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Assessment of Water Quality and Nutrient Uptake of Azolla in Different Fertilizers

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Abstract: The improvement of water quality through aquatic plants' nutrient uptake is one of the crucial elements in achieving a sustainable ecosystem. Water quality is affected by a variety of variables, including dissolved oxygen concentrations, nutrient overabundance, bacterial populations, salt content, and the number of suspended particles in the water. This experiment was performed to evaluate the biological and biochemical characteristics of azolla using various fertilizers (compost food waste, cow dung, liquid fertilizer, mushroom block fertilizer). It also examined the ability and effectiveness of aquatic macrophytes, *Azolla pinnata* to absorb amounts of nutrients including phosphate, potassium, and nitrogen ammonia in a nutrient-rich medium. The water quality during the cultivation period, including its turbidity, pH, and the temperature was monitored. The biological and biochemical characteristics of azolla have been assessed in a variety of fertilizers, including cow dung, liquid fertilizer, mushroom block fertilizer, and compost food waste, at various concentrations of 5g, 10g, and 15g. The study findings indicated that the most suitable fertilizer for azolla growth is cow dung fertilizer and the least suitable fertilizer is liquid fertilizer. The rapid growth of azolla between days 15 to 25 were observed for the cow dung fertilizer at all concentration as compared to control and other fertilizers. Water quality for each parameter increase over time. The ability of azolla to absorb nutrients shows great potential for the phosphorus element when compared to potassium and nitrogen uptake. To reduce the amount of excessive phosphorus and potassium in water, azolla is a great candidate for eco-friendly phytoremediation.

Keywords: Aquatic plants, water quality, nutrient uptake, fertilizer, *Azolla pinnata*



1. Introduction

The world's water quality has been significantly impacted by population increase, unplanned urbanization, rapid industrialization, various human activities, and the careless exploitation of natural water resources [1]. Due to a rise in agricultural production, fertilizer runoff is one of the main causes of poor water quality. Agricultural runoff contains a complex variety of pollutants, such as nitrates, ammonium, phosphorus compounds, heavy metals, and permanent organic pollutants [2]. Agriculture-related non-point source pollution has long been regarded as a significant element influencing the degree of eutrophication [3]. According to [4], in order to achieve sustainable development and to ensure the long-term health, well-being, and prosperity of people living in the Danube River Basin, it is crucial to conserve and improve the water quality and environment of the basin for the healthy life

There are numerous modern and conventional methods for improving water quality. The most environmentally benign method for phytoremediation is the exploitation of aquatic plants. The mechanism includes the uptake of contaminants by roots, accumulation in bodily tissues, decomposition, and transformation of pollutants into less hazardous forms [5]. According to [6] the ability of aquatic plants to absorb, store, and collect excess water nutrients may be a crucial element in reducing stress and enhancing the management of wetlands. For example, the high water P removal efficiency (above 95%) has been observed in two floating aquatic species, *Ludwigia peloides* and *Hydrocharis dubia* [7]. A study by [8], observed that *Salvinia cucullata* and *Ipomoea aquatica* are suitable candidate species to be used in constructed wetland systems to absorb N from polluted water. According to [9] an ecosystem's biotic and abiotic components are deeply linked by energy and numerous exchanges, necessary to keep a constant ongoing balance.

Aquatic plants are also known to be responsible for nutrient uptake. They take up dissolved nutrients and store them as biomass, which is then used in subsequent processes like burial or grazing by the consumer, slowing the re-release of inorganic nutrients into the environment [10]. A study by [11], demonstrated that compared to consistently nutrient-rich invasive alien aquatic plants (IAAP) ecosystem, aquatic macrophytes are more responsive to nutrient inputs in unaffected areas. Therefore, it may be possible to use *Lemna minor* and *Azolla pinnata*, two aquatic plants with high protein content to remove Se and Zn from wastewater while also providing dietary proteins and fertilizers that are enriched with micronutrients [12].

Azolla pinnata has been known to be one of the most important sources of sustainable animal feed due to its high protein source, rich in secondary metabolites, and eco-friendly [13]. According to a study by [14], azolla cultures can produce significant quantities of protein that are used to enhance the diets of animals. Supplying fertilizer is one of the factors that should be taken into account to promote the growth of azolla. The application of fertilizer to increase plant biomass is closely related to nutrient uptake. According to [15], up to 60 days, when an increase in uptake capacity was noticed, fertilization treatments indicated an increase in nutrient uptake for all components in King grass.

The primary goal of this research is to evaluate the biological and biochemical characteristics of azolla using various fertilizers (compost food waste, cow dung, liquid fertilizer, mushroom block fertilizer). In this experiment, we examined how efficient the uptake of nitrogen, phosphorus, and potassium components by aquatic plants *A. pinnata*. It is also important to identify the type of fertilizer that can influence the growth of azolla.

2. Methods

2.1 Experimental setup

The experiments were conducted in a greenhouse located at Eco-Pro Training Centre, Kebun Abi, Perlis from 1st May 2022 until 31st July 2022. The average day and night temperatures were 33 °C and 25 °C respectively. A total of thirteen rectangular plastic containers, with a dimension of 35cm width, 45cm length, and 12cm depth were used for the experiments with 10L of nutrient-rich medium with a total of 130g of azolla with 10g as in Figure 1. The containers were divided into four different treatments: (i) commercial liquid fertilizer (LF), (ii) cow dung fertilizer (CD), (iii) compost food waste fertilizer (C),

(iv) mushroom block fertilizer (M) ranging from 5 - 15g and (v) control, no fertilizer (B) as in Table 2. Each group had duplicate containers. Before experiments, all containers were filled with 10g of paddy mud, and 10L of tap water was mixed as a medium for azolla cultivation. The water had been left for a week to eliminate chlorine. The initial water quality parameters such as temperature (T), pH, electrical conductivity (EC), total dissolved solids (TDS), Chemical Oxygen Demand (COD), Phosphate (P), Nitrogen (N), Potassium (K) and Turbidity (t) were recorded in Table 2. Nutrient-rich water samples were collected from each container at various intervals (0 days, 5 days, 10 days, 15 days, 20 days, 25 days, and 30 days) by taking 10ml samples in a sample bottle at different locations across the containers. The aliquots of each sample were filtered.

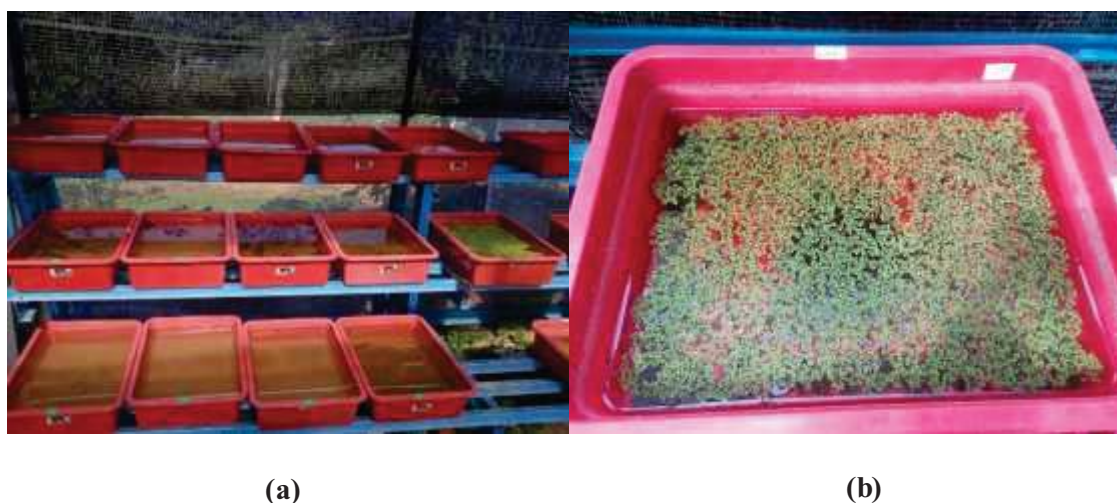


Figure 1. Experimental set up taken on first day (a) all containers (b) *Azolla pinnata*

Table 1. Various fertilizer treatments with different concentration

Treatments	Water with mud and cow dung fertilizer.	Water with mud and compost food waste fertilizer	Water with mud and liquid fertilizer	Water with mud and mushroom block fertilizer
Different concentration	5g (CD5) 10g (CD10) 15g (CD15)	5g (C5) 10g (C10) 15g (C15)	50L (LF5) 100L (LF10) 150L (LF15)	5g (MB5) 10g (MB10) 15g (MB15)

2.2 Chemical analysis

The NO₃⁻ and NH₃⁻ were determined using the Nessler method. The PO₄³⁻ used Acid Persulfate Digestion Method while K used the Tetraphenylborate method determined using the Hach Laboratory spectrophotometer (Hach, DR3900, USA). The other physical and chemical characteristics including T, EC, pH, salinity, and total dissolved solids (TDS) were obtained using a portable multimeter (Soonda, TDS-8B, China).

Table 2. The initial parameters for all water samples treatments

Treatment	T (°C)	pH	EC (µs/cm)	TDS (ppm)	COD (mg/L)	Phosphorus (mg/L)	Nitrogen ammonia (mg/L)	Potassium (mg/L)	Turbidity
LF5	30.3	7.7	204	79	124	0.58	0.12	0.1	1.435
LF10	30.3	7.8	194	96	169	0.23	0.03	0.1	3.655
LF15	30.4	7.6	196	98	20	0.11	0.1	0.2	3.02
C5	31.5	7.5	210	104	18	0.8	0.14	0.1	2.495
C10	31.1	7.5	218	108	188	1.05	0.43	0.2	4.28
C15	30.9	7.5	244	117	23	1.2	0.5	0.2	4.38
CD5	30.6	7.6	208	104	221	1.85	0.23	0.3	2.96
CD10	30.3	7.7	218	109	133	1.45	0.13	0.4	6.59
CD15	30.1	7.7	232	116	39	2.66	0.08	0.1	2.55
MB5	30.3	7.6	220	110	48	0.88	0.34	0.3	1.22
MB10	30.6	7.5	260	130	339	1.81	0.35	0.2	2.775
MB15	30.7	7.7	260	130	111	1.82	0.47	0.4	3.23
B	30.3	7.6	192	96	176	0.19	0.51	0.4	2.945

*Commercial Liquid fertilizer (LF), Compost fertilizer (C), Cow dung (CD), Mushroom block fertilizer (M), Blank (B).

3. Results and Discussion

3.1 Visual observation

In this study, 10 g of azolla was harvested in 13 different mediums. Initially, the color of the azolla shows that this plant is green in all parts, leaves range in width from 5 to 8 mm while the azolla root was measured between 7 and 9 millimeters in length. After 30 days, the result indicated that there was an increment in the characteristic observed in all treatments except for blank (B). This was confirmed as there was an increment in terms of average leaves' width and root's length from (5 - 8) mm to (4 - 10) mm for leaves while (7 - 9) mm to (10 - 23) mm for roots. Most of the azolla in the different fertilizers were able to grow and reproduce. However, the azolla that grows in different types of medium fertilizer also undergoes a change of color from green to dark green or green to yellow.

According to Figure 2, azolla growth in the liquid fertilizer is at its lowest point, covering only 450 cm² of the container's surface area towards the end of the sixth week. Thus, it is confirmed that liquid fertilizer is not suitable for azolla growth. It is also indicated that the most suitable fertilizer for azolla growth is cow dung fertilizer. The highest azolla growth was observed with cow dung fertilizer, where the area cover increased quickly by week five.

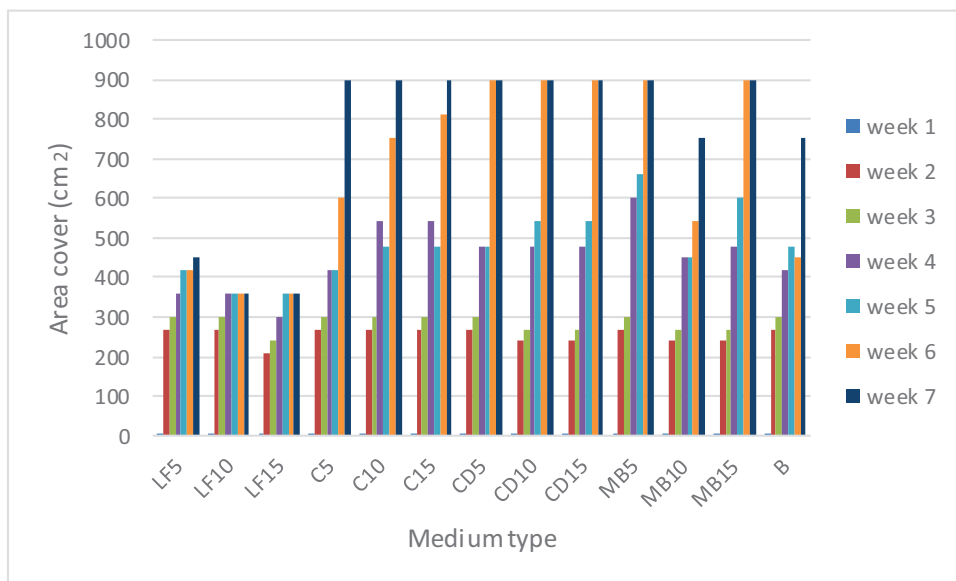


Figure 2. Azolla growth in different fertilizer

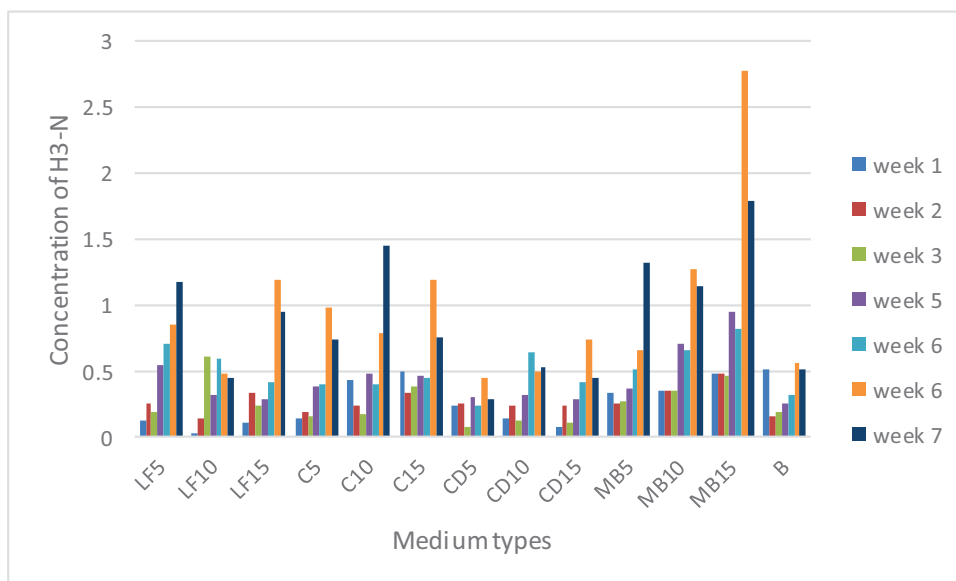


Figure 3. Concentration of nitrogen ammonia (NH₃-N) in different fertilizer

The concentration of nitrogen ammonia for each medium fertilizer was recorded in Figure 3. MB15 has a higher contains of nitrogen ammonia (2.78 mg/L) compared to other fertilizers because it is the most important source for mushroom growth cultivation. Nitrogen ammonia decreases over time due to the absorption of azolla during the growth rate. NH₃-N content in the fertilizer show increasing reading after day 25 might be due to the increasing nitrogen ammonia content in the azolla tissue. This is due to NH₃-N from the water fertilizer in the container being absorbed out of the tissue back into the water for stability purposes. According to [16], biological mechanisms in nutrient uptake and the presence of ammonia uptake were responsible for NH₃-N elimination. Additionally, it shows the build-up of high nitrification bacteria as a result of their proliferation. The inorganic nitrogen will be absorbed by plants

as NH_3 - or NH_4^+ ions with the help of bacteria. Furthermore, due to its role as nitrogen-fixing especially in paddy crops, it is known to be a biofertilizer. The nitrogen-fixing cyanobacterium *Anabaena azollae* is hosted as a symbiont in the dorsal leaves of the water fern azolla, which is regarded as a powerful N_2 fixer [17]. According to [18], *A. pinnata* act as a bio-stimulant for enhancing the length, and several pods in mungbean. This study suggests that *A. pinnata* is not effective at absorbing $\text{NH}_3\text{-N}$ from its environment.

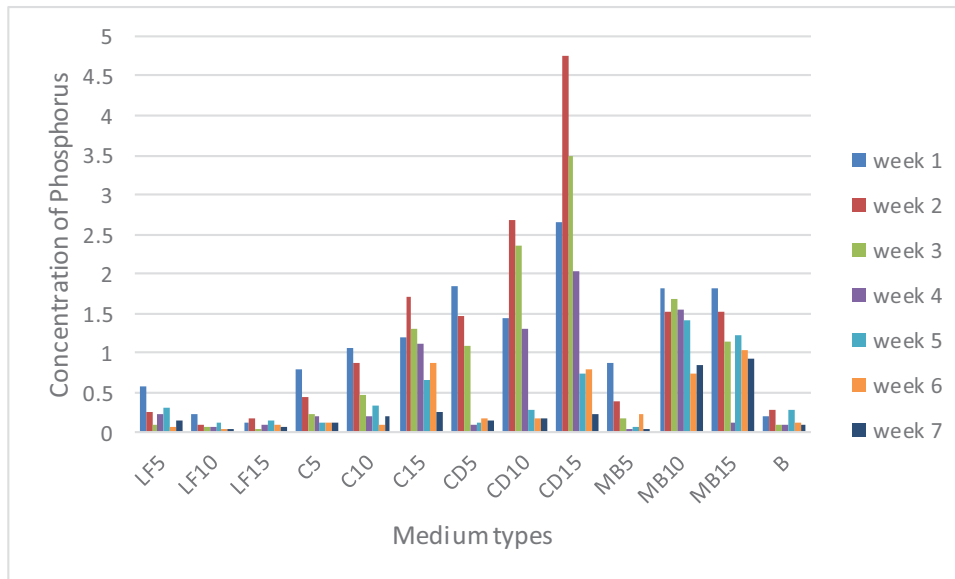


Figure 4. Concentration of phosphorus in different medium

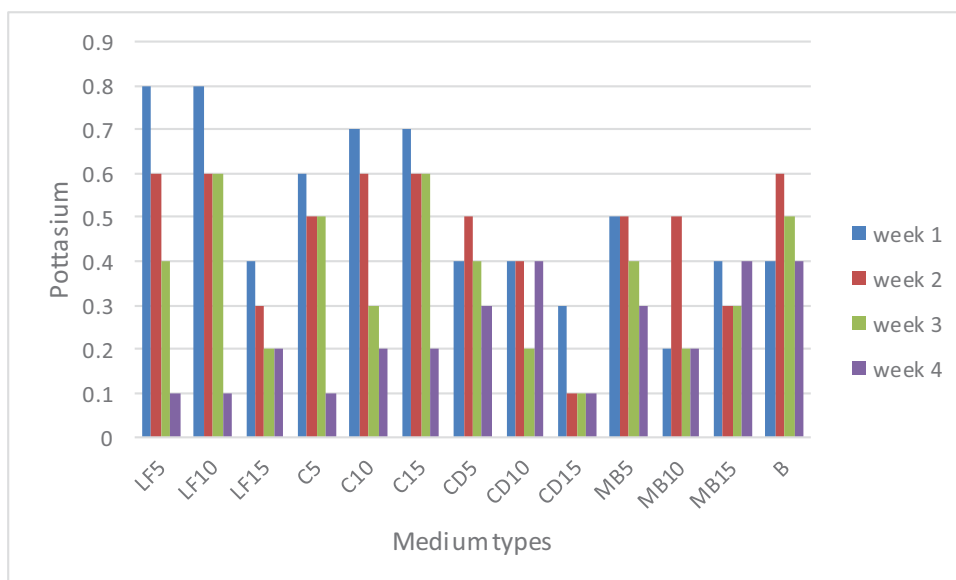


Figure 5. Concentration of potassium in different medium

Based on Figure 4, cow dung at all concentrations has higher phosphorus content compared to other fertilizers. Cow dung is rich in nutrients and includes a lot of organic materials. It contains 3 percent nitrogen, 2 percent phosphorus, and 1 percent potassium. Phosphorus nutrient uptake by azolla in cow dung increases with concentration. According to [19], the biomass of *A. filiculoides* grew 2.2 times in the 0.5 and 2 mol P l⁻¹ treatments, but increased 3.4 times in the 10, 50, and 100 mol P l⁻¹ treatments. Cow dung is the most suitable fertilizer for the azolla as compared to other fertilizers. In the natural environment, eutrophication often occurs due to the accumulation of phosphorus.

In Figure 5, potassium contents decrease over time in all medium types due to the absorption of azolla during growth except for mushroom block fertilizer which gives no reduction even at a high concentration of 15g. Potassium nutrient uptake by azolla was found to be higher in medium type 5g liquid fertilizer and 5g food waste compost at week 4, indicating that these aquatic plants will only absorb K at a particular level. This is similar to the finding by [20], by controlling photosynthesis, C and N metabolising enzyme activities, nitrate assimilation gene activities, and nitrate transport, a sufficient K supply of 6 mM can enhance photoassimilate transfer from leaves to roots and increase nitrogen use efficiency (NUE).

From Table 3, we could see that higher dosages of liquid fertilizer (LF15) do not provide a suitable environment for azolla to absorb phosphorus more efficiently, the same goes for higher dosages of mushroom block fertilizer (MB15). As we can observe azolla are excellent for phosphorus absorption. Almost all the removal efficiency for phosphorus is more than 50% except LF 15 with 45.45% and MB15 with 48.35%. Nutrient uptake for LF15, CD15, and MB10 is 100% after 30 days. The lowest nutrient removal for potassium is LF5 with only 3%.

Table 3. Nutrient removal efficiency by azolla

Treatment	Nitrogen Ammonia	Phosphorus	Potassium
LF5	8.75%	72.41%	3%
LF10	13%	86.96%	5%
LF15	8.5%	45.45%	100%
C5	4.21%	85%	4%
C10	2.35%	80.95%	10%
C15	33.33%	78.33%	4%
CD5	21.74%	92.43%	10%
CD10	3.08%	87.59%	50%
CD15	4.625%	91.73%	100%
MB5	2.88%	96.59%	25%
MB10	2.26%	53.59%	100%
MB15	2.81%	48.35%	25%
B	100%	52.63%	10%

Turbidity rose and decline throughout the trial. The eutrophication event that took place in those inorganic mediums was to blame for this. Numerous factors are known to affect azolla's growth. The average temperature for all the mediums for 30 days was around 25°C until 33°C. Based on the result obtained, it can be concluded that a greenhouse's temperature is suitable for the growth of azolla. According to [21], the findings showed that the maximum air temperature between 31.37 and 33.74°C, minimum temperature between 22.81 and 23.34°C, and a water temperature between 32.6 and 34.4°C was found to increase the biomass productivity of azolla, whereas air temperature above 35.2°C caused the azolla colonies to starve to death.

In a water culture experiment, *A. pinnata* was grown under natural light at three pH levels (4.5, 6.5, and 7.5). The increasing pH levels produced a reduction in azolla growth. Plant growth was highest at pH 4.5 and worst at pH 7.5, indicating that these plants thrive in acidic environments. For this project, the average pH value for all samples is between 7 and 9. The pH value shows that the water medium was slightly alkaline. This shows that the pH sample is slightly not suitable for azolla growth. According to [22], the absorption of ammonia and nitrate compounds was what caused the pH of water containing organic fertilizer to decrease because of the high quantity of nutrients that caused the algal bloom, the pH of water containing inorganic fertilizer increased. This is what makes the pH value fluctuate increasing and decreasing every day as the result of the reaction of eutrophication.

4. Conclusion

Azolla shows greater absorption of phosphorus at all concentrations. For potassium, the uptake efficiency depends on the appropriate concentration. Azolla is a great nitrogen provider, not a nitrogen remover. The most suitable fertilizer to use in azolla growth is cow dung fertilizer. The growth rate for each dosage of cow dung fertilizer (5g, 10g, and 15g) shows a fast growth rate within days 15 to 25. Water quality for each parameter increase over time. Phosphorus shortage, insect/snail damage, high temperature, harsh sunshine, or pesticide usage are all common causes of azolla's slow growth.

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