EnvironmentAsia
The international journal by the Thai Society of Higher Education Institutes on Environment

EnvironmentAsia 18 (Special Issue) (2025) 85-102
DOI 10.14456/ea.2025.25
ISSN 1906-1714; ONLINE ISSN: 2586-8861

Investigating *Azolla microphylla* in Domestic Wastewater Treatment and Its Potential for Biological Carbon Capturing

Nyein Nyein[1] and Chuleemas Boonthai Iwai[1,2*]

[1]Department of Soil Science and Environment, Faculty of Agriculture, Khon Kaen University, Thailand
[2] Integrated Land and Water Resource Management Research and Development Center in Northeast Thailand, Khon Kaen University, Thailand

*Corresponding author: chuleemas1@gmail.com

Abstract

The challenges of climate change, increased greenhouse gas emissions, water pollution, and environmental quality degradation have intensified the necessities for ecological sustainability, lower operating expenses, and nature-based solutions. The aquatic plant; Azolla is highly suggested to apply in bioremediation process as an ecological treatment method. The present study aimed to investigate the potential of *Azolla microphylla* in intergrading the phytoremediation of domestic wastewater treatment, biomass growth for biological carbon capturing, and its nutrient content for generating valuable by-products. The experiment was conducted by using a completely randomized design with four treatments. After the experimental period, the highest reduction of biological oxygen demand (BOD) 75.9% and chemical oxygen demand (COD) 72.9% were observed in domestic wastewater (DW). The highest nutrient removal of nitrate nitrogen (NO_3^-) 46.5% and orthophosphate (PO_4^{3-}) 62.5% was observed in DW. The reduction in the concentration of treatments were significant compared to controls at a significance level of $p < 0.05$. The maximum carbon sequestration rate of 1.36 g CO_2 per g of dry weight for Azolla was observed in DW. This study was conducted to optimize the potential of *A. microphylla* in promoting the Bio-Circular-Green economy model, that aims to utilize renewable biological resources and transform into valuable by-products, to contribute in water pollution management, to eliminate atmospheric carbon dioxide, to keep a balance among the economy, society, and environment.

Keywords: *Azolla microphylla*; Domestic wastewater; Biological carbon assimilation; Circular economy

1. Introduction

The increased population and rapid urbanization of the world has led to a problem in terms of environmental health, which effects to the well-being and livelihoods of primarily impoverished individuals. Among other contaminants, water pollution is a significant issue that requires considerable attention. The volume of domestic wastewater generated has reached substantial levels, and the management of wastewater disposal remains a considerable challenge for many countries. Insufficiently treated domestic wastewater significantly contributes to water pollution, resulting in the degradation of freshwater and marine ecosystems, the spread of waterborne infections, and increase of water scarcity in many areas (Arivukkarasu and Sathyanathan, 2023; Fahad *et al.*, 2019; Seetasang and Iwai, 2024). Domestic wastewater is a major contributor of human-generated wastewater and typically classified into two distinct groups: black water and grey water (Widyarani *et al.,* 2022). Domestic wastewater comprises biodegradable organic substances, supplementary organic and inorganic elements, metals, nutrients, and bacteria, which contribute to oxygen depletion, eutrophication, and harmful consequences in aquatic environments (Fahad *et al.,* 2019). Effectively managing and treating domestic wastewater is essential for ensuring public health and the environment (Seetasang and Iwai, 2024).

Developing nations have less advanced infrastructure for wastewater management compared to developed nations (Arivukkarasu and Sathyanathan, 2023). Diverse methodologies have been investigated and implemented for the treatment of domestic wastewater, encompassing adsorption, membrane filtration, electrochemical processes, nano catalysis technologies, and ion exchange (Sangamnere *et al.,* 2023; Thao *et al.,* 2023). Nevertheless, existing wastewater treatment methods present many constraints, including the necessity for high financial investment, physical requirements, and energy consumption, prompting the investigation of alternatives that can overcome these challenges (Anand *et al.,* 2017; Prabakaran *et al.,* 2022; Taghilou *et al.,* 2023). Authenticated, cost-effective, and eco-friendly phytoextraction methods are more appropriate than conventional treatment approaches, which may not be acceptable for smaller regions (Arivukkarasu and Sathyanathan, 2023). Eco-friendly methods are crucial inside the novel technologies and strategies evaluated in wastewater treatment. Biological methods have recently acquired recognition for the elimination of hazardous and other harmful substances (Rajasulochana and Preethy, 2016). Some plants can absorb and retain high concentrations of nutrients and heavy metals, this phenomenon is known as phytoremediation and can be applied in wastewater treatment. Phytoremediation approaches, that do not necessitate external energy, enable macrophyte-based treatment both economically feasible and sustainable (Jayasundara, 2022; Sayanthan *et al.,* 2024; Yadav *et al.,* 2018).

The accessible information about global warming indicates that this issue has grown widespread globally. Therefore, the implementation of novel and more effective treatment approaches, including biological treatments, is essential for reducing greenhouse gas emissions (Goli *et al.,* 2016). Carbon capture and storage (CCS) and carbon capture and utilization (CCU) are the two technologies designed to capture atmospheric CO_2 (Gayathri *et al.,* 2021). There are two main types of global carbon dioxide storage methods: physical and biological (Bhola *et al.,* 2014). Adherence to biobased and circular economy concepts will lead to reduced carbon emissions by promoting the use of renewable resources and minimizing waste generation. This will enable a transition to a more sustainable economic model, thereby advancing toward the achievement of long-term environmental goals and reducing the effects of climate change (Ugya *et al.,* 2024). Plants and microorganisms function as a natural carbon dioxide filter. Various biomolecules, including carbohydrates, proteins, and lipids, are synthesized by the biological carbon fixation process during photosynthesis. Biomass serves as an alternative method for sequestering obtained CO_2. Diverse species, including plants and microorganisms such as bacteria, fungus, yeast, and algae, participate in the conversion of CO_2 into biomass (Gayathri *et al.,* 2021). Azolla plants are widely recognized for being beneficial in promoting greener and more environmentally friendly practices, as well as enhancing quality and supporting a bio-based economy compared to traditional methods (Korsa *et al.,* 2024; Nyein and Iwai, 2024).

Azolla is a small floating plant species and it belongs to the family *Azollacea.* Azolla species are the smallest but most economically significant macrophytes that float on the water's surface. Azolla is distinctive since it is among the fastest-growing plants, capable of doubling its coverage area within 2 to 4 days. The growth rate increase within an ideal pH range of 6.5 - 7.5, at temperatures up to 30 ºC, and relative 83% (Sood *et al.,* 2012; Taghilou *et al.,* 2023). Azolla fern is indigenous to Asia, America and African countries (Prabakaran *et al.,* 2022).

In recognition of its relevance to sustainability of environment, Azolla is a recognized biomedical product of worldwide relevance (Korsa *et al.,* 2024). Previous research and reviwe articles indicated that the utilization of Azolla is an efficient and practical method for wastewater treatment. Many studies have determined that Azolla has significant capacity for nutrient and heavy metal absorption in water, accompanied by substantial biomass output (Amare *et al.,* 2018; Jayasundara, 2022; Kollah *et al.,* 2016; Prabakaran *et al.,* 2022; Soman *et al.,* 2018). Azolla exists in a symbiotic association with nitrogen-fixing cyanobacteria. The capacity of Azolla to fix atmospheric nitrogen enables this fern to thrive in aquatic environments with deficient or low nitrogen levels (Prabakaran *et al.,* 2022; Sood *et al.,* 2012). Many literatures reported that Azolla was a source of protein-rich animal feed for fish, duck, cattle, and poultry, feedstock for biofuel production, and the potential utilization in biofertilizer applications and green manure facilitating a circular economy (Bocchi and Malgioglio, 2010; Brouwer *et al.,* 2018; Datta, 2011; Kollah *et al.,* 2016; Razavipour *et al.,* 2018). Azolla provides as a substantial protein supply, effectively reducing a portion of the nutritional requirements for livestock, poultry, and fish production, functioning as an economical dietary supplement (Korsa *et al.,* 2024; Nyein and Iwai, 2024). The generation of biofuel from Azolla presents a viable sustainable alternative to significantly decrease reliance on non-renewable fossil fuels and alleviate greenhouse gas emissions (Nikkhah *et al.,* 2024). Azolla's quick growing ability enables its utilization for absorping substantial quantities of atmospheric CO_2 as biomass, that can then be sequestered to eliminate atmospheric CO_2 from the active carbon cycle. The Eocene Azolla grow episode contributed to atmospheric CO_2 accumulation and the consequent cooling of the Earth, exemplifying Azolla's efficacy as a rapid CO_2 sequester (Korsa *et al.,* 2024; Nyein and Iwai, 2024).

Many studies focused on using *A. pinnata* and *A. filiculoides* as a promosing in wastewater treatment, animal feeding, soil and agricultural management. There is a lack of comprehensive study on the integrated assesment of Azolla in wastewater treatment, its biomass production for carbon assimilation, and its benefit to circular economy. The aims of present study are to investigate the potential of *A. microphylla* in domestic wastewater treatment, to compare its biomass production for atmospheric carbon sequestration and to analyze nutritional content of *A. microphylla* for efficient application. The novelty of this study lies an integrated assessment of the efficacy of *A. microphylla* which provides valuable by-products for sustainable circular economy, economic benefits and environmentally sound solutions.

2. Methodology

2.1 Study location and materials

The research was conducted at the laboratory of Department of Soil Science and Environment, Khon Kaen University, Thailand. The climatic condition is characterized as temperature range between 17 - 36 ºC, relative humidity of 63 - 65% and the lowest rainfall throughout the study period. *Azolla microphylla* that was cultured for experimental purpose at the Department of Soil Science and Environment was employed in the experiment. Sample of domestic wastewater from a wastewater treatment plant at Khon Kaen University was used. Khon Kaen University had an estimated population of 49,063 personnel in fiscal year 2023. The generated wastewater from faculties, residentials, and cafeteria are treated in domestic wastewater treatment plant constructed in the university. The experiment compared the effectiveness of *A. microphylla* in wastewater treatment and atmospheric carbon sequestration. A model experiment completely randomized design (CRD) was used with 4 treatments, 3 replications; domestic wastewater with and without *A. microphylla* and tap water with and without *A. microphylla.* The experiment was arrangement with a total of 12 containers (57 cm diameter x 22 cm heigh). The containers were filled with 16 liters each of domestic wastewater and tap water. The fresh *A. microphylla*; 143 g was inoculated as treatments and the containers without *A. microphylla* were performed as controls. Experiment was set up under polyhouse conditions and the containers were covered with polynet to avoid pest infestation. The experiment was carried out for two weeks, as Azolla has a doubling phase of around 2 to 5 days; hence, the growth rate can decrease after this duration. Within two weeks, the maximum growth and biomass production were optimized for effective wastewater treatment and enhanced carbon sequestration.

2.2 Water quality analysis

Prior to the experiment, baseline samples were collected to evaluate the overall conditions of water. Physiochemical properties of water and growth characters of Azolla were determined every week of experimental period. The collected samples at the end of the sampling occasion were taken to the laboratory for further investigation. The water temperature, pH, and electrical conductivity (EC) were assessed by utilizing a multi-parameter device (LAQUA Model PC 210), according to the laboratory and field methods (APHA, 2017). The data analytical measurements and methods for physiochemical parameters of water are presented in Table 1. The percentage reduction R (%) can be determined by using the following equation (Bokhari *et al.*, 2016).

Percentage removal (R%) =
(Ci - Co / Ci) *100

Where, Ci = initial residual concentration, Co = final residual concentration.

2.3 Growth rate and nutrient content Analysis

The growth of Azolla is crucial for wastewater treatment and source of nutrients and protein. The initial biomass weight of Azolla was recorded before inoculation. The increased weight, specific growth rate (SGR), absolute growth rate (AGR) and doubling time (DT) were determined at each sampling occasion. Initial and final content of total Kjeldahl nitrogen (TKN), total phosphate (TP), total potassium (TK), organic carbon (OC), crude protein in dried Azolla were analyzed. The Kjeldahl nitrogen (TKN) was analyzed using a catalyst in accordance with the Kjeldahl technique (ICARDA, 2013). Crude protein was assessed according to AOAC section 954.01, utilizing the Kjeldahl method for animal feed analysis (AOAC, 1990). The total phosphorus (TP) content of Azolla was analyzed by Spectrophotometric vanadium phosphomolybdate method (FAO, 2008) and total potassium (TK) was analyzed by the flame photometer, atomic absorption spectrophotometer (FAO, 2008). Organic carbon (OC) content was examined by following the standard rapid dichromate wet oxidation method of Walkley and Black (1934). The growth rate was calculated by using the method of Brouwer *et al.,* (2018) and Sarkar *et al.,* (2023).

Table 1. Data analytical methods for physiochemical parameters of water

Parameters	Methods	References
TSS	Glass fiber filtration disk and drying at temperature range of 103 °C to 105 °C	(APHA, 2017)
TDS	Dried at 180 ± 20 °C	(APHA, 2017)
BOD	Azide modification method at temperature 20°C in 5day	(APHA, 2017)
COD	Potassium dichromate digestion, close reflux method	(APHA, 2017)
NO_3^-	Brucine method	(USEPA, 1971)
PO_4^{-3}	Automated Ascorbic Acid Reduction method	(APHA, 2017)

Note: TSS = total suspended solid, TDS = total dissolved solid, BOD = biological oxygen demand, COD = chemical oxygen demand, NO_3^- = Nitrate nitrogen, PO_4^{-3} = Orthophosphate

Absolute growth rate (g/d) = (W1 – W2)/ (t1 – t2)

Where, W1 and W2 are Azolla mean weight at time t1 and t2.

OC (%) = [(Vb − Vs) × N × 0.3]/ Wt × 1.33

Where,
N = Normality of $(NH_4)\ 2SO_4•FeSO_4•6H_2O$ solution (about 0.5 N),
Vb = $(NH_4)2SO_4•FeSO_4•6H_2O$ solution volume to titrate the blank (ml),
Vs = $(NH_4)2SO_4•FeSO_4•6H_2O$ solution volume to titrate the sample (ml),
Wt = Sample weight (g)

2.4 Analysis on carbon capturing of A. microphylla

The total organic carbon (TOC) in the experiment is ascertained from the total dry weight of *A. microphylla*. Analyzing the carbon content would result in a direct assessment of its carbon sequestration rate (Hamdan and Houri, 2022). The CO_2 to carbon (C) ratio is calculated based on the weight of CO_2 (CO_2 = 44, C = 12). The TOC weight of Azolla is multiplied by 3.6663 and expressed in grams of CO_2 to determine the carbon dioxide sequestered by *A. microphylla* in the experiment (Sarkar *et al.,* 2023).

2.5 Statistical Analysis

All the data acquired from the experiment were inputted and analyzed using Excel and SPSS (Statistical Package for Social Sciences-SPSS 25 statistical program). A one-way analysis of variance (ANOVA) was utilized to assess the statistical analysis of mean concentration of physiochemical properties of water and *A. microphylla*. Tukey's post hoc test was applied to calculate the pairwise comparison of mean concentration and significance level was considered at 95% confidence interval ($p < 0.05$). The R software package (R 4.4.1) was utilized to conduct Pearson's correlation coefficient and principal component analysis (PCA) on the variables and treatments of the experiment (R Core Team, 2023).

3. Results and Discussion

3.1 Physiochemical properties of water

The physiochemical properties of water in the experiment are present in Table 2 and 3. The initial water temperature ranged from 30.13 to 30.97 °C. Within 7 days experiment, temperature of water tended to decrease and it ranged from 25.47 to 28.70 °C. The water temperature increased gradually with the range between 30.03 and 33.40 °C within 14 days (Table 2). The changes in temperature may be ascribed to the effects of plant and external temperature conditions, performing a crucial role in the experimental setup (Muvea *et al.,* 2019). The effective decline of water temperature in the treatments might be attributed the biomass growth of Azolla and it covers the water surface from the light penetration (Kimani *et al.,* 2020). The study results were supported by Shah *et al.* (2015) who found that macrophytes exhibited their peak performance within 15 - 38 °C, that was optimal for plant efficacy in treating wastewater. The pH range during phytoremediation is a critical, hence it can enhance the nutrient uptake (Rizwana *et al.,* 2014). The initial pH values were 7.48 in TW and 8.80 in DW. The average pH concentration demonstrated a significant difference between treatments and controls ($p < 0.05$). pH values in experiment tended to decrease between 6.88 and 8.21 within 7 days and, became constant between 7.47 and 7.72 at 14 days experiment (Table 3). Korsa *et al.* (2024) observed that the rapid growth was obtained with a pH of 7. Azolla is remarkably pH insensitive and exhibits ideal growth within a pH range of 4-10 (Van Hove, 1989) Variations in water acidity can impact nutrition availability. Under alkaline situations, ammonium ions can decompose into ammonia, which is not absorbable by plants (Sudiarto *et al.,* 2019). The pH range of 7 to 9 promotes microbial activity that reduces COD and BOD concentration in the wastewater (Dipu *et al.,* 2011). The pH range of our study results were within the limit for irrigation 6.5 - 8.5 that was recommended by FAO (1985) and USEPA (2012). The results of water temperature and pH levels were suitable for the plant growth and provide a favorable condition for its mechanism.

The concentrations of Electrical conductivity (EC), TDS and TSS were observed with decreased pattern in treatments while comparing to controls. ANOVA showed the concentrations of EC, TDS, TSS in treatments and controls are significantly difference ($p < 0.05$). At initial concentrations of EC were 189.93 µS/cm in TW and 279.43 µS/cm in DW. The treatments showed significant reduction and EC decreased from 189.83 to 76.57 µS/cm in TW, and from 279.43 to 114.43 µS/cm in DW. Within 14 days experiment, the significant EC reduction was observed 59.7% in TW and 59.0% in DW, respectively (Figure 1). EC ranged from 76.57 to 195.5 µS/cm were in lined with the permissible limit of FAO (1985) and USEPA (2012), whereas, the recommended EC concentration for irrigation was 700 and 300 µS/cm, respectively. The decrease in EC in the treatment systems might be attributed to the decrease in ion mobility resulting from the elimination of dissolved particles, including chloride, nitrates, phosphates, sodium, magnesium, and calcium, by the aquatic plants from the effluent (Dembere *et al.,* 2023). EC reduction during the phytoremediation signifies the substantial absorption of nutrients by *A. microphylla.*

The concentrations of Total Dissolved Solids (TDS) and Total Suspended Solids (TSS) during the experimental period are presented in Table 2. In TDS and TSS removal, treatments showed significant reduction compared to controls. The initial TDS were 153.33 and 276.00 mg/L in TW and DW, respectively. The treatments removed TDS 53.9% in TW and 53.1% in DW (Figure 1). Within 14 days experiment, TSS reduction ranged from 16 to 8.00 mg/L in TW and from 26.67 to 10.67 mg/L in DW. The significant reduction of TSS 60% was observed in DW compared to others. Similar previous study found that 54.09% EC reduction and 68.77% TDS removal by *A. pinnata* in industrial wastewater (Kumar *et al.,* 2020). Within 14 days, our finding results of TDS from experimental units were below the recommended limit for agricultural irrigation < 450 mg/L and 0 - 2000 mg/L as reported in FAO (1985) and USEPA (2012). The decrease in EC and TDS in the control units can be attributed to the formation and/or adsorption of several nutritional elements, including as nitrate, phosphate, sulfate, calcium, sodium, and potassium, as reported by Verma and Suthar, (2014). The removal process of TSS in wastewater mostly involved filtering and sedimentation. The TDS and TSS reduction may be ascribed to the reduction of suspended particles, especially organic matter, an increase in solubility and a substantial decrease in BOD (Amare *et al.,* 2018). *A. microphylla* facilitates the sedimentation of dissolved and suspended solids and nutrients removal.

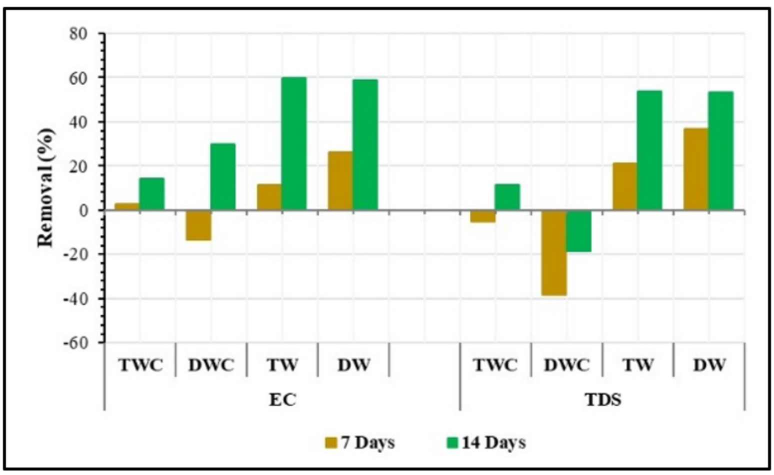

Figure 1. Percentage reduction of EC and TDS in treatments and controls the throughout experimental period

The statistical study of dissolved oxygen indicates a significant difference between treatments and controls ($p < 0.05$). DO concentrations were 7.30 and 6.63 mg/L in TW and DW at initial sampling. The concentration of DO observe within 14 days were 7.43 mg/L in TW and 7.63 mg/L in DW, whereas the mean DO values in the controls are 7.27 and 7.23 mg/L, respectively (Table 3). The photosynthetic processes enhance DO levels in water, therefore promoting aerobic conditions in wastewater. This support aerobic bacterial activity and eventually decreases BOD and COD concentrations (Rizwana *et al.,* 2014). DO level in the control may have been influenced by the presence of algae and other microorganisms which could have contributed to oxygen production (Muvea *et al.,* 2019).

Table 3 indicates the average concentrations of BOD and COD in the experiment. ANOVA analysis on average concentrations of BOD and COD revealed significant difference between treatments and controls ($p<0.05$). At initial sampling, BOD concentration ranged from 0.57 to 28.67 mg/L and COD concentration ranged from 69.33 to 124.45 mg/L. The BOD removal 75.9% was found in DW whereas, 47.7% of BOD removal in DWC. Within 14 days experiment, the percentages of COD removal 28.2%, 64.3%, 30.8% and 72.9% were observed in TWC, DWC, TW and DW, respectively (Figure 2). The percentage reduction observed in the control was lower than that in the treatment. The BOD and COD reductions in the treatments suggest that the presence of *A. microphylla* can enhance the organic biodegradation. The finding results were consistence to prior study that reported BOD removal 74.0% and COD removal 94.6% by A. pinnata in wastewater (Ugya et al., 2017). The suspended and associated microbial growth is responsible for the elimination of soluble BOD. The reduction of BOD and COD concentrations due to bioremediation can be ascribed to biological processes, specifically microbial activity, which is enhanced by the optimal conditions provided by Azolla. (Amare *et al.,* 2018). The plants provide habitat for various decomposing bacteria in the root area and facilitate the transfer of oxygen to their root zones and rhizomes (Sehar *et al.,* 2015).

The study showed that *A. microphylla* can be applied to enhance nutrient uptake from the wastewater. The removal efficiency of NO_3^- tended to increase with the time period (Table 3). Within 14 days, the efficient removal of NO_3^- 46.5% (0.71 - 0.38 mg/L) was observed in DW, whereas, 20.5% in TWC, 32.0% in DWC, 34.1% in TW. The results of present study were supported by prior study of Forni *et al.* (2001) who reported that reduction of NO_3^- 40.5% in well water and 78% in sewage water by *A. filiculoides* treatment. *Hendriks et al.* (2023) observed that Azolla has minimal efficacy in removing any of the generated NO_3^-. This phenomenon is most likely attributed to its symbiotic relationship with the cyanobacterium Nostoc *azollae*,

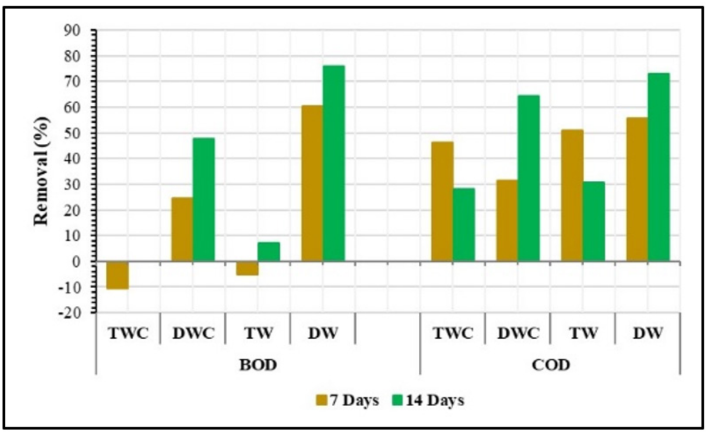

Figure 2. Percentage reduction of BOD and COD in treatments and controls the throughout experimental period

which effectively fixes nitrogen from the atmosphere. The nitrogen removal process in wetland treatment systems involve the absorption of nitrogen by plants and their associated microbes, sediment deposition, ammonia emission, and the processes of nitrification and denitrification (Korner *et al.,* 2003; *Marimon et al.,* 2013). Nitrogen removal by the primary long-term process of nitrification/denitrification relies on the presence of organic carbon. Nitrogen assimilation comprises a range of biological activities that transform inorganic nitrogen forms into organic molecules functioning as fundamental components for cells and tissues (Amare *et al.,* 2018). In the present study, NO_3^- concentrations at the end of sampling ranged from 0.29 to 0.48 mg/L and the results were below the recommended limit for agricultural irrigation < 5 and 10 mg/L as reported in FAO, (1985) and USEPA (2012). The initial PO_4^{3-} concentration was 0.32 mg/L in DW and the PO_4^{3-} concentration in TW was below the detection level. The concentrations of PO_4^{3-} ranged from 0.32 to 0.12 mg/L in DW and from 0.32 to 0.53 mg/L in DWC. The reduction of PO_4^{3-} 62.5% (0.32 - 0.12 mg/L) was observed in DW compared to control. The removal percentage of PO_4^{3-} was similar to the result reported by Ugya *et al.,* (2017) who stated that the effective removal of phosphate by 64.1% within 21 days in the treatment of textile wastewater by using *A. pinnata*. After 14 days experiment, the concentrations of PO_4^{3-} in DWC and DW were ranged from 0.12 to 0.53 mg/L and the results were in lined with the permissible limit 2 mg/L for agricultural irrigation (FAO, 1985).

3.2 Correlation and Principal Component Analysis among physiochemical properties of water

Figure 3 presents a correlation among the physiochemical properties of water and Person's correlation analysis was performed to evaluate the relationship. EC-TDS-TSS-BOD-NO_3^--PO_4^{3-} indicated a strong positive correlation (r = 0.71 - 0.96). This significant relation might be related to nutrients and

Table 2. Physiochemical parameters of water during the experimental period (n = 3)

Parameters	Period (Day)	Control		Treatment	
		TWC	DWC	TW	DW
Temperature (°C)	0	30.13 ± 0.06^b	30.97 ± 0.12^a	30.13 ± 0.06^b	30.97 ± 0.12^a
	7	28.70 ± 0.36^a	28.37 ± 0.15^a	25.47 ± 0.29^b	26.37 ± 0.15^b
	14	30.80 ± 2.88	33.40 ± 0.85	30.03 ± 0.80	30.37 ± 0.47
EC (µS/cm)	0	189.83 ± 1.59^b	279.43 ± 2.35^a	189.83 ± 1.59^b	279.43 ± 2.35^a
	7	185.13 ± 4.48^c	316.67 ± 1.25^a	168.47 ± 4.03^d	206.67 ± 5.11^b
	14	162.83 ± 4.48^b	195.50 ± 5.05^a	76.57 ± 3.21^d	114.43 ± 3.20^c
TDS (mg/L)	0	153.33 ± 2.31^b	276.00 ± 4.00^a	153.33 ± 2.31^b	276.00 ± 4.00^a
	7	161.33 ± 2.31^c	381.33 ± 2.31^a	121.33 ± 4.62^d	174.67 ± 2.31^b
	14	136.00 ± 4.00^b	326.67 ± 4.62^a	70.67 ± 2.31^c	129.33 ± 2.31^b
TSS (mg/L)	0	16.00 ± 4.00^b	26.67 ± 2.31^a	16.00 ± 4.00^b	26.67 ± 2.31^a
	7	32.00 ± 4.00^b	60.00 ± 4.00^a	10.67 ± 2.31^c	14.67 ± 2.31^c
	14	28.00 ± 0.00^b	57.33 ± 2.31^a	8.00 ± 0.00^c	10.67 ± 2.31^c

Note: Means shown by distinct letters within the same row are significantly different ($p < 0.05$), Initial concentration indicated 0 Day. TWC = tap water without Azolla, DWC = domestic wastewater without Azolla, TW = tap water with Azolla, DW = domestic wastewater with Azolla pollutants loading of domestic wastewater. The electrical conductivity, an attribute mostly influenced by dissolved salts, may function as an indirect estimate of total dissolved solids. These dissolved ions subsequently function as the conductors for the electric current. The relationship between EC and TDS was further evidenced by a positive correlation found in correlation investigation. Saalidong *et al.* (2022) observed that the raised TDS signifies an ideal pH, whereas the rising EC reveals a non-optimal water pH. The negative relation of DO-BOD-COD suggests the lower DO level might be associated with higher BOD and COD with an increased oxygen consumption in biochemical reactions. PCA was performed on physiochemical properties of water and treatment to identify the significant parameters affecting on water quality and possible pollution source. The scree plot of PCA among physiochemical properties of water indicates two factors for variables and PC1 explained 63.3% while, PC2 explained 24.3% of the total variances. PC1 captured strong positive loading of EC, TDS, TSS, BOD, NO_3^-, PO_4^{3-} (Figure 4). Whereas, positive moderate loading of DO and pH was found in PC2 and COD was negatively captured in PC2. COD measures the quantity of organic materials present in the water, indicating nutrient contamination from anthropogenic sources, including eutrophication resulting from sewage water and agricultural practices (Li *et al.,* 2014).The variables loading on PC1 were inorganic and contaminants that consume oxygen, which may be related to influences from domestic wastewater, nitrogen and phosphorus from point source discharges (Yang *et al.,* 2020). The domestic wastewater treated with *A. microphylla* showed less loading of nutrients and pollutants compared to its control.

Table 3. Physiochemical parameters of water throughout the experimental period (n = 3)

Parameters	Period (Day)	Control		Treatment	
		TWC	DWC	TW	DW
pH	0	7.48 ± 0.05^b	8.80 ± 0.06^a	7.48 ± 0.05^b	8.80 ± 0.06^a
	7	7.40 ± 0.10^b	8.21 ± 0.02^a	6.88 ± 0.09^d	7.21 ± 0.02^c
	14	7.50 ± 0.10^c	7.47 ± 0.06^c	7.56 ± 0.04^{ab}	7.72 ± 0.04^a
DO (mg/L)	0	7.30 ± 0.00^a	6.63 ± 0.15^c	7.30 ± 0.00^a	6.63 ± 0.15^c
	7	7.30 ± 0.00^b	6.97 ± 0.12^c	7.50 ± 0.00^b	7.93 ± 0.21^a
	14	7.27 ± 0.06^c	7.23 ± 0.06^c	7.43 ± 0.06^b	7.63 ± 0.06^a
BOD (mg/L)	0	0.57 ± 0.06^b	28.67 ± 1.15^a	0.57 ± 0.06^b	28.67 ± 1.15^a
	7	0.63 ± 0.06^c	21.67 ± 1.44^a	0.60 ± 0.17^c	11.33 ± 0.65^b
	14	0.57 ± 0.06^a	15.00 ± 2.50^a	0.53 ± 0.06^a	6.89 ± 0.38^b
COD (mg/L)	0	69.33 ± 5.34^b	124.45 ± 3.08^a	69.33 ± 5.34^b	124.45 ± 3.08^a
	7	37.33 ± 5.34^c	85.33 ± 0.00^a	34.11 ± 5.58^c	55.11 ± 3.08^b
	14	49.78 ± 3.08^a	44.45 ± 3.08^a	48.00 ± 0.00^a	33.78 ± 3.08^b
NO_3^- (mg/L)	0	0.44 ± 0.01^b	0.71 ± 0.03^a	0.44 ± 0.01^b	0.71 ± 0.03^a
	7	0.41 ± 0.02^b	0.61 ± 0.01^a	0.37 ± 0.01^c	0.40 ± 0.02^{ab}
	14	0.35 ± 0.06^b	0.48 ± 0.04^a	0.29 ± 0.03^b	0.38 ± 0.02^b

Note: Means shown by distinct letters within the same row are significantly different ($p < 0.05$), Initial concentration indicated 0 Day. TWC = tap water without Azolla, DWC = domestic wastewater without Azolla, TW = tap water with Azolla, DW = domestic wastewater with Azolla

The domestic wastewater and tap water in the treatment distinguished for improved water quality with the reductions of pollutants and nutrients concentration. When *A. microphylla* was applied in wastewater medium, Azolla significantly showed its capacity for polishing wastewater and water quality improvement.

3.3 Biomass growth, growth rate and doubling time of A. microphylla

In the investigation on the growth aspect of *A. microphylla*, increased biomass production was observed in both DW and TW. The initial biomass inoculated in the

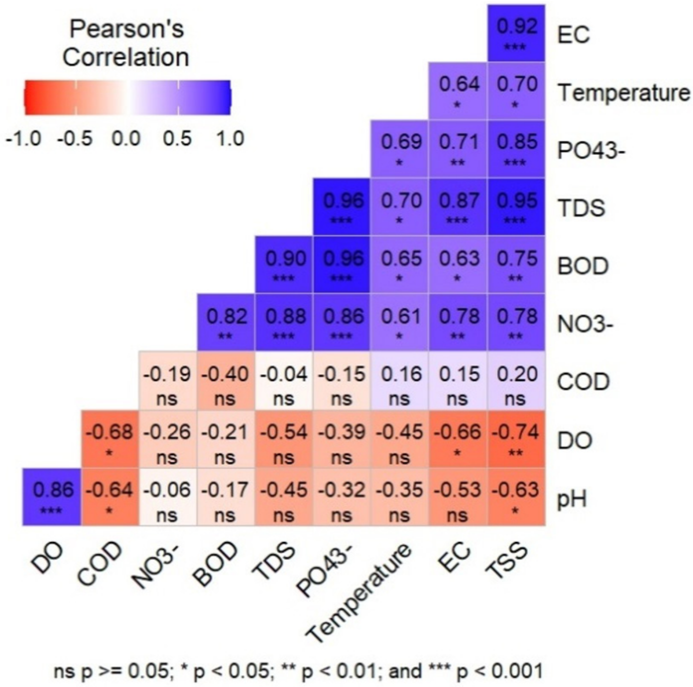

Figure 3. Person's correlation coefficient among the physical and chemical properties of water examined by R program

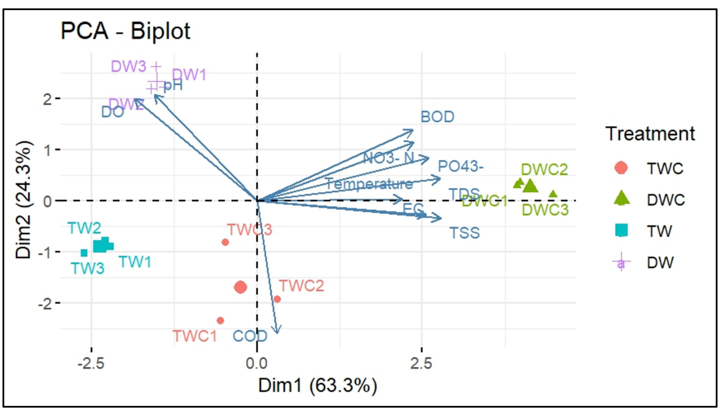

Figure 4. PCA – Biplot among the physical and chemical properties of water and treatment examined by R program

experiment were 143 g and the biomass production increased gradually with the experimental period. Biomass increased from 143 to 442.87 g in TW over a period of 14 days while, 143 to 407.63 g increased in DW. The low biomass build up was found in DW compared to TW. The growth performance of Azolla may differ based on the type of water sources, influencing the quantity of plants produced, biomass yield and growth rate. This variation may be ascribed to several factors, including the fragmentation process, nutrient availability and mineral content (Nordiah *et al.,* 2012). The dry weight increased from 6.07 g to 13.5 g in TW and to 12.5 g in DW during a period of 14 days. The doubling time of *A. microphylla* were found 4.9 and 8.6 days in TW and 5.7 and 9.3 days in DW during 14 days period. The growth rates of Azolla in both water sources were inversely related with time period. Azolla grown in TW showed absolute growth rate (AGR) 34.73 and 21.42 g/d while, 27.2 and 18.9 g/d in DW within 7 and 14 days, respectively. Otherwise, specific growth rates (SGR) 6.16% and 5.25% were found in TW and DW within 7 days. It was found that the growth rate tended to decrease with the time progress. This represents the typical pattern for short-lived plants, such as Azolla, which attains peak growth in the initial week, followed by a drop as the plant grows and finally died (Nordiah *et al.,* 2012). The high biomass density of *Azolla* may lead to its death and decomposition, resulting in a decline in effectiveness after its full development.

3.4 Nutrient content of A. microphylla

The nutritional content of *A. microphylla* grown in TW and DW was presented in Table 4. Within 14 days experiment, higher crude protein and nutrient content were observed in Azolla grown in DW when compared to TW. Wastewater includes three essential macronutrients: nitrogen, phosphorus, and potassium, as well as several micronutrients necessary for plant development (Rebi *et al.,* 2021). The nutrient available from domestic wastewater might led to extract and degrade in the tissue of *A. microphylla*. Azolla has potential to absorb nutrients from the wastewater and convert them to carbohydrate and protein for the plant growth. The initial percentage of TKN, TP, TK and C/N ratio content were 2.89%, 0.30%, 0.81%, and 7.78% in Azolla dry weight basis. An increase of 3.06% in TKN, 0.33% in TP, 0.99% in TK, and a C/N ratio of 12.28% were observed in Azolla grown in DW (Table 4). In this study, OC content of *A. microphylla* ranged from 22.38 to 37.04% in DW while 22.38 to 34.26% in TW. Our findings indicated that *A. microphylla* contains TKN 3.06 %, TP 0.33 %, and TK 0.99 % on dry weight basis. The initial crude protein content was 18.04%. The content of crude protein increased from 18.04% to 19.15% in DW and from 18.04% to 18.96% in TW. These findings were supported by previous studies that reported 21 - 23% crude protein in *A. pinnata.* and 0.36 - 0.48% TP in *A. filiculoides* (El-Shafai *et al.,* 2016; Kumar and Chander, 2017; Lay and Iwai, 2022).

The observed results may be attributed to capacity of Azolla to carry out nitrogen fixation in the presence of nitrogenase during symbiotic interactions with Anabaena blue-by Handajani (2011). The presence of Azolla led to increase plant nitrogen absorption compared to nitrogen removal from the effluent, suggesting that the Azolla-Nostoc symbiosis facilitated nitrogen reduction from the atmosphere. Hendriks *et al.,* (2023) demonstrated that nearly all of the nitrogen taken by Azolla was derived from atmospheric nitrogen fixation. Azolla serves as a promising biofertilizer due to its substantial nitrogen-fixing capacity. Many species of Azolla may possess distinct compositions (Azab and Soror, 2020). Azolla substantially enhances the availability of nitrogen fertilizers for cultivating crops and has been utilized as an organic nitrogen fertilizer to increase production for centuries (Korsa *et al.,* 2024). The prior research demonstrated that substituting 25% of urea-nitrogen with Azolla biofertilizer provides the farmers an economical way to substantially enhance nitrogen use efficiency and production, while effectively minimizing nitrogen loss in intensive rice cultivation system (Yao *et al.,* 2018). In this study, nutritional content of *A. microphylla* is well recorded and it is widely marketed as a high-quality protein source

18.04 - 19.15%. Although the growth media includes a low concentration of nitrogen, Azolla species are capable of synthesizing nitrogen molecules and converting them into protein form. Its high protein and mineral content are essential for livestock feeding, lowering feed costs, and increasing productivity.

3.5 Biological carbon capturing of A. microphylla

Carbon dioxide is a fundamental component of the carbon cycle and a primary source for plant photosynthesis. Biomass is generated from atmospheric CO_2 by a sequence of metabolic events within photosynthetic organisms (Gayathri *et al.,* 2021). Figure 5 presents the biological carbon capturing of *A. microphylla* during the experiment. At the beginning of experiment, the CO_2 absorption in *A. microphylla* in dry weight basis was 4.96 g of CO_2. Within 7 days, carbon capturing rates of 14.32 and 13.28 g CO_2 were observed in TW and DW. Whereas, *A. microphylla* captured 16.96 and 16.99 g CO_2 in TW and DW within 14 days. The carbon sequestration rates were 126 to 136% of dry weight of Azolla in the experimental period. The results indicated a carbon sequestration rate of 1.36 g CO_2 per g of dry weight Azolla throughout experimental period. Our study results were supported by the previous work on carbon sequestration rate 154 to 164% of *A. pinnata* (dry weight) (Sarkar *et al.,* 2023). In line with our hypothesis, the growth of *A. microphylla* led to increase biological CO_2 sequestration and transform as its biomass while drastically reduce in greenhouse gas. Azolla's rapid growth enables its use as a mean to absorb substantial atmospheric CO_2 as biomass, which can be sequestered to entirely eliminate carbon from the active carbon cycle.

This study demonstrates that *A. microphylla* has promise for domestic wastewater treatment and atmospheric carbon sequestration. It can be explained that the phytoremediation capability of *A. microphylla* is promising in the nutrients and pollutants removal form the domestic wastewater. Azolla provides a favorable condition for microorganisms that facilitate

Table 4. Nutrient content of *A. microphylla* before and after experiment (n = 3)

Parameters	Initial Content	Final Content	
		A. microphylla grown in TW	*A. microphylla* grown in DW
TKN (%)	2.89 ± 0.20	3.03 ± 0.08	3.06 ± 0.12
TP (%)	0.30 ± 0.01	0.32 ± 0.09	0.33 ± 0.03
TK (%)	0.81 ± 0.04	0.84 ± 0.03	0.99 ± 0.01
Crude Protein (%)	18.04 ± 1.26	18.96 ± 0.51	19.15 ± 0.76
C/N ratio (%)	7.78 ± 0.61	11.30 ± 0.38	12.28 ± 0.91
OC (%)	22.38 ± 0.83	34.26 ± 1.32	37.04 ± 1.25

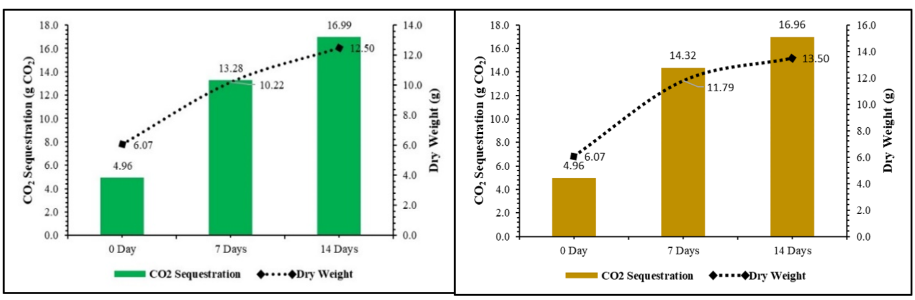

Figure 5. Biological carbon capturing of *A. microphylla* grown in (a) domestic wastewater (b) and tap water

the removal of suspended solids, dissolved solids, various pollutants and nutrients. The wastewater treatment utilizing Azolla is an efficient and cost-effective option, as they offer lower operational, and maintenance costs compared to traditional systems, effectively removing pollutants and ensuring water reuse for irrigation and other purposes. In this study, the high biomass production, high growth rate and minimum doubling time of *A. microphylla* were observed in TW while, high nutritional content was observed DW. The biomass growth of *A. microphylla* revealed a carbon sequestration rate of 1.36 g CO_2 per g of dry weight of Azolla. The result suggests the rapid growth enables its application to capture substantial quantities of atmospheric CO_2 as biomass, which can eliminate carbon from the active carbon cycle. Azolla characterized biological carbon sequestration as the most cost-effective and ecologically sustainable alternative.

Utilizing Azolla as biofertilizer has been recognized as more effective than inorganic fertilizers in reducing greenhouse gas emissions. Azolla as biofertilizer ensures cost-effectiveness by using easily available solar energy and atmospheric nitrogen (Kollah *et al.,* 2016). In this study, the nutrient content of *A. microphylla* revealed 3.06% TKN, 0.33% TP, and 0.99% TK on a dry weight basis. The crude protein 19.15% of Azolla is well recorded in this study, and it is marketed as a high protein source and easily digestible in animal. Feeding Azolla to animals such as goats, chickens, ducks, cows, and pigs enhances the weight gain, egg production, and milk yield. The cultivation of Azolla can assist the farmers with lowering livestock feeds supplementary costs, improving productivity and economic returns.

The challenges of Azolla under large-scale conditions are its high biomass production, rapid growth rate, and adaptability to environmental conditions. It might have constraints to control the biomass growth under scale up cultivation. Therefore, a complete comprehension of the natural habitat conditions of Azolla is required for optimal management. The prior study suggested that the peak growth of Azolla was achieved in the first week, with optimal growth (Lay and Iwai, 2022; Nordiah *et al.,* 2012). Nikkhah *et al.,* 2024) hypothesized that the harvesting and valorization of Azolla mitigate the adverse impacts of this invasive fern on wetland ecosystems while enhancing the regional circular economy by utilizing Azolla as a feedstock for the production of sustainable products, including biofuel and compost. Azolla employs a bio-based economy rather than conventional methods, providing an ecologically sustainable solution for a longer and healthy environment.

4. Conclusion

This study investigates *A. microphylla* through a comprehensive approach of domestic wastewater treatment, biomass production for atmospheric carbon sequestration and nutritional enrichment for effective utilization. Experimental findings reveal that *A. microphylla* have various roles in wastewater treatment, including nutrient removal and heavy metal absorption. The relevance of this research lies in its contribution to resource recovery from wastewater, including water reuse, and nutrient recycling, hence presenting potential for strengthening a circular economy principle. *A. microphylla* exhibited significant atmospheric carbon sequestration and explored the beneficies of Azolla's biomass in contributing the greenhouse gas reduction in the current global warming. Azolla exhibits remarkable nitrogen fixation and, making it suitable for using as green manure to enhance soil nutrition in agricultural operations at tropical regions. It supports organic farming practices that improve the farmers' income and reduce negative impact on the environment. Its ecological sustainability make it particularly appropriate for developing nations, providing an efficient solution for water pollution management while producing valuable by-products. Further research is essential to investigate the interesting potential of nitrogen fixation and carbon sequestration among different species of Azolla. This study concludes that *A. microphylla* is potentially useful as a phytoremediator, a source of nutrients for sustainable agriculture, and a high-protein food for animals. In contrast, it suggests its efficacy for developing cricular economy, and green economy by generating valuable by-products and services that are eco-friendly and require minmal resource input, while mitigating the global warming.

Acknowledgment

The authors would like to express sincere thanks to Khon Kaen University Scholarship (ASEAN and GMS Countries' Personnel 2023), Thailand and the Integrated Land and Water Resource Management Research and Development Center in Northeast Thailand, Khon Kaen University, Thailand.

References

Amare, E., Kebede, F., & Mulat, W. (2018). Wastewater treatment by Lemna minor and *Azolla* filiculoides in tropical semi-arid regions of Ethiopia. Ecological Engineering, 120(May), 464–473. https:// doi.org/10.1016/j.ecoleng.2018.07.005

Anand, S., Bharti, S., Barman, S., & Kumar, N. (2017). Macrophytes for the Reclamation of Degraded Waterbodies with Potential for Bioenergy Production. March, 1–472. https:// doi.org/10.1007/978-981-10-3084-0

AOAC, (1990). (n.d.). AOAC: Official Methods of Analysis (K. Helrich (ed.); 15th ed., Vol 1). Association of Official Analytical Chemists, Inc., 1(Volume 1).

APHA. (2017). Standard Methods for the Examination of Water and Wastewater. American Public Heath Association, American Water Works Association, Water Environment Federation, 23rd Editi, 1546pp.

Arivukkarasu, D., & Sathyanathan, R. (2023). Phytoremediation of domestic sewage using a floating wetland and assessing the pollutant removal effectiveness of four terrestrial plant species. H2Open Journal, 6(2). https://doi.org/10.2166/ h2oj.2023.032

Azab, E., & Soror, A. S. (2020). Physiological behavior of the aquatic plant *Azolla* sp. In response to organic and inorganic fertilizers. Plants, 9(7), 1–13. https:// doi. org/10.3390/plants9070924

Bhola, V., Swalaha, F., Ranjith Kumar, R., Singh, M., & Bux, F. (2014). Overview of the potential of microalgae for CO2 sequestration. International Journal of Environmental Science and Technology, 11(7), 2103–2118. https:// doi.org/10.1007/ s13762-013-0487-6

Bocchi, S., & Malgioglio, A. (2010). *Azolla*-Anabaena as a Biofertilizer for Rice Paddy Fields in the Po Valley, a Temperate Rice Area in Northern Italy. International Journal of Agronomy, 2010, 1–5. https:// doi.org/10.1155/ 2010/152158

Bokhari, S. H., Ahmad, I., Mahmood-Ul-Hassan, M., & Mohammad, A. (2016). Phytoremediation potential of Lemna minor L. for heavy metals. International Journal of Phytoremediation, 18(1), 25–32. https://doi.org/ 10.1080/ 1522 6514.2015.1058331

Brouwer, P., Schluepmann, H., Nierop, K. G. J., Elderson, J., Bijl, P. K., van der Meer, I., de Visser, W., Reichart, G. J., Smeekens, S., & van der Werf, A. (2018). Growing *Azolla* to produce sustainable protein feed: the effect of differing species and CO2 concentrations on biomass productivity and chemical composition. Journal of the Science of Food and Agriculture, 98(12), 4759–4768. https://doi.org/ 10.1002/jsfa.9016

Datta, S. N. (2011). Culture of *Azolla* and its efficacy in diet of Labeo rohita. Aquaculture, 310(3–4), 376–379. https: //doi.org/http://dx.doi.org/10.1016/j. aquaculture.2010.11.008

Dembere, B. (2023). Phytoremediation Potentials of *Azolla* filiculoides L . and Lemna minor L . for Heavy Metals from Soft Drink Factory Effluent : The Case of Hawassa Millennium Pepsi Cola. https:// dx.doi.org/10.4314/jasem.v27i7.36

Dipu, S., Kumar, A. A., & Thanga, V. S. G. (2011). Phytoremediation of dairy effluent by constructed wetland technology. Environmentalist, 31(3), 263–278. https:// doi.org/ 10.1007/ s 10669-011-9331-z

El-Shafai, S. A., Abdelfattah, I., Nasr, F. A., & Fawzy, M. E. (2016). Lemna gibba and *Azolla* filiculoides for sewage treatment and plant protein production. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 7(2), 1869–1876.

Fahad, A., Saphira Mohamed, R. M., Radhi, B., & Al-Sahari, M. (2019). Wastewater and its Treatment Techniques: An Ample Review. Indian Journal of Science and Technology, 12(25), 1–13. https://doi.org/10.17485/ijst/2019/v12i25/146059

FAO. (1985). Guidelines: land evaluation for irrigated agriculture. Food and Agriculture Organization of the United Nations, Rome, Italy:

FAO. (2008). Guide to laboratory establishment for plant nutrient analysis, FAO fertilizer and plant nutrition bulletin 19. (Vol. 19).

Forni, C., Chen, J., Tancionil, L., & Grilli Caiola, M. (2001). Evaluation of the fern *Azolla* for growth, nitrogen and phosphorus removal from wastewater. Water Research, 35(6), 1592–1598. https://doi.org/10.1016/S0043-1354(00)00396-1

Gayathri, R., Mahboob, S., Govindarajan, M., Al-Ghanim, K. A., Ahmed, Z., Al-Mulhm, N., Vodovnik, M., & Vijayalakshmi, S. (2021). A review on biological carbon sequestration: A sustainable solution for a cleaner air environment, less pollution and lower health risks. Journal of King Saud University - Science, 33(2), 101282. https://doi.org/10.1016/j.jksus.2020.101282

Goli, A., Shamiri, A., Talaiekhozani, A., Eshtiaghi, N., Aghamohammadi, N., & Aroua, M. K. (2016). An overview of biological processes and their potential for CO2 capture. Journal of Environmental Management, 183, 41–58. https://doi.org/10.1016/ j.jenvman.2016.08.054

Hamdan, H. Z., & Houri, A. F. (2022). CO2 sequestration by propagation of the fast-growing *Azolla* spp. Environmental Science and Pollution Research, 29(12), 16912–16924. https://doi.org/ 10.1007/s11356-021-16986-6

Handajani, H. (2013). Optimation of Nitrogen and Phosphorus in *Azolla* Growth as Biofertilizer. Makara Journal of Technology, 15(2), 142. https:// doi.org /10.7454/mst.v15i2.931

Hendriks, L., Smolders, A. J. P., van den Brink, T., Lamers, L. P. M., & Veraart, A. J. (2023). Polishing wastewater effluent using plants: floating plants perform better than submerged plants in both nutrient removal and reduction of greenhouse gas emission. Water Science and Technology, 88(1), 23–34. https://doi.org/10.2166/wst.2023.203

Hove, C. Van. (1989). Azoll and its multiple uses with emphasis on Africa.

ICARDA. (2013). Methods of Soil, Plant, and Water Analysis: A manual for the West Asia and North Africa region. International Center for Agricultural Research in the Dry Areas, 1–244.

Jayasundara, P. (2022). Wastewater Treatment by *Azolla*: A review. Diyala Agricultural Sciences Journal, 14(1), 40–46. https://doi.org/10.52951/ dasj. 22140105

Kimani, S. M., Kanno, T., Tawaraya, K., & Cheng, W. (2020). Floating *Azolla* Cover Influences Evapotranspiration from Flooded Water Surfaces. Wetlands, 40(5), 1425–1432. https:// doi.org/10.1007/s13157-020-01282-9

Kollah, B., Patra, A. K., & Mohanty, S. R. (2016). Aquatic microphylla *Azolla*: a perspective paradigm for sustainable agriculture, environment and global climate change. Environmental Science and Pollution Research, 23(5), 4358–4369. https://doi.org/10.1007/s11356-015-5857-9

Korner, S., Vermaat, J. E., & Veenstra, S. (2003). The Capacity of Duckweed to Treat Wastewater: Ecological Considerations for a Sound Design. J. Environ. Qual. 32:1583-1590.

Korsa, G., Alemu, D., & Ayele, A. (2024). *Azolla* Plant Production and Their Potential Applications. International Journal of Agronomy, 2024, 1–12. https://doi.org/10.1155/2024/1716440

Kumar, G., & Chander, H. (2017). A Study on the Potential of *Azolla* pinnata as Livestock Feed Supplement for Climate Change Adaptation and Mitigation. Asian Journal of Advanced Basic Sciences, 5(2), 65–68. https://www. researchgate.net/publication/320444318_A_Study_on_the_Potential_of_*Azolla*_pinnata_as_Livestock_Feed_Supplement_for_Climate_Change_Adaptation_and_Mitigation

Kumar, V., Kumar, P., Singh, J., & Kumar, P. (2020). Potential of water fern (*Azolla* pinnata R.Br.) in phytoremediation of integrated industrial effluent of SIIDCUL, Haridwar, India: removal of physicochemical and heavy metal pollutants. International Journal of Phytoremediation, 22(4), 392–403. https://doi.org/10.1080/15226514.2019.1667950

Lay, P., & Iwai, C. B. (2022). Recycling of Piggery Effluent for *Azolla microphylla* Production and Application in Vermiculture for Enhancing Nutrients Quality and Driving BCG Economy Model. EnvironmentAsia, 15(Special Issue), 68–86. https://doi.org/ 10.14456 / ea.2022.23

Li, X., Li, P., Wang, D., & Wang, Y. (2014). Assessment of temporal and spatial variations in water quality using multivariate statistical methods: a case study of the Xin'anjiang River, China. Frontiers of Environmental Science and Engineering, 8(6), 895–904. https:// doi.org/10.1007/s11783-014-0736-z

Marimon, Z. A., Xuan, Z., & Chang, N. Bin. (2013). System dynamics modeling with sensitivity analysis for floating treatment wetlands in a stormwater wet pond. Ecological Modelling, 267, 66–79. https://doi.org/10.1016/ j.ecolmodel.2013.07.017

Muvea, F. M., Ogendi, G. M., & Omondi, S. O. (2019). Nutrient removal efficiency by floating macrophytes; Lemna minor and *Azolla* pinnata in a constructed wetland. Global Journal of Environmental Science and Management, 5(4), 415–430. https:// doi.org/10.22034/gjesm.2019.04.02

Nikkhah, F., Ramazanian, M. R., & Rosentrater, K. A. (2024). Prioritizing the valorization strategies of an invasive fern (*Azolla*) in a wetland. Bioresource Technology Reports, 26(March), 101847. https://doi.org/ 10.1016/ j.biteb.2024.101847

Nordiah, B., Mula Harah, Z., Japar Sidik, B., & N Wan Hazma, W. (2012). *Azolla* pinnata Growth Performance in Different Water Sources. Pakistan Jownal of Biological Sciences 15 (13): 621-628, 2012. https:// doi.org/ 10.3923/ pjbs.2012.621.628

Nyein, N., & Iwai, C. B. (2024). Using *Azolla microphylla* in investigating different agro-wastewaters treatment and its biomass growth for carbon sequestration. Results in Engineering, 25(November 2024), 103865. https:// doi.org/10.1016/j.rineng.2024.103865

Prabakaran, S., Mohanraj, T., Arumugam, A., & Sudalai, S. (2022). A state-of-the-art review on the environmental benefits and prospects of *Azolla* in biofuel, bioremediation and biofertilizer applications. Industrial Crops and Products, 183(March), 114942. https:// doi.org/10.1016/j.indcrop.2022.114942

R Core Team 2023. (n.d.). R: A language and environment for statistical computing (version 4.4.1). R Foundation for Statistical Computing.https://www.r-project.org/.

Rajasulochana, P., & Preethy, V. (2016). Comparison on efficiency of various techniques in treatment of waste and sewage water – A comprehensive review. Resource-Efficient Technologies, 2(4), 175–184. https:// doi.org/10.1016/j.reffit.2016.09.004

Razavipour, T., Moghaddam, S. S., Doaei, S., Noorhosseini, S. A., & Damalas, C. A. (2018). *Azolla* (*Azolla* filiculoides) compost improves grain yield of rice (Oryza sativa L.) under different irrigation regimes. Agricultural Water Management, 209(November 2017), 1–10. https://doi.org/10.1016/ j.agwat.2018.05.020

Rebi, A., Rehman, T., Wahaj, M., Raza, H. A., Usman, M., Gul, N., Bano, M., Khan, Z., Ghazanfar, S., Irfan, M., Naz, M., & Ahmed, M. I. (2021). Impact of Sewage Water on Human Health and Agricultral Land: A Review. Annals of the Romanian Society for Cell Biology, 25 (7), 1366–1376. https://www. annalsofrscb.ro/ndex.php/journal/ article/view/10462

Rizwana, M., Darshan, M., & Nilesh, D. (2014). Phytoremediation of Textile Waste Water Using Potential Wetland Plant: Eco Sustainable Approach. International Journal of Interdisciplinary and Multidisciplinary Studies, 1(4), 130–138. http:// www. ijims.com

Saalidong, B. M., Aram, S. A., Otu, S., & Lartey, P. O. (2022). Examining the dynamics of the relationship between water pH and other water quality parameters in ground and surface water systems. PLoS ONE, 17(1 1), 1–17. https://doi.org/10.1371/journal.pone.0262117

Sangamnere, R., Misra, T., Bherwani, H., Kapley, A., & Kumar, R. (2023). A critical review of conventional and emerging wastewater treatment technologies. Sustainable Water Resources Management, 9(2), 1–26. https://doi.org/10.1007/s40899-023-00829-y

Sarkar, A., Bhakta, J. N., Bubai Bhakta, & Ohnishi, K. (2023). Evaluating growth-dependent enhanced carbon dioxide sequestration potential of *Azolla* pinnata using cattle wastes (cow dung and cow urine). Heliyon, 9(3). https://doi.org/10.1016/j.heliyon.2023.e14610

Sayanthan, S., Hasan, H. A., & Abdullah, S. R. S. (2024). Floating Aquatic Macrophytes in Wastewater Treatment: Toward a Circular Economy. Water (Switzerland), 16(6). https://doi.org/10.3390/w16060870

Seetasang, S., & Iwai, C. B. (2024). Enhancing domestic wastewater treatment: Integrating vermifiltration and biochar for heavy metal and microplastic reduction and by-product utilization. Case Studies in Chemical and Environmental Engineering, 11(August 2024), 101025. https:// doi.org/10.1016/j.cscee.2024.101025

Sehar, S., Sumera, Naeem, S., Perveen, I., Ali, N., & Ahmed, S. (2015). A comparative study of macrophytes influence on wastewater treatment through subsurface flow hybrid constructed wetland. Ecological Engineering, 81, 62–69. https://doi.org/ 10.1016/ j.ecoleng.2015.04.009

Shah, M., Hashmi, H. N., Ghumman, A. R., & Zeeshan, M. (2015). Performance assessment of aquatic macrophytes for treatment of municipal wastewater. Journal of the South African Institution of Civil Engineering, 57(3), 18–25. https://doi.org/10.17159/2309-8775/2015/v57n3a3

Soman, D., V, A., & Arora, A. (2018). Bioremediation of Municipal Sewage Water With *Azolla microphylla*. International Journal of Advanced Research, 6(5), 101–108. https://doi.org/10.21474/ijar01/7012

Sood, A., Uniyal, P. L., Prasanna, R., & Ahluwalia, A. S. (2012). Phytoremediation potential of aquatic macrophyte, Azolla. Ambio, 41(2), 122–137. https://doi.org/10.1007/ s13280-011-0159-z

Sudiarto, S. I. A., Renggaman, A., & Choi, H. L. (2019). Floating aquatic plants for total nitrogen and phosphorus removal from treated swine wastewater and their biomass characteristics. Journal of Environmental Management, 231 (October 2018), 763–769. https:// doi.org/10.1016/j.jenvman.2018.10.070

Taghilou, S., Peyda, M., & Mehrasbi, M. (2023). A review on the significance of *Azolla* for water and wastewater treatment. Desalination and Water Treatment, 293, 138–149. https:// doi.org/ 10.5004/dwt.2023.29528

Thao, N. T. P., Nga, N. T. T., Kim, H. T. T., Kien, T. T., Hieu, T. T., Thang, N. V., Tri, N. L. M., & Le, T. H. (2023). Combination of biochar filtration and ozonation processes in livestock wastewater treatment and application for soil cultivation. Case Studies in Chemical and Environmental Engineering, 7(December 2022), 100286. https://doi.org/ 10.1016/ j.cscee.2022.100286

Ugya, A. Y., & Imam, T. . (2015). The Efficiency of Eicchornia crassipes in the Phytoremediation of Waste Water from Kaduna Refinery and Petrochemical Company\n. IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT), 9(1), 43–47. https://doi.org/10.9790/ 3008-10147680

Ugya, A. Y., Sheng, Y., Chen, H., & Wang, Q. (2024). Microalgal bioengineering: A futuristic tool for carbon capture. Results in Engineering, 24(September), 102990. https://doi.org/10.1016/ j.rineng.2024.102990

USEPA. (1971). Method 352 . 1 : Nitrogen , Nitrate (Colorimetric , Brucine) by Spectrophotometer.

USEPA. (2012). Guidelines for Water Reuse. Development, 26(September), 252. http://www.epa.gov/nrmrl/pubs/625r04108/625r04108.pdf

Verma, R., & Suthar, S. (2014). Synchronized urban wastewater treatment and biomass production using duckweed Lemna gibba L. Ecological Engineering, 64, 337–343. https:// doi.org/10.1016/j.ecoleng.2013.12.055

Walkley, A., & Black, I. A. (1934). An examination of the degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. In Soil Science (Vol. 37, Issue 1, pp. 29–38). https://doi.org/10.1097/00010694-193401000-00003

Widyarani, Wulan, D. R., Hamidah, U., Komarulzaman, A., Rosmalina, R. T., & Sintawardani, N. (2022). Domestic wastewater in Indonesia: generation, characteristics and treatment. Environmental Science and Pollution Research, 29(22), 32397–32414. https://doi.org/10.1007/s11356-022-19057-6

Yadav, K. K., Gupta, N., Kumar, A., Reece, L. M., Singh, N., Rezania, S., & Ahmad Khan, S. (2018). Mechanistic understanding and holistic approach of phytoremediation: A review on application and future prospects. Ecological Engineering, 120(June), 274–298. https://doi.org/10.1016/j.ecoleng.2018.05.039

Yang, W., Zhao, Y., Wang, D., Wu, H., Lin, A., & He, L. (2020). Using principal components analysis and idw interpolation to determine spatial and temporal changes of Surfacewater quality of Xin'Anjiang river in huangshan, china. International Journal of Environmental Research and Public Health, 17(8), 1–14. https:// doi.org/10.3390/ijerph17082942

Yao, Y., Zhang, M., Tian, Y., Zhao, M., Zeng, K., Zhang, B., Zhao, M., & Yin, B. (2018). *Azolla* biofertilizer for improving low nitrogen use efficiency in an intensive rice cropping system. Field Crops Research, 216(July 2017), 158–164. https://doi.org/10.1016/ j.fcr.2017.11.020