



Research Article

The Potential Application of Diatom-aided Constructed Floating Wetlands for Domestic Wastewater Treatment

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Abstract

Urbanization and industrialization have resulted in the generation of a large quantity of wastewater. The substantial amount of resources and capital requirement is driving the growing demand for sustainable, eco-friendly, and cost-effective treatment systems. In this context, extensive research is underway on Constructed Floating Wetlands (CFWs) for wastewater treatment. The present study attempts to treat real-domestic wastewater using diatoms and four different macrophytes, including *Eichhornia crassipes*, *Salvinia molesta*, *Lemna minor*, and *Pistia stratiotes*. Chemical oxygen demand (COD), phosphates, and total nitrogen (TN) were monitored to assess the ability of individual plants, diatoms, and their combinations to treat wastewater. COD, phosphate, and TN removal efficiencies varied from 56.4% to 86.5%, 64.8% to 99.1%, and 85% to 96.2% respectively, with the plants and their combination with diatoms. A 4-fold increase in wet biomass of the seeded plants and their roots was observed. This increased biomass and root length indicates the acclimatization of plants to the wastewater. The plant biomass produced during the treatment can be a potential substrate for bioenergy and bio-fertilizer production.

Keywords: Acclimatization, Constructed floating wetlands, Diatoms, Domestic wastewater treatment, Macrophytes

1 Introduction

Wastewater generation and its subsequent treatment for pollutant removal have become challenging in developing countries. Only 20% of wastewater generated is treated globally, while 80% is discharged directly into the environment without proper treatment. In the case of middle- to low-income countries, 62–92% of the generated wastewater is discharged without treatment [1].

In countries with large populations and poor sanitation, the discharge of untreated wastewater is one of the main causes of the spread of waterborne diseases. Continued research concerning removing pollutants from wastewater has led to the development of various physico-chemical and biological processes. Conventional treatment technologies such as activated sludge processes are energy intensive with limited nutrient removal capacity. They also need high capital for their construction, operation and maintenance,



particularly in remote areas [2]. Recently, constructed wetland (CW) treatment (phytoremediation) and phycoremediation are being investigated for their utilization in wastewater treatment [3]–[5]. Phytoremediation with constructed wetlands for wastewater treatment works on the natural treatment processes. They are widely adapted due to their cost-effectiveness, adaptability, efficient nutrient removal, long-term sustainability, habitat for a large number of species, additional aesthetic values, and other environmental benefits [2], [6]–[9]. Constructed floating wetlands (CFWs) are one of the categories of constructed wetlands which use rooted plants (macrophytes) growing on the top of the water (free-floating on the mat) rather than being rooted in the slit. These systems have an added advantage to withstand water flow fluctuations [10], [11]. CFWs have better treatment efficiency compared to conventional ponds [9]. Macrophytes accumulate, transform, and degrade the pollutants in the wastewater [2]. The dense roots of the macrophytes help them to bioaccumulate pollutants and provide more surface area for bacterial biofilm formation and pollutant adsorption [10]. The factors to be considered while selecting plants for treatment include climatic conditions, type of wastewater to be treated, and target pollutants to be degraded or removed [12].

Many studies have been conducted globally to understand hydrophytes' potential for treating different wastewater through CFWs. Research has been conducted to assess the feasibility of CFWs for the treatment of stormwater, urban runoff, improving the water quality of lakes and rivers, agricultural wastewater, aquaculture effluent, and primary or secondary effluents of industries, with only a few studies targeting raw domestic wastewaters [6], [13]. Prajapati *et. al.*, [9] have assessed the performance of *Phragmites australis*, *Azolla filiculoides*, *Pistia stratiotes*, *Lemna minor*, and *Lactuca sativa* plants in a floating wetland system for treatment of domestic wastewater and reported that *Pistia stratiotes* shown the maximum nutrient removal. The subsurface and the root zones of the CFWs can host different microalgal and bacterial species. The microalgae present in the subsurface zone can increase the overall pollutant removal efficiency. The present work assesses the ability of four different macrophytes i.e., *Eichhornia crassipes*, *Salvinia molesta*, *Lemna minor*, and *Pistia stratiotes* to remove organic carbon and nutrients from real domestic wastewater. Further, the advantages of algae-assisted macrophytes over

macrophytes alone for the removal of pollutants from domestic wastewater were investigated.

2 Materials and Methods

2.1 Reactors configuration and wastewater

Two batch experiments were conducted in glass reactors with dimensions 2 ft × 1.5 ft × 1.5 ft (L×W×H) and a working volume of 100 L each. To avoid external light interference, the reactor side walls were wrapped with aluminum foil.

For this study, real wastewater collected from the collection tank of an activated sludge-based sewage treatment plant (STP) on the campus of the Indian Institute of Technology Hyderabad (IITH) was used. The collected wastewater was mixed and filtered through 2.36 mm prior to its use in the test reactors. The pH of the wastewater fed into the reactors in the first and second batch experiments was 7.1 and 6.8, respectively. The COD, phosphate, and TN values of wastewater fed into reactors during the first and second batch experiments were 99.1, 11.6, 24.6 mg/L, and 128, 10, and 16.6 mg/L, respectively.

2.2 Hydrophytes and culture

Primarily, the study investigated four individual hydrophytes' ability to treat domestic wastewater. The hydrophytes used in this study were *Eichhornia crassipes*, *Salvinia molesta*, *Lemna minor*, and *Pistia stratiotes*. The *Eichhornia crassipes*, *Salvinia molesta*, and *Pistia stratiotes* macrophytes used in the study were purchased from a nursery, while *Lemna minor* was collected from a lake near IITH campus. The factors considered while selecting these species for the study included: 1. their suitability under the Indian condition, 2. their availability in the natural water environment, and 3. their biomass production potential, which makes the end products of the treatment a valuable raw material for bioenergy production and agricultural activities. The diatom seed used for this study was initially collected from the Manjeera Reservoir located in the Sangareddy District of the State of Telangana, India, and was cultured in the laboratory before inoculation.

2.3 Experimental methodology

During the first stage, which lasted for 30 days, experiments were conducted to assess the ability of individual hydrophytes to remove COD, phosphate,

and TN from the wastewater. The sub-surface zone of the test reactors was continuously mixed using a 12 W submersible pump with a water flow capacity of 400 L/h. In the second set of experiments, heterotrophic macrophytes (50 g *Lemna minor*, 100 g *Pistia stratiotes*, 150 g *Salvinia molesta*, and 150 g *Eichhornia crassipes*), and Diatom-assisted heterotrophic macrophytes, were investigated for COD, phosphate and TN removal. Total chlorophyll, biomass production, and root length were monitored during the study period. Figure 1 shows the schematic of the experimental setup, while Figure 2 shows the photos of the reactors. The experiments were conducted in an open area under natural sunlight. The side walls of the reactors were covered with aluminum foil to allow sunlight to enter only from the top. In the second stage of the experiment, the advantages of diatom additions to constructed floating wetlands for wastewater treatment were assessed. During this batch experiment, two 10-W, 2-ft blue LED strips were used as a source of illumination, which provided a light intensity of 440 $\mu\text{mol}/\text{m}^2/\text{s}$ in the diatom-aided reactors.

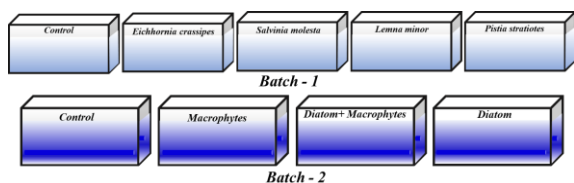


Figure 1: Schematic representation of the experimental setup; Floating treatment wetlands (FTWs) batch reactors used in the present study Batch 1- Control and individual macrophytes, Batch 2- Control, heterotrophic macrophytes (M), diatom-aided heterotrophic macrophytes (M+D), and diatom (D) reactor.

2.4 Analytical methods

All the chemical reagents used were analytical grade purchased from the Merck group. The samples' COD was analyzed using the closed reflux method [14]. Phosphate concentration was analyzed using the 930 compact Ion Chromatography (Make: Metrohm). The TN was measured using a TOC-TNM-L analyzer (Make: Shimadzu). Total chlorophyll was analyzed using the acetone spectrophotometric method (Make: Lab India, UV/VIS 3000+) [14]. During the study, the wet weight of biomass was measured gravimetrically. The plant root growth was measured in length using a graduated scale.

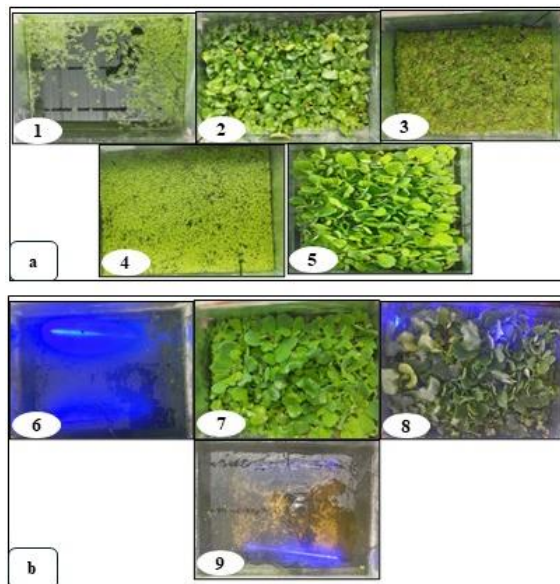


Figure 2: (a) Batch 1 reactors (1- Control, 2- *Eichhornia crassipes*, 3- *Salvinia molesta*, 4- *Lemna minor*, 5- *Pistia stratiotes*), (b) Batch 2 reactors (6- Control, 7- M, 8- M+D, 9- D).

2.5 Statistical analysis

One-way Analysis of Variance (ANOVA) was used to evaluate the efficacy of various treatment combinations for the removal of COD, phosphate, and TN. ANOVA and post-hoc Tukey's honestly significant difference (Tukey's HSD) tests were conducted at a significant level of 0.05 using SPSS Statistics software (Version 30.0.0.0 (172)).

3 Results and Discussions

3.1 Performance of Macrophytes for COD, Phosphates, and Total Nitrogen Removal

The COD removal profile during the 30 days of the first batch study is shown in Figure 3(a). After 30 days, *Eichhornia crassipes* showed the highest COD removal efficiency of 78.3%, while *Lemna minor* showed the lowest COD removal efficiency of 56.4% among the four macrophytes investigated. COD removal efficiencies of 67.9% and 71.3% were observed in *Salvinia molesta* and *Pistia stratiotes* reactors, respectively. A similar COD removal efficiency of 78% has been reported by Gabr *et. al.*, [12] during wastewater treatment in a pilot-scale reactor with *Pistia stratiotes* plants. The average COD

removal reported in their study with different plants in FTW varied between 61% and 80%. In another study by Sayanthan *et al.*, [15] treating domestic wastewater, COD removal efficiency of 89.9% and 89.1 % has been reported with *Pistia stratiotes* and *Eichhornia crassipes*. The maximum COD removal rates obtained for *Eichhornia crassipes*, *Salvinia molesta*, *Lemna minor*, and *Pistia stratiotes* macrophytes were 3.04, 2.72, 2.27, and 3.0 mg/L/day, respectively. COD removal in the control reactor exposed to atmospheric air was observed to be 35.9%. This reduction may be attributed to bacteria present in the wastewater and the microalgal growth observed in the reactor. In a similar study, Bauer *et al.*, [16] also reported microalgae growth in control reactors. Domestic wastewater contains organic pollutants, including nutrients. The presence of these nutrients, along with light exposure, creates favorable conditions that result in the proliferation of microalgae in control reactors. Solar photo-oxidation can also lead to an improvement of water quality in the control reactor [16].

The second batch experiment was conducted to investigate the effect of heterotrophic macrophytes (M), Diatom-aided heterotrophic macrophytes (M+D), and diatoms (D) alone for the degradation of COD, phosphates, and TN from wastewater. During the second batch study, all the reactors were illuminated with blue lights to aid diatom growth. The temperature in the reactors varied between 22 °C and 24 °C. In the light-illuminated reactors, the temperature was observed to increase by 1.5 to 2 °C. Figure 3(b) illustrates the COD removal profile observed during the second batch study. The COD removal efficiency of the control reactor in the second batch experiments was higher than the COD removal observed with the control reactor in the first batch. Furthermore, the addition of light has resulted in higher algal growth in the second batch control reactor, contributing to enhanced removal. A higher COD removal efficiency of 83.2% and a maximum COD removal rate of 8.83 mg/L/day were observed in the reactors with heterotrophic macrophytes compared to those with individual macrophytes, suggesting the advantages of heterotrophic macrophytes over individual macrophytes for wastewater treatment. Adding diatoms to heterotrophic macrophytes (M+D reactor) further increased the COD removal efficiency and maximum COD removal rate. The M+D reactor showed the highest COD removal efficiency of 86.5% and a maximum COD removal rate of 8.93 mg/L/day. Reactors with only diatoms (D) showed a COD

removal efficiency of 76.9% and a maximum removal rate of 7 mg/L/day. In both the first and second-batch studies, one-way ANOVA for COD removal showed that the reactor performance has a statistically significant difference from the control reactor with p -value < 0.0037 . The COD removal efficiency obtained in the present study was slightly higher than the reported literature. For instance, Prajapati *et al.*, [9] reported COD removal efficiency in the range of 50 and 57% for plants such as *Phragmites australis*, *Azolla filiculoides*, *Pistia stratiotes*, *Lemna minor*, and *Lactuca sativa*. In another study, the COD removal efficiencies of 47.2% to 65% for primary influent and 45.6% to 76.8% for secondary influent have been reported by Ali *et al.*, [17] in treating domestic wastewater using CFWs. The primary cause of COD removal in macrophyte reactors is due to phytoremediation [15]. Bacterial activity in the root zones of macrophytes also contributes to the effective degradation of pollutants from wastewater.

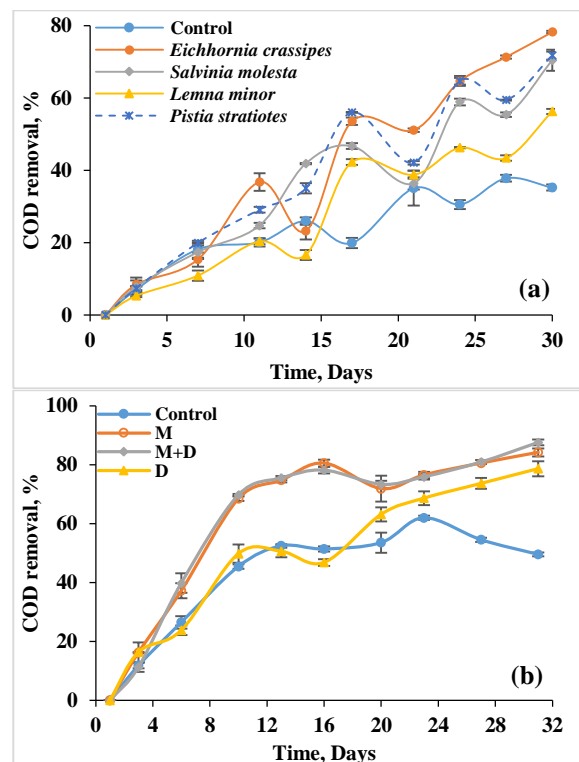


Figure 3: COD removal profile during the study period. (a) COD removal rate in control and individual macrophytes, (b) COD removal rate in control, M, M+D, and D reactor.

The phosphate removal profile in the batch reactors during the study is presented in Figure 4. In the first batch experiments with macrophytes, phosphate removal efficiencies were 65.2, 99.9, 88.2, 64.9, and 71.4% for control, *Eichhornia crassipes*, *Salvinia molesta*, *Lemna minor*, and *Pistia stratiotes*, respectively (Figure 4(a)). The phosphate removal in *Eichhornia crassipes*, *Salvinia molesta*, and *Pistia stratiotes* was significantly higher (p -value < 0.001) than in the control. Meanwhile, no significant difference (p -value = 0.216) was observed between *Lemna minor* and the control. In the present study, similar to COD removal, *Eichhornia crassipes* showed the maximum phosphate removal, while *Lemna minor* showed the lowest during 30 days of reactor operation. Gabr *et al.*, [12] have reported a phosphorus removal of 89% while treating the wastewater with *Pistia stratiotes* plants. In the second set of batch experiments, phosphate removal efficiencies for control, M, M+D, and D reactors were observed to be 52.2, 99.1, 98.8, and 98.8%, respectively (Figure 4(b)). No significant variation in phosphate removal was observed among the three reactors (M, M+D, and D). However, a significant difference (p -value < 0.05) was observed in phosphate removal between control and the M, M+D, and D reactors. Very high phosphate removal has been obtained in the present study when compared with other studies assessing phosphate removal in CFWs. For instance, Ali *et al.*, [17] observed a phosphate removal of 36% and 64% for *Phragmites australis* and *Iris pseudacorus*, and 35% and 63% removal for *Azolla filiculoides* and *Lemna minor*.

TN removal efficiencies were 51.5, 96.2, 94.7, 89.5, and 85%, for control, *Eichhornia crassipes*, *Salvinia molesta*, *Lemna minor*, and *Pistia stratiotes*, respectively (Figure 5(a)). In the present study, similar to COD and phosphate removal, *Eichhornia crassipes* showed maximum TN removal. During the second batch study, the maximum TN removal efficiencies for the control, M, M+D, and D reactors were observed to be 82.87, 95.22, 93.93, and 85.22% respectively. In a study by Moortel *et al.*, [18], a TN removal efficiency of 42% has been reported for wastewater treatment with FTW. In another study by Rigotti *et al.*, [19], TN removal efficiency has been observed in control reactor treating synthetic wastewater. Bauer *et al.*, [16] and Gao *et al.*, [20] also reported a slightly higher TN removal efficiency in the control reactors, which was attributed to the development of microalgae. One-way ANOVA for TN removal showed that all macrophytes had a

significant difference (p -value < 0.0001) from the control reactor. In the case of TN removal during the second batch study, the M and M+D reactors showed significant differences (p -value < 0.02) from the D reactor.

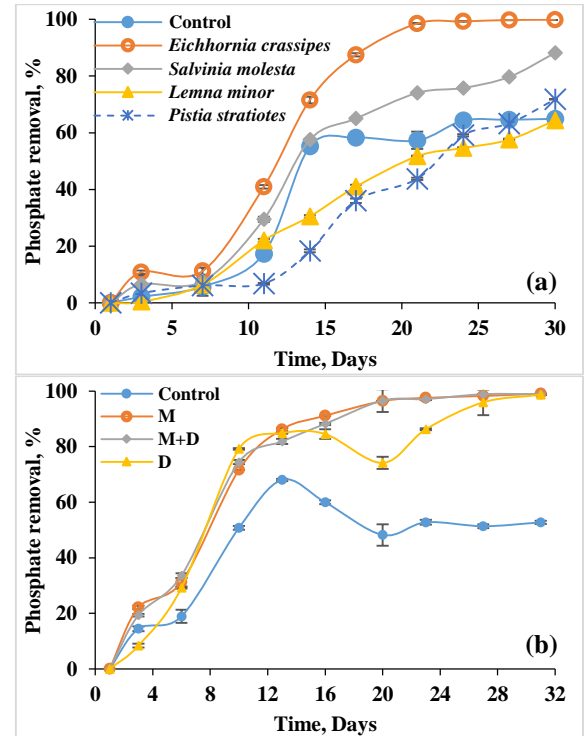


Figure 4: Phosphate removal during the study period. (a) phosphate removal efficiency in control and individual macrophytes, (b) phosphate removal efficiency in control, M, M+D, and D reactor.

3.2 Biomass production, root growth, and total chlorophyll

The wet biomass and root growth of the plants were measured during the study. The changes in biomass and root growth are presented in Figure 6(a) and (b), and Figure 7, respectively. The *Salvinia molesta* plants produced higher biomass among the four species investigated. *Eichhornia crassipes*, which showed higher COD and phosphate removal, produced lower biomass than *Salvinia molesta*. The biomass produced by *Eichhornia crassipes* was 6.4 times higher than the initial wet weight added at the beginning of the experiment. *Lemna minor* and *Pistia stratiotes* wet biomass increased by 7 and 5.7 times the initial weight added into the reactors at the beginning of the experiments when used individually for wastewater treatment.

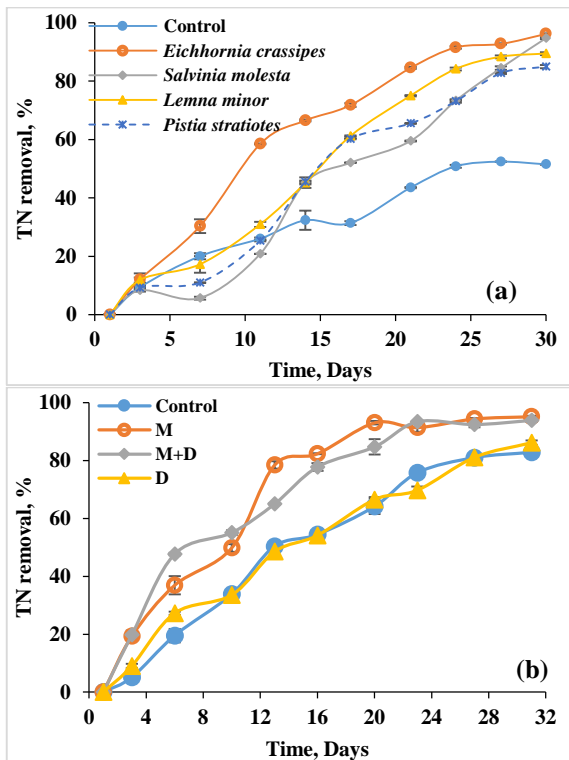


Figure 5: TN removal during the study period. (a) TN removal efficiency in control and individual macrophytes, (b) TN removal efficiency in control, M, M+D, and D reactor.

During the second batch of experiments with heterotrophic macrophytes and diatom-aided heterotrophic macrophytes, the plant's biomass yield was increased by 5.21 and 4.53 times. The macrophytes biomass produced in the diatom-aided macrophytes reactor was less than the biomass produced by macrophytes alone. The possible reason could be the lack of organic materials due to the uptake by diatoms. Both diatom-aided and heterotrophic macrophytes showed very high COD and total nitrogen removal rates compared to the individual macrophytes in the present study. This shows the advantages of synergistic effects of the species over the individual macrophytes. Mixed macrophytes have been reported to perform better in pollutant removal than individual macrophytes [21], [22]. This enhancement arises from increased biodiversity, which fosters a more effective microbial community. Diverse root structures allow for better nutrient access

at various water depths. Furthermore, mixed microphytes are less vulnerable to seasonal variations.

Roots length at the beginning and end of the experiment are presented in Figure 7. The performance of CFWs mainly depends on the development of dense and large root systems, which directly take the organic pollutants. In the case of *Eichhornia crassipes*, which showed maximum COD, phosphate, and TN removal, root length was increased by 28.4 cm. *Pistia stratiotes*, which showed the lowest biomass production in the reactor, had their root length increase by 24.8 cm, which was almost equal to that of *Eichhornia crassipes*. In the case of *Salvinia molesta*, which showed the second highest removal for COD, phosphate and total nitrogen had a low increase in root length by 13.6 cm when compared with *Eichhornia crassipes* and *Salvinia molesta*. Root zones play a significant role in the uptake of pollutants from macrophytes. In the present study, reactors with macrophytes showed higher pollutant removal and increased plant biomass and root growth. In a study by Bauer *et al.*, [16] authors reported an average root length of 20 ± 4.6 cm in their study with *Typha domingensis*. The roots of the plants provide space for microbial communities, which helps develop the symbiotic relationships between plants and bacteria, which helps in improved nutrient removal [23]. Figures 8 and 9 present the pictures of plant roots in the reactors and the comparative pictures of plant roots' length on the first and 30th day of the batch study, respectively.

Studies have reported the advantage of root zones in the development of biofilms and providing sufficient area for bacterial adsorption onto plant roots, aiding direct or indirect uptake by plants and degradation of pollutants by bacteria [8], [24], [25]. The symbiotic relationship between bacteria and plant's root zone enhances pollutant degradation and increases the efficiency of floating wetlands treatment [26]. Increased root zones also lead to increased dissolved oxygen in wastewater via root oxygenation, favoring aerobic bacterial degradation of pollutants [10]. The increase in plant biomass and their roots confirms the acclimatization of plants to wastewater. The reduced COD and phosphate values indicate the plants' ability to treat domestic wastewater. Thus, the removal of COD and phosphates can be majorly attributed to plant uptake alone or by the interaction of bacteria with plants in wastewater.

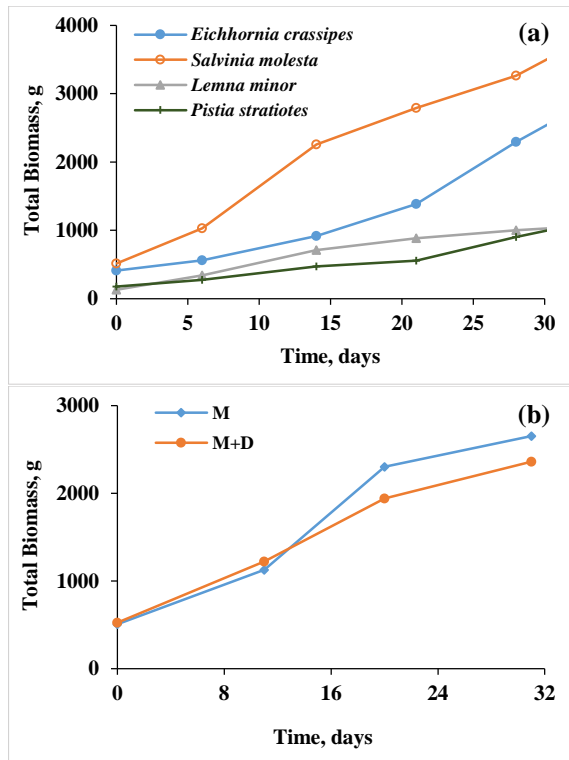


Figure 6: Plant biomass production during the study period. (a) wet biomass in control and individual macrophytes reactors, (b) wet biomass in M and M+D reactors.

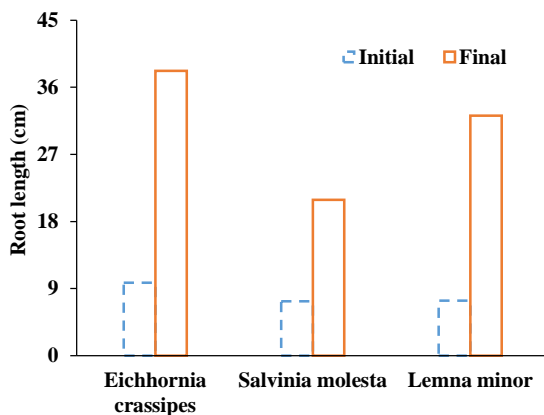


Figure 7: Root length of the plants at the beginning and end of batch 1 experiments.

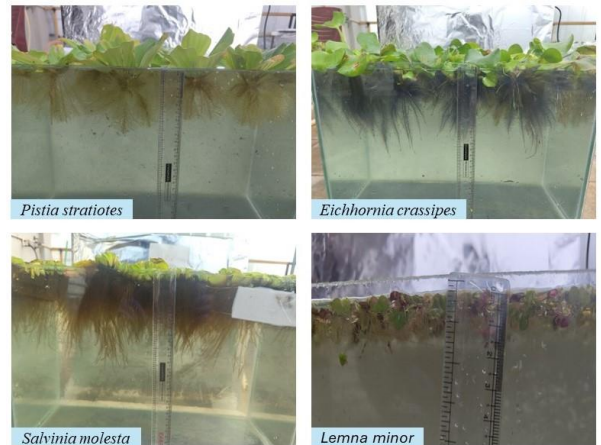


Figure 8: Pictures of plant roots during the study.



Figure 9: Pictures presenting the comparison of roots of a) *Eichhornia crassipes* and b) *Pistia stratiotes* taken at the beginning and end of the study.

The total chlorophyll profile of the reactors in batch I and batch II has been shown in Figure 10(a) and (b), respectively. In the first batch experiments, a maximum chlorophyll value of 8.38 mg/L was observed in the control reactor whereas the *Lemna minor* reactor had a maximum chlorophyll content of 2.6 mg/L. It can be observed that there is no increase in chlorophyll content in *Eichhornia crassipes*, *Salvinia molesta*, and *Pistia stratiotes* reactors,

indicating that there is no algal growth in the reactors. The maximum algal growth in the control reactor could be due to the direct sunlight facilitating the algal growth [16], [20]. The higher algal growth in *Lemna minor* reactor compared to other macrophytes could be due to the smaller root zone facilitating light diffusion favoring algal growth. The chlorophyll content in all the reactors in the second batch experiment was higher than that of the reactor in the first batch experiment, indicating that there is a significant growth of microalgae. In the second batch experiment, the reactors were provided with blue light illumination favoring diatom growth. The symbiotic relationship between the rootzone bacteria and microalgae enhances the pollutant removal. A higher concentration of chlorophyll-C (0.4–5.1 mg/L) among total chlorophyll confirms the presence of diatoms in these reactors.

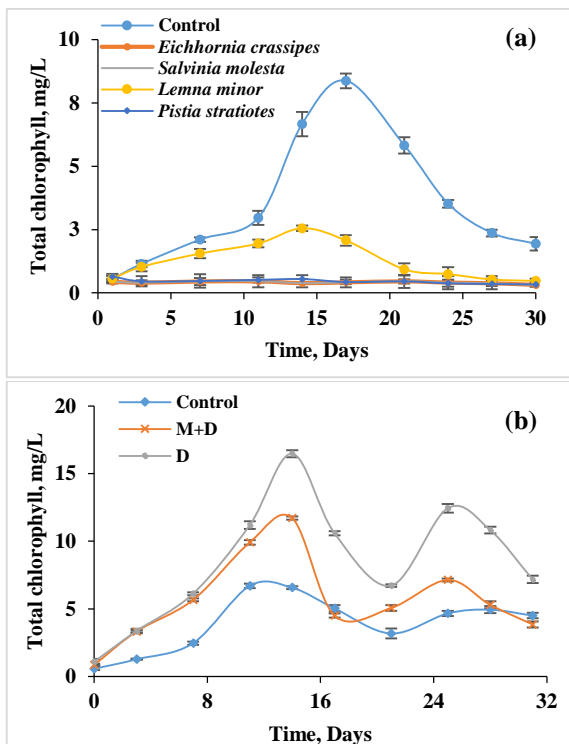


Figure 10: Total Chlorophyll changes during the study period. (a) Total Chlorophyll changes in control and individual macrophytes reactor, (b) Total Chlorophyll changes in control, heterotrophic macrophytes and diatom-aided macrophytes reactors.

The plant biomass produced is rich in nutrients and carbon and can be utilized as a source of nutrients

for agricultural applications. Wetland biomass is best suitable to produce high-quality bio-fertilizers. However, there are concerns about the uptake of micropollutants and heavy metals by macrophytes, which further need to be investigated to ensure that micropollutants do not enter the food cycle. Biomass from wetlands can be converted into bio-adsorbent, i.e., into activated carbon (AC) [2]. Biochar produced from wetlands plants biomass rich in nutrients is used for soil amendment [27]. The biomass produced could be a highly suitable substrate for bio-energy conversion and the synthesis of several value-added products, adding value to the treatment system and contributing to a circular economy.

4 Conclusions

The present study investigated the macrophytes' ability to treat wastewater in floating treatment wetlands. The combination of heterotrophic macrophytes showed enhanced pollutant removal compared to the individual macrophytes. Floating treatment wetlands could be a cost-effective and sustainable technology for domestic wastewater treatment. The rich lignocellulosic biomass produced during the treatment has significant potential in bioenergy and biofertilizer applications contributing to a circular economy. Further studies on the interaction between bacteria and plant roots in the CFWs can help in understanding the removal mechanisms of pollutants. Also, future research may focus on employing CFWs at pilot and field scales for domestic wastewater treatment.

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Author Contributions

M.K.K.: data analysis, interpretation, writing original draft and editing; R.K.O.: data analysis, interpretation, writing original draft and editing; R.T.: methodology, investigation, data curation; M.S.: reviewing and editing; A.T.: reviewing and editing; D.B.: conceptualization, research design, funding acquisition, project administration, reviewing and editing. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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