

Phytoremediation of Domestic Wastewater Using Aquatic Plants and Their Characterization: A Sustainable Approach for Wastewater Treatment

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Abstract: Water pollution caused by domestic wastewater discharge poses significant environmental challenges, necessitating sustainable and efficient treatment solutions. This study explores the potential of aquatic plants, specifically *Azolla microphylla* and *Lemna minor* for the phytoremediation of domestic wastewater. The removal efficiency of these plants was evaluated based on various factors including plant type, duration, weight and solution pH with a focus on the removal of both organic and inorganic compounds, such as ammoniacal nitrogen, total phosphate, color, and turbidity. The findings demonstrated the effectiveness of both species in removing these pollutants, with *Lemna minor* exhibiting superior performance. The highest removal efficiencies for ammoniacal nitrogen, color, and turbidity were 82.0%, 74.3%, and 77.4%, respectively, achieved with 7g of *Lemna minor* in 300 ml of domestic wastewater at natural solution pH. Pre- and post-phytoremediation characterization was conducted using X-ray diffraction (XRD), thermogravimetric analysis (TGA), and scanning electron microscopy (SEM) incorporated with energy-dispersive X-ray spectroscopy (EDX) to analyze the structural and compositional changes in the plant biomass. This study highlights the potential of *Azolla microphylla* and *Lemna minor* as natural phytoremediators for wastewater treatment, contributing to sustainable waste management.

Keywords: *Azolla microphylla*; Domestic wastewater; *Lemna minor*; Phytoremediation

1. Introduction

Over the past decade, the water crisis has emerged as a critical concern in Malaysia, particularly in the state of Selangor. It is approximately about 8.32 million of citizens staying in Selangor and Federal Territories of Kuala Lumpur, which accounting for about 26% population in Malaysia. The estimated gross domestic production per capita in Selangor is \$43,625 (Taweesan et al., 2023). Approximately 10% of the urban population is served by the public sewerage system in Kuala Lumpur, while the majority relies on non-sewered sanitation systems. The effluents from these systems are directly discharged into open drains or nearby water bodies (Taweesan et al., 2023). Meanwhile, domestic wastewater from rural areas does not undergo any treatment before releasing back into the environment, resulting in significant environmental harm. The composition of domestic wastewater can be divided into three main categories: black water, yellow water, and grey water. The wastewater that contains pathogens can be categorized as black water, while urine and wastewater rich in nutrients can be categorized as yellow water. Meanwhile, the wastewater contains cleaning agents that can be categorized as grey water. In

developing countries, critical ecological issues including compromised water quality and biodiversity loss were attributed to the untreated wastewater as the research found that 90% of domestic wastewater was discharged without a proper treatment (Werkneh & Gebru, 2023).

According to Indah Water Konsortium (IWK), majority of sewage treatment plants in the country utilize primary treatment systems such as septic tanks and Imhoff tanks followed by oxidation ponds with low-cost operating requirements in the secondary stage (Ariffin & Sulaiman, 2015). No tertiary systems are in place to remove excessive nutrients such as nitrogen and phosphate, thereby, the discharge sewage fail to meet regulatory effluent standards due to these limitations in treatment processes. This has created an urgent need to develop solutions for the safe management of domestic wastewater to achieve the targets outlined in Sustainable Development Goal No. 6 (SDG6), as highlighted in the WHO/UNICEF report (Unicef, 2023), which focuses on water, sanitation, and hygiene. The targets aim to improve the conditions of unsafely managed sanitation by 2030. If domestic wastewater is not properly treated before being discharged into natural water bodies, it can contribute to water eutrophication, exacerbating the problem (Al-dhawi et al., 2023). In many countries, including urbanized areas in Southeast Asia, only 20% wastewater treatment is considered safely managed. Alarmingly, approximately 2.8 billion population still rely on low-standard sanitation facilities, leading to significant negative impacts and the spread of fecal pathogen infections.

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Consequently, the integration of municipal domestic wastewater treatment with holistic resource utilization has emerged as an urgent concern that requires immediate attention (Wang et al., 2022).

Phytoremediation techniques have been employed in the treatment of weak wastewater to reduce the organic substances in the wastewater and slow down eutrophication. This emerging approach in wastewater treatment offers a promising and sustainable alternative by harnessing the natural capabilities of aquatic plants to remediate polluted water. Phytoremediation offers numerous benefits such as minimal costs and energy needs, straightforward operation and maintenance, ecological sustainability, and reliance on natural processes (Mustafa & Hayder, 2021; Tan et al., 2023). Recent research has revealed that specific species of aquatic macrophytes can tolerate stressful conditions and high concentrated pollutants in the water sources (Ansari et al., 2020). Floating aquatic plants, such as duckweeds species are commonly utilized in wastewater treatment since duckweeds are cost-effectiveness and ease to access. Duckweeds belong to a small family of aquatic floating plants or macrophytes, comprising 37 species distributed worldwide (Appenroth et al., 2018). Different species display varying patterns of growth and development even when grown in the similar medium. Duckweeds are known to be among the fastest-growing aquatic plants globally, with short doubling times (1.24 days) on nutrient-rich waters (Coughlan et al., 2022). Under favourable growth conditions, they can rapidly cover the entire water surface within a few days. Under suitable environmental conditions, the yearly production of duckweed has been documented to be 55 tons per hectare of dry biomass (Yang et al., 2021). Meanwhile, the *Azolla* plants have a rapid growth rate with the doubling time of 3 to 10 days (Thiruvengkatachari et al., 2021). This characteristic is attributed to its adaptability to different aquatic environments, making it another potential aquatic plant for phytoremediation. *Azolla microphylla* exhibits nitrogen-fixing ability due to symbiotic association with cyanobacteria through its endosymbiont, enabling growth with minimal nutrient requirements. It has been estimated that *Azolla* spp. can fix about 1.1 kg nitrogen·ha⁻¹·day⁻¹ and has been used as green manure as fertiliser to enhance rice production by about 20% (Prasad et al., 2016).

While there has been significant research conducted related to phytoremediation, limited attention has been given to the phytoremediation of domestic wastewater with the use of *Azolla microphylla* and duckweed species for reducing pollutants in domestic wastewater. Therefore, this study seeks to evaluate the effectiveness of both plant species in removing a wide range of contaminants from domestic wastewater, including chemical oxygen demand (COD), ammonia, nitrate, nitrite, and total phosphate. The effects of various operating parameters, such as plant type, treatment duration, plant weight, and solution pH, on phytoremediation efficiency were further discussed.

2. Methodology

2.1 Sampling of Wastewater

The raw domestic wastewater was collected from Kajang Utama Regional Sewage Treatment Plant HLT010 (2.972° N,

101.792° E). The wastewater samples were kept at 4 °C before being used in the experiment. Sodium hydroxide pellet (99.9%) and hydrochloric acid (37%) were purchased from Sigma-Aldrich and used as received without further purification.

2.2 Preparation of Accumulator Plants

The preparation of floating macrophytes followed the methodology described by Wibowo *et al.* (2023). The plants used in the experiment, including *Azolla microphylla* and *Lemna minor*, were purchased from the marketplace as free-floating specimens. Prior to experimentation, the roots of plants were cleaned under the running tap water to remove the attached soil and dirt. Then, the plants were placed in a basin of tap water and allowed to acclimatize for a week prior to the start of the sampling study. The tap water in the basin was changed weekly to maintain healthy growth of macrophytes and part of the biomass was removed to ensure enough space for further growth. Any dead leaves were removed prior to the experiment.

2.3 Characterization of Accumulator Plant

The plant morphology and elemental analysis for *Azolla microphylla* and *Lemna minor* were analysed by a Hitachi S-3400 scanning electron microscope with energy dispersive X-ray (EDX). The crystalline structure of the plants was determined via X-ray diffraction (XRD) using a Shimadzu XRD-6000. Additionally, thermogravimetric analysis (TGA) was conducted with a Perkin Elmer STA8000 to evaluate the thermal behavior of the plants.

2.4 Analysis of Wastewater

The physicochemical characteristics of domestic wastewater were determined at the beginning (raw) and monitored daily throughout the treatment period. During phytoremediation of domestic wastewater, various physicochemical conditions were observed including pH, turbidity, ammoniacal nitrogen, total phosphate, colour, and chemical oxygen demand (COD) using colorimetric methods. Prior to the liquid sample analysis, all wastewater samples before and after phytoremediation were filtered through a 0.45-micron filter to remove suspended solids, except for the turbidity analysis. The measurements were taken in triplicate, and the average removal efficiency was calculated using Equation (1), as described by Wibowo et al. (2023).

$$\text{Removal efficiency (\%)} = \frac{W_i - W_e}{W_i} \times 10 \quad (1)$$

where W_i = influent concentration and W_e = effluent concentration

The analytical methods for domestic wastewater was adopted and modified from Goren et al. (2021) and Worku et al. (2024). The solution pH was performed using a pH meter (Sartorius- PB-10 Basic Bench Top pH Meter). Turbidity, ammoniacal nitrogen, total phosphate, colour, and chemical oxygen demand (COD) were measured using colorimetric method by a potable DR 890 colorimeter (HACH, Loveland, Co., USA). The unit of turbidity was measured in Formazin Attenuation Units (FAU), where 1 FAU = 1 NTU = 1 FTU and conducted in the range of 21 to 1000 FAU under program number 95. The total phosphate of the wastewater samples was assessed by the method PhosVer® 3 with acid persulfate digestion method using program number 82. The

analysis was conducted for a detectable range of 0.06 to 3.50 mg/L PO_4^{3-} were used. The ammoniacal nitrogen ($\text{NH}_3\text{-N}$) analysis of the wastewater samples was performed by AmVer™ Salicylate Test 'N Tube™ Method and detected at high range of 0.4 to 50.0 mg/L $\text{NH}_3\text{-N}$ under program number 67.

The COD analysis of the wastewater samples was performed using United States Environmental Protection Agency (USEPA) reactor digestion method (8000) under Program No. 16. The COD digestion reagent vials for detectable range of 3 to 150 mg/L low range COD were used and samples were preheated to 200 °C in a DRB 200 reactor. The true colour of filtered initial and final wastewater sample was performed according to platinum-cobalt standard method at 420 nm, under program number 19. The removal of suspended solids in the wastewater sample was conducted using 0.45 micron-filtration. The results followed the standard of 1 colour unit being equal to 1 mg/L platinum as chloroplatinate ion.

2.5 Parameter Studies during Phytoremediation

The phytoremediation study investigated the effects of different hyperaccumulator plants (*Azolla microphylla* and *Lemna minor*), remediation duration (1, 2, and 3 days), plant weights (5, 10, 15, 20, and 25 g), and solution pH (3, 5, 7, 9, and 11) on domestic wastewater. Each experiment used approximately 10 g (± 0.5 g) of plant in 300 ml of wastewater under ambient conditions and natural pH, with controls lacking plants. For pH adjustment, 0.1 M HCl and 0.1 M NaOH were used. Optimal parameters from each stage were carried forward to the rest of the parameter studies.

3. Results and discussion

3.1 Characterization of Accumulator Plant

Figure 1(a) shows the thermal behaviours of *Azolla microphylla* and *Lemna minor* over a broad temperature range (30 to 900 °C). The aquatic plants contain cell-wall components such as cellulose, hemicellulose, and lignin. It was observed that *Azolla microphylla* and *Lemna minor* underwent three phases of weight loss, below 200, 200-400 and above 400 °C. Osman et al. reported that the weight loss was attributed to the removal of moisture, decomposition of the hemicellulose followed by lignin pyrolysis decomposition above 600 °C. Miranda et al. (2016) reported that the weight lost happened at above 200 was denoted to the fractionation of carbohydrates, proteins and lipids. Meanwhile,

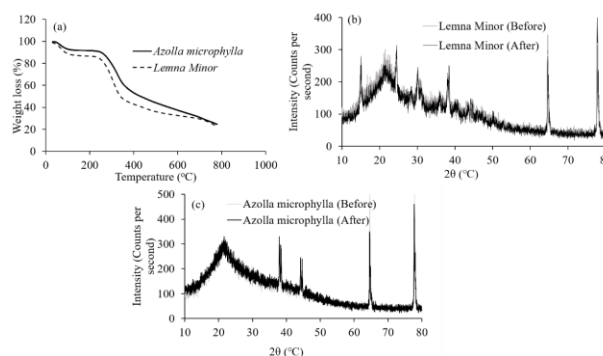


Figure 1. (a) Thermal analysis and XRD patterns of (b) *Lemna minor* (c) *Azolla microphylla* before and after phytoremediation.

the further weight loss was primarily due to the gasification of the carbonaceous residue, where highly non-volatile carbon compounds vaporized to produce carbon monoxide and carbon dioxide. Insignificant weight loss of *Azolla microphylla* and *Lemna minor* samples likely indicate the presence of minor quantities of lignin-like compounds.

The volatile content of the samples was accessed by calculating the percentage of weight lost between 120 and 650 °C (primary volatiles) and between 650 and 950 °C (secondary volatiles). The ash content (%) of the sample was determined by measuring the percentage of solids remaining after the combustion process. Based on the proximate analysis of the plant samples, it was found that the moisture loss from *Lemna minor* (11 %) was higher compared to *Azolla microphylla*, (7 %). The total volatiles for *Azolla microphylla* and *Lemna minor* were 96 % and 91 %, respectively. This indicated that *Azolla microphylla* was having higher secondary volatiles compared to *Lemna minor*. *Azolla* species have been reported to be rich in proteins, essential amino acids, vitamins A and B12, beta-carotene, growth-promoting intermediates, and minerals such as calcium, phosphorus, potassium, iron, copper, and magnesium (Muradov et al., 2014). The high nutrient content, substantial phosphorus (P) and nitrogen (N), coupled with its low lignin levels, renders *Azolla* an effective nutrient feed for livestock.

Figure 1(b) and Figure 1(c) present the XRD patterns of both *Lemna minor* and *Azolla microphylla* before and after phytoremediation. The patterns exhibit significant noise, underscoring the high degree of amorphousness characteristic of these aquatic plants. *Lemna minor* shows the XRD peaked at about 15.7 and 22.8°, corresponding to the (110) planes typical of cellulose and starch components. According to Azwar et al. (2022), duckweed species exhibits low crystallinity and drying could modifying the crystalline structure and resulting in a transformation from cellulose I to cellulose II. Meanwhile, *Azolla microphylla* demonstrated a broad peak observed between $2\theta = 10\text{--}30^\circ$ was corresponding to the (002) plane of amorphous carbon in lignocellulosic compounds (Puglia et al., 2024). This broad peak might be attributed to the lignocellulosic content, primarily composed of cellulose, hemicellulose, and lignin. In

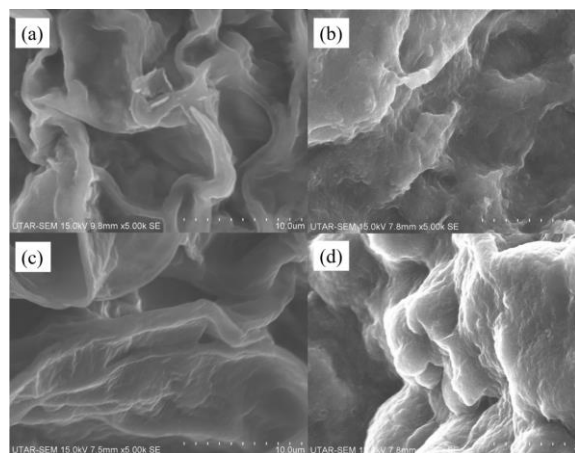


Figure 2. SEM Images of *Lemna minor* (a) before (b) after phytoremediation and *Azolla microphylla* (c) before (d) after phytoremediation.

natural lignocellulosic structures, cellulose exhibits both crystalline and amorphous regions, while hemicellulose and lignin are entirely amorphous. It is interesting to highlight that both *Lemna minor* and *Azolla microphylla* samples showed no observable changes in terms of XRD peak intensities at 2θ values before and after phytoremediation.

Figure 2(a) and Figure 2(b) demonstrate the surface morphology of *Lemna minor* before and after phytoremediation of domestic wastewater. It could be observed the fresh leaf structure of *Lemna minor* had deep pores and free space, which might provide more surface area and facilitate the biosorption process. The surface of *Azolla microphylla* also demonstrated a similar structure as *Lemna minor* as shown in Figure 2(c), exhibited free spaces and a large surface area, enhancing its capacity for nutrient biosorption in domestic wastewater. In addition, *Azolla microphylla* was also known for the small, hair-like structures on its leaves called trichomes which could provide a large surface area for absorption of nutrients and pollutants in water (Akhtar et al., 2021). However, shrinking on leaf structures could be observed on the plant after phytoremediation due to the drying process of leaf prior to SEM analysis as shown in Figure 2(b) and Figure 2(d).

Elemental analysis for the fresh plant samples and after phytoremediation were also conducted to identify the elemental compositions. Based on Table 1, the results showed that the increment of phosphorus (1 to 2 wt%) and nitrogen (2 to 3 wt%) by *Azolla microphylla* after phytoremediation of domestic wastewater, indicating nutrient uptake had occurred. A similar observation was observed with *Lemna minor*. The result showed increment of phosphorus (3 to 4 wt%) and nitrogen (2 to 5 wt%). The findings from this study indicated a higher composition of element nitrogen and phosphorus in the *Lemna minor* compared to *Azolla microphylla*. Previous research highlighted the high tolerance of *Lemna minor* to high levels of ammonium ions in the wastewater (Britto & Kronzucker, 2002). Furthermore, *Lemna minor* was reported to have the ability to transport nitrogen and effectively to accumulate large amount of nitrogen within its biomass (Guo et al., 2020). These reports correlated with the findings of this study where *Lemna minor* showed higher removal efficiency than *Azolla microphylla* in terms of ammoniacal nitrogen and total phosphorus.

Table 1: EDX analysis of *Azolla microphylla* and *Lemna minor* before and after phytoremediation

	Weight percentage (%)			
	C	O	N	P
<i>Lemna minor</i>				
Before	57.4	37.1	2.0	3.5
After	51.2	38.8	5.4	4.6
<i>Azolla microphylla</i>				
Before	69.7	29.1	2.5	2.7
After	53.5	39.0	3.6	3.9

3.2 Parameter Studies

Figure 3(a) shows the phytoremediation ability of domestic wastewater by using *Azolla microphylla* and *Lemna minor*. It was observed that *Lemna minor* performed better than *Azolla microphylla* in terms of removal efficiency of the domestic wastewater. The removal efficiency of domestic wastewater

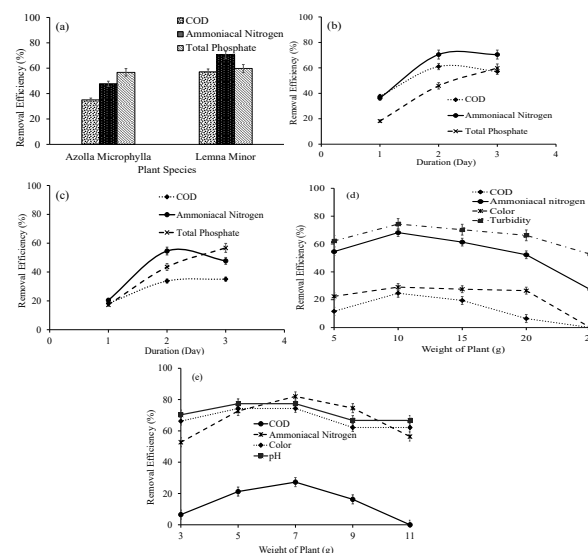


Figure 3. Effect of (a) plant species, duration on (b) *Lemna minor* (c) *Azolla microphylla* (d) plant weight of *Lemna minor* (e) solution pH during phytoremediation (Domestic wastewater volume = 300 ml, plant weight = 10 g ($\pm$ 0.5 g), ambient conditions, natural solution pH, treatment duration = 3 days).

using *Lemna minor* on the harvested day in terms of ammoniacal nitrogen, total phosphate and COD, were 70.5 %, 59.8 % and 57.1 %, respectively. The findings also indicated that *Lemna minor* possessed deeper spaces and larger surface area as compared to *Azolla microphylla*, which aids in the better absorption of nutrients (i.e. ammoniacal nitrogen and total phosphate) from the domestic wastewater.

The results were compared with findings from the literature review on the improvement of water quality. The findings were consistent with historical data, demonstrating the efficient absorption of nitrogen and phosphorus components in swine wastewater by *Azolla microphylla* and *Lemna minor* (Muradov et al., 2014). Another study compared the effectiveness of both plants in treating heavy metal wastewater. Polechońska et al. (2022) reported that *Lemna* species could uptake more K, Mg and Na than *Azolla* species, but the content of Cd, Cr, Cu, Fe, Ni, and Pb in *Azolla* species was higher than in *Lemna* species. The same authors proved that the metal accumulation index for *Lemna* species was higher than *Azolla* species. *Lemna* species, characterized by submerged reduced roots and fronds that are approximately 50% submerged, were advantageous for absorption, whereas only the bottom layer of *Azolla* species had direct contact with the contaminated water, which impacted its performance.

Figure 3 (b) and (c) show that phytoremediation treatment duration had a positive effect on the removal efficiency of

ammoniacal nitrogen, total phosphate, and COD for both *Lemna minor* and *Azolla microphylla*, respectively. At the beginning of the experiment, both plants could achieve similar removal efficiency. However, the findings revealed that *Lemna minor* outperformed *Azolla microphylla* in achieving a higher removal efficiency from day 2 onwards. On the second day of the experiment, *Lemna minor* exhibited a higher removal efficiency of domestic wastewater in terms of total phosphate, ammoniacal nitrogen, and COD, that were 45.9%, 70.5%, and 61%, respectively. On day 3, the COD removal efficiency by *Lemna minor* was reduced from 61% to 57.1%.

The fluctuation results on the COD removal result was aligned with the current observations. Nesan et al. (2020) reported that the increase in COD values may be due to several factors: the plant's adaptation ability under saline conditions, the induction of organic cell death and lysis by high salinity, resulting in the release of organic substances into the medium, or the excretion of organic substances by the plantlets in response to biotic stress caused by difference in salinity levels between the plants and surroundings. Furthermore, the changes in leaves colour from greenish to pale yellow indicated plant cell death due to high exposure of nitrogen ammonia in the wastewater which had a chlorination effect on the plants. Kumar & Deswal (2020) found that duckweed species showed better phosphorus removal efficiency (70.21% on day 13) and COD removal (62.19%) for rice mill wastewater as compared to *Salvinia* species (61.38% and 60%). They claimed that the negative growth rate of *Salvinia* starting on day 10 was caused by the presence of periphyton in the *salvinia* tub.

Lemna minor was chosen to investigate the optimal weight during phytoremediation of domestic wastewater and results are shown in Figure 3 (d). It was found that the optimal weight for the specific conditions was 10 g of *Lemna minor*. In this study, the highest removal efficiency of domestic wastewater using 10 g of *Lemna minor* in terms of ammoniacal nitrogen, COD, colour and turbidity were 68.2 %, 24.7 %, 29.1 % and 74.3 %, respectively. The increase in *Lemna minor* biomass from 5 g to 10 g resulted in enhanced removal efficiencies, attributed to the substantial increase in nutrient uptake capacity of the macrophyte. However, further increases in biomass beyond 20 g were observed could reduce phytoremediation efficiency. Several factors contributed to this decline, including nutrient depletion, plant overcrowding, and increased oxygen demand, all of which negatively impacted the phytoremediation capability of *Lemna minor*. Consequently, an optimal biomass of 10 g was selected for subsequent parameter studies.

The effect of solution pH of 3, 5, 7, 9 and 11 during phytoremediation of domestic wastewater using *Lemna minor* was evaluated, and the results are demonstrated in Fig. 3 (e). Consideration must be given to the effect of solution pH on plant growth during extended restoration processes to ensure stable development. It was observed the *Lemna minor* could bioaccumulate higher removal efficiency when the solution pH ranged from 5 to 7. The duckweed species requires a pH of 5–9, 60 mg/L of nitrogen and at least 1 mg/L of phosphorus for optimal growth (Ekperusi et al., 2019). Ekperusi et al. (2019) and Heitzman et al. (2024) reported that the optimal duckweeds growth rates in

laboratory happened in the range of 6.5–7.5. Meanwhile, the same authors pointed out that an adequate supply of nitrogen, phosphorus, potassium and other essential nutrients is required for optimal duckweed growth. Thus, most of the available form of nitrogen and phosphorous would be readily bioaccumulated by *Lemna minor* at this optimal condition.

Jones et al. (2023) reported that the ammonia (NH_3) proportion increases with rising water pH. NH_3 is more toxic to duckweed due to its lipid-solubility, which allows it to penetrate cell membranes, while the ionized form is less harmful. Thus, elevated water pH leads to the formation of ammonia in the solution, which can be toxic and prone to volatilization (Ekperusi et al., 2019). Acidifying the media to shift the $\text{NH}_4^+ : \text{NH}_3$ equilibrium allows duckweed to grow in higher concentrations of ammoniacal nitrogen. However, it was found that extremely acidic condition could lead to stress on the plant, which negatively impact plant's cell membrane permeability, reducing the photosynthesis of the plant and its growth rate. Furthermore, the solubility of phosphorus and nitrogen decreased at highly acidic conditions which reduces the uptake of these nutrients by the plant.

5. Conclusion

This study investigated the phytoremediation of domestic wastewater using *Lemna minor* and *Azolla microphylla*. The SEM images showed deep pores in both plants, indicating significant surface area for nutrient bioaccumulation. The EDX analysis confirmed higher nitrogen and phosphorus levels in the plants post-phytoremediation, with *Lemna minor* exhibiting greater nutrient uptake than *Azolla microphylla*. Several parameters were assessed, including plant type, duration, weight, and solution pH. *Lemna minor* outperformed *Azolla microphylla* in phytoremediation efficiency, achieving 45.9% total phosphate, 70.5% ammoniacal nitrogen, and 61.0% COD removal after three days. Factors such as nutrient depletion, plant overcrowding, and increased oxygen demand affected *Lemna minor*'s performance. Optimal conditions for *Lemna minor* were found at a pH of 5 to 7, with ammoniacal nitrogen removal efficiency ranging from 72.7% to 82.0% and COD removal from 21.3% to 27.3%. Extreme solution pH conditions (below pH 3 or above pH 9) could reduce removal efficiency due to plant stress and free ammonia toxicity. The best results were achieved at phytoremediation of domestic wastewater using 10 g of *Lemna minor* for about two days at a solution pH of 7. This research demonstrates the effectiveness of *Lemna minor* and *Azolla microphylla* in phytoremediation which contributing to sustainable wastewater treatment practice.

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