Application of Azolla pinnata in Decentralized Constructed Wetlands for Rural Domestic Wastewater Treatment

Amira M. Awad
Zagazig University
Salah M. Dahdouh
Zagazig University
Mohamed S. Abu-Hashim
Zagazig University
Soha S.M. Mostafa
sohaaasayed@yahoo.com

Agricultural Research Center

Research Article

Keywords: Azolla pinnata, constructed wetlands-VFCW, wastewater phytoremediation, COD, BOD, nutrient recovery, heavy metals removal, rural areas

Posted Date: June 9th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9943104/v1

Additional Declarations: No competing interests reported.

Abstract

This study evaluates the efficiency of *Azolla pinnata* in a vertical flow constructed wetland (VFCW) for decentralized wastewater treatment in rural areas. The experimental program monitored the planted VFCW over a 25-day period. Results demonstrated substantial reductions in chemical oxygen demand (COD) and biological oxygen demand (BOD_5), with maximum removal efficiencies of 78% and 72%, respectively, coinciding with peak plant growth, followed by slight increases due to biomass senescence. Nutrient removal was significant, with orthophosphate ($P\text{-}PO_4^{3-}$) reduced by 90.2%, nitrate nitrogen ($NO_3^-\text{-}N$) by 90.4%, and ammonium nitrogen ($NH_4^+\text{-}N$) by 84.5%. Heavy metals, including Fe, Zn, Cu, B, and Mn, were effectively sequestered, reaching minimum concentrations before minor increases due to plant decomposition. These findings indicate that *A. pinnata* enhances microbial activity, nutrient uptake, and heavy metal stabilization, providing a sustainable, low-cost, and environmentally friendly solution for wastewater treatment in rural settings.

1. Introduction

Vertical flow constructed wetlands (VFCWs) are engineered to replicate natural wetland processes, including sedimentation, filtration, adsorption, and microbial degradation, enabling the removal of organic matter, nutrients, and pathogens from wastewater[1,2]. The efficiency of VFCWs depends on hydraulic retention time, media type, and the presence of vegetation and microbial communities. Studies report removal rates of 60–95% for organic matter and nutrients, and up to 99% for pathogens, highlighting VFCWs as eco-friendly and efficient wastewater treatment systems[2,3].

Decentralized wastewater treatment systems are increasingly preferred in rural and peri-urban areas due to their cost-effectiveness, scalability, and adaptability to small communities lacking centralized infrastructure[4]. Plant-based treatment approaches are particularly advantageous because they combine pollutant removal with low operational costs, natural nutrient assimilation, and enhancement of microbial activity within the system[5,6].

The choice of substrate is critical; sand and gravel mixtures with appropriate grading and porosity enhance hydraulic conductivity, oxygen transfer, and microbial activity, improving pollutant removal[7]. While conventional wastewater treatment methods such as electrocoagulation can be costly and energy-intensive, phytoremediation using aquatic macrophytes offers a sustainable alternative with strong biosorption potential and low operating costs[4,5].

Among macrophytes, *Azolla* species have demonstrated remarkable ability to remove both organic and inorganic pollutants, including nitrogen, phosphorus, heavy metals, and emerging contaminants such as dyes and bisphenol A[8,9,10,11]. *Azolla* is a small, free-floating aquatic fern known for its rapid growth and high nutrient uptake capacity[8,9]. Its ability to assimilate nitrogen, phosphorus, and various heavy metals makes it particularly suitable for phytoremediation applications in constructed wetlands. In addition, the extensive root system of *Azolla pinnata* provides a favorable surface area for microbial colonization, enhancing the biodegradation of organic pollutants and improving overall wastewater treatment efficiency[6,10]. These characteristics justify the selection of *A. pinnata* as a cost-effective, sustainable, and environmentally friendly biological agent for the treatment of agricultural and domestic wastewater in vertical flow constructed wetlands. Effective management of *Azolla* biomass is essential due to potential bioaccumulation, with safe disposal or conversion to bioenergy recommended [12,13]. Moreover, *Azolla* can serve as a nutrient-rich fertilizer when cultivated in uncontaminated water [14,15].

This study aimed to evaluate the efficacy of *Azolla pinnata* in a pilot-scale VFCW for agricultural wastewater treatment, assessing its performance in removing heavy metals and key chemical parameters, and examining the biomass produced to determine the plant's suitability for sustainable phytoremediation.

2. Results

2.1 Effect of *Azolla pinnata* remediation on pH, EC, and Major Ions in Wastewater

The chemical characteristics of wastewater treated with *Azolla pinnata* over a 25-day period are summarized in Table 1. Electrical conductivity (EC) values ranged from 2.95 ± 1.97 dS/m on day 1 to 3.06 ± 2.29 dS/m on day 25, with the lowest value observed on day 15 (1.52 ± 4.98 dS/m).

The pH of the effluent remained stable throughout the experiment, varying between 6.70 ± 4.01 and 7.02 ± 1.17. Major cations exhibited significant temporal variations. Calcium concentrations decreased from 8.24 ± 1.45 meq/L on day 1 to 3.51 ± 8.8 meq/L on day 10, and then varied slightly until day 25.

Table (1): Changes in water Chemical analysis at the outlet after treatment with *Azolla*							
Parameters	**Days**						**F values**
	1	**5**	**10**	**15**	**20**	**25**	
EC(dSm^{-1})	**2.95** ± 1.97^{A}	**2.66** ± **2.01**^{A}	**2.35** ± **1.06**^{A}	**1.52** ± 4.98^{A}	**2.93** ± 1.06^{A}	**3.06** ± **2.29**^{A}	**0.96**^{NS}
pH	**6.96 ± 1.81**^{A}	**6.9 ± 2.4**^{A}	**6.82 ± 2.09**^{A}	**6.70 ± 4.01**^{A}	**7 ± 2.08**^{A}	**7.02 ± 1.17**^{A}	**0.26**^{NS}
Ca^{+2}($meqL^{-1}$)	**8.24 ± 1.45**^{A}	**8.16 ± 2.18**^{A}	**7.35 ± 8.78**^{AB}	**3.51** ± 8.8^{B}	**8.15 ± 9.07**^{A}	**8.41 ± 1.52**^{A}	**1.97**^{NS}
Mg^{+2}($meqL^{-1}$)	**8.20 ± 1.33**^{A}	**7.73 ± 1.95**^{A}	**7.12** ± 9.26^{AB}	**3.06 ± 8.88**^{A}	**8.09 ± 1.51**^{A}	**8.88 ± 1.49**^{A}	**2.13**^{NS}
Na^{+1}($meqL^{-1}$)	**12.07 ± 9.66**^{A}	**9.68 ± 1.54**^{AB}	**8.04 ± 2.3**^{B}	**7.69 ± 1.09**^{B}	**12.07 ± 1.23**^{A}	**12.21 ± 1.46**^{A}	**2.93**^{NS}
K^{+1}($meqL^{-1}$)	**1.08 ± 3.18**^{A}	**1.06** ± 2.54^{A}	**1.02 ± 4.57**^{A}	**1.01 ± 4.25**^{A}	**1.08 ± 5.19**^{A}	**1.11** ± 1.73^{A}	**0.01**^{NS}
HCO^{-} ^{3}($meqL^{1}$)	**15.5 ± 1.89**^{A}	**14.96 ± 2.08**^{A}	**13.94 ± 1.15**^{A}	**6.62 ± 1.25**^{B}	**15.5 ± 3.5**^{A}	**15.51 ± 2.32**^{A}	**2.81**^{NS}
Cl^{-1}($meqL^{-1}$)	**12.54 ± 2.02**^{AB}	**10.34 ± 6.7**^{AB}	**8.37 ± 3.81**^{AB}	**7.36** ± 2.05^{B}	**12.34 ± 1.75**^{AB}	**12.88** ± 1.60^{A}	**1.78**^{NS}
$SO4^{-2}$($meqL^{-1}$)	**1.55 ± 7.18**^{A}	**1.33 ± 1.3**^{A}	**1.22** ± 1.33^{A}	**1.29 ± 4.94**^{A}	**1.55 ± 5.07**^{A}	**2.22 ± 3.62**^{A}	**0.68**^{NS}
Values are means of triplicate reading and standard error (± SE). Different superscripts in the same row indicate significant differences ($P < 0.05$)							

Magnesium followed a similar trend, ranging from 8.20 ± 1.33 meq/L on day 1 to 3.06 ± 8.88 meq/L on day 10. Sodium concentrations decreased from 12.07 ± 9.66 meq/L on day 1 to 7.69 ± 1.09 meq/L on day 15, with a subsequent increase towards the final sampling day.

Potassium levels remained relatively stable, ranging from 1.01 ± 4.25 to 1.11 ± 1.73 meq/L throughout the study period.Among the anions, bicarbonate concentrations decreased from 15.5 ± 1.89 meq/L on day 1 to 6.62 ± 1.25 meq/L on day 10, then gradually increased to 15.5 ± 2.32 meq/L by day 25. Chloride concentrations ranged from 7.36 ± 2.05 meq/L to 12.88 ± 1.60 meq/L over the treatment period. Sulfate concentrations varied slightly, ranging from 1.22 ± 1.33 to 2.22 ± 3.62 meq/L. These results demonstrate the temporal dynamics of both major cations and anions during the 25-day treatment of wastewater using *Azolla pinnata* in a vertical flow constructed wetland system. The data provide a quantitative basis for evaluating the treatment efficiency of this system with respect to ionic composition of the effluent.

2.2 Removal of heavy metals from sewage water using *Azolla pinnata*

The treated sewage water in **Table 2** shows the concentrations of five heavy metals copper (Cu), iron (Fe), boron (B), zinc (Zn), and manganese (Mn) at the system outlet. The lowest concentrations for Cu, B, Fe, Zn, and Mn were observed on day 15, measuring 0.01 ± 2.08 mg L^{-1}, 0.07 ± 2.51 mg L^{-1}, 0.11 ± 4.61 mg L^{-1}, 0.01 ± 1.15 mg L^{-1}, and 18 ± 1.52 mg L^{-1}, respectively. By day 25, concentrations increased slightly due to the atrophy and decay of *Azolla pinnata*. Cu, B, Fe, and Mn levels remained relatively stable among water sources, while Zn showed measurable variation.

Table (2): Changes in treated wastewater heavy metals content

Parameters	Days						F values
	1	5	10	15	20	25	
CU(mgL^{-1})	0.01 ± 2.3^{A}	0.01 ± 6.66^{A}	0.01 ± 4.58^{A}	0.01 ± 2.08^{A}	0.01 ± 1.15^{A}	0.01 ± 2.3^{A}	0.14 ^{NS}
Br(mgL^{-1})	0.08 ± 2.08^{A}	0.08 ± 1.15^{A}	0.08 ± 3.05^{A}	0.07 ± 2.51^{A}	0.08 ± 1.52^{A}	0.08 ± 6.5^{A}	0.02 ^{NS}
Fe(mgL^{-1})	0.85 ± 1.3^{A}	0.21 ± 9.29^{AB}	0.19 ± 2.3^{AB}	0.11 ± 4.61^{B}	0.85 ± 3.54^{A}	0.85 ± 3.9^{A}	4.58^{NS}
Zn(mgL^{-1})	0.02 ± 5.77^{AB}	0.02 ± 2.64^{AB}	0.01 ± 2.08^{B}	0.01 ± 1.15^{B}	0.02 ± 2.08^{AB}	0.02 ± 8.81^{A}	9.97*
Mn(mgL^{-1})	19.17 ± 1.91^{A}	18.01 ± 1.53^{A}	18.01 ± 2.31^{A}	18 ± 1.52^{A}	19.22 ± 5.07^{A}	19.32 ± 3.16^{A}	0.05^{NS}

Values are means of triplicate reading and standard error (± SE). Different superscripts in the same row indicate significant differences (P < 0.05)

2.3 Orthophosphate ($P\text{-}PO_4^{3-}$), nitrate (NO_3^--N) and ammonium (NH_4-N) removal efficiency in the system

The concentrations of phosphate (PO_4) and nitrate (NO_3^--N) in wastewater treated with *Azolla pinnata* are presented in Fig. 1.

Phosphate levels decreased from an initial 1.20 mg/L to 0.60 mg/L at the sampling points, while nitrate nitrogen concentrations declined from 3.70 mg/L to 2.70 mg/L over the treatment period. The reductions in both nutrients correspond with the activity of *Azolla pinnata*, indicating its role in nutrient uptake and assimilation. Phosphate showed moderate variability across sampling points, whereas nitrate nitrogen remained relatively uniform, reflecting consistent nitrogen removal by the plant.

The concentrations of ammonium nitrogen (NH_4-N) in wastewater treated with *Azolla pinnata* over a 25-day period (Fig. 2) decreased from 42.00 mg/L on day 1 to 36.50 mg/L on day 10, reaching a minimum of 35.40 mg/L on day 15. Subsequently, concentrations increased to 38.90 mg/L on day 20 and 41.40 mg/L on day 25.

These results demonstrate that *Azolla pinnata* effectively reduced the concentrations of key nutrients in wastewater, confirming its role in nutrient regulation within the treatment system. The data provide a detailed quantitative account of temporal variations in orthophosphate (P-PO_4^{3-}), nitrate (NO_3^--N), and ammonium (NH_4^+-N), highlighting the dynamic changes in nutrient levels over the course of the treatment period.

2.4 Removal efficiency of *organic pollution* by *Azolla pinnata*

The removal efficiency of organic matter in wastewater treated with *Azolla pinnata* was evaluated over a 25-day treatment period in a vertical flow constructed wetland (Fig. 3). BOD concentrations decreased from 733.0 ± 46.6 mg/L at day 1 to 642.0 ± 68.0 mg/L at day 15, indicating a substantial reduction of organic load. Similarly, COD levels declined from 803.0 ± 31.6 mg/L at day 1 to 697.0 ± 60.9 mg/L at day 15. Both parameters showed a slight increase after day 15, with BOD reaching 725.0 ± 147.2 mg/L and COD 783.0 ± 72.0 mg/L at day 25.

The observed reductions demonstrate the high efficiency of A. *pinnata* in removing organic matter from wastewater within vertical flow constructed wetlands. The maximum decrease at day 15 suggests that this represents the optimal treatment period under the applied experimental conditions, after which senescence or nutrient saturation may lead to a slight rebound in pollutant concentrations. These findings confirm that *Azolla pinnata* effectively removed COD and BOD from wastewater, with an average removal rate of 14.8% and 14.1%, respectively, in the VFCW planted with *Azolla pinnata* for the treatment of wastewater.

3. Discussion

The vertical flow constructed wetland (VFCW) planted with *Azolla pinnata* demonstrated pronounced removal of dissolved ions, nutrients, organic matter, and selected heavy metals over the 25-day experimental period. Electrical conductivity (EC) declined by approximately 48% by day 15, reflecting effective ion removal during the peak growth phase of *A. pinnata*, with a subsequent slight increase attributed to senescence and decomposition of biomass, releasing previously assimilated ions[6,16]. Calcium and magnesium exhibited reductions of 57% and 63%, respectively, indicating efficient cation removal through plant bioaccumulation and adsorption. Sodium decreased moderately, whereas potassium remained stable, demonstrating selective ion uptake by *A. pinnata*[17,18]. Bicarbonate and chloride concentrations exhibited substantial reductions by day 15, whereas sulfate remained relatively stable, with a minor increase observed toward day 25. These patterns reflect differences in anion bioavailability and chemical behavior within the vertical flow constructed wetland (VFCW), in agreement with prior Azolla-mediated studies[19,20]. The initial declines in anion concentrations contributed to the observed decrease in EC, while the subsequent increases after day 15 likely resulted from senescence and decomposition of *A. pinnata* biomass, releasing previously assimilated ions back into the water. Heavy metals are persistent contaminants in wastewater that pose significant environmental and health risks due to their toxicity and potential for bioaccumulation. In the present study, treatment with *Azolla pinnata* effectively stabilized the concentrations of copper (Cu), boron (B), iron (Fe), and manganese (Mn) throughout the experimental period, whereas zinc (Zn) exhibited measurable fluctuations. The lowest concentrations of these metals were generally observed around day 15, followed by minor increases by day 25, likely due to senescence and decomposition of *A. pinnata* biomass, which released accumulated metals back into the water column. These observations corroborate previous findings demonstrating that free-floating macrophytes such as *A. pinnata* are highly effective in phytoremediation, capable of removing substantial amounts of heavy metals within short treatment periods [21,22]. Earlier studies also reported efficient removal of iron and copper from polluted water by *A. pinnata* [23], confirming the species' capacity to uptake, sequester, and stabilize heavy metals in aquatic environments. *Kumar et al.*[17]reported that *Azolla pinnata* effectively accumulated copper (Cu) and other heavy metals in wastewater treatment systems, thereby reducing their bioavailable fractions. *Abdelwahab et al.*[19] demonstrated the removal of Zn, Cu, Ni, and Cr by *A. pinnata*, highlighting its potential for heavy metal remediation across diverse wastewater matrices. Kumar[18] observed significant reductions of heavy metals in industrial wastewater treated with *A. pinnata*, further supporting its phytoremediation efficacy. Additionally, Akhtar *et al.*[20] confirmed the species' capacity to remove copper through adsorption and bioaccumulation mechanisms. Collectively, these studies underscore the suitability of *A. pinnata* as a biological agent for heavy metal stabilization and the enhancement of effluent quality in constructed wetland systems.

Nitrate removal represents a critical component of wastewater treatment due to its potential adverse effects on aquatic ecosystems and human health when present at elevated concentrations. In the present study, *A. pinnata* exhibited a progressive decrease in nitrate ($N\text{-}NO_3^-$) concentrations (Fig. 1), achieving a maximum removal efficiency of 90.4%. This enhanced nitrate removal can be attributed to the physiological characteristics of *A. pinnata*, including its efficient absorption and assimilation of nitrate. The transient increase in ammonium nitrogen ($N\text{-}NH_4^+$) observed on day 15 (Fig. 1) reflects active nitrogen transformation processes, facilitated by the extensive root system of *A. pinnata*, which provides a large surface area for microbial denitrification[6]. Variability in nitrate uptake rates may be influenced by root architecture and oxygen availability within the system[24]. The overall ammonium nitrogen removal efficiency reached 84.5%, indicating effective nitrogen regulation by the VFCW system.

Phosphate ($P\text{-}PO_4^{3-}$) removal in the vertical flow constructed wetland (VFCW) was primarily facilitated through adsorption and precipitation within the filter media, direct uptake by emergent biomass, and accumulation within the plant tissues[25]. Soluble phosphorus moved with the water flow, whereas particulate-bound phosphorus was retained through filtration and interception within the substrate [26]. In this study, *Azolla pinnata* achieved average phosphate removal efficiencies of 89.8% by day 15 and 90.2% by day 25, demonstrating its capacity to regulate phosphorus levels effectively. These results confirm that *A. pinnata* provides a cost-effective and efficient biological strategy for nutrient removal in constructed wetland systems. The primary mechanism underlying organic matter reduction was predominantly microbial, involving aerobic and anaerobic decomposition processes. Sedimentation and filtration between the substrate layers further contributed to organic load attenuation [27,28]. The observed decrease in COD concentrations can be attributed to these biological processes, which are enhanced by microbial colonization facilitated by the extensive root system of *A. pinnata*[29]. The well-developed root network increases surface area for microbial growth and nutrient cycling, while the broad leaves enhance water interaction and gas exchange, promoting microbial activity. These morphological and physiological traits render *A. pinnata* highly adapted to aquatic environments, improving its efficiency in nutrient uptake and phytoremediation. Additionally, environmental factors such as pH and light intensity further influence its nutrient assimilation and overall treatment performance. Previous studies have demonstrated that unplanted constructed wetlands are capable of achieving substantial BOD_5 removal efficiencies, typically ranging from 60% to 90%, depending on system design and hydraulic loading rate [30]. The absence of vegetation in such systems generally limits microbial activity and oxygen transfer, resulting in lower BOD_5 removal compared to planted wetlands [31]. Nonetheless, the present study confirms that unplanted VFCWs can still provide effective BOD_5 attenuation, suggesting their potential as a cost-efficient alternative in specific wastewater treatment scenarios. Similarly, COD removal in unplanted VFCWs has been reported to range from 70% to 98%, depending on wastewater type and system configuration [32, 33]. These reductions are primarily attributed to adsorption and biodegradation mediated by biofilms established on the gravel media. Consequently, unplanted VFCWs represent a viable low-cost technology for the treatment of wastewater with high organic loads. Temporal trends in the current study showed that effluent pH decreased until day 15, reaching a minimum of 6.70 ± 4.01, followed by an increase due to senescence and decomposition of *Azolla pinnata* biomass (Kumar et al., 2022). Electrical conductivity (EC) exhibited a similar pattern, declining to 1.52 ± 4.98 dS/m by day 15 and rising thereafter, reflecting the release of previously assimilated ions from decaying plant material[6]. Nutrient concentrations exhibited patterns consistent with the growth and senescence of *Azolla pinnata*. Orthophosphate (PO_4^{3-}) decreased during the initial 15 days, followed by an increase attributable to phosphorus release from decaying plant tissues [25,26]. Nitrate nitrogen (NO_3^--N) reached a minimum at day 15 and subsequently increased, corresponding with the decomposition of *A. pinnata* and the release of nitrogenous compounds (Kumar et al., 2022; 24). Ammonium nitrogen (NH_4^+-N) concentrations increased around day 15 relative to earlier measurements, indicating nitrogen release from plant biomass[6]. Similarly, heavy metals such as Fe and Zn attained their lowest concentrations near day 15 and rose by day 25, reflecting the liberation of accumulated metals during plant decay[32,33]. These observations confirm that the post-day 15 increases in multiple water quality parameters resulted from the senescence and structural changes in *A. pinnata*, highlighting the necessity of periodic harvesting to sustain optimal treatment efficiency in vertical flow constructed wetlands.

4. Materials and methods

4.1. Experimental design

The *Azolla pinnata* strain utilized in this study was sourced from the Microbiology Department, Soils, Water, and Environment Research Institute (SWERI), Agricultural Research Center (ARC), Giza, Egypt. The *A. pinnata* biomass was introduced on the surface of the soil within the vertical flow constructed wetland (VFCW). Throughout the experiment, water levels were maintained at a consistent height of 5 cm above the soil surface. The experiment was conducted over a period of 25 days, with effluent samples collected at 5-day intervals to assess various water quality parameters. At the conclusion of the study, *Azolla* biomass was harvested from the soil surface, weighed, and subsequently dried in a laboratory oven at 60°C until a constant dry weight was achieved.

2.2. Pilot plant design

A single pilot-scale vertical flow constructed wetland (VFCW) unit was employed in this study. The unit comprised the following main components: a 1 m³ open-top international bulk tank (dimensions: 1 × 1 × 1 m), a water distribution system constructed from PVC pipes, hoses, and elbow fittings, and a PVC drainage system including a vertical aeration pipe. The wetland bed consisted of stratified filling material with layers of coarse gravel, fine gravel, and fine sand. The unit was also equipped with an outlet tap for sample collection, a sampling valve, and a separate valve to regulate the inlet flow rate (Fig. 4).

The cover layer of the wetland bed consisted of 150 mm of gravel with a grain size ranging from 16 to 32 mm. Beneath this, a sand filter layer with a thickness of 300 mm and a grain size of 0–4 mm was placed, followed by a gravel filter layer 200 mm thick with a grain size of 2–8 mm. At the base, a gravel drainage layer 200 mm thick with a grain size of 16–32 mm was installed (Fig. 5).

To ensure accurate and consistent measurements, the experimental protocol adhered to the American Water Works Association Standard Methods for Water and Wastewater[34], with the latest version developed during the course of this study. The experimental program maintained a constant hydraulic loading rate of 150 L/m². Raw wastewater was introduced in equal volumes every four hours, corresponding to 25 L/m² per feeding, six times per day. A primary sedimentation tank served as a constant-head reservoir to regulate a uniform flow rate, with the clarified water collected from this tank and delivered to the wetland unit (Fig. 6).

The raw wastewater was introduced into the sedimentation tank at a rate higher than the requirement of the wetland unit, ensuring a sufficient supply for the system. Any excess wastewater was discharged through a constant-height weir (Fig. 7). Influent and effluent samples from the unit were collected and analyzed for key water quality parameters, including COD and BOD_5, in accordance with the American Water Works Association Standard Methods for Water and Wastewater[34].

2.3. Chemical analysis for parameters and elements

Chemical characterization of the sewage wastewater was conducted to determine pH, electrical conductivity (EC), and the concentrations of major anions, including carbonate (CO_3^{2-}), bicarbonate (HCO_3^-), chloride (Cl^-), and sulfate (SO_4^{2-}), as well as major cations, including calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^+), and potassium (K^+). Nutrient salts, specifically nitrate-nitrogen (NO_3-N) and ammonia-nitrogen (NH_4-N), were also quantified. Trace elements, including copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn), were analyzed according to the Standard Methods for the Examination of Water and Wastewater[35] both before and after inoculation with *Azolla pinnata*.

2.4. Statistical analysis

Statistical analysis was performed on the data obtained using tools such as Statistical Package for Social Scientists, SPSS (version 20) and Microsoft Excel to carry out Duncan multiple regression test (DMRT), least square difference (LSD) and analysis of variance ANOVA at 95% confidence interval ($P < 0.05$).

5. Conclusions

This study demonstrated that a vertical flow constructed wetland (VFCW) planted with *Azolla pinnata* represents an effective and sustainable approach for treating real domestic wastewater. The system exhibited significant improvement in water quality, enhancing the removal of organic matter, as indicated by COD, and key nutrients, including ammonium nitrogen, nitrate nitrogen, and phosphate. The VFCW also contributed to the reduction of selected heavy metals, particularly Fe and Zn, highlighting the role of *A. pinnata* in nutrient uptake and metal accumulation. Treatment efficiency was strongly influenced by the growth stage of *A. pinnata*. Most parameters, including pH, electrical conductivity, phosphate, nitrate, ammonium, Fe, and Zn, showed optimal performance around day 15 of the treatment period. Beyond this stage, increases in these parameters were observed, likely due to plant senescence, biomass decomposition, and the release of previously absorbed nutrients, ions, and metals back into the wastewater. These findings indicate that periodic harvesting of *A. pinnata*, especially around the peak treatment period, is essential to maintain high removal efficiency and prevent secondary pollution. Integrating *A. pinnata* into a VFCW provides a promising, low-cost, and environmentally sustainable technology for domestic wastewater treatment, particularly suitable for rural, peri-urban, and small- to medium-scale applications.

Declarations

Author Contributions

Conceptualization was performed by S.S.M.M., M.S.A-H., S.M.D., and A.M.A. Data sampling, analysis, and investigations were conducted by S.S.M.M. and A.M.A. The original draft of the manuscript was prepared by S.S.M.M., M.S.A-H., and S.M.D. Review and editing of the manuscript were finalized by S.S.M.M., M.S.A-H., S.M.D. and A.M.A. Supervision and funding acquisition were provided by S.S.M.M., S.M.D., and

M.S.A-H. The final version of the manuscript was revised by S.S.M.M. and M.S.A-H. All authors have read and approved the published version of the manuscript.

Funding:

no funding was received for this study. Not applicable for grant number.

Author Contribution

Author Contributions: Conceptualization was performed by S.S.M.M., M.S.A-H., S.M.D., and A.M.A. Data sampling, analysis, and investigations were conducted by S.S.M.M. and A.M.A. The original draft of the manuscript was prepared by S.S.M.M., M.S.A-H., and S.M.D. Review and editing of the manuscript were finalized by S.S.M.M., M.S.A-H., S.M.D. and A.M.A. Supervision and funding acquisition were provided by S.S.M.M., S.M.D., and M.S.A-H. The final version of the manuscript was revised by S.S.M.M. and M.S.A-H. All authors have read and approved the published version of the manuscript.

Acknowledgement

The authors gratefully acknowledge the Soils, Water and Environment Research Institute (SWERI), Agricultural Research Center (ARC), Egypt, for providing the infrastructure, technical support, and institutional facilities that enabled this study, particularly through its expertise in pilot-scale studies and research on Azolla pinnata in constructed wetland systems. The authors also thank the Department of Soil Science, Faculty of Agriculture, Zagazig University, Egypt, for providing access to the decentralized water purification system and assistance in establishing the applied experimental trials.

Data Availability

All data generated or analyzed during this study are included in this published article and its supplementary files. Additional datasets are available from the corresponding author upon reasonable request.

References

1. Vymazal, J. Removal of nutrients in various types of constructed wetlands. *Sci. Total Environ.***380**, 48–65 (2007).
2. Wu, H., Zhang, J., Ngo, H.H., Guo, W., Hu, Z., Liang, S., Fan, J. & Liu, H. A review on the sustainability of constructed wetlands for wastewater treatment: Design and operation. *Bioresour. Technol.***175**, 594–601 (2015).

3. Babatunde, A.O., Zhao, Y.Q., Zhao, X.H. & Li, X.M. Constructed wetlands for environmental pollution control: a review of developments, research and practice in Ireland. *Environ. Sci. Pollut. Res.* **17**, 531–551 (2010).
4. Taghilou, S., Peyda, M. & Mehrasbi, M. A review on the significance of Azolla for water and wastewater treatment. *Desal. Water Treat.* **293**, 138–149 (2023). https://doi.org/10.5004/dwt.2023.29528
5. Choudhury, A.T.M.A. & Kennedy, I.R. Phytoremediation: Using plants to clean up soils and water contaminated with toxic metals and other pollutants. *Appl. Environ. Microbiol.* **87**, e01068-21 (2021).
6. Muvea, F., et al. Nutrient removal efficiency by floating macrophytes; *Lemna minor* and *Azolla pinnata* in a constructed wetland. *Glob. J. Environ. Sci. Manag.* **5**, 415–430 (2019).
7. Chen, Z., Chen, Z. & Yu, W. Effects of porous media properties on the removal of organic matter and nitrogen. *Water Sci. Technol.* **76**, 1305–1312 (2017).
8. Kochi, L.Y., Freitas, P.L., Maranho, L.T., Juneau, P. & Gomes, M.P. Aquatic macrophytes in constructed wetlands: A fight against water pollution. *Sustainability* **12**, 9202 (2020). https://doi.org/10.3390/su12219202
9. Nedjimi, B. Phytoremediation: a sustainable environmental technology for heavy metals decontamination. *SN Appl. Sci.* **3**, 286 (2021). https://doi.org/10.1007/s42452-021-04301-4
10. Jayasundara, P. Wastewater treatment by Azolla: A review. *Diyala Agric. Sci. J.* **14**, 40–46 (2022). https://doi.org/10.52951/dasj.22140105
11. Prabakaran, S., Mohanraj, T. & Arumugam, A. A state-of-the-art review on the environmental benefits and prospects of Azolla in biofuel, bioremediation and biofertilizer applications. *Ind. Crops Prod.* **183**, 114942 (2022). https://doi.org/10.1016/j.indcrop.2022.114942
12. Rai, P.K. Phytoremediation of heavy metals in contaminated soil and water: Recent developments. In *Environmental Bioremediation Technologies*, 99–122 (Springer, Cham, 2020).
13. Thakur, I.S. & Kumar, M. Phytoremediation approaches for heavy metals and organic pollutants: A sustainable solution for environmental remediation. *Curr. Pollut. Rep.* **7**, 160–171 (2021).
14. Shawky, S.M., Mostafa, S.S.M. & AbdEl-All, A.A.M. Efficacy of some algal species, Azolla and compost extract in controlling root knot nematode and its reflection on cucumber growth. *Bull. Fac. Agric., Cairo Univ.* **60**, 443–459 (2009).
15. Bhuvaneshwari, S., Saravanan, A., Karthikeyan, R., Vinosha, M., Senthilkumar, K., Subramanian, S. & Sivamani, S. Azolla: A potential biofertilizer for sustainable agriculture. *Agronomy* **12**, 874 (2022).
16. Osei, R.A., Abagale, F.K. & Konate, Y. Exploitation of indigenous bamboo macrophyte species and bamboo biochar for faecal sludge treatment with constructed wetland technology in the Sudano-Sahelian ecological zone. *Water* **8**, 12 (2022).
17. Kumar, V., Kumar, P., Singh, J. & Kumar, P. 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. *Int. J. Phytoremediation* **22**, 392–403 (2020a).

18. Kumar, V., Kumar, P., Singh, J. & Kumar, P. 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. *Int. J. Phytoremediation***22**, 392–403 (2020b).
19. Abdelwahab, N., Faysal, A., Shaban, M., Amin, A.A. & Hamed, S.M. Green remediation strategy using *Azolla pinnata*: Heavy metals removal and wastewater ultra-purification. *Egypt. J. Aquat. Biol. Fish.***29**, 17–38 (2025).
20. Akhtar, M.S., Aslam, S., Ditta, A., Albalawi, B.F.A., Oki, Y. & Nakashima, Y. Interspecific variability in growth characteristics and phytoremediation of Cu by free-floating Azolla macrophytes. *Sustainability***15**, 497 (2022).
21. Divya, S., Richa, G. & Archana, T. Potential of duckweed (*Lemna minor*) for removal of lead from wastewater by phytoremediation. *J. Pharm. Res.***5**, 1578–1582 (2012).
22. Nuzhat, S., Ashok, K.P., Azra, N.K. & Basharat, M. Heavy metal accumulation by *Azolla pinnata* of Dal Lake ecosystem, India. **1**, 8–12 (2015).
23. Jain, S.K., Vasudevan, P. & Jha, N.K. Removal of some heavy metals from polluted water by aquatic plants: Studies on duckweed and water velvet (*A. pinnata*). *Biol. Wastes***28**, 115–126 (1989).
24. Nyameasem, J. Diverse forage production systems and their potential for greenhouse gas mitigation. Ph.D. dissertation, Ghana (2021).
25. Yang, Y., et al. Influence of application of manganese ore in constructed wetlands on the mechanisms and improvement of nitrogen and phosphorus removal. *Ecotoxicol. Environ. Saf.***170**, 446–452 (2019).
26. Thalla, A.K., et al. Performance evaluation of horizontal and vertical flow constructed wetlands as tertiary treatment option for secondary effluents. *Appl. Water Sci.***9**, 1–9 (2019).
27. Carranzo, I.V. Standard methods for examination of water and wastewater. In *Anales de Hidrología Médica*, Universidad Complutense de Madrid (2012).
28. Zhu, T., et al. Comparison of performance of two large-scale vertical-flow constructed wetlands treating wastewater treatment plant tail-water: Contaminants removal and associated microbial community. *J. Environ. Manag.***278**, 111564 (2021).
29. Krishna, M.M., et al. Wastewater treatment from dairy farm by using *Azolla pinnata*. *Pharma Innov. J.*, 1190–1193 (2022).
30. Ji, G., Li, W., Li, S., Xu, X., Li, X., Zhang, J. & Liu, Y. Influence of hydraulic loading rate and plant species on COD and BOD removal in a constructed wetland. *J. Environ. Manag.***222**, 291–298 (2018).
31. Gao, S., Xu, D., Sun, X., Zeng, C., Chen, X. & Liu, X. Effects of vegetation on microbial communities in constructed wetlands for wastewater treatment. *Sci. Total Environ.***694**, 133590 (2019).
32. Korkusuz, E.A., Ayaz, S.C., Alkan Uçkun, N. & Tünay, O. Domestic wastewater treatment with VFCWs: A review. *Environ. Sci. Pollut. Res.***26**, 25768–25784 (2019). https://doi.org/10.1007/s11356-019-05687-8

33. Zhang, L., Wang, X., Zhang, Y., Li, Y. & Dong, R. Performance and microbial community of livestock wastewater treatment: Effect of hydraulic loading rate and unplanted period. *Bioresour. Technol.* **271**, 56–64 (2019). https://doi.org/10.1016/j.biortech.2018.09.130
34. American Water Works Association. *Standard Methods for the Examination of Water and Wastewater*, 23rd ed. American Public Health Association, American Water Works Association, Water Environment Federation (2017).
35. APHA. *Standard Methods for the Examination of Water and Wastewater*, 14th ed. American Public Health Association, Washington, D.C. (1995).

Figures

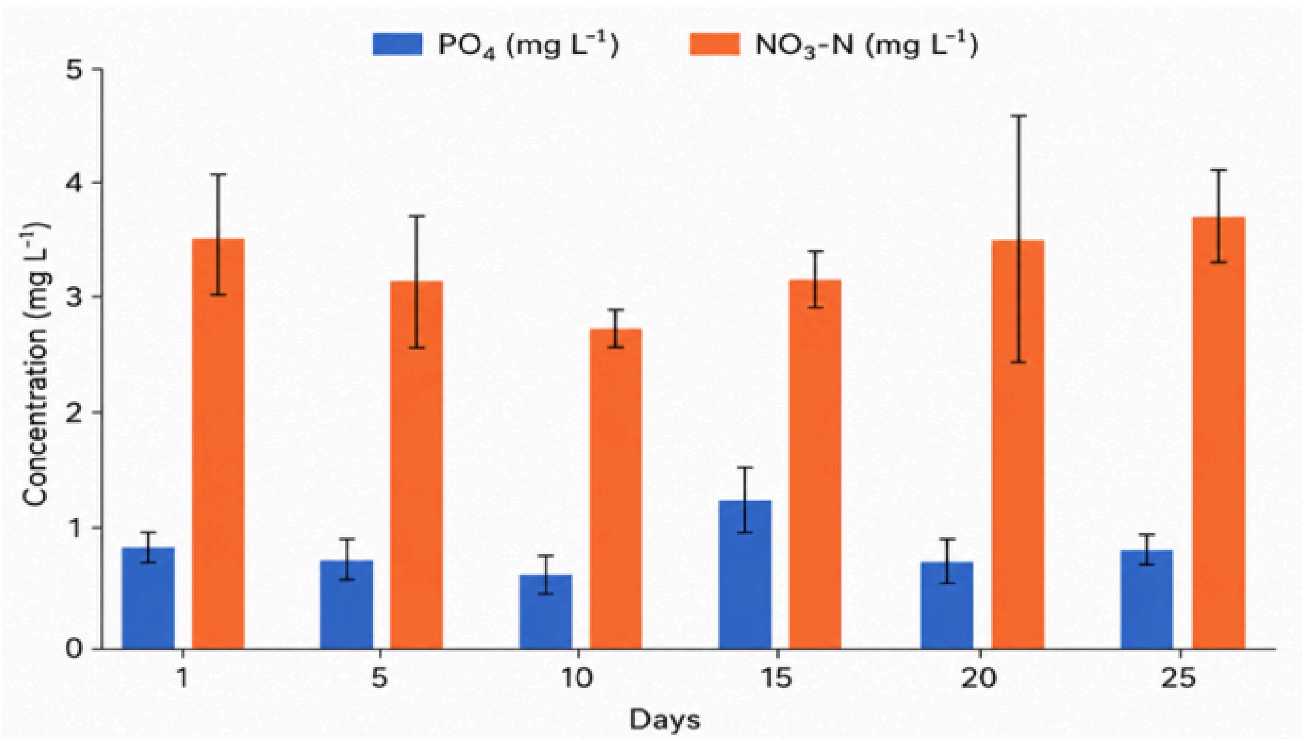

Figure 1

Impact of *Azolla* on nitrate (NO_3-N^-) and phosphate (PO_4-P) removal

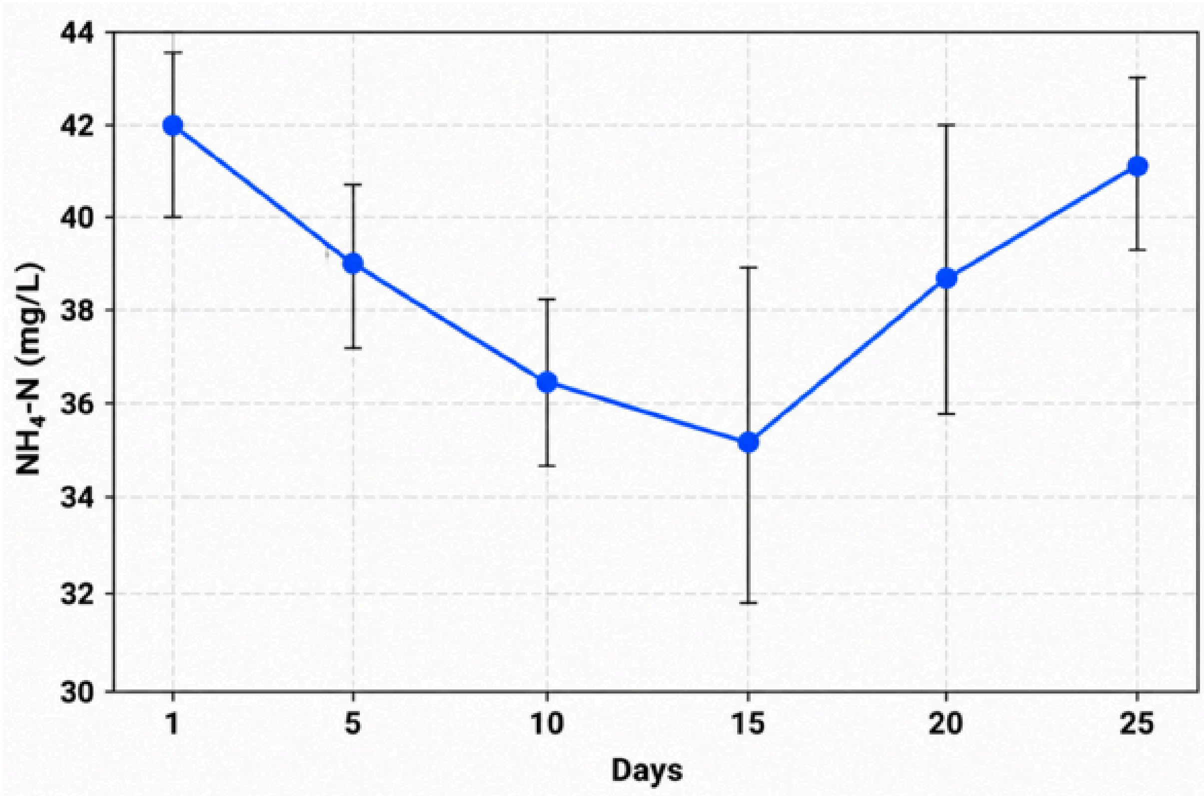

Figure 2

Impact of *Azolla* on ammonium (NH_4-N^-) removal

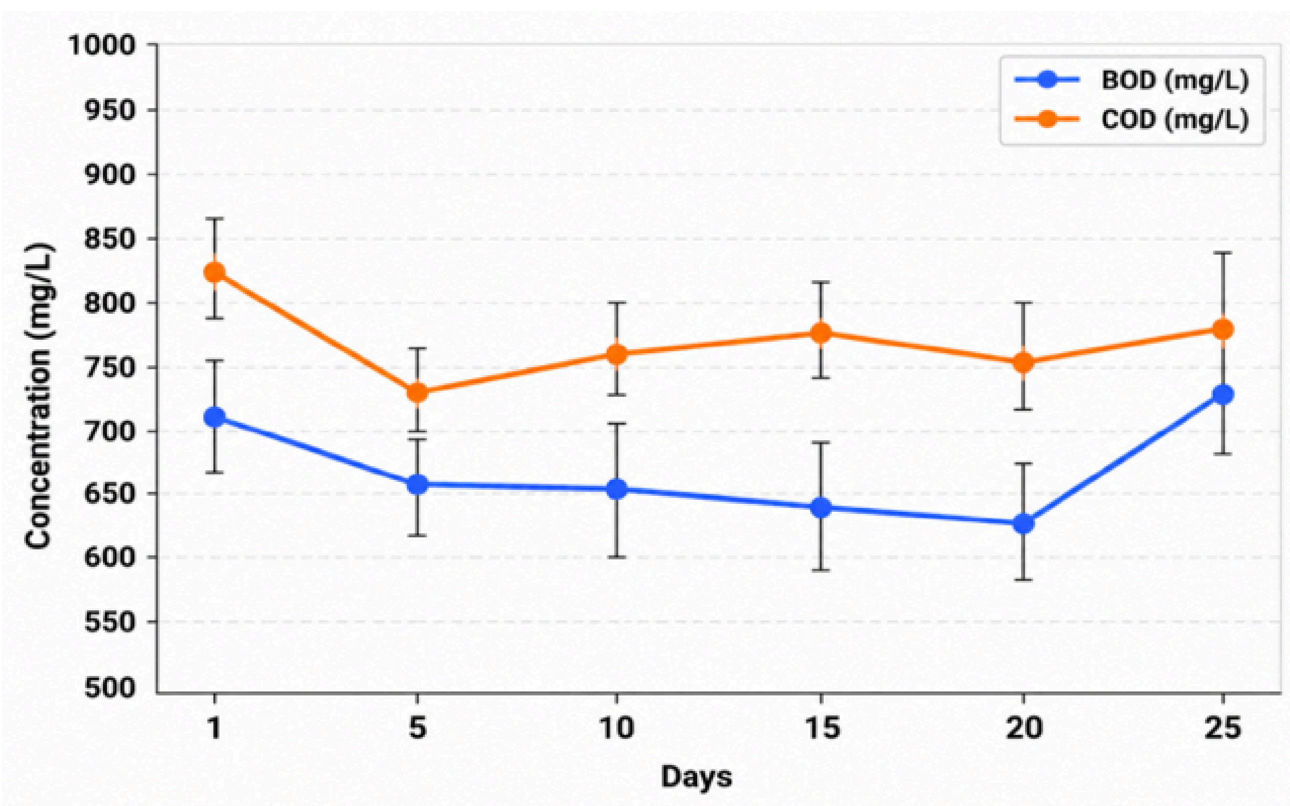

Figure 3

Removal Efficiency of BOD and COD in Wastewater Using *Azolla pinnata* in a Vertical Flow Constructed Wetland System (VFCW)

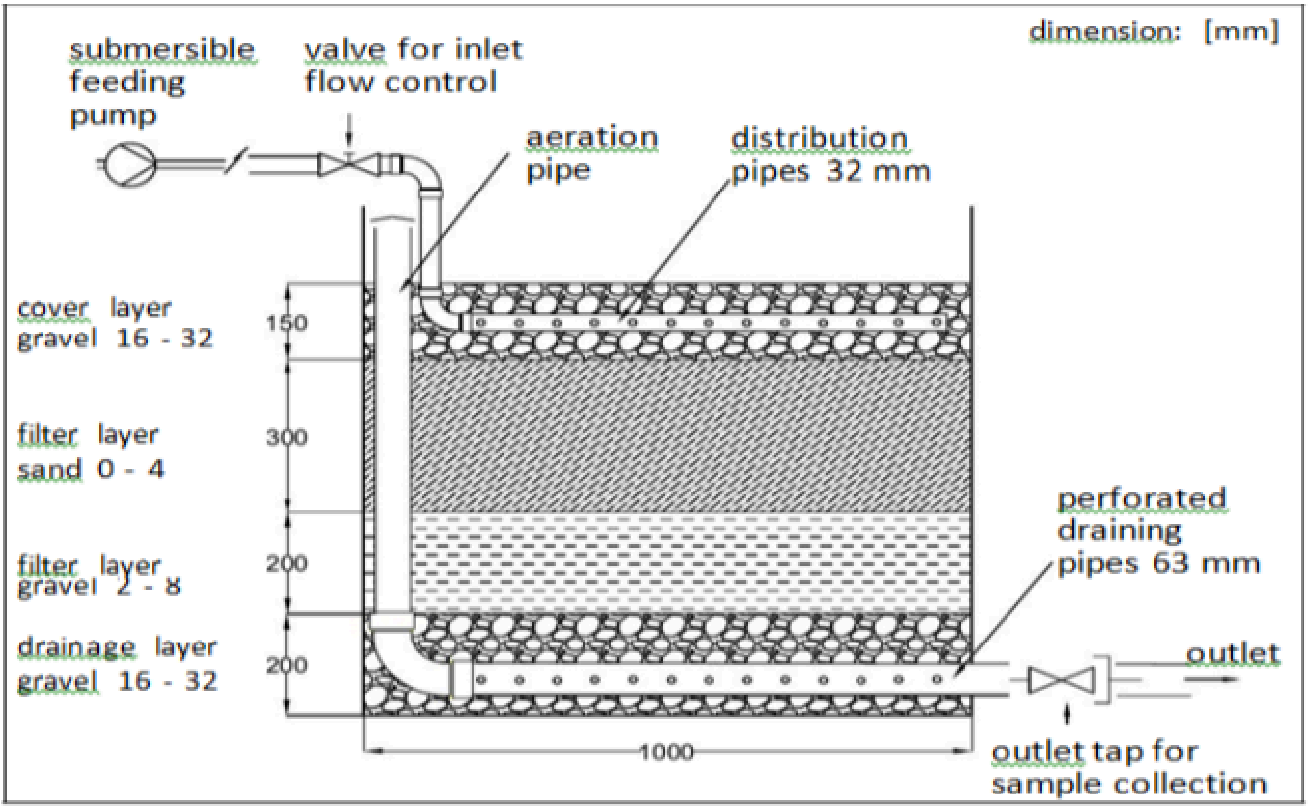

Figure 4

Technical drawing illustrating the cross section of a vertical filter unit. Shown filter layers, the drainage system, the distribution system and the vertical aeration pipe.

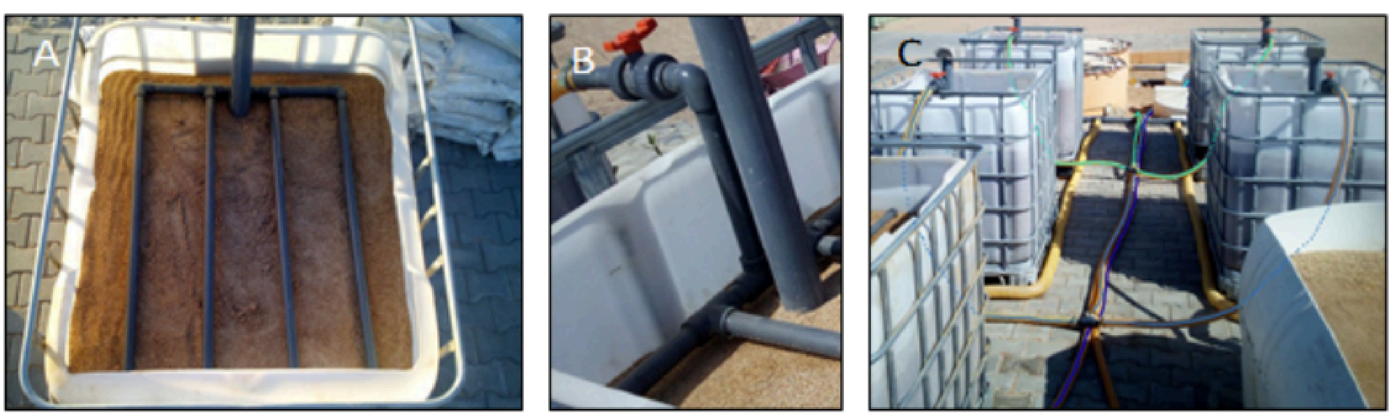

Figure 5

VFCW Units materials and method of installation

Figure 6

Wastewater source in Alqenayat wastewater treatment plant, Zagazig, Sharqia Governorate , Egypt.

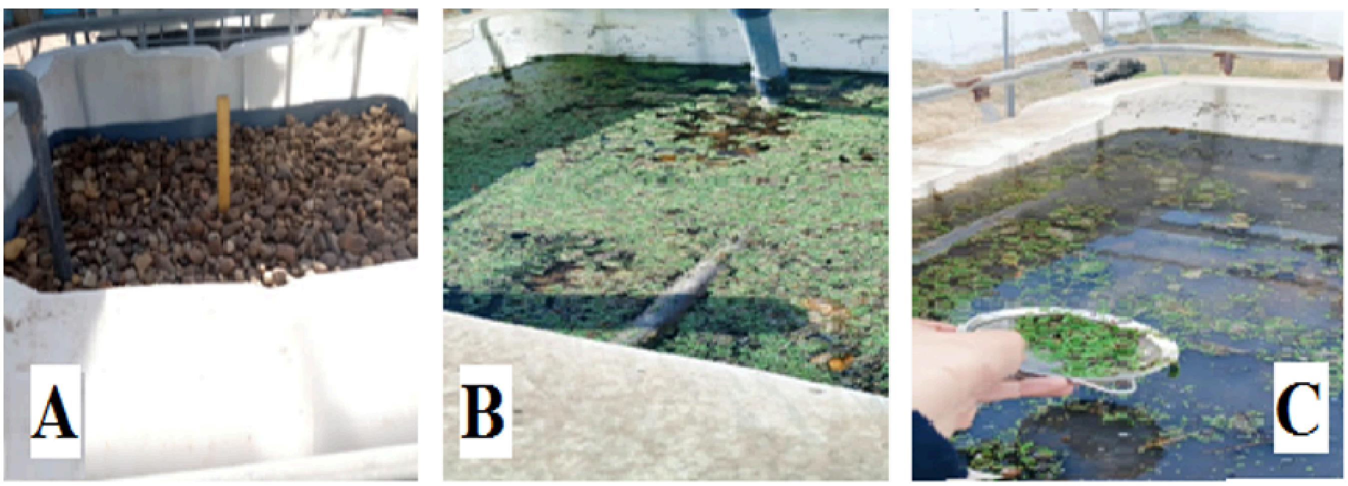

Figure 7

Growth and development of *Azolla pinnata* in the constructed wetland unit: **(**A) Wetland unit prior to *A. pinnata* application; (B) *Azolla pinnata* coverage after 15 days; (C) *Azolla pinnata* harvesting after 25 days