



Water quality improvement in aquaculture: phytoremediation for closed-system fish farming using *Azolla pinnata* and *Lemna minor*



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Received : 01/06/2025 ; Accepted : 26/09/2025

Abstract

Lemna minor and *Azolla pinnata* were tested for their ability to improve water quality during red tilapia culture. Three ponds were present: Pond A contained *Azolla*, Pond B contained duckweed (*Lemna minor*), and Pond C was devoid of aquatic vegetation (control). The experiment was designed to analyse the capacity of the two plant species to filter aquaculture water for pH, temperature, electrical conductivity, salinity, ammonium, phosphate, nitrite, and nitrate concentrations and compare the results with the standards established with the WHO. At the end of the experiment (7 weeks, from March to May 2023) Weight gain varied from 460 to 508 grams; pollutant eliminations were recorded in amounts for the *Azolla* basin: nitrite 63%, nitrate 71%, and phosphate 42%. For the duckweed basin, 90% phosphate, 57% ammonium, 71% nitrite, and 68% nitrate were removed. The ability of these aquatic plants to purify water maintained optimal quality by eliminating excess waste products and other contaminants from the aquaculture system.

KEYWORDS: *Lemna minor*, *Azolla pinnata*, Red tilapia, Wastewater, Closed-systems.

HIGHLIGHTS

Closed systems also have a high tendency to accumulate nutrients and wastes, which must be effectively managed to maintain suitable water quality for fish to grow sustainably. This study proposes a means of phytoremediation to treat fishpond water in a closed-loop system. The capacity of two possible plant species, *Lemna minor* and *Azolla pinnata*, to purify the water in fishponds or

aquaculture systems with typical red tilapia stocks will be assessed. In the aquaculture industry, this process leads to a circular economy and a sustainable practice of lowering chemical water treatment.

1.INTRODUCTION

Aquaculture, mainly fish farming, helps food security as it enhances and diversifies the diet of a population. Therefore, fish farming is a new activity and among the Algerian projects that is very promising in terms of utilising certain zones and meeting the requirement of water from a closed system, i.e., recycling wastewater [1]. This includes fish farming plus crop production in an enclosed space, producing both fish and agricultural produce, a system that leads to a sustainable and efficient agricultural system. Azolla and Lemna are genera of floating aquatic ferns; they have diverse similarities in their rapid growth. They have various additional advantages, as they are used in agriculture as biofertilizers and feed supplements for livestock and fish, contributing to sustainable agriculture [2].

Tilapia is a freshwater fish and one of the most popular fish worldwide. It adapts well to intensive farming because it can grow quickly and multiply rapidly. Hence, with respect to tilapia, the first thing that water provides to the fish is life. This indicates that quality water is very important to the well-being of tilapia. When it comes to its life, no chemical or physical pollution should occur in the quality of water. Any pollution leads to deficiency in the fish in its environmental system, which reduces the ability to live according to its natural role [3]. Similarly, when fish excretion passes pollution and increases to a high percentage of pollution (i.e., ammonium above its normal level), fish can also overdose themselves with their density. In addition, unrefined feed pellets and fish excretion are the siltation in the water column, which produces suspended matter, which will lead to the accumulation and decomposition of organic matter, ultimately poisoning fish, causing loss of appetite, physical stress, decreasing ability to move, and ultimately death of the fish [4]. Thus, the quality of water must be kept in check for tilapia in order to keep them healthy and maintain sustainable aquaculture practices to ensure their viability, productivity, and welfare in aquaculture systems. The earliest observation on the impact of aquatic macrophytes was recorded by Mitzner (1978)[5], who observed an increase in alkalinity after a 91% biomass reduction of aquatic macrophytes by grass carp in a lake in Iowa.

The research by Leslie et al. (1983)[6] also indicated that in Florida lakes, removal of aquatic macrophytes could influence some water quality parameters. Crutchfield et al. (1992)[7] studied the establishment and ecological effect of redbelly tilapia (*Tilapia zillii*) in a vegetated cooling reservoir located in South Carolina, USA. The redbelly tilapia was introduced to the reservoir accidentally. Akhtar et al. (2023)[8] had as its conclusion that Azolla can be an effective means for water purification, especially in contexts of significant heavy metals such as copper.

This research is about the phytoremediation of fishpond waters in a closed-loop system. The study investigates the effectiveness of two plant species, *Lemna minor* and *Azolla pinnata*, to improve the water quality of fishponds containing standard red tilapia. The research is gastronomic and is

conducted at the experimental desert aquaculture station of the National Research and Development Centre in Fisheries and Aquaculture in Ouargla Province, southeast Algeria.

2.MATERIALS AND METHODS

2.1.MATERIALS

The ponds were designated as A, B, and C, and the designation was based on configuration depending on the types of plants used or the absence of plants. For our experiment, we used plastic tanks (boxes) with dimensions of Length : 34 cm, Width: 23 cm, Height: 16 cm, with a volume of 14 liters. As for the aquariums used in our experiment, they each had a capacity of 200 liters. and that was stocked with 30 fingerlings. The dimensions of the aquariums were: Height of 50 cm, Width of 50 cm, Length of 90 cm. Each aquarium had 200 grams of each species of macrophyte added to the aquaculture system. In the experimental use of *Lemna minor* and *Azolla pinnata* as a crop plant, the environmental conditions and experimental parameters were specific conditions. These conditions were controlled, and conditions enhanced in order to recreate optimal natural environments, or enhance growth in the experimental set up to allow for robust cultivation of *Lemna minor*.

Lemna minor and *Azolla pinnata* were grown best under abundant light, which can be from adequate artificial lighting in managed systems. Temperatures were maintained between 15-30°C, with pH maintained ideally between 6 to 8. The plant grew in freshwater or slightly brackish water. To support growth, it strongly benefitted from balanced nutrient inputs such as nitrogen, phosphorus, and potassium, which are generally added using aquatic fertilizers.

The macrophytes: *Lemna minor* and *Azolla pinnata* were gathered locally from water channels in a rural area in sidi khaled (Ouled Jellal) , Algeria . *Lemna minor* is duckweed, or lesser duckweed, which is identifiable by its fronds which are small, rounded, with a single vein and *Azolla* has a unique morphology . its leaves are small, rounded, usually bright green with reddish or brown coloration when exposed to sunlight.

Members of the Cichlidae family are abundant in Africa, which primarily contain freshwater and brackish waters, One hundred and eighty red tilapia (*Oreochromis* sp.) fingerlings (**Figure1**) were used in this study, with each aquarium housing 30 individuals. The fingerlings were supplied by CNRDPA, Ouargla station. After collection, they were housed in storage tanks and allowed to acclimate for one week before the first experiment was performed.

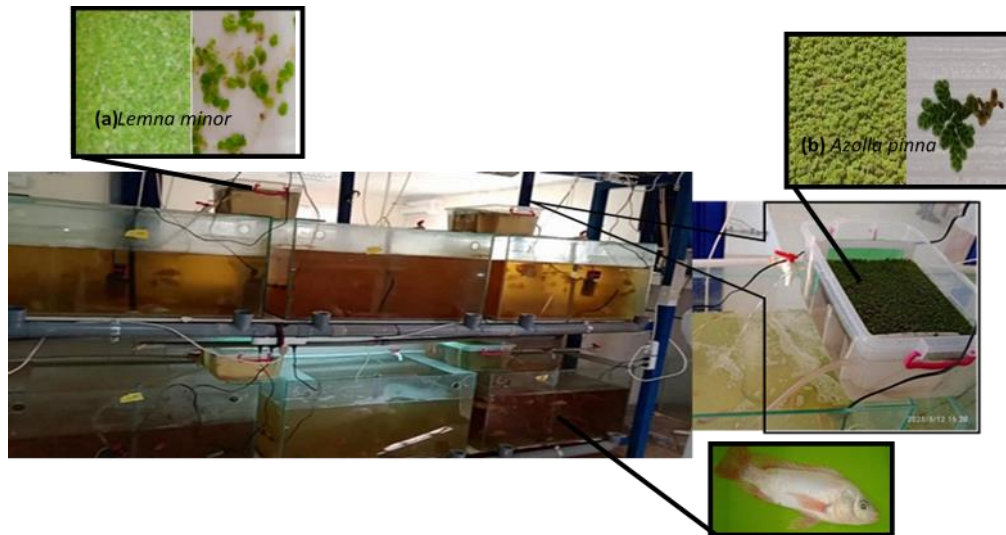


Figure 1.Experimental setup (Original, 2023): (a)*Azolla pinnata*, (b)*Lemna minor*

2.2. EXPERIMENTAL METHOD:

A big aerator was used for each aquarium, with a hose and a diffuser, and a thermostat was set to 26°C to heat the water to 28°C. To keep the breeding circle sanitary and avoid the growing of bacteria on food remnants, the bottoms of the aquariums were syphoned, and the trims were cleaned and rinsed thoroughly before feeding the fish to minimise the deposition of any fatty matter left by the food. This was done with a card having details specifying the name of the aquarium and type of sample: basin A: azolla; basin B: duckweed; basin C: control.

The aquaponic system is a closed-loop, sustainable method of aquaculture. The yokes were under the same conditions (e.g., temperature, oxygen, light, capacity). There were three containers above each aquarium. The first was filled with *Azolla*, the second was filled with duckweed, and the third was a control to monitor the efficacy of the plant filters. Each aquaponic tank was designed with two holes for water inlet and outlet. The inlet hole was attached to a hose and a filtration pump.

2.3. ANALYTICAL METHOD:

The physicochemical parameters were measured with precision. Temperature was recorded daily using a thermometer, following the guidelines set forth in ISO 5667-4:2016[9]. The pH meter was used following the procedures outlined in ISO 10523:2008[10]. A conductivity meter assessed electrical conductivity as per ISO 7888:1985[11]. Phosphate concentrations were measured by combining 100 mL of the sample solution with 10 mL of a reagent mixture containing R1: ammonium molybdate, R2: 2.5 mol/L H₂SO₄, R3: ascorbic acid solution, and R4: potassium antimony tartrate solution. After allowing the mixture to react for 5 minutes, the absorbance was measured at 885 nm, following the guidelines outlined in ISO 6878:2004[12].

3. RESULTS AND DISCUSSION

3.1.TEMPERATURE, PH, ELECTRICAL CONDUCTIVITY AND SALINITY :

As shown in **Figure2**, the temperature data collected from the *Azolla pinnata*, *Lemna minor*, and Control aquariums over an eight-week period produced some significant highlights. The temperatures were generally within the advisable range of $25.7 - 30 \pm 0.1^{\circ}\text{C}$, although there were instances of higher temperatures. The *Azolla* and *Lemna minor* aquariums tended to follow similar temperature trends during most weeks which indicates a closely related thermal environment in these respective systems. The Control aquarium exhibited a thermal environment that was similarly consistent to the optimum range, although some small variation was observed. Throughout the period of the experiment, the pH values increased over time in the *Lemna minor* aquarium. Conversely, in the *Azolla pinnata* aquarium, the pH values decreased consistently, starting in the second week and showing the second highest pH detected over the various sampling times of 7.50 ± 0.1 , as compared to the lowest pH of 6.20 ± 0.01 recorded in the *Lemna minor* aquarium. Both of these pH values are permissible.

At the outset of Week 1, *Azolla* started with a pH of 7.5 ± 0.1 (above the upper limit of the desirable range), *Lemna minor* started at a pH of 6.5 (within the desired range), and the Control had a pH of 7.15 ± 0.01 (also within the acceptable range). In each of the following weeks, there were variations in all of the aquariums which reflected changes in environmental factors and/or the metabolic processes of the organisms.

While recording data weekly, we discovered that variations in conductivity occurred. The electrical conductivity of *Azolla* ranged from 3.78 ± 0.2 ms/cm to 6.75 ± 0.2 ms/cm, with changes likely influenced by temperature variations of 20.4°C to 32°C and nutrient uptake. Conductivity for *Lemna minor* ranged from 3.15 ± 0.02 ms/cm to 6.95 ± 0.05 ms/cm, being affected by temperature changes of 27°C to 29.9°C and nutrients availability. The Control aquarium had a conductivity range from 3.89 ± 0.01 to 6.20 ± 0.2 ms/cm. While they generally followed similar trends to the planted aquariums, there were small differences that likely related to nutrient cycling and water management.

Salinity across all aquariums was likewise consistently within $5 \pm 0.2\text{‰}$, which is uniform for the study zone sat within 1-8‰. In spite of all aquariums showing stable temperatures and pH values consistent with the parameters established above, it should be noted that Aquarium A and Aquarium B had higher EC readings suggesting perhaps greater ion concentration or salinity could have affected plant growth, with Aquarium C as a control group having more stable conditions, providing a more balanced setting to grow the plants. Overall, while temperatures were generally consistent with FAO guidelines above during most of the duration of this study; some periods did exceed upper limits, despite being somewhat generally maintained within an acceptable range (FAO, 2020). Throughout the course of this experiment, although there were some temperature fluctuations in all aquariums, it can be concluded that temperature and time, although probably had a slight impact, did not significantly detrimentally affect the overall, stability in the aquariums life.

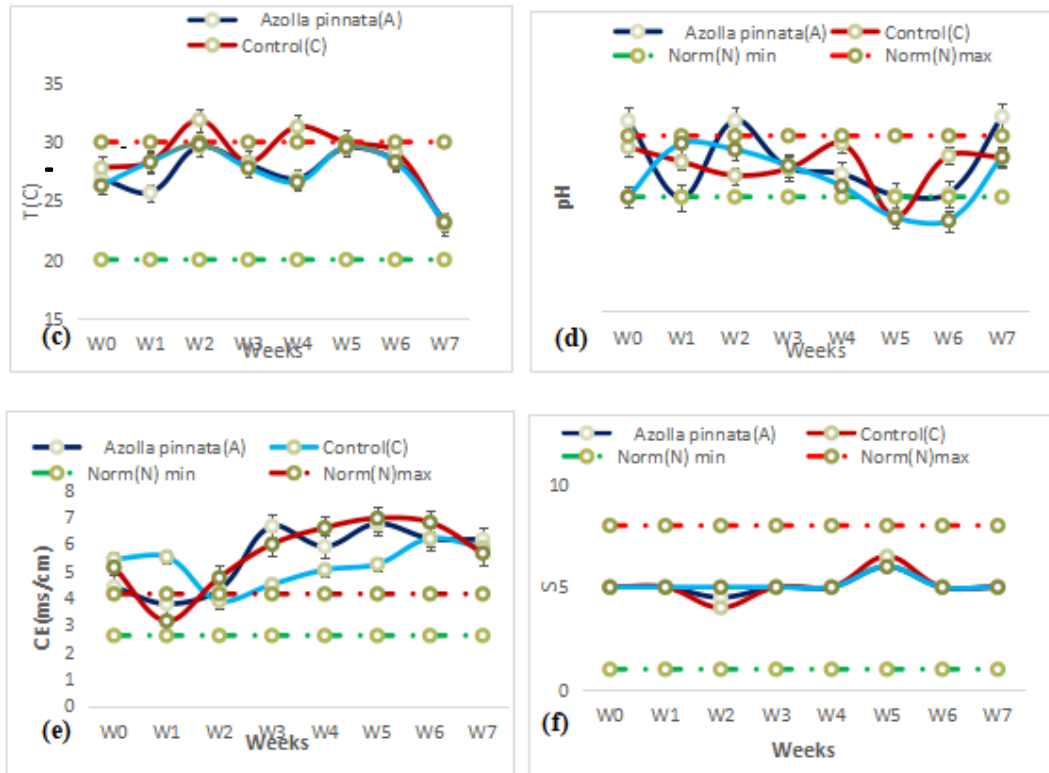


Figure 2. Depicts the changes in the aquariums: Over time (weeks), (c) Temperature, (d) pH, (e) Electrical conductivity and (f) salinity values (‰).

3.2. NITRITE (N-NO_3^-), PHOSPHATE (P-PO_4^{3-}), AMMONIUM (N-NH_4^+) AND NITRATES (N-NO_2^-):

Nitrogen compounds are essential for biological structures, as nitrogen comes from our food and the waste produced by tilapia. The transformation of nitrogen within a fish pond ecosystem is depicted in **Figure 3**. The main source of nitrogen entering the system is fish meal, which contains proteins that the fish metabolize. The breakdown of these proteins results in the excretion of ammonia ($\text{NH}_3/\text{NH}_4^+$), the most toxic form of nitrogenous waste. Through a process called nitrification, ammonia-oxidizing bacteria (AOB) first convert ammonia into nitrite (NO_2^-), and then nitrite-oxidizing bacteria (NOB) further oxidize nitrite into the less harmful nitrate (NO_3^-). This transformation is further supported by denitrification.

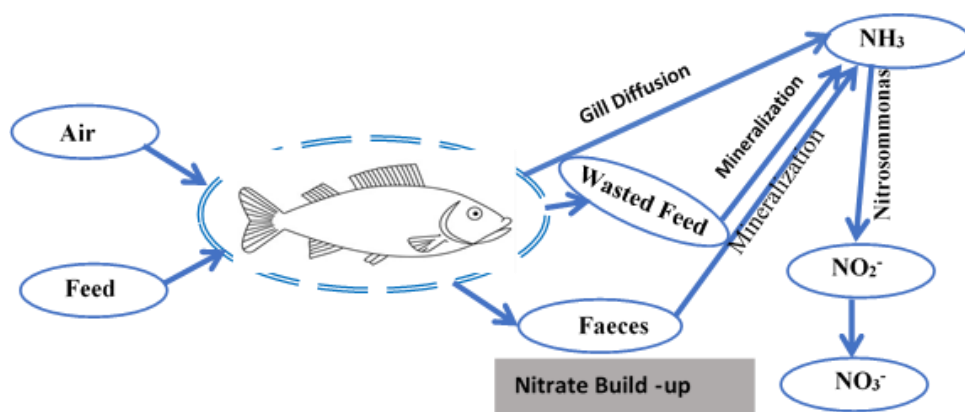


Figure.3: The Nitrogen Cycle in a Fish Pond: From Feed to Nitrogenous Products.

As can be observed from **Figure 4(i) and (i')**, it can be seen that the removal efficiency of ammonium ions was greater in the duckweed pond than in the Azolla tank. The highest removal efficiency seen was at 68.48% for the Lemna minor aquarium, while Azolla had a maximum of 71.39%. There is also an obvious lower efficiency similar to the control, so it couldn't remove too much ammonium. The efficiency difference for phosphate ion removal from fish water between Azolla pinnata and duckweed (Lemna minor). In the first few weeks, Lemna minor had the highest purification rate at 89.77%, while Azolla had the slowest purification process at 42.10%.

the duckweed pond had a higher ammonium ion removal efficiency than the Azolla tank. Azolla had a maximum removal efficiency of 71.39%, while Lemna minor aquariums had the highest removal efficiency of 68.48%. It was also unable to remove too much ammonium due to a noticeable decrease in efficiency, which is comparable to the control. **Figure 4(g) and (g')** illustrates the efficiency difference between Azolla pinnata and duckweed (Lemna minor) in removing phosphate ions from fish water. In the initial weeks, the purification rate for Lemna minor was the highest at 89.77%, while the purification process's reaction performance for Azolla was the slowest, reaching a maximum of 42.10%. Lemna minor showed better results because of its greater need for ammonium among nutrients, thus it can take up and clean ammonium from the water more effectively. Azolla gave more mixed results most probably because it does not rely on ammonium as much, hence lower yields of purification. This variability underscores the necessity for knowledge about the nutritional requirements of plant species when attempting to purify water. In **Figure 4(j) and (j')**, NO_3^- purification rate in the Azolla aquarium increases from week one to the last week. The duckweed aquarium has its best shot at purification in the earlier weeks falling 71% thereafter, meaning that duckweed is a fast absorber of nitrates through the process of purification.

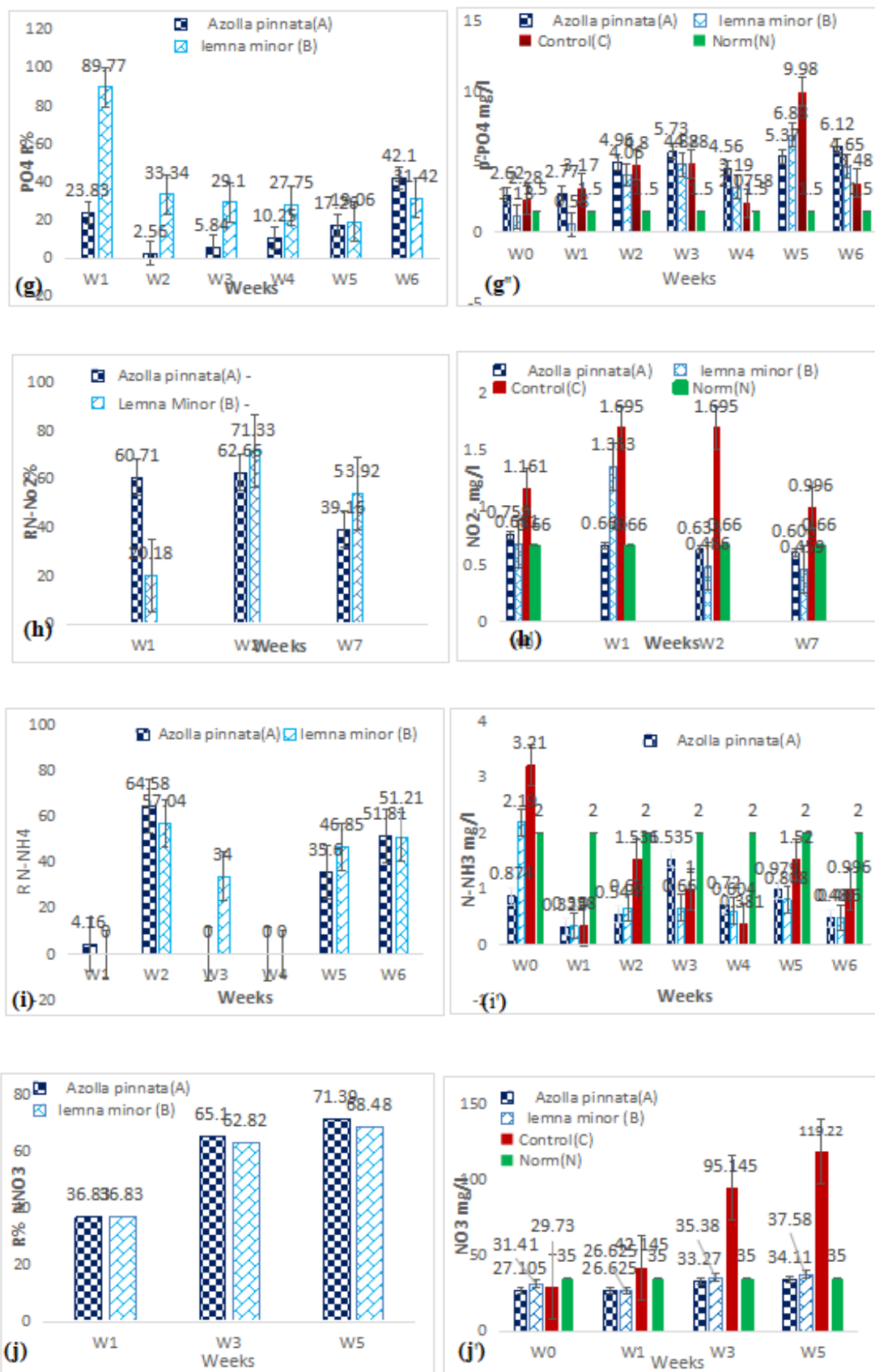


Figure 4: Shows how the aquarium's element levels have changed: (g,g') N-NO₃⁻, (h,h') P-PO₄³⁻, (i,i') N-NH₄⁺, (j,j') N-NO₂⁻

3.3. BIOLOGICAL ANALYSES (WEIGHT AND RATES)

Comparing the Azolla and Lemna minor aquariums to the control group aquarium, the experiment results show a significant difference in growth rates. The Azolla aquarium's weight increased from 380 ± 0.5 mg/g at the start of the experiment to 840 ± 0.3 mg/g at the end, increasing the Azolla's daily growth rate to 16.15%. The Lemna minor aquarium started out weighing 380 ± 0.2 mg/g and ended up weighing 888.5 ± 0.2 mg/g. This translated into a daily growth rate that was just 16.145% faster than Azolla's. Lemna minor and Azolla both showed a very significant degree of overall growth, with growth rates roughly equal. At the start, the control aquarium had 380 ± 0.11 mg/g and finished at a final weight of 857 ± 0.11 mg/g, which gave it a daily growth rate not incredibly lower than Azolla's of 16.06%. It showed the least growth due to the absence of any plants to help facilitate nutrient absorption and enhance the environment as plants would have provided. Both Azolla and Lemna minor achieved approximately equal growth rates, with the Lemna minor having a slightly better overall average, suggesting that plants have critical importance in amplifying the environment to support the ecology of aquatic organisms. From **Figure5** it appears that the growth of the fish across all three aquariums has remained incredibly close (15 to 16 grams) since the beginning and even throughout the day.

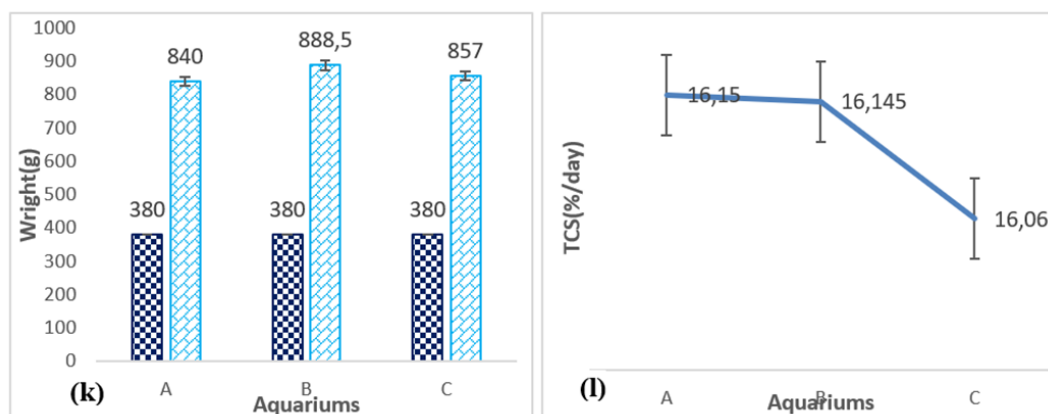


Figure 5: Shows the evolution of growth rate(k)and weight, (l) of red tilapia over the course of a single day.

In just three to four months, tilapia raised under intensive farming conditions can weigh between 150 and 200 grams [13], according to numerous studies. Factors influencing tilapia growth rate include temperature, feeding schedule, stocking density, and water quality. Ultimately, these constitute essential standards that aquaculture professionals must adhere to.

4.A COMPARATIVE REVIEW

Table 1 presents the results of various studies investigating the use of different macrophyte species for removing pollutants and nutrients from multiple types of wastewater, including stormwater, fish pond discharges, and aquaculture effluent. Research by **Nurul et al. (2020)**[14] highlights the

considerable benefits of employing cultured aquatic plant systems for treating wastewater from fish farms.

It was found that water hyacinth (*Eichhornia crassipes*) had the best removal efficiencies for phosphorus, up to 80%, while water lettuce (*Pistia stratiotes*) had the highest removal efficiency for nitrogen, at 89.5% removal. When it came to heavy metals, water hyacinth was the most effective at removing copper (90%), lead (88%), and zinc (92%).

One aspect of the studies that was very clear was the consistently high nutrient removal efficiency of duckweed (*Lemna minor*). In one study, Perniel *et al.* in (1998)[15] showed duckweed removed up to 95% total nitrogen and 80% total phosphorus from stormwater detention ponds.

Furthermore, the study by Ferdoushi *et al.*(2008)[16] study demonstrated that duckweed (*Lemna minor*) in combination with *Azolla pinnata* removed 74% total nitrogen and 69% total phosphate from the water in fish ponds. Collectively, it appears duckweed is an excellent vessel for nutrient uptake[17].

The use of duckweed (*Lemna minor*) combined with *Azolla* was evaluated in freshwater catfish ponds. Its application showed a remarkable improvement in the water quality parameters of ammonia concentration, total suspended solids (TSS), turbidity, and chemical oxygen demand (COD).

Table 1: Comparative Summary of Nutrient Removal Efficiency by Aquatic Macrophytes for Wastewater Treatment in Different Aquaculture Systems .

Authors	Wastewater	Macrophytes	% Removal		
			N-NO ₃ ⁻	P-PO ₄ ⁻	Other
Nurul <i>et al.</i> (2020)	Aquaculture wastewater	Centella asiatica, Ipomoea aquatica, Salvinia molesta, Eichhornia crassipes, Pistia stratiotes	90%	80%	Copper (90%), lead (88%), and Zinc (92%)
Perniel <i>et al.</i> (1998)	Stormwater detention ponds	Lemna minor	95%	80%	-
Ferdoushi <i>et al.</i> (2008)	Fish Ponds (Rohu , Catla, Mrigal , Thai sharpunti)	Lemna minor, Azolla pinnata	74%	69%	-
Yin <i>et al.</i> (2021)	Fish Ponds (Freshwater catfish)	Lemna, Azolla	-	-	81%N-NH ₄ , 75%TSS,88% Turbidity,71% COD
This work	Fish Ponds (Red Tilapia)	Lemna minor, Azolla pinnata	71%	90%	65%N-NH ₄

Azolla pinnata and *Lemna minor* (duckweed) have shown great potential for improving water quality in aquaculture ponds. They were effective at removing ammonium, phosphate, nitrite, and nitrate.

The study showed specifically that duckweed (*Lemna minor*) was more efficient for phosphate removal, where it demonstrated reductions of up to 90%, compared to Review. *Azolla* acquired a higher rate of nitrite removal from the water. Both plants maintained the experimental conditions suitable for the removal of nitrate (up to 71%) and ammonium (up to 65%), which represent the highest sources of difficulty reported by fish farmers.

5.CONCLUSION

The seven-week experiment, conducted from February 27 to May 7, aimed to demonstrate that the aquatic plants *Lemna minor* and *Azolla pinnata* could purify aquaculture water in tanks at a national centre for fisheries and aquaculture. This was achieved by reducing pollutant elements (N-NH₃, N-NO₂⁻, N-NO₃⁻, P-PO₄⁻³) and monitoring physical parameters such as temperature, pH, salinity, and electrical conductivity.

The results indicate that the concentrations of phosphorus, nitrate, and nitrite have increased while the concentration of ammonium has decreased. The purification efficiency data are as follows: PO₄⁻³ at 89.5%, NH₄⁺ at 51%, NO₃⁻ at 27%, and NO₂⁻ at 43.13% for the *Lemna minor* aquarium, and PO₄ at 17.25%, NH₄⁺ at 31.17%, NO₃⁻ at 62.52%, and NO₂⁻ at 44.38% for the *Azolla* aquarium.

These findings indicate that duckweed was more effective in purifying phosphorus and ammonium, while *Azolla* was more effective in purifying nitrites and nitrates. These aquatic plants have proven their effectiveness in purifying aquariums at a lower cost compared to other techniques used.

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