E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 76.37
(GIF) Impact Factor: 0.549
IJFAS 2023; 11(3): 35-39

www.fisheriesjournal.com
Received: 25-01-2023
Accepted: 09-03-2023

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Rhizofiltration potential of floating aquatic macrophytes (FAMs) in Nile Tilapia (*Oreochromis niloticus* L.) tanks

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DOI: https://doi.org/10.22271/fish.2023.v11.i3a.2805

Abstract
Currently additional sustainable ways to mitigate the degradation of water quality are being researched all over the world. Phytoremediation is one of the serious efforts towards sustainability. The main objective of this study was to assess the rhizofiltration potential of water lettuce (*Pistia stratiotes*), common duckweed (*Lemna minor*) and mosquito fern (*Azolla pinnata*) on the removal of total ammonia-nitrogen (TAN) in circular outdoor tanks. Physico-chemical water quality parameters such as dissolved oxygen (DO), temperature and pH were also monitored. It was observed that the mean initial TAN concentration in all treatments were statistically comparable. In the final TAN concentration, tank with *L. minor* had the highest TAN concentration which was significantly different to tanks with *P. stratiotes* and *A. pinnata*. Likewise, tanks with *L. minor* had the lowest removal efficiency. For the DO concentration, it was observed that DO in all treatments was depleted in the first one week of the study. However, on the ninth day of the study, DO started to rise. Tanks with *P. stratiotes* had shown the highest trend among treatments. Mean temperature and pH in all treatments were statistically comparable. For the relationship of root length and TAN removal efficiency of floating aquatic macrophytes, it was observed that there was a strong positive correlation. These results show that the utilization of these floating aquatic macrophytes as bioremediator in small-scale tilapia production are feasible. In addition, other physico-chemical water quality parameters such as phosphorus, total dissolved solids, alkalinity, hardness, nitrate, and nitrite can be conducted.

Keywords: Nile tilapia, water quality, floating aquatic macrophytes, phytoremediation

Introduction
Nitrogen-rich effluent from intensive land-based aquaculture may negatively affect the water quality and other environmental factors (Robinson *et al*., 2018) [14]. Water quality problems can often be as severe as those of water availability, but less attention has been paid to them, particularly in developing countries. Aquifer depletion caused by overuse is common because many countries lack sufficient water supply to meet demand. Moreover, the scarcity of water is accompanied by deterioration in the quality of available water due to heavy pollution load and environmental degradation (Srivastava *et al*., 2008) [14].
The treatment of organic waste remains one of the key sustainability challenges facing the growing global aquaculture industry (Robinson *et al*., 2018) [14]. Nutrient removal is essential for aquaculture for reuse of the water (Srivastava *et al*., 2008) [16]. Bioremediation – the biological treatment of waste streams and pollutants – is a widely established process, increasingly applied by a broad range of industries operating within varied environmental and ecological settings and constraints (Robinson *et al*., 2015) [13]. In general, bioremediation technologies are bacteria driven, with selective stimulation of the degrading activities of endogenous microbial populations, a fundamental concept underpinning the approach (Colleran 1997; Robinson *et al*., 2015) [7, 13].
Phytoremediation is a natural wastewater treatment system driven in large part by free energy such as sunlight and wind and has proven to be a financially smart investment in controlling pollutants (Nakphet *et al*., 2016) [11].
Overall, phytoremediation or bioremediation is a result of the following properties of the aquatic plants that are used: phytotransformation, rhizosphere bioremediation, phyto-stabilization, phytoextraction, or rhizofiltration (Schnoor, 1997) [15].

However, phytoremediation may require more time to achieve satisfactory results than a complex bio filter setup, and plant species potentially useable in phytoremediation processes have different pollutant removal efficiencies because plants have different growth rates and absorption characteristics (Mei *et al*. 2014; Peng *et al*. 2008) [10, 12]. Brix and Schierup (1989) [4] and Brix (1997) [3] pointed out that no research data were then available which was clearly able to demonstrate a significant difference in purification capacity between different macrophytes commonly used in constructed wetlands wastewater treatment systems and under identical hydraulic and design conditions. Leto *et al*. (2013) [9] later showed that not only the use but also the choice of plant species significantly influenced wastewater treatment processes with regards to all chemical, physical, and microbiological parameters. Hence, this study aimed to assess the rhizofiltration potential of floating aquatic macrophytes (FAMs) on the removal of Total Ammonia Nitrogen (TAN) in Nile tilapia tanks.

Materials and methods

Experimental Fish: One hundred eighty pieces (180) of Nile tilapia fingerlings size #24 with initial weight of 0.14±0.03g were used in this study. Fish were obtained from the Freshwater Aquaculture Center, Central Luzon State University (FAC-CLSU), Science City of Muñoz, Nueva Ecija. The FAC Selected Tilapia (FaST) strain was used as the strain of Nile tilapia in this study. The fish were placed in a tank and acclimated for 2 weeks.

Experimental Plant: Floating aquatic macrophytes (FAMs): water lettuce (*Pistia stratiotes*) (Treatment 1), Common duckweed (*Lemna minor*) (Treatment 2), and Mosquito fern (*Azolla pinnata*) (Treatment 3), were used in this study. Both *P. stratiotes* and *A. pinnata* were obtained from FAC-CLSU, while *L. minor* was sourced out from the Bureau of Fisheries and Aquatic Resources - National Freshwater Fisheries Technology Center, Science City of Muñoz, and Nueva Ecija. The FAMs were initially propagated in separate tanks to suffice the needed biomass of plants in the study.

Experimental Set-up: Nine (9) circular outdoor concrete tanks with an area of 0.32 m^3 were used in this study. The experiment followed a Completely Randomized Design (CRD) which was replicated thrice. The tanks were cleaned and dried for 24 hours. After drying, the tanks were filled with water until reaching 0.40 m water depth.

Experimental Procedure: Chicken manure was acquired from Farnacio Poultry, Brgy. Maligaya, Science City of Muñoz, Nueva Ecija, Philippines. Eighty (80) grams of chicken manure were applied per tank as per the suggested one ton per hectare fertilization rate by Freshwater Aquaculture Center. Another 80 g of chicken manure was applied after the day of initial fertilization in order to reach the TAN concentration required by the experiment. Upon reaching TAN concentration of ~2.40 mg/L (El-Sherif and El-Feyk, 2008) [8], 20 fish were stocked in each tank. After stocking, the experimental plants were placed covering 50% of the total surface area of the tank. Fish were fed twice a day at 10% of their body weight.

Monitoring of Physico-chemical Water Parameters: During the experimental period, physico-chemical water quality parameters such as dissolved oxygen (DO), temperature, pH and total ammonia nitrogen (TAN) were monitored. DO, temperature and pH were monitored using a multi-parameter. In the determination of TAN concentration from water, UV-Vis spectrophotometer that is capable of operating at 630 nanometers (nm) was used in the analysis. The following reagents were prepared: Oxidizing Solution, 20 mL of bleach was mixed with 80 mL of ammonia-free distilled water; Manganous Sulfate Solution, 50 mg of $MnSO_4*H_2O$ was dissolved in 100 mL of ammonia-free distilled water; Phenate Solution, in 100 mL of ammonia-free distilled water, 2.5 g of NaOH and 10 g of phenol were dissolved; and Standard Ammonium Chloride Solution, 0.3 mg/L of Nitrogen, 1.9079 g of NH_4Cl was dissolved in 500 mL ammonia-free distilled water to give 1,000 mg/L of total ammonia-nitrogen. With a volumetric pipet, 5 mL of the 1,000 mg/liter solution was transferred into a 500-mL volumetric flask and diluted to volume with ammonia-free distilled water to give a 10 mg/L solution of total ammonia-nitrogen. 15 mL of the 10 mg/L solution was then pipetted into a 500-mL volumetric flask and diluted to volume with ammonia-free distilled water to give a 0.3 mg/L solution of total ammonia-nitrogen. The 0.3 and 10 mg/L solutions were made fresh before the analysis.
For the TAN analysis, 25 to 50 mL of the water sample was filtered through Whatman No. 42, or equivalent, filter paper. 10 mL of the filtered sample was pipetted into a 50-ml beaker. One drop of $MnSO_4*H_2O$ solution, 0.5 ml of oxidizing solution, and 0.6 ml of phenate solution were then added to the solution. The solution was stirred and allowed for maximum color development. The solution was then transferred to a 1-cm cuvette. With the spectrophotometer at 630 nm, blank sample was read and set at 0.0 absorbance (100% transmittance). The absorbance of the standard and the samples was then read, respectively.

Statistical Analysis: The normality of data was assessed before proceeding to parametric testing. All statistical analyses were performed at the 0.05 probability level**.** Data were analyzed using One-way Analysis of Variance (ANOVA) and treatment means were compared using Least Significant Difference (LSD). The relationship of root length and TAN removal efficiency was analyzed using Pearson Correlation Coefficient. Data analyses were performed using SPSS version 20 for Windows.

Results and Discussions

Total Ammonia Nitrogen

Table 1 presents the initial and final concentration of TAN (mg/L) and removal efficiency (%) of floating aquatic macrophytes (FAMs) during the whole duration of the study. One-way Analysis of Variance revealed that the mean initial concentration of TAN in all treatments were statistically comparable ($p>0.05$). In the final concentration of TAN, Treatment 2 with 1.19±0.10 mg/L had the highest TAN which was significantly different to Treatment 1 and 3 with 0.28±0.02 mg/ L and 0.41±0.07 mg/L, respectively. The latter two treatments were not significantly different ($p>0.05$). For the removal efficiency of FAMs, Treatment 1 with 88.33±1.19 % had the highest removal efficiency followed by Treatment 3 with 82.97±2.32 %. T1 and T3 were statistically comparable ($p>0.05$). However, Treatment 2 had the lowest removal efficiency with 49.77±4.97 % which was significantly different ($p<0.05$) to T1 and T3.

Table 1: Mean total ammonia nitrogen concentration (mg/L) of water and mean removal efficiency of FAMs

Treatment	Mean (±SD)		
	Initial Concentration	Final Concentration	Removal Efficiency (%)
T1	2.43±0.06[a]	0.28±0.02[b]	88.33±1.19[b]
T2	2.44±0.18[a]	1.19±0.10[a]	49.77±4.97[a]
T3	2.40±1.00[a]	0.41±0.07[b]	82.97±2.32[b]

Note: Means in a column superscripted with different letters are significantly different at 5% ($p<0.05$)

In this study, water lettuce had the highest removal efficiency of TAN. According to Aoi and Hayashi (1996) [2], water lettuce has the capacity to reduce ammonium ions from the water as it utilizes NH_4–N prior to NO_3–N as nitrogen source and does not switch on the utilization of NO_3– N until NH_4–N gets consumed entirely. On the other hand, several literatures (Al-Nozaily *et al*., 2000; Cheng *et al*., 2002; El-Shafai *et al*., 2004) [1,5,6] reported the use of duckweed for water quality improvement and nutrient removal. However, in this study, duckweed performed inefficiently as compared to water lettuce and mosquito fern.

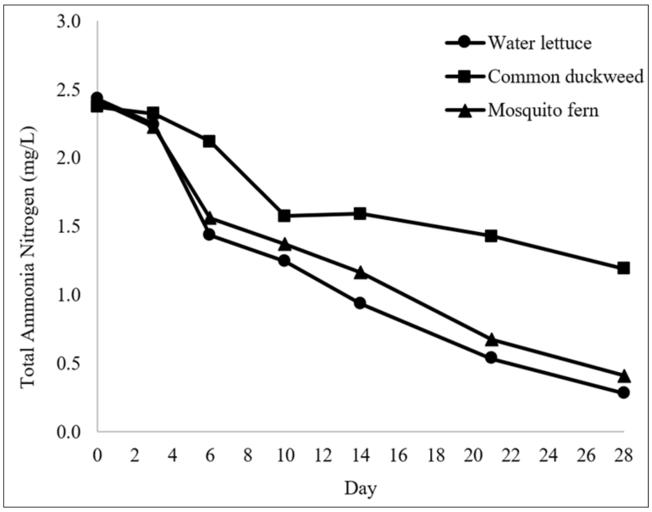

Fig 1: Total Ammonia Nitrogen of culture water

Figure 1 shows the trend of TAN concentration per treatment during the study. It shows that from the initial concentration of ~2.4 mg/L, all treatments exhibited decrease in TAN concentration after placing the floating aquatic macrophytes (FAMs) which were used as phytoremediation agents. T1 (*Pistia stratiotes*) had shown highest decrease in TAN concentration, followed by T3 (*Azolla pinnata*). However, T2 (*Lemna minor*) had the lowest decrease in TAN. *P. stratiotes* had a removal efficiency of 88.33±1.19 %, followed by *Azolla pinnata* with 82.97±2.32 % and *Lemna minor* with 49.77±4.97%.

Dissolved Oxygen

Table 2 presents the mean dissolved oxygen (DO) concentration (mg/L) of water during the whole duration of the study. One-way Analysis of Variance revealed that the mean DO concentration in Treatment 1 (0.99±1.06 mg/L) was significantly different ($p<0.05$) than Treatment 2 (0.58±0.62 mg/L) and Treatment 3 (0.67±0.68 mg/L). However, the mean DO concentration in the latter two treatments were statistically comparable ($p>0.05$).

Table 2: Mean dissolved oxygen concentration (mg/L) of culture water

Treatment	Mean (±SD)
T1	0.99±1.06[a]
T2	0.58±0.62[b]
T3	0.67±0.68[b]

Note: Means in a column superscripted with different letters are significantly different at 5% ($p<0.05$)

Figure 2 shows the trend of dissolved oxygen (DO) per treatment. It was observed that DO was depleted in the first one week of the study. However, on the ninth day of the study, DO started to rise. T1 had shown the highest trend among treatments. This could be attributed to increased biological oxygen demand (BOD) in the system because of the organic matter introduced to the experimental units which was used to increase the TAN concentration of the water. According to Huddleston *et al.* (2000), BOD and ammonia are directly proportional which signifies that as the ammonia increases, BOD also increases. Thus, in this study, depleted DO was observed because of increased BOD.

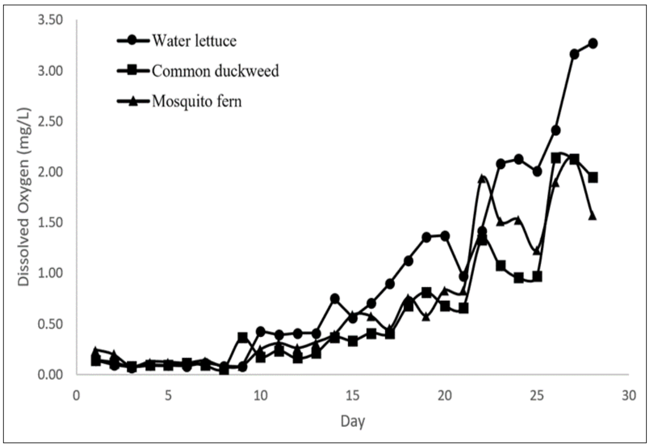

Fig 2: Dissolved oxygen (mg/L) of culture water

Temperature

Table 3 presents the mean temperature (°C) of water during the whole duration of the study. One-way Analysis of Variance revealed that the mean temperature in all treatments (T_1= 26.10±1.81; T_2= 26.29±1.80; T_3= 26.10±1.80) were not significantly different ($p>0.05$).

Table 3: Mean temperature (°C) of culture water

Treatment	Mean (±SD)
T1	26.10±1.81[a]
T2	26.29±1.80[a]
T3	26.10±1.80[a]

Note: Means in a column superscripted with different letters are significantly different at 5% ($p<0.05$)

Figure 3 shows the trend of temperature (°C) per treatment during the study. It was observed that temperature was somehow constant per treatment. However, it was observed that T2 had higher mean temperature than T1 and T2 both in the morning and afternoon.

Factors affecting this result may be attributed to the size of the floating aquatic macrophytes. Water lettuce and mosquito fern are larger than common duckweed when it comes to leaf blade size. Also, the leaves of water lettuce and mosquito fern were protruding upwards whereas the leaves of the common duckweed were just lying flat in the water surface.

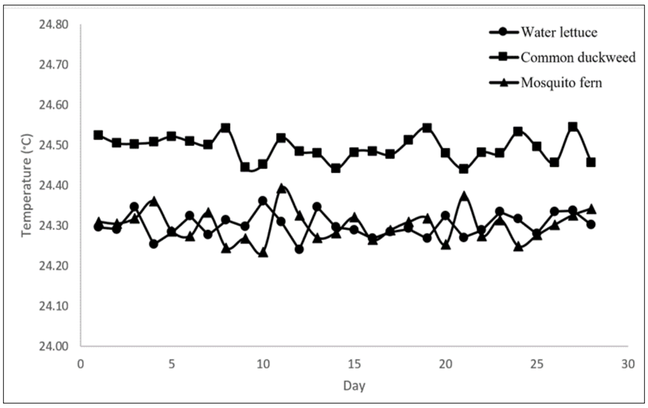

Fig 3: Temperature (°C) of culture water

pH

Table 4 presents the mean pH of water during the whole duration of the study. One-way Analysis of Variance revealed that the mean pH in all treatments (T_1= 8.01±0.28; T_2= 8.01±0.29; T_3= 7.99±0.32) were not significantly different (p>0.05).

Table 4: Mean pH of culture water

Treatment	Mean (±SD)
T_1	8.01±0.28^a
T_2	8.01±0.29^a
T_3	7.99±0.32^a

Note: Means in a column superscripted with different letters are significantly different at 5% (p<0.05)

Figure 4 shows the trend of pH of water per treatment during the study. It was observed that pH readings were constant, lying roughly between 7.70 to 8.40.

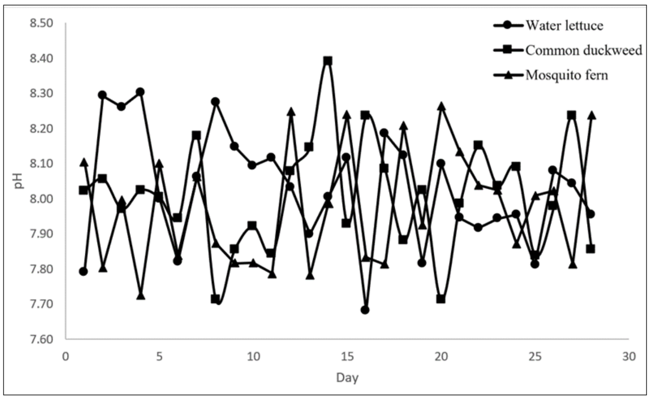

Fig 4: pH of culture water

Relationship of root length and TAN removal efficiency

Figure 5 shows the relationship of root length (cm) and TAN removal efficiency (%) of floating aquatic macrophytes (FAMs). It was observed that there was a significant strong uphill linear relationship (r= 0.903, p<0.01) between root length and TAN removal efficiency of FAMs. This signifies that as the root length increases, TAN removal efficiency also increases. This can be attributed to the increment of surface area as the root length increases for the absorption of excessive nutrients in the water. However, root density may also affect the result of this study since *Pistia stratiotes* and *Azolla pinnata* have denser root system than that of *Lemna minor*.

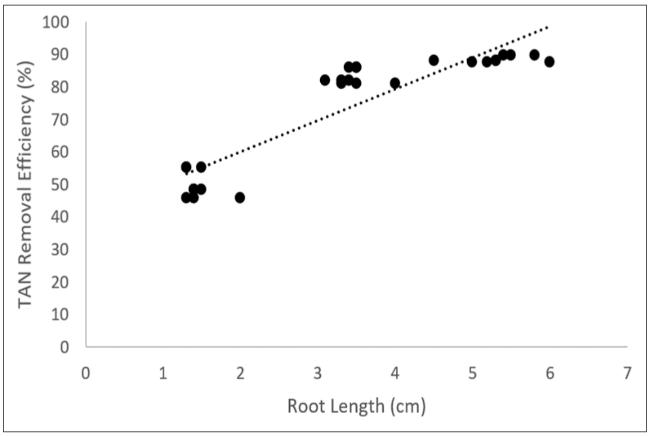

Fig 5: Relationship of TAN removal efficiency and root length

Conclusion

The study concluded that water lettuce and mosquito fern performed better than duckweed in the removal of TAN in the water. Water lettuce performed better than the two FAMs in terms of improvement of dissolved oxygen concentration. Further, fish in the tanks treated with water lettuce had the highest survival rate. Therefore, water lettuce has more potential for rhizofiltration than mosquito fern and duckweed.

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