Weight of fish biomass at the end of the experiment (g), Wd: Weight of fish that died during the study (g), and F: Amount of feed given (g).
- Feed Efficiency, calculated from: $FE = ((W_t + W_d) - W_o) / F \times 100\%$, according to Zonneveld et al., (1991) [14].

2.3. Data Analysis

Tables and graphs present the data on average values of peat water quality parameters, absolute weight, absolute length growth, survival, FCR, and Siamese catfish feed efficiency obtained in the study. The data was subjected to analysis of variance (ANOVA), and a significant difference ($P < 0.05$) indicates an effect of Azolla biomass on peat water quality. To further examine the differences in each treatment, it is necessary to conduct the Student Newman-Keuls test.

3. RESULTS AND DISCUSSIONS

Figures 1, 2 and Table 1 display the results of measuring the average value of physical and chemical parameters of peat water in the Siamese catfish breeding media. Table 1 presents the average measurement values at the end of the experiment for the four treatments (AM0, AM1, AM2, and AM3). The parameters listed include Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Turbidity, Brightness, Temperature, pH, Dissolved Oxygen (DO), Free CO₂, Nitrate, and Orthophosphate. Quality standards for each parameter are given in the second column, which is followed by the average measurement values for each treatment. The Table allows for a comparison of the measurements for each parameter among the different treatments.

3.1. Peat Water Physical Parameters

TSS (Total Suspended Solids) measures the amount of suspended matter in the water with values above 80 mg L⁻¹ considered below standard. The TSS values for the control (AM0) ranged from 86 mg L⁻¹ to 175 mg L⁻¹ which indicate a poor physical quality. The application of Azolla reduced TSS levels in peat water. The decrease in the TSS value of peat water occurred on the 7th, 14th, 21st, and 28th days until 60 days on all treatments (Figure 1). Treatment AM1 (20 g m⁻² A. microphylla) had the lowest effect, reducing TSS to 55.33 mg L⁻¹, while AM3 (60 gm⁻²) was the best treatment reducing TSS from 83.67 mgL⁻¹ to 26.33 mgL⁻¹. Based on the Analysis of Variance test, A. microphylla had a significant effect on the reduction of TSS in peat water in the maintenance of Siamese catfish (*P. hypophthalmus*) ($P < 0.05$) with the best treatment found in the AM3 treatment. The

accumulation of TSS values reduced light penetration, suppressing the photosynthetic activity of phytoplankton, algae, and macrophytes. As a result, DO concentrations will decrease and fish growth will be disrupted [15]. The TSS value is inversely proportional to the survival of Siamese catfish. Correspondingly, AM0 is the treatment had the lowest fish survival rate.

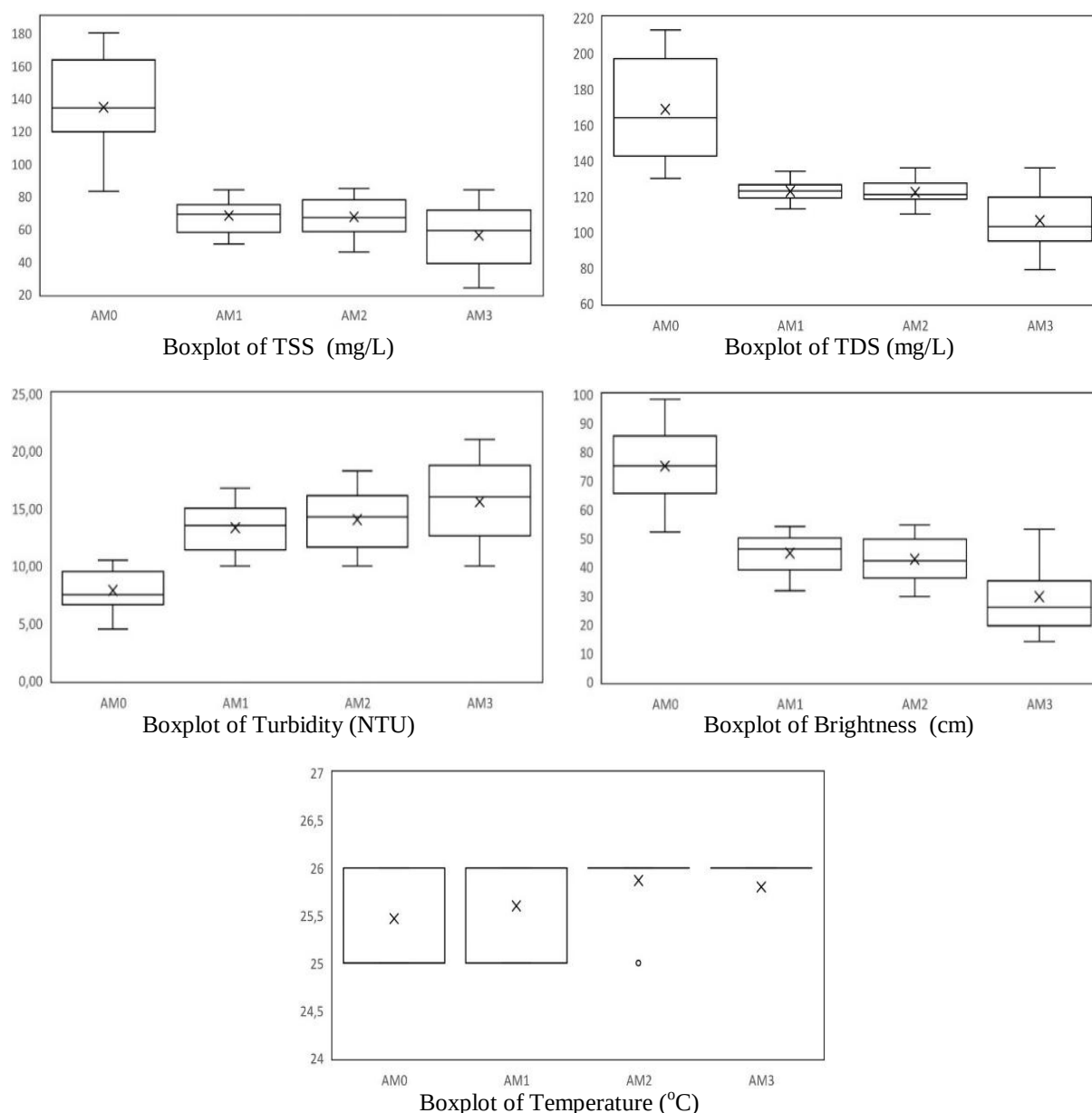


Figure 1. Boxplots of physical parameters of peat water for catfish cultivation.

Similar results were found for TDS (Total Dissolved Solids). The average TDS measurement value for the control (AM0) was 168.73 ± 1.17 , which was due to the high dissolved organic matter content of the peat water. Azolla treatment decreased TDS from day 7 to day 60. until 60 days AM3 was the best treatment, reducing TDS from 131 mg L^{-1} to 81.67 mg L^{-1} . Statistical test confirmed the significant decrease ($p < 0.05$). Azolla can mitigate the concentration of TDS in peat water-raised catfish by absorbing dissolved solids through its root hairs and transporting them through its xylem and phloem networks [7, 16]. Azolla also forms a mutualistic symbiosis with cyanobacteria microorganisms, particularly *Anabaena azollae*, which reside in the dense roots of Azolla and contribute to reducing TDS by promoting the activity of decomposing microorganisms [17, 18].

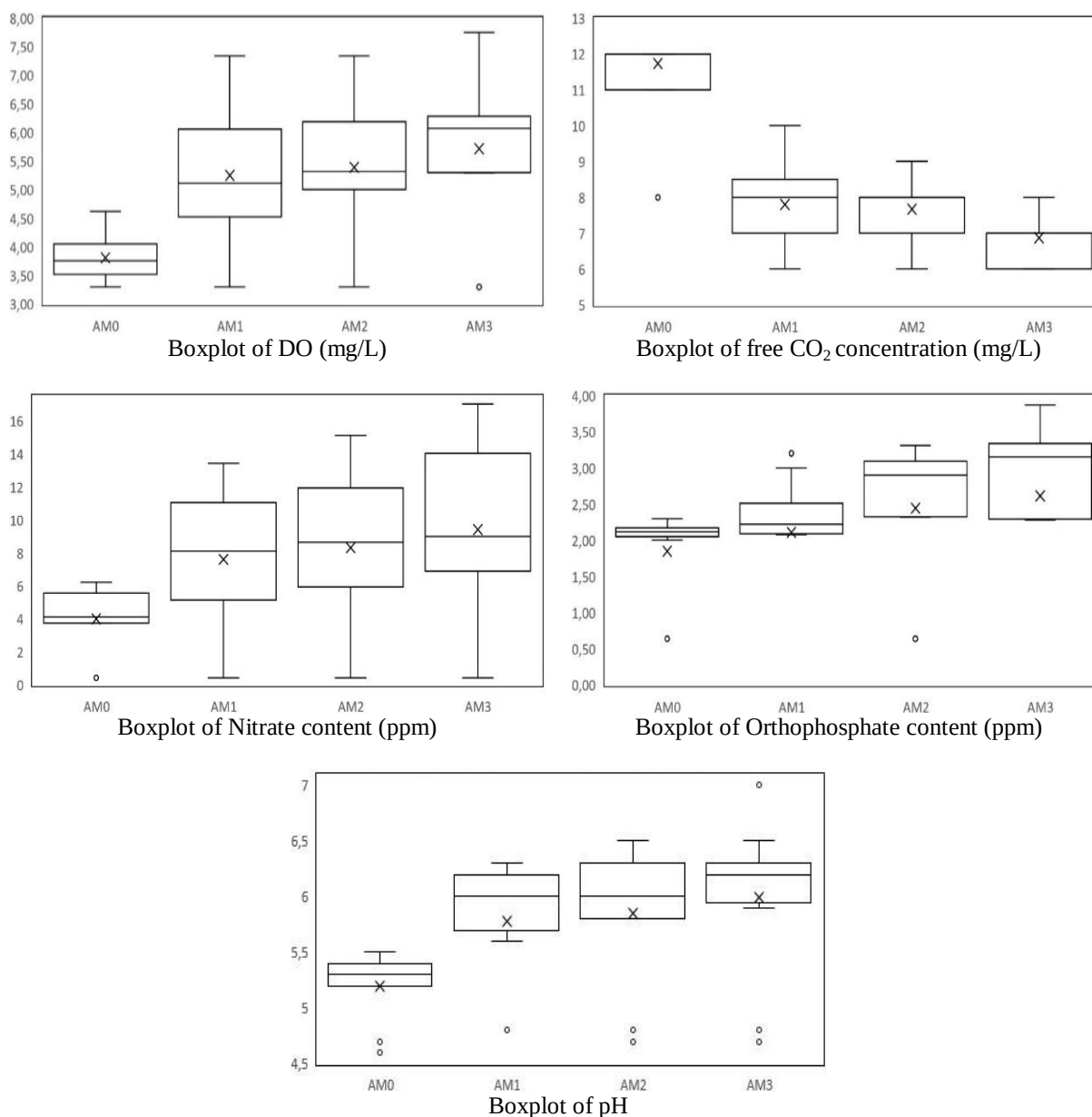


Figure 2. Boxplots of chemical parameters of peat water for catfish cultivation.

The Azolla treatment also, affected turbidity and brightness. The control had an average measurement turbidity of 75.02 ± 3.70 NTU, and brightness of 7.86 ± 0.30 cm, indicating poor water quality due to high suspended solids and organic matter. The AM3 treatment lowered turbidity to 29.66 ± 0.53 and increased brightness level to 15.58 ± 0.20 . The brightness of water is negatively correlated with its turbidity. Azolla is effective in reducing turbidity as its fibrous roots are capable of absorbing suspended solids present in the water. Nevertheless, temperature was not affected by treatments. The water temperature was optimal for Siamese catfish at 25-28°C and mostly controlled by the weather.

3.2 Peat Water Chemical Parameters

The control (AM0) had the lowest pH measurement value of 5.20 ± 0.02 , indicating that the water was acidic due to organic matter accumulation. Azolla treatment increased water pH compared to control ($p < 0.05$) from 5.70 ± 0.02 (AM1), 5.77 ± 0.05 (AM2), 5.92 ± 0.07 (AM3). Lower pH values are associated with lower dissolved oxygen which affects photosynthesis, respiration of aquatic animals, and decomposition of organic matter. Photosynthesis from Azolla could reduce the acid content in water.

Table 1. Physical and chemical parameters of peat water.

Parameters	Unit	Quality standard*	Average measurement values			
			AM0	AM1	AM2	AM3
TSS	mg/L	25 – 80 mg/L (good)	134.46 ±	68.26 ±	67.53 ±	56.13 ±
		81 – 400 mg/L (unhealthy)	5.14 ^c	2.20 ^b	1.10 ^b	1.81 ^a
TDS	mg/L	500 – 1200 mg/L	168.73 ± 1.17 ^c	122.80 ± 1.20 ^b	120.86 ± 1.50 ^b	106.46 ± 3.58 ^a
Turbidity	NTU	2 – 30 (desirable)	75.02 ± 3.70 ^c	44.61 ± 0.25 ^b	42.42 ± 0.51 ^b	29.66 ± 0.53 ^a
Brightness	cm	25 – 40 cm	7.86 ± 0.30 ^a	13.30 ± 0.05 ^b	14.03 ± 0.11 ^c	15.58 ± 0.20 ^d
Temperature	°C	25 – 30°C (optimal)	26.3	26.3	26.5	26.5
pH	-	6 – 9 (good)	5.20 ± 0.02 ^a	5.70 ± 0.02 ^b	5.77 ± 0.05 ^b	5.92 ± 0.07 ^c
DO	mg/L	DO > 5 mg/L (very good)	3.81 ± 0.14 ^a	5.25 ± 0.04 ^b	5.39 ± 0.10 ^b	5.71 ± 0.03 ^c
Free CO ₂	mg/L	CO ₂ > 10 mg/L	11.73 ± 0.31 ^c	7.80 ± 0.53 ^b	7.67 ± 0.12 ^b	6.87 ± 0.12 ^a
Nitrate	ppm	5 – 50 ppm	4.00 ± 0.06 ^a	7.60 ± 0.03 ^b	8.30 ± 0.12 ^c	9.41 ± 0.08 ^d
Orthophosphate	ppm	5 – 50 ppm	1.85 ± 0.05 ^a	2.11 ± 0.03 ^b	2.44 ± 0.01 ^c	2.61 ± 0.12 ^d

*According to Boyd and Tucker (1992) [19].

Numbers within a row with the same letter indicate no statistical difference between treatments at $p < 0.05$.

DO (Dissolved Oxygen), measures the amount of oxygen dissolved in the water with values >5 mg L⁻¹ considered as a good quality. The control had the lowest DO value of 3.81±0.14 mg L⁻¹ and highest free CO₂ concentration of 11.73±0.31 mg L⁻¹, indicating poor water quality. Azolla treatments increased DO and decreased free CO₂ significantly with the AM3 treatment had the highest DO value of 5.71±0.03 mg L⁻¹ and CO₂ concentration of 6.87±0.12 mg L⁻¹. The DO content increased and decrease in CO₂ during the experimental period was due to the photosynthesis process by *A. microphylla* which utilized CO₂. In addition, *A. microphylla* could host cyanobacterium *Anabaena azollae* that have the ability to fix nitrogen from the atmosphere and simultaneously release oxygen into the water. Moreover, the rise in dissolved oxygen levels can be attributed to oxygen diffusion from the surrounding air and photosynthesis.

The addition of *A. microphylla* resulted in an increase in water nitrate content, with the control group measuring at 4 ppm and the highest concentration observed in AM3 at 9.41 ppm. As the quantity of *A. microphylla* increased, the nitrate content also rose. Nitrate is formed through the nitrification process, where aerobic bacteria oxidize NO₂ to NO₃. Nitrate plays a crucial role in supporting the growth of aquatic plants and phytoplankton. *A. microphylla* plays host to bacteria that can fix nitrogen from the air, enabling the nitrification process to occur. Azolla is a floating pteridophyte that is symbiotic with the cyanobacterium *Anabaena Azollae* [20].

The nitrate concentration remained below a critical level of 50 ppm, which is generally considered acceptable for freshwater fish species with low toxicity. However, it's worth noting that seawater fish species tend to be more sensitive to the presence of nitrates in the water.

The addition of *A. microphylla* treatment led to an increase in orthophosphate levels, compared to the control group's measurement of 1.85 ppm. The highest orthophosphate content was observed in AM3, reaching 2.61 ppm. Orthophosphate represents the predominant inorganic form of phosphate in the phosphate cycle. The availability of orthophosphate in water is influenced by factors such as microbial decomposition of organic materials, fertilization, and rainfall that carries phosphorus dust from the air [21]. As aquatic organisms, particularly phytoplankton, utilize orthophosphate as a nutrient source, its levels tend to decrease over time.

3.3 Peat Water Biological Parameters

Table 2 shows the abundance of different taxa of phytoplankton and zooplankton as a function of Azolla treatments. The total abundance of phytoplankton and zooplankton increased with increasing concentration of *A. microphylla*. The most abundant taxa of phytoplankton are *Synedra* sp. and *Chlorococcum* sp., while the most abundant taxa of zooplankton are *Pleodarina* sp. and *Monostyla* sp.

Table 2. Types and average abundance of plankton (ind.L⁻¹) found during the study.

Taxa	Treatment (ind. L ⁻¹)			
	AM0	AM1	AM2	AM3
PHYTOPLANKTON				
Bacillariophyceae				
<i>Climacospheia moniligera</i>	278	0	111	0
<i>Cocconeis</i> sp.	1002	946	946	1225
<i>Nitzschia acicularis</i>	501	278	390	445
<i>Synedra</i> sp.	1058	1058	1113	1336
Charophyceae				
<i>Closterium</i> sp.	0	111	0	390
Chlorophyceae				
<i>Chlorococcum</i> sp.	501	1002	1113	1392
<i>Scenedesmus</i> sp.	334	724	668	1280
Cyanophyceae				
<i>Anabaena</i> sp.	0	557	724	891
Total	3674	4676	5066	6958
ZOOPLANKTON				
Protozoa				
<i>Pleodarina</i> sp.	278	501	501	557
Rotifera				
<i>Brachionus bidentate</i>	0	111	167	167
<i>Monostyla</i> sp.	0	223	445	501
<i>Philodina roseola</i>	223	111	223	278
Total	501	946	1336	1503
Total Abundance	4175 ± 1002 ^a	5622 ± 674 ^b	6401 ± 255 ^b	8461 ± 347 ^c

The types of plankton found during the research were 6 classes consisting of 4 classes of phytoplankton and 2 classes of zooplankton. Four classes of phytoplankton found were: Bacillariophyceae, Charophyceae, Chlorophyceae, and Cyanopyceae. While the 2 classes of zooplankton were Protozoa and Rotifera. The total types of plankton found in the research were 12 species. The most common types of plankton found were in the rearing medium treated with *A. microphylla* biomass (AM1, AM2, and AM3), namely 11 species. Meanwhile, the fewest types of plankton were found in the control, namely only 8 species. There were not many types of plankton found during this study because the plankton that grew and developed in the rearing medium came from nature, namely peat water itself without additional plankton seeds and also without fertilizer application.

The treatment of *A. microphylla* resulted in a higher abundance of plankton compared to the control (AM0). The introduction of *A. microphylla* biomass had an influence on the quality of nutrients, especially the nitrate content, resulting in a high abundance of plankton in the rearing medium. Overall, the total abundance of both phytoplankton and zooplankton increased with increasing *A. microphylla* concentration. The highest total abundance of both phytoplankton and zooplankton was found in treatment AM3, with a total abundance of 8,461 individuals per liter, which was significantly higher than the other three treatments. This indicates that the presence of *A. microphylla* had a positive effect on the growth of both phytoplankton and zooplankton.

In terms of phytoplankton, Chlorophyceae had the highest abundance in all treatments, followed by Bacillariophyceae and Charophyceae. The highest abundance of Chlorophyceae was found in treatment AM3, with a total abundance of 1,392 individuals per liter. *Cocconeis* sp. was the most abundant species of phytoplankton, with the highest abundance found in treatment AM3. This species is known to be an important food source for zooplankton.

For zooplankton, Protozoa had the highest abundance in all treatments, followed by Rotifera. The highest abundance of Protozoa was found in treatment AM3, with a total abundance of 557 individuals per liter. Pleodarina sp. was the most abundant species of zooplankton, with the highest abundance found in treatment AM3.

In summary, the results of the table suggest that the addition of *A. microphylla* to the cultivation media had a positive effect on the growth of both phytoplankton and zooplankton, with the highest abundance found in treatment AM3. This could be due to the availability of nutrients and organic matter provided by *A. microphylla*.

The results of species diversity index obtained from the study are presented in Figure 3.

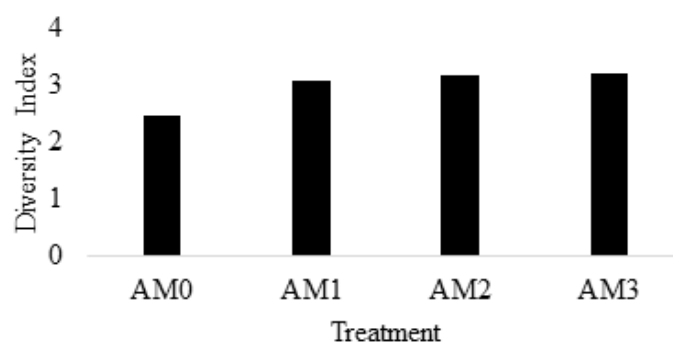


Figure 3. Average plankton diversity index during the study.

According to Figure 3, the AM1, AM2, and AM3 treatments increased the plankton diversity with Shannon diversity H' values to greater than 3. The control had a medium diversity with an average H' value of 2.48. H' is affected by water quality and nutrient availability, with poor water quality leading to reduced plankton diversity.

The results of species dominance index are presented in Figure 4. The AM3 treatment had the lowest dominance index value, with an average species dominance (C) value of 0.121, indicating a stable water condition without any dominating organism. This suggests that the dosage of *A. microphylla* biomass used in the study was within the tolerance limit of plankton in the medium. The dominance index is used to assess the dominance of biota groups in habitat and the quality of water conditions, and a lower index indicates a more stable aquatic community [13, 22].

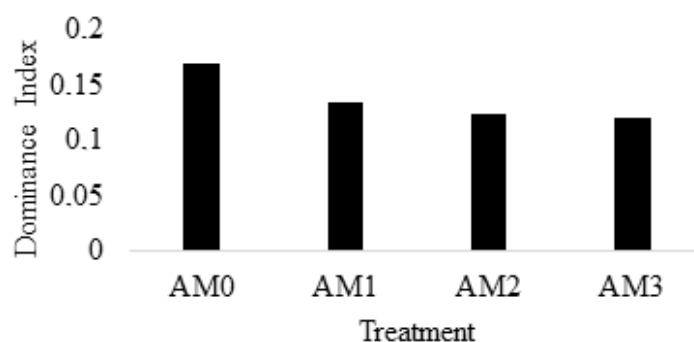


Figure 4. Average dominance index of plankton types during the study.

3.4 Siamese Catfish Growth Parameters

Table 3 shows the growth of Siamese catfish (*P. hypophthalmus*) as a function of treatments. The average measurements for the absolute weight (in grams per head), absolute length (in centimeters per head), survival rate (SR in percentage), feed conversion ratio (FCR), and feed efficiency (FE in percentage) are presented. The highest values for all growth parameters were observed in the AM3 treatment. The FCR was the lowest in the AM3 treatment, while the FE was the highest. The SR increased with increasing doses of *A. microphylla* biomass.

Table 3. Growth of siamese catfish.

Growth of catfish	Unit	Average result of measurement/analysis			
		AM0	AM1	AM2	AM3
Absolute weight	g/head	1.80 ± 0.03 ^a	2.05 ± 0.13 ^b	2.05 ± 0.12 ^b	2.39 ± 0.01 ^c
Absolute length	cm/head	1.50 ± 0.10 ^a	1.57 ± 0.06 ^a	1.93 ± 0.15 ^b	2.50 ± 0.20 ^c
Survival rate (SR)	%	75.00 ± 5.00 ^a	78.33 ± 2.89 ^a	81.67 ± 7.64 ^a	93.33 ± 2.89 ^b
Feed conversion ratio (FCR)	-	1.38 ± 0.05 ^b	1.22 ± 0.06 ^{ab}	1.25 ± 0.12 ^{ab}	1.14 ± 0.01 ^a
Feed efficiency (FE)	%	72.73 ± 2.39 ^a	82.36 ± 3.67 ^{ab}	80.47 ± 7.35 ^{ab}	88.01 ± 0.25 ^b

Based on the results (Table 3), it is observed that the highest absolute fish weight and length were recorded in the AM3 treatment, indicating that the addition of 60 g m⁻² A. microphylla biomass to the rearing medium had the most significant effect on the growth of catfish. This includes the largest absolute weight (2.39 g/head) or 33% increase compared to control and an average fish length value of 2.50 cm or 67% increase compared to control.

The SR, FCR and EP values in the AM3 treatment were also the highest among all treatments. The high SR value indicates a high 93±3% survival rate of the fish in the AM3 treatment compared to the control 75±5%. While the low FCR value or high FE value indicates that the fish efficiently converted the feed into body mass (88% efficiency at AM3 compared to 75% under control). The FCR value is affected by water quality. Hasibuan et al., (2023) showed FCR increased (became less efficient) there was more carbon dioxide in the water [23].

The environmental conditions of the cultivation media and protein utilization from feed also affect the FCR value by adapting to a greater environment and using less energy for growth. It is important to note that the FCR value is affected by various factors, including water quality and protein utilization from feed. The high FE value observed in the AM3 treatment indicates that the fish efficiently converted the feed into body mass, which can be attributed to the optimal environmental conditions provided by the A. microphylla biomass addition. The high SR value also suggests that the addition of A. microphylla biomass positively affected the survival rate of the fish.

Overall, the results suggest that adding A. microphylla biomass to the peat water as a rearing medium at a dose of 60 g m⁻² positively affects the growth and survival of Siamese catfish, as well as their feed efficiency. Water quality is the main factor affecting fish growth [11]. good water quality can support fish growth, metabolic rate, and energy assimilation [10].

3.5 Overall discussion

Azolla is a fast-growing water fern that can grow in low-quality water and is known for its high growth rates and bioremediation efficiency [24]. Azolla biomass is rich in proteins, essential amino acids, vitamins, and minerals, making it a useful nutrient feed for fishes. Azolla has been successfully used as green manure in rice fields in Asia and Africa and as a feed supplement for aquatic animals. Azolla has a symbiotic relationship with a nitrogen-fixing cyanobacterium, *Anabaena azollae*. The rate of nitrogen fixation of A. azollae is estimated to be significantly higher than the nitrogen fixation rate of legumes [25]. Azolla has been used as a supplement and even substitute for fish feed, which can reduce the cost of production and is considered an eco-friendly and sustainable resource. Azolla can produce a large amount of nitrogen, and its protein can be converted into a high-quality edible protein for fish [26]. In India, incorporating Azolla as feed, reduced the cost of feed, with a cost-saving up to 25%. While in Bangladesh, a 25% feed substitute with Azolla can maintain Thai silver barb fish growth and product quality and could contribute significantly to a higher profit margin [27]. Research also found Azolla can be used to remediate wastewater by reducing the concentration of nitrogen and phosphorus by absorbing and assimilating them into its biomass [28].

This study further explores the benefit of Azolla to improve peat water quality. Peat water had high levels of TSS and TDS which can reduce light penetration, suppress photosynthetic activity, and decrease DO concentrations, which in turn can disrupt fish growth and survival. This study shows that

Azolla at 60 g m⁻² can reduce TDS, turbidity and increase the dissolved oxygen concentration in the water, improving the water's overall quality and supporting aquatic life. Azolla can absorb suspended and dissolved solids through its root hairs and transport them through its xylem and phloem networks, forming a mutualistic symbiosis with cyanobacteria and promote the activity of decomposing microorganisms. Azolla can also reduce turbidity by absorbing suspended solids present in the water. The increase in pH and DO levels is attributed to the photosynthesis process by Azolla.

These incremental benefits ultimately lead to clearer water and a healthier aquatic ecosystem. Further Azolla could be used to control nutrient enrichments in peat water that has been used extensively for fish production. Mosha (2018), showed that Azolla is one of the high-biomass and protein-producing aquatic plants that can be used as a direct fish feed or food component as an alternative protein source [29]. Due to higher crude protein content (13%: 30%) and critical amino acid composition (rich in lysine) compared to other green forage crops and aquatic macrophytes, Azolla has gained its significance in aquaculture, and o a larger percentage of nutrient composition on a dry weight basis and other elements such as nutrients, chlorophyll, carotenoids, amino acids, and vitamins.

However, further research is needed to determine whether increasing Azolla dose greater than 60 g m⁻² can still provide an optimal condition. The dose and duration of *A. microphylla* biomass addition need further examination and to evaluate its potential effects on other important aspects, such as fish health and nutrient composition. An economic analysis on the profitability of Azolla addition would further support livelihood analysis.

4. CONCLUSIONS

The study investigated the effects of Azolla microphylla on the physical and chemical parameters of peat water used for raising Siamese catfish. Total Suspended Solids (TSS), decreased in all Azolla treatments from day 7 to day 60, with AM3 (60 g m⁻²) being the most effective treatment in reducing TSS from 83.67 mg L⁻¹ to 26.33 mg L⁻¹. Total Dissolved Solids (TDS) also decreased in all Azolla treatments, with AM3 being the best treatment, reducing TDS from 131 mg L⁻¹ to 81.67 mg L⁻¹. Turbidity and brightness improved in all Azolla treatments, with AM3 being the most effective. Water pH increased in all Azolla treatments, and dissolved oxygen (DO) increased significantly, with the AM3 treatment having the highest DO value of 5.71±0.03 mg L⁻¹. *A. microphylla* addition increased the availability of nitrate and orthophosphate in water, which is beneficial for aquatic organisms. The results suggest that Azolla microphylla can improve the physical and chemical quality of peat water and create a favorable environment for the growth of Siamese catfish. Consequently, the AM3 treatment had the most significant effect on the growth of catfish, resulting in the highest absolute weight and length, survival rate, feed conversion ratio, and feed efficiency.

In summary, incorporating Azolla microphylla in peat water fish rearing systems offers multiple advantages. It helps improve physical and chemical water quality by removing excess nutrients, provides a cost-effective feed source for fish, and generates economic opportunities through the sale of harvested biomass. Harnessing the potential of Azolla microphylla promotes a symbiotic relationship between aquaculture, water quality improvement, and economic growth for local farmers.

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