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# Physicochemical and Biological Contribution of Native Macrophytes in the Constructed Wetlands to Treat Municipal Wastewater: A Pilot-Scale Experiment in a Sub-Tropical Climate Region

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**Abstract:** In this study, the physicochemical and biological contributions of different macrophytes in horizontal sub-surface flow constructed wetlands (HSSF-CWs) to treat low-strength municipal wastewater operated at high hydraulic loads under a sub-tropical climatic region is investigated. Out of the four identical beds, three were planted with locally available macrophytes (*P. australis*, *Sagittaria*, and *Iris*), whereas one bed was kept as a control. The beds were filled with media and operated in parallel continuously for eight months, with increasing the surface loading rate (SLR) from 0.19 to 2.78 m day$^{-1}$. The results indicate that the planted beds performed significantly ($p < 0.01$) better to remove TSS (70% to 78%), BOD$_5$ (66% to 77%), COD (59% to 75%), NO$_3$-N (56% to 64%), NH$_4$-N (41% to 69%), TN (36% to 41%), and TP (44% to 61%) as compared to the unplanted bed for the same parameters (48%, 39%, 40%, 33%, 18%, 20%, and 29%, respectively). The presence of macrophytes in HSSF-CWs was found to be highly significant. The average relative growth rate (RGR) was observed in the order of *P. australis* (0.0086 day$^{-1}$) > *Sagittaria* (0.0061 day$^{-1}$) > *Iris* (0.0059 day$^{-1}$). When compared to the performances of the species used, *Sagittaria* and *P. australis* produced better results than *Iris*. The investigations on biomass showed that *Sagittaria* yielded higher production, followed by *P. australis* and *Iris*. The proportions of uptake by the macrophytes were found to be 9.3%, 6.3%, and 3.9% of mass N removal, and 7.6%, 5.1%, and 4.4% of mass *p* removal in *Sagittaria*, *P. australis*, and *Iris*, respectively. This study contributes to the effective response to the environment, which validates a major role of macrophytes and their disparate response to pollutant removal processes by different species from municipal wastewater through HSSF-CWs.

**Keywords:** experimental botany; native macrophytes; municipal wastewater; sub-tropical climate; constructed wetlands

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## 1. Introduction

Natural processes have always been recognized as one of the best methods to clean the water flow through rivers, lakes, streams, and wetlands in a more cost-effective and sustainable way. When these processes, such as wetlands, are designed in an engineered way, a variety of wastewaters such as surface run-off, domestic wastewater, industrial effluent, and agriculture wastewater can be effectively treated to improve the water quality. Therefore, this technology has been accepted as one of the most reliable and preferred methods for wastewater treatment. It is a proven method to deal with a variety of polluted waters in many parts of the world, particularly in Europe, Australia, and North American countries [1–5]. The technology is also gaining a wide range of popularity in developing countries such as India and its regions, where climatic conditions are favorable. The main advantages of the CWs are a relatively low-capital investment, high removal efficiencies, easy to implement, and hassle-free operation and maintenance [6–8]. Additionally, CWs can actively contribute to achieving the UN Sustainable Development Goals.

Constructed wetlands (CWs) are engineered systems designed and constructed to utilize the natural functions of wetland vegetation, soil media, and their associated microbial assemblages for wastewater treatment within a more controlled environment [9]. Based on the water flow regime and the type of macrophytic growth, CWs may be classified into three groups: free water surface flow CWs (FWS CWs), subsurface flow CWs (SSF CWs), and hybrid systems [9,10]. Amongst others, the HSSF-CWs system is one of the most common types of SSF wetland systems used in many parts of the world.

In this system, wastewater is maintained at a constant depth and flows horizontally below the surface along the length through granular medium, macrophyte roots, and rhizomes [11]. Generally, the substrate in this system is permanently flooded with water, and treatment of pollutants occurs using the interconnection of various removal processes, i.e., chemical, microbial, and physical [9,12].

HSSF-CWs have good removal in terms of solids and organic matter. Studies showed the TSS removal in HSSF-CWs within the range of 29–80% [13–18]. Some researchers reported the removal of $BOD_5$ and COD in the range of 19–70% and 27–66%, respectively [13,15,19–21]. However, several studies reported comparatively higher removal of $BOD_5$ and COD, such as 78–87% and 71–77%, respectively [16,18,22]. Similarly, the removal of ammonium and total nitrogen was recorded with disparate figures in the previous studies. Some studies showed the removal of $NH_4$-N and TN within the range of 16–38% [14,15,20,23,24] and 22–40% [14,20,24,25], respectively, whereas some researchers reported relatively higher removal rates of $NH_4$-N and TN within the range of 38–59% [14,16,25,26] and 45–59% [16,22,26,27], respectively. Moreover, the removal of phosphorus in HSSF-CWs was reported in the literature from 13% to 64% [14–16,19,20,22,23,26].

The vegetation or plants inter alia, commonly called macrophytes that are used in the CWs, are often considered as one of the key factors for the removal of the pollutants. Several research studies on the role of macrophytes in the wetland system have been carried out [28–31]. Most of the studies have shown that the planted beds have produced higher removal efficiencies than the non-planted beds in the removal of suspended solids, organics, and nutrients [8,32,33]. Studies have also proved that the main processes are enhanced by the presence of macrophytes, including filtration and sedimentation by root structures, microbial decomposition of pollutants due to oxygen transported to the root zone, and their capacity for nutrient uptake [5,34,35]. However, there are contrasting findings by some of the researchers. The literature suggests that the presence of the macrophytes has no significant effect on the treatment performance of the wetlands [36–39]. In some cases, a small improvement existed for the removal of organics and a measurable enhancement was exhibited in the removal of nutrients with vegetated beds [40,41].

Studies have also shown that the presence of macrophytes is more important than the selected species planted in the wetlands for the remediation of pollutants. Calheiros et al. [23] and Carballeira et al. [42] discussed in their findings that different species used in their experimental beds have not had an effect on the removal efficiencies for organic matter and nutrients. However, few researchers demonstrated higher removal efficiencies on the use of different species [34,43,44]. The above findings are contrary to each other, and therefore further study is warranted to investigate the effectiveness of the macrophytes in their presence and the type of species.

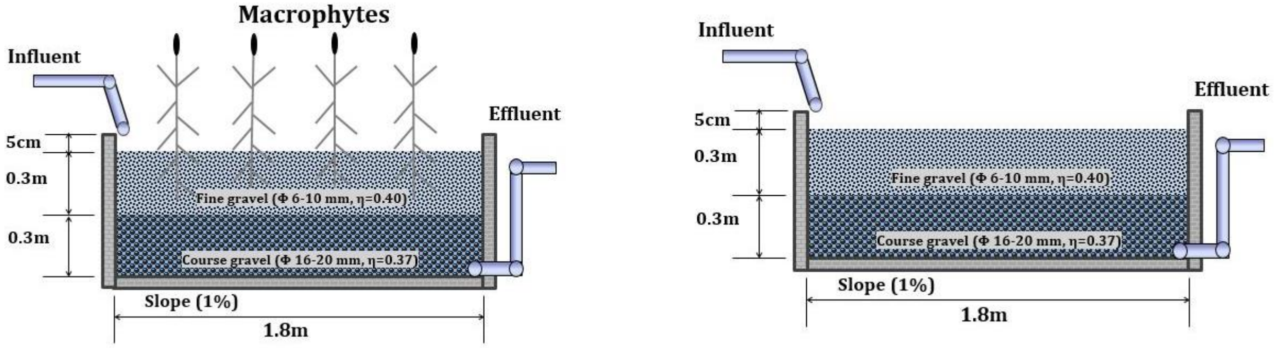
Most of the previous studies have investigated the role of macrophytes operated at relatively lower hydraulic loads. Therefore, this paper presents the outcomes of a study that assessed the physicochemical and biological contributions of *P. australis*, *Sagittaria*, and *Iris* in HSSF-CWs to treat low-strength municipal wastewater operated at high loading rates in sub-tropical climatic conditions, which prevail in the northern plains of India.

## 2. Materials and Methods

### 2.1. Description of the Pilot Set-Up

The existing pilot set-up installed in the Department of Civil Engineering, Aligarh Muslim University, Aligarh (28°10′ N and 78°36′ E), Uttar Pradesh, was used for this study. The region of the study site falls within the sub-tropical conditions. The average rainfall intensity in this area is 640 mm and the temperatures ranges in between 20 °C and 41 °C in summer, and 7 °C and 13 °C in the winter season [45]. The study presented in this paper was started in October 2018.

The pilot set-up comprises a sedimentation tank of 750 L capacity, followed by the pilot wetlands. The influent wastewater was the combination of the effluent from a septic tank and the wastes from the departmental buildings, which is similar to the nature of the domestic wastewater accompanied by the discharge from the laboratories. The collective mixed wastewater was fed into a holding tank employing an electric pump and delivered to wetland beds by gravity. A slope of 1% was provided along the flow direction from the inlet to the outlet of all beds. All the beds were constructed with brick masonry works with leakage-proof plaster at the inner and outer walls. The four parallel beds have the same dimensions and substrate media but different macrophyte species. Each bed (1.8 × 0.6 × 0.6 m) was filled with washed dual-gravel media having a combined porosity of 38.5%, containing an upper layer of fine gravel (size: 6–10 mm, depth: 0.3 m, porosity: 0.40) and a lower layer of coarse gravel (size: 16–20 mm, depth: 0.3 m, porosity: 0.37) (Figures 1 and 2). The porosity of each media layer was calculated by obtaining the displaced amount of water to a known-volume container when filled with the used media up to the top level. The ratio of the displaced amount to the total volume of water represented the porosity of the media.



(**a**) (**b**)

**Figure 1.** Schematic section of the planted (**a**) and unplanted (**b**) beds of HSSF-CWs.

Out of four beds, three were planted with locally available macrophytes, namely, *Phragmites australis* (W1), *Sagittaria sagittifolia* (W2), and *Iris* spp. (W3), whereas one bed was kept as unplanted and used as a control (W4) (Figure 2). The saplings of each macrophyte of 12–16 cm high had a macrophyte density of 11 macrophytes per meter square. Initially, up to the maturation level, macrophytes were irrigated with tap water to support the macrophyte growth.

**Figure 2.** Profile photo of the four beds of HSSF-CWs: W1 (*P. australis*), W2 (*Sagittaria*), W3 (*Iris*), and W4 (Control).

The wastewater was applied continuously on each bed at the hydraulic loading rates of 0.19, 0.56, 1.11, and 2.78 m $\mathrm{day}^{-1}$ for eight months (each load for two months). The corresponding hydraulic retention times (HRTs) were 29.94, 9.98, 4.99, and 2 h, respectively. The hydraulic loading rate in each bed was measured from the average flow rates at the inlet and outlet using a measuring cylinder, stopwatch, and plastic collecting bottle. The theoretical HRT of the beds at different hydraulic loading rates was estimated by Equation (1):

$$\mathrm{HRT\ (day)} = \frac{\eta \times \mathrm{L} \times \mathrm{D} \times \mathrm{W}}{\mathrm{Q}} \tag{1}$$

where L, D, and W are the length, depth, and width of the beds in meters, η is the porosity of the used media, and Q is the flow of water in $\mathrm{m}^3\ \mathrm{day}^{-1}$.

### *2.2. Monitoring and Analyses*

#### 2.2.1. Wastewater Monitoring and Analysis

Wastewater sampling was performed continuously from influent and effluent of the different HSSF-CWs beds at a frequency of twice per week for eight months (February–September 2019). On-site parameters pH, dissolved oxygen (DO), and temperature were measured directly by grab sampling by using the probe-based HACH instrument (HQ40 d). The parameters including total nitrogen (TN), total phosphorus (TP), and nitrate nitrogen ($NO_3$-N) were determined by Spectrophotometric methods 10,072, 10,210, and 10,049, respectively, by using the HACH DR 6000. The analysis of 5-day biological oxygen demand ($BOD_5$), chemical oxygen demand (COD), total suspended solids (TSS), and ammonium nitrogen ($NH_4$-N) was performed as per the Standard Methods [46].

#### 2.2.2. Macrophyte Monitoring and Analysis

The macrophyte heights were measured monthly for twelve months (October 2018–September 2019) and the corresponding relative growth rate (RGR) was calculated to describe the growth condition of *P. australis, Sagittaria,* and *Iris*. The length of stems was measured using tape by taking one arbitrarily chosen shoot from the inlet, middle, and outlet of each bed, and selected shoots were assigned a particular number code. The macrophytes were visually checked weekly on a regular basis. The relative growth rate (RGR) was calculated by Equation (2):

$$\text{RGR} = (\text{In } H_2 - \text{In } H_1)/(T_2 - T_1) \tag{2}$$

where W1 and W2 are the average macrophyte heights in cm at time $T_1$ and $T_2$ in days [47].

All the macrophytes in the HSSF-CWs beds (WI, W2, and W3) were excavated at the end of the whole study. To obtain dry weight, the biomass of aboveground (top, leaves and stem) and belowground (bottom, roots and rhizomes) were separated. The corresponding fractions of samples were sorted and washed to remove attached sediments, and carefully rinsed in the laboratory with distilled water, and later on dried in an oven at 70 °C until a constant weight was obtained. All dried samples were grounded individually and passed through a 70-mesh screen (0.21 mm size). Then, the samples were digested and analyzed for TN and TP content according to the methods of Lindner [48] and Fiske and Subbarow [49], respectively.

### 2.3. Evaluation of Monitored Data

All the data analyses were performed using Microsoft Excel XP version 2016 and OriginPro version 2018b (OriginPro, Northampton, Massachusetts, USA). To investigate the difference between planted and unplanted HF-CWs and the effectiveness of the macrophytes, one-way analysis of variance (ANOVA) at a 95% significance level was used, reported as not significant at $p > 0.05$, significant at $p < 0.05$, and highly significant at $p < 0.01$.

Pollutant removal performance was calculated from Equation (2). The dry biomass produced per unit area and the nutrient concentrations in the macrophyte were determined as an average of all the samples. Macrophyte uptake or nutrients' accumulation for N and $p$ were estimated from Equation (3) by multiplying the total dry biomass of the system by the specific ratio of nutrients per dry biomass:

$$(\%)\text{R} = \frac{(\text{Ci} - \text{Ce})}{\text{Ci}} \times 100 \tag{3}$$

$$N_{\text{total}} = \text{DW} \times \text{C} \tag{4}$$

where R is the percent of pollutant removal, Ci is the influent concentration (mg/L), Ce is the effluent concentration (mg/L), DW is the dry biomass of macrophytes (kg DW/$m^2$), C is the mean content of N or $p$ in the macrophytes (mgN/g DW or mgP/g DW), and N is the nutrient uptake content by the biomass of macrophytes (g/$m^2$).

## 3. Results and Discussion

### 3.1. Characteristics of Influent and Effluent Wastewater

Each bed was continuously loaded with the real wastewater during the whole study period with four different surface loading rates, increasing as 0.19, 0.56, 1.11, and 2.78 m $\text{day}^{-1}$. Each load was operated for a period of two months. Figure 3 shows the variation in the loading rates of influent with mean and standard deviations. The high fluctuation in the organic loading rate was observed due to increasing the surface loading rate. The loading rate to the beds ranged to 28.5–910.9 g TSS $m^{-2}$ $\text{day}^{-1}$, 7.2–190.6 g $BOD_5$ $m^{-2}$ $\text{day}^{-1}$, 18.5–438.2 g COD $m^{-2}$ $\text{day}^{-1}$, 0.2–7.1 g $NO_3$-N $m^{-2}$ $\text{day}^{-1}$, 1.5–65.6 g $NH_4$-N $m^{-2}$ $\text{day}^{-1}$, 3.3–100.0 g TN $m^{-2}$ $\text{day}^{-1}$, and 0.4–7.1 g TP $m^{-2}$ $\text{day}^{-1}$. Most of the total influent nitrogen (mean value 29.6 g $m^{-2}$ $\text{day}^{-1}$) was in the form of ammonium nitrogen (18.8 g $m^{-2}$ $\text{day}^{-1}$).

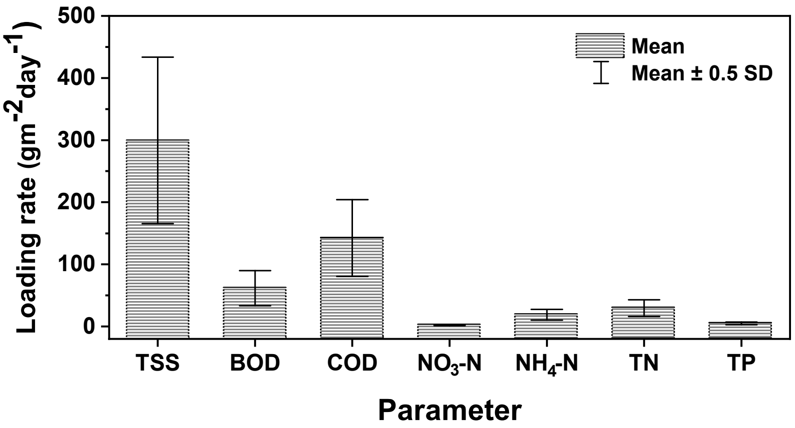


**Figure 3.** The pollutant loading rates for HSSF-CWs. The error bars denote ±one standard error of the mean.

Table 1 shows the mean characteristics of wastewater concentrations with standard deviations in influent and effluent for water quality parameters in different HSSF-CWs (W1, W2, W3, and W4). During the whole study period, the pH of influent ranged between 6.3 and 9.8, with an average of 7.7, showing nearly neutral nature. The effluent pH in planted beds (W1, W2, and W3) showed significant differences ($p < 0.05$) as compared to the influent pH. However, the effluent pH in the unplanted bed (W4) showed insignificant differences ($p > 0.05$). The fluctuation of temperature in influent ranged between 11.4 and 41.8 °C during the whole study period, with an average temperature of 29.0 °C (Table 1). DO concentration in influent was observed between 0.74 and 3.41 mg/L, with an average of 1.40 mg/L during the whole study period. The effluent DO from all beds showed insignificant differences ($p > 0.05$), however effluent DO values were slightly higher than influent values. It may be due to higher organic loads that require more DO because the oxygen released by the macrophyte's root has been fully utilized by microorganisms in the biological process.

The mean concentrations with standard deviation in influent and effluent of the HSSF-CWs beds are shown in the table. The fluctuation in influent and effluent concentrations of TSS, $BOD_5$, COD, $NO_3$-N, $NH_4$-N, TN, and TP in different beds can be seen in Figure 4. During the whole study period, HSSF-CWs received high fluctuations in inlet concentrations for TSS (147.9–330.9 mg/L), $BOD_5$ (38.7–68.6 mg/L), COD (93.9–160.9 mg/L), $NO_3$-N (0.88–2.79 mg/L), $NH_4$-N (6.9–23.6 mg/L), TN (16.3–36.0 mg/L), and TP (2.13–6.48 mg/L).

### *3.2. Removal of Solids*

The results of the performance of different beds for water quality parameters are shown in Table 1. The TSS concentration before and after treatment and the removal rates of different beds showed high fluctuation (Figures 4 and 5). The level of TSS concentration in all beds decreased significantly ($p < 0.01$) from influent to effluent. The planted beds (70–83%) showed a significantly ($p < 0.01$) higher removal of TSS when compared to the unplanted bed (48%) (Tables 1 and 2). This may be attributed to the fact that the macrophytes' rooting biomass provided more effective TSS filtration and complimentarily contributed to microbial degradation of the organic portion of TSS [36].

**Table 1.** Mean influent and effluent concentrations of pollutants with standard deviations and removal efficiencies in W1, W2, W3, and W4 (mean ± SD, $n = 100$).

| Parameter | Influent | WI | | W2 | | W3 | | W4 | |
|---|---|---|---|---|---|---|---|---|---|
| | | Effluent | RE % | Effluent | RE % | Effluent | RE % | Effluent | RE % |
| TSS | 259.1 ± 49.75 *<br>(148–331) # | 55.84 ± 13.13<br>(37–101) | 78 | 41.7 ± 15.23<br>(10–87) | 83 | 73.79 ± 13.92<br>(48–96) | 70 | 130.73 ± 19.95<br>(85–189) | 48 |
| $BOD_5$ | 51.07 ± 5.23<br>(38.7–68.6) | 11.61 ± 5.07<br>(3.2–28.27) | 77 | 11.81 ± 5.08<br>(4.55–27.65) | 77 | 17.34 ± 5.109<br>(8.45–31.92) | 66 | 30.68 ± 8.08<br>(20.11–52.69) | 39 |
| COD | 123.4 ± 13.76<br>(94–161) | 31.38 ± 6.66<br>(16–49) | 74 | 30.12 ± 7.41<br>(14–49) | 75 | 50.21 ± 7.75)<br>(35–69) | 59 | 73.13 ± 15.51<br>(42–108) | 40 |
| $NO_3$-N | 1.74 ± 0.42<br>(0.88–2.79) | 0.68 ± 0.25<br>(0.24–1.38) | 61 | 0.62 ± 0.25<br>(0.10–1.34) | 64 | 0.77 ± 0.28<br>(0.23–1.36) | 56 | 1.17 ± 0.37<br>(0.41–2.12) | 33 |
| $NH_4^+$-N | 15.84 ± 3.84<br>(6.9–23.6) | 4.97 ± 1.74<br>(1.5–10.3) | 66 | 4.58 ± 1.85<br>(1.1–10.7) | 69 | 8.97 ± 1.91<br>(4.3–15.4) | 41 | 12.95 ± 3.10<br>(5.6–20.8) | 18 |
| TN | 25.16 ± 4.14<br>(16.3–36) | 14.54 ± 3.56<br>(6.6–23.7) | 41 | 14.70 ± 4.04<br>(5.2–25.10) | 41 | 16.00 ± 4.35<br>(6.6–27.4) | 36 | 19.96 ± 3.90<br>(11.60–31.10) | 20 |
| TP | 4.20 ± 0.93<br>(2.13–6.48) | 2.07 ± 0.53<br>(1.09–3.23) | 50 | 1.63 ± 0.52<br>(0.69–3.00) | 61 | 2.34 ± 0.61<br>(1.02–3.78) | 44 | 2.96 ± 0.71<br>(1.36–4.39) | 29 |
| pH | 7.68 ± 0.67<br>(6.28–9.76) | 7.58 ± 0.63<br>(6.1–9.58) | - | 7.68 ± 0.62<br>(6.12–9.21) | - | 7.75 ± 0.62<br>(6.52–9.89) | - | 7.75 ± 0.62<br>(6.60–9.93) | - |
| DO | 1.40 ± 0.41<br>(0.74–3.41) | 1.45 ± 0.49<br>(0.56–3.42) | - | 1.46 ± 0.53<br>(0.58–3.46) | - | 1.45 ± 0.48<br>(0.63–3.28) | - | 1.28 ± 0.45<br>(0.34–3.25) | - |
| T | 29.01 ± 5.98<br>(11.39–41.78) | 28.66 ± 6.87<br>(9.90–41.34) | - | 28.50 ± 6.93<br>(7.10–41.56) | - | 28.52 ± 6.91<br>(7.10–41.56) | - | 28.86 ± 6.25<br>(11.39–41.79) | - |

* Mean (average) values with standard deviation; # Min and max values of pollutants concentration; RE: removal efficiency (%). TSS: total suspended solids; $BOD_5$: 5-day biological oxygen demand; COD: chemical oxygen demand; $NO_3$-N: nitrate nitrogen; $NH_4^+$-N: ammonium nitrogen; TN: total nitrogen; TP: total phosphorus; DO: dissolved oxygen; T: temperature. All the values are in mg/L except pH and temperature (°C).

The comparison among the species showed significant differences ($p < 0.01$) in the order of the beds planted, with *Sagittaria* (83%) > *P. australis* (78%) > *Iris* (70%). This may be attributed to the denser rooted biomass cover provided by the macrophytes. The unplanted bed obtained a significant amount of TSS reduction, which may be attributed to sedimentation and filtration processes. Low-porosity gravel media is effective for filtration as it filtered and trapped the solids in the wetland system to allow better removal [50].

In this study, the planted beds produced higher TSS removal than in previous research [13,14,17]. However, Katsenovich et al. [27] studied similar wetlands planted with *Brachiaria* and reported higher TSS removal (87%) than in the present study, which may be attributed to the lower surface loading. The highest removal (83%) obtained from this study in W2 is in agreement with other researchers [19,20], who reported almost the same TSS removal (82%) for the same wetland system. From the present results, it was revealed that the presence of macrophytes and types of species play an essential role in the reduction of solids; however, the presence of macrophytes had more of an effect than the choice of species.

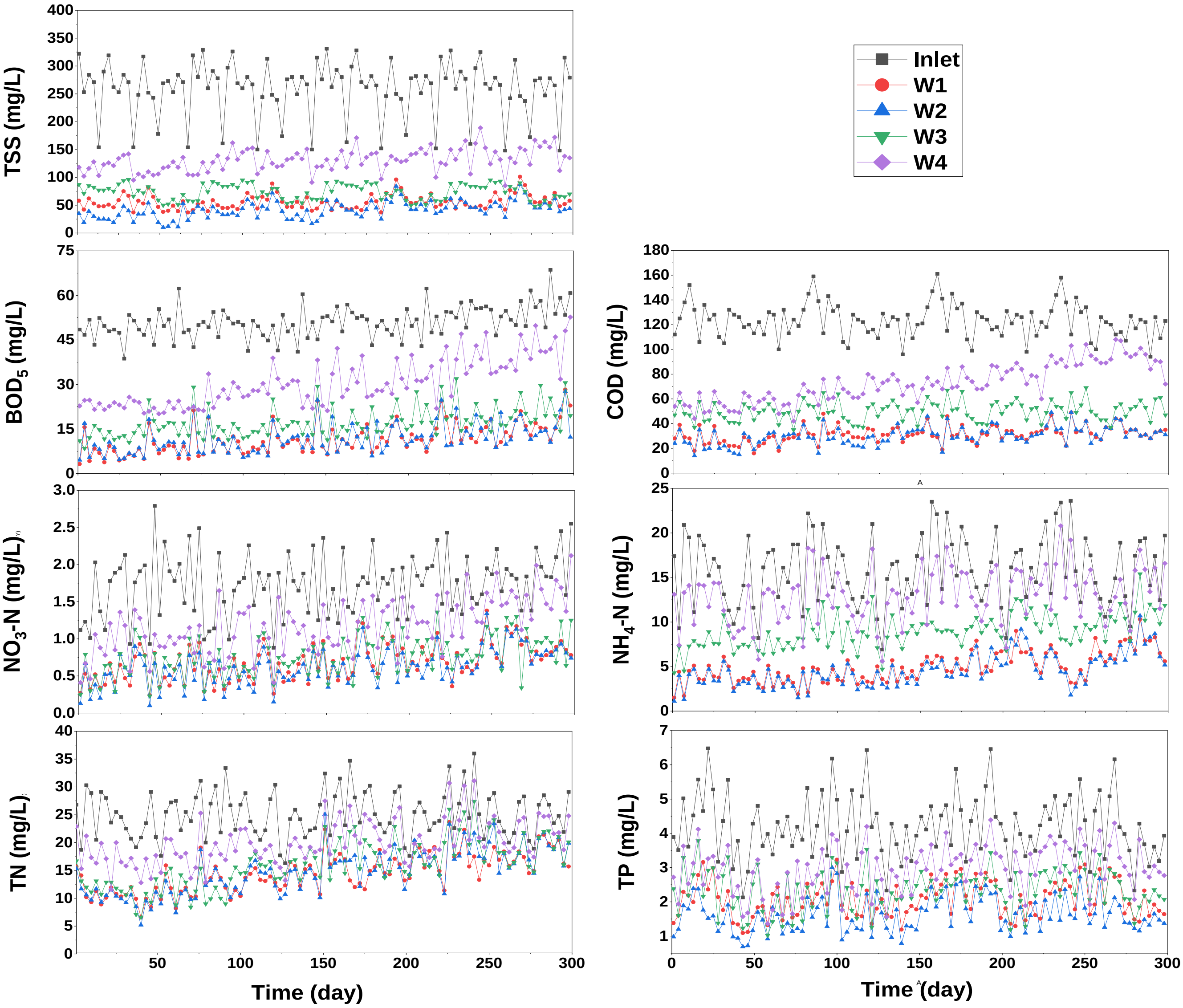


**Figure 4.** Fluctuations in the concentrations of TSS, $BOD_5$, COD, $NO_3$-N, $NH_4$-N, TN, and TP before and after the treatment in different HSSF-CWs.

### 3.3. Removal of Organic Matter

Mean concentrations with standard deviations of the influent and effluent and their fluctuations in the different beds can be seen in Figure 4 and Table 1. All the beds showed significant differences ($p < 0.01$) between the level of organic concentration in the inlet and the outlet. The planted beds obtained a significantly ($p < 0.01$) higher removal of $BOD_5$ and COD than the unplanted bed (Table 2). The removal rates of $BOD_5$ and COD ranged from 66% to 77% and 57% to 75% in the planted beds and 39% and 40% in the unplanted bed, respectively (Table 1). This indicated that the possible positive influence of macrophytes on organic matter removal may be attributed to the biological degradation of organic matter due to the increased oxygen supply by the rooted biomass as compared to the unplanted wetland. Additionally, the beds planted with *P. australis* and *Sagittaria* obtained significantly ($p < 0.01$) better performance than *Iris* in terms of $BOD_5$ and COD. This may be attributed to the large number of adventitious root systems. However, there was no significant difference ($p > 0.05$) observed between the beds planted with *P. australis* and *Sagittaria* (Table 2).

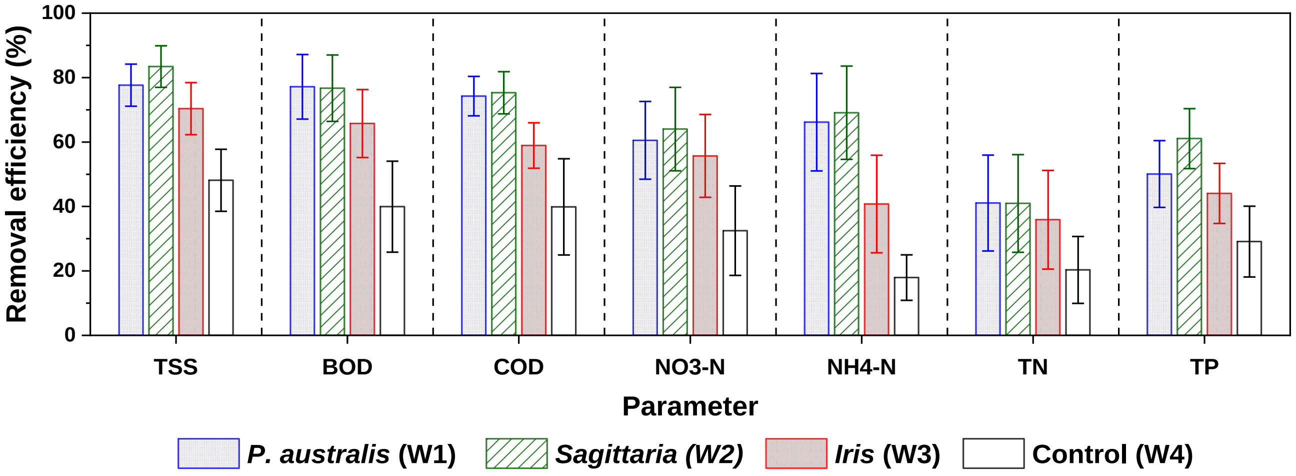


**Figure 5.** The performance of different macrophytes in the removal of TSS, $BOD_5$, COD, $NO_3$-N, $NH_4$-N, TN, and TP.

**Table 2.** One-way analysis of variance (ANOVA) check for the influence of different macrophytes on the removal efficiencies of pollutants during the whole study period in HSSF-CWs.

| Pollutant | ANOVA Test (*p*-Value) | | | | | | |
|---|---|---|---|---|---|---|---|
| | *p* vs. S | *p* vs. I | S vs. I | *p*, S and I | *p* vs. C | S vs. C | I vs. C |
| TSS | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| $BOD_5$ | 0.7639 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| COD | 0.2491 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| $NO_3$-N | 0.0490 | 0.0000 | 0.0000 | 0.0001 | 0.0000 | 0.0000 | 0.0000 |
| $NH_4$-N | 0.1865 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| TN | 0.9088 | 0.0138 | 0.0200 | 0.0208 | 0.0000 | 0.0000 | 0.0000 |
| TP | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

*p*: *P. australis*; S: *Sagittaria*; I: *Iris*; C: control.

From the results, it was indicated that *P. australis* and *Sagittaria* provided more aerobic as well as filtration facilities than *Iris* for better removal of organic matter. The removal rates from the present study for $BOD_5$ and COD (57–77%) are in agreement with those of other researchers [19,20,22], who reported 58–78%, and higher than those of some previous studies [15–27], where they were reported within a range between 38% and 62%.

The findings of this study revealed that the presence of the macrophytes and their choice play a significant role in the removal of organic matter. However, the type of species in the wetland system had less of an effect than that of its presence.

### 3.4. Removal of Nitrogen

The ammonification, nitrification, denitrification, microbial degradation, adsorption, volatilization, and uptake are the main processes involved in the removal of nitrogen [6,51,52]. However, in HSSF-CWs systems, nitrification/denitrification processes are mainly responsible for nitrogen removal [53]. The volatilization process can also remove the ammonium nitrogen if a higher pH (>9) is available in the wetland system [16]. However, in this study, the average pH was measured as 7.7.

During the whole study period, it was observed that the nitrogen ($NO_3$-N, $NH_4$-N, and TN) concentration was significantly different ($p < 0.01$) from influent to effluent. The removal efficiencies of nitrogen in individual beds can be seen in Table 1 and Figure 5. The removal rates of $NO_3$-N, $NH_4$-N, and TN in planted beds were 56–64%, 41–69%, and 36–41%, respectively, whereas in the unplanted bed they were 33%, 18%, and 20%, respectively. All the planted beds performed significantly ($p < 0.01$) better when compared with the unplanted bed for the removal of $NO_3$-N, $NH_4$-N, and TN, indicating that the positive effect of the macrophytes may be attributed to the increased oxygen provided by rooted biomass as compared to an unplanted wetland. The comparison among the species demonstrated a significant difference ($p < 0.05$), where *P. australis* and *Sagittaria* exhibited better removal compared to *Iris*, which may be due to the large number of adventitious rooted biomass. However, *Sagittaria* and *P. australis* demonstrated no significant differences ($p > 0.05$) between them for the removal of nitrogen.

The results obtained in this study were within the range reported by Tanaka et al. [13] and Li et al. [54] for $NO_3$-N (39–65%) and $NH_4$-N (32–75%), and almost similar to the researchers who reported 40% removal of TN [14,25]. However, higher TN removal (51%) was reported by Jozwiakowski et al. [26], which may be due to the relatively lower organic loads.

The results revealed that macrophytes play an essential role in the wetland system for the abatement of nitrogen from wastewater. The selection of appropriate species can also significantly improve the nitrogen removal.

### *3.5. Removal of Phosphorus*

The mechanism of phosphorus removal mainly included the macrophyte uptake, adsorption, and precipitation, in which the macrophytes' growth is responsible for up to 10% removal of phosphorous [52,55]. The fluctuation in influent and effluent TP concentrations can be observed in Figure 1. The TP concentration in all beds was demonstrated as significantly different ($p < 0.01$) from influent to effluent throughout the study period. TP removal demonstrated significant differences ($p < 0.01$) between planted and unplanted beds (Table 2). It indicated a possible positive effect of macrophytes that may be attributed to their uptake. Moreover, the comparison among the species also demonstrated significant differences ($p < 0.01$), presenting the TP removal in the order of *Sagittaria* (61%) > *P. australis* (50%) > *Iris* (44%) (Table 1). However, the unplanted bed achieved a sufficient amount of TP reduction (29%), which may be attributed to adsorption by substrate media and partly by precipitation. The results revealed that the presence of macrophytes and their appropriate selection play an important role in the reduction of phosphorus.

### *3.6. Plants Analysis*

#### 3.6.1. Macrophytes' Growth

Usually, the macrophytes' growth is considered very essential in the contribution of the removal of organic matter and nutrients in the treatment processes. All the macrophytes were fed with tap water for one month for their growth and then fed with diluted real wastewater over three months for acclimatization. At the start of the study, the shoots' density of macrophytes was 11 shoots/$m^2$. The increment in the number of shoots was recorded mainly due to the availability of nutrients in wastewater. The progressive increases in densities of macrophytes were recorded with time, from 11 to 87 shoots/$m^2$ for *P. australis*, 11 to 47 shoots/$m^2$ for *Sagittaria*, and 11 to 61 shoots/$m^2$ for *Iris*. This was mainly attributed to available space and nutrients to grow in the newly established wetlands.

In all planted beds, each macrophyte (*P. australis*, *Sagittaria*, and *Iris*) grew well in dual-gravel media-based HSSF-CWs when loaded with the real wastewater and produced a dense macrophyte cover with large biomass. Figure 6 shows the monthly changes in macrophyte heights with corresponding relative growth rates (RGR). All species followed the trend of gradually increasing in height and decreasing in RGR with the passage of time during the entire period. *P. australis* grew significantly ($p < 0.05$) much faster than other macrophytes and reached a maximum height of 287 cm, from 16 cm.

The corresponding RGR was also observed as optimum and decreased from 0.0157 to 0.0023 $day^{-1}$. The maximum height of *Sagittaria* (99 cm) was recorded higher than *Iris* (76 cm). The RGR of *Sagittaria* was observed higher than *Iris* during the growing period October 2018 to February 2019, whereas *Iris* showed higher values of RGR for the growing period March–September 2019 (Figure 6). The RGR values were recorded as 0.0010–0.0138 and 0.0014–0.0112 day$^{-1}$ for *Sagittaria* and *Iris*, respectively. The highest average relative growth rate was observed in *P. australis*, followed by *Sagittaria* and *Iris*, presenting the values 0.0086, 0.0061, and 0.0059 day$^{-1}$, respectively.

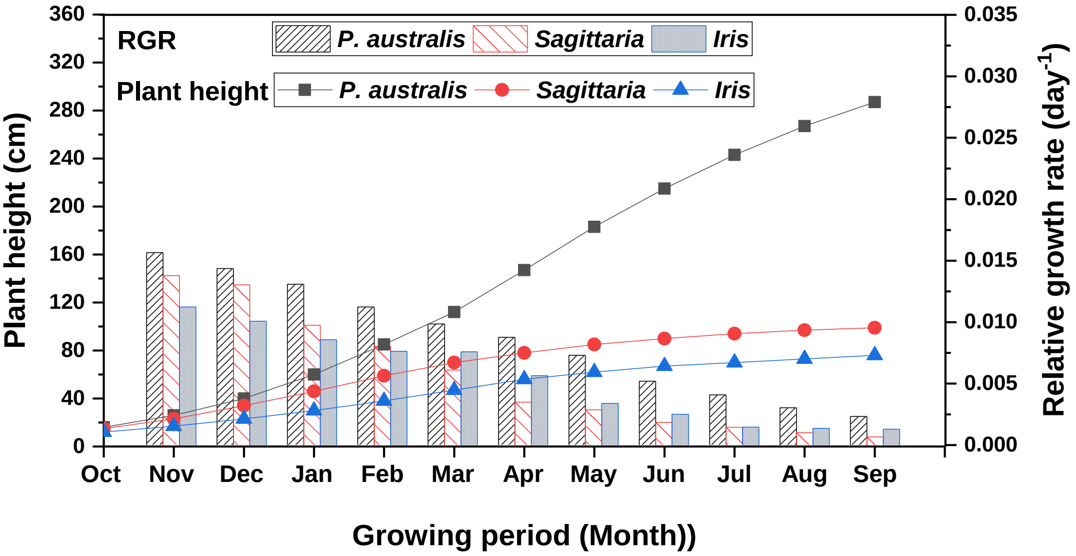


**Figure 6.** Monthly development in shoot height of different macrophytes and their relative growth rates (RGR) in HSSF-CWs.

### 3.6.2. Biomass Production and Uptake Capacity

After harvesting the macrophytes at the end of the study, *Sagittaria* yielded the highest dry biomass (9.25 kg DW/m$^2$), followed by *P. australis* (5.89 kg DW/m$^2$) and *Iris* (5.37 kg DW/m$^2$) (Table 3). The aboveground and belowground biomass amounted to 4.27 and 1.62 kg DW/m$^2$ for *P. australis*, 5.47 and 3.78 kg DW/m$^2$ for *Sagittaria*, and 1.91 and 3.46 kg DW/m$^2$ for *Iris*, respectively (Figure 2). From the results, the R/S (root/shoot) ratio (1.8) for *Iris* indicated high underground biomass, however, *Sagittaria* and *P. australis* showed R/S ratios less than one, indicating higher biomass aboveground. *Sagittaria* significantly ($p < 0.05$) outperformed *P. australis* and *Iris*. The biomass yield from these species was higher than the figures reported by Ennabili et al. [3] (2–3 kg DW/m$^2$), Konnerup et al. [53] (3.13 kg DW/m$^2$), and Vymazal [56] (0.79–5.1 kg DW/m$^2$). Abou-Elela et al. [57] reported a biomass yield of 4.46–5.18 kg DW/m$^2$, which was lower than that in the present study for *Sagittaria* and almost near that of *P. australis* and *Iris*.

Figure 7 provides a comparison of TN and TP accumulation ability of different macrophytes in HF-CWs beds after harvesting. The average N content in *P. australis*, *Sagittaria*, and *Iris* for the aboveground and belowground parts was found to be 34.47 and 27.28 mg/g, 31.69 and 29.95 mg/g, and 21.32 and 18.43 mg/g, respectively (Table 3). Similarly, $p$ content was 5.26 and 4.89 mg/g, 6.38 and 5.97 mg/g, and 4.39 and 4.13 mg/g, respectively. N and $p$ accumulations in aboveground and belowground for the macrophytes can be seen in Table 3 and Figure 7. The root system of *P. australis* and *Sagittaria* showed higher N and $p$ accumulation than the shoot system, however, *Iris* showed the opposite. The highest N and $p$ accumulation was recorded in *Sagittaria* (287.17 and 57.43 g/m$^2$), followed by *P. australis* (193.31 and 30.68 g/m$^2$) and *Iris* (104.39 and 22.85 g/m$^2$), respectively. N and $p$ accumulation in aboveground biomass of macrophytes in this study were higher than that in another study, which reported the accumulation of 5.3–58.7 and 0.7–5.5 g/m$^2$, respectively [58]. These results are in line with those of Angassa et al. [31], who reported N and $p$ accumulation in aboveground biomass of 8.6–1376.0 and 2.3–324.0 g/m$^2$, respectively.

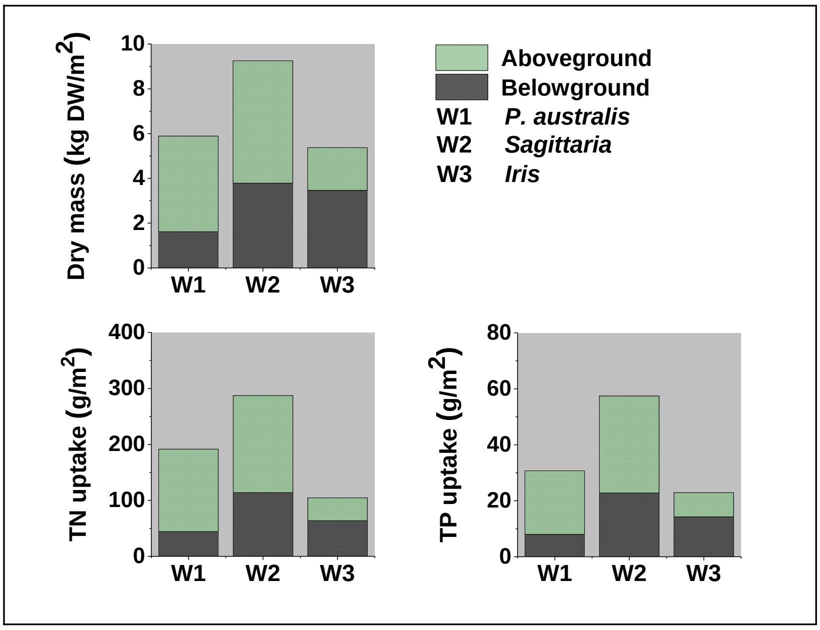


**Figure 7.** Biomass production and nutrient accumulation by macrophytes in HSSF-CWs.

**Table 3.** Contribution of macrophyte uptake to nutrient removal in HSSF-CWs.

| Parameter | Wetland Bed | Influent (g/m$^2$) | Effluent (g/m$^2$) | Aboveground Biomass | | | Belowground Biomass | | | Total N (g/m$^2$) | Others (g/m$^2$) | Macrophytes Uptake (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | DM | C | N | DM | C | N | | | |
| TN | W1 | 7113.19 | 4183.98 | 4268.63 | 34.47 | 147.14 | 1618.10 | 27.28 | 44.14 | 193.31 | 2737.90 | 6.3 |
| | W2 | 7113.19 | 4211.68 | 5467.42 | 31.69 | 173.26 | 3783.37 | 29.95 | 113.31 | 287.17 | 2614.34 | 9.3 |
| | W3 | 7113.19 | 4572.52 | 1911.61 | 21.32 | 40.74 | 3458.91 | 18.43 | 63.75 | 104.39 | 2436.28 | 3.9 |
| TP | W1 | 1171.26 | 584.81 | 4268.63 | 5.26 | 22.45 | 1618.10 | 4.89 | 7.91 | 30.68 | 555.77 | 5.1 |
| | W2 | 1171.26 | 456.09 | 5467.42 | 6.38 | 34.88 | 3783.37 | 5.97 | 22.59 | 57.43 | 657.74 | 7.6 |
| | W3 | 1171.26 | 655.32 | 1911.61 | 4.39 | 5.23 | 3458.91 | 4.13 | 14.29 | 22.85 | 493.09 | 4.4 |

DW: Dry weight (g/m$^2$); C: Mean nutrient concentration in macrophytes biomass (mg/g); N: Amount of nutrient uptake by the macrophytes (g/m$^2$); Others: Amount of nutrient removal due to adsorption by substrates and microbial processes, calculated by subtracting the amount of nutrients removed by the macrophytes from the total amount removed in the system.

An analysis of TN and TP mass balance was conducted for the different planted HSSF-CWs throughout the study (Table 3). The total influent load to each bed was 7.11 kg/m$^2$ for TN and 1.17 kg/m$^2$ for TP. TN and TP effluent by drainage was 4.18 and 0.58 kg/m$^2$ for W1, 4.21 and 0.46 kg/m$^2$ for W2, and 4.57 and 0.66 kg/m$^2$ for W3, respectively. Total net accumulation was evaluated by multiplying the TN or TP content of macrophytes with the entire dry biomass at the end of the experiment. The net total macrophyte uptake was 193.31 g N/m$^2$ and 30.68 g p/m$^2$ in *P. australis*, 287.17 g N/m$^2$ and 57.43 g p/m$^2$ in *Sagittaria*, and 104.39 g N/m$^2$ and 22.85 g p/m$^2$ in *Iris* (Table 3 and Figure 7).

TN loss due to microbial nitrification, denitrification, and substrate physical sorption in HF-CWs beds was estimated at 2.74 kg/m$^2$ for W1, 2.61 kg/m$^2$ for W2, and 2.43 kg/m$^2$ for W3. Loss of TP due to microbial assimilation and main substrate physical sorption was 0.56, 0.66, and 0.49 kg/m$^2$ for W1, W2, and W3, respectively. The comparison of macrophyte uptake for N and *p* with mass removal obtained in the planted beds during the entire experiment (Table 3) indicates that the uptake by *Sagittaria* was the highest, accounting for 9.3% of mass N removal and 7.6% of mass *p* removal. The proportion of uptake was 6.3% of mass N removal and 5.1% of mass *p* removal by *P. australis* and 3.9% of mass N removal and 4.4% of mass *p* removal by *Iris*.

## 4. Conclusions

Based on eight months of regular monitoring and investigation on the effect of the presence of macrophytes and their impact on the removal of contaminants from low-strength municipal wastewater in the horizontal sub-surface flow constructed wetland, the following conclusions were drawn:

- The planted beds demonstrated significantly ($p < 0.01$) higher removal of TSS (70–78%), $BOD_5$ (66–77%), COD (59–75%), $NO_3$-N (56–64%), $NH_4$-N (41–69%), TN (36–41%), and TP (44–61%) as compared to the unplanted bed, which showed the removal of 48%, 39%, 40%, 33%, 18%, 20%, and 29%, respectively.
- All species showed significant differences ($p < 0.05$) throughout the study. *P. australis* and *Sagittaria* exhibited better performance than *Iris*. *Sagittaria* performed better than *P. australis* in the removal of TSS, $NO_3$-N, and TP, however, for $BOD_5$, COD, $NH_4$-N, and TN, no significant differences ($p > 0.05$) were found.
- The highest biomass production was found in *Sagittaria* (9.6 kg DW/$m^2$), followed by *P. australis* (5.9 kg DW/$m^2$) and *Iris* (5.4 kg DW/$m^2$). Total N and $p$ accumulation were recorded in the order of *Sagittaria* (287.2 and 57.4 g/$m^2$) > *P. australis* (193.3 and 30.7 g/$m^2$) > *Iris* (104.4 and 22.9 g/$m^2$), respectively. The contributions due to uptake of *Sagittaria, P. australis,* and *Iris* to mass N and $p$ removal were 9.3% and 7.6%, 6.3% and 5.1%, and 3.9% and 4.4%, respectively. Macrophyte species demonstrated significant differences ($p < 0.01$), showing the effectiveness in the production of biomass and nutrient accumulation in the order of *Sagittaria* > *P. australis* > *Iris*.
- This study has revealed that the presence of macrophytes and their choice is an important aspect to obtain higher removal efficiencies in terms of solids, organic matter, and nutrients. It has further revealed that careful selection of species with extensive root networks and large biomasses can also increase the removal of nutrients in HSSF-CWs.

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## References

1. Jinadasa, K.B.S.N.; Tanaka, N.; Mowjood, M.I.M.; Werellagama, D.R.I.B. Free water surface constructed wetlands for domestic wastewater treatment: A tropical case study. *J. Chem. Ecol.* **2006**, *22*, 181–191. [CrossRef]
2. Kadlec, R.H.; Wallace, S. *Treatment Wetlands*, 2nd ed.; CRC Press Taylor and Francis Group: Boca Raton, FL, USA, 2009.

3. Konnerup, D.; Koottatep, T.; Brix, H. Treatment of domestic wastewater in tropical, subsurface flow constructed wetlands planted with *Canna* and *Heliconia*. *Ecol. Eng.* **2009**, *35*, 248–257. [CrossRef]
4. Zhang, D.Q.; Jinadasa, K.B.S.N.; Gersberg, R.M.; Liu, Y.; Ng, W.J.; Tan, S.K. Application of constructed wetlands for wastewater treatment in developing countries-a review of recent developments (2000–2013). *J. Environ. Manag.* **2014**, *141*, 116–131. [CrossRef] [PubMed]
5. Vymazal, J. The use of constructed wetlands for nitrogen removal from agricultural drainage: A review. *Sci. Agric. Bohem.* **2017**, *48*, 82–91. [CrossRef]
6. Wei, Z.; Ji, G. Constructed wetlands, 1991–2011: A review of research development, current trends, and future directions. *Sci. Total Environ.* **2012**, *441*, 19–27. [CrossRef]
7. Vymazal, J. Constructed wetlands for wastewater treatment. *Water Res.* **2010**, *2*, 530–549. [CrossRef]
8. Verma, R.; Suthar, S. Performance assessment of horizontal and vertical surface flow constructed wetland system in wastewater treatment using multivariate principal component analysis. *Ecol. Eng.* **2018**, *116*, 121–126. [CrossRef]
9. Jing, S.R.; Ling, J.F.; Wang, T.W.; Lee, D.Y. Microcosm wetlands for wastewater treatment with different hydraulic loading rates and macrophytes. *J. Environ. Qual.* **2002**, *31*, 690–696. [CrossRef]
10. Vymazal, J.; Kropfelova, L. Growth of *Phragmites australis* and *Phalaris arundinacea* in constructed wetlands for wastewater treatment in the Czech Republic. *Ecol. Eng.* **2005**, *25*, 606–621. [CrossRef]
11. Zhou, Q.; Zhu, H.; Bañuelos, G.; Yan, B.; Liang, Y.; Yu, X.; Cheng, X.; Chen, L. Effects of vegetation and temperature on nutrient removal and microbiology in horizontal subsurface flow constructed wetlands for treatment of domestic sewage. *Water Air Soil Pollut.* **2017**, *228*, 95. [CrossRef]
12. Angassa, K.; Leta, S.; Mulat, W.; Kloos, H.; Meers, E. Evaluation of pilot-scale constructed wetlands with *Phragmites karka* for phytoremediation of municipal wastewater and biomass production in Ethiopia. *Environ. Process.* **2019**, *6*, 65–84. [CrossRef]
13. Button, M.; Nivala, J.; Weber, K.P.; Aubron, T.; Müller, R.A. Microbial community metabolic function in subsurface flow constructed wetlands of different designs. *Ecol. Eng.* **2015**, *80*, 162–171. [CrossRef]
14. Toscano, A.; Marzo, A.; Milani, M.; Cirelli, G.L.; Barbagallo, S. Comparison of removal efficiencies in mediterranean pilot constructed wetlands vegetated with different macrophyte species. *Ecol. Eng.* **2015**, *75*, 155–160. [CrossRef]
15. Camacho, J.V.; Martínez, A.D.L.; Gómez, R.G.; Sanz, J.M. A comparative study of five horizontal subsurface flow constructed wetlands using different plant species for domestic wastewater treatment. *Environ. Technol.* **2007**, *28*, 1333–1343. [CrossRef]
16. Shelef, O.; Gross, A.; Rachmilevitch, S. Role of plants in a constructed wetland: Current and new perspectives. *Water* **2013**, *5*, 405–419. [CrossRef]
17. Karathanasis, A.D.; Potter, C.L.; Coyne, M.S. Vegetation effect on fecal bacteria BOD, and suspended solid removal in constructed wetlands treating domestic wastewater. *Ecol. Eng.* **2003**, *20*, 157–169. [CrossRef]
18. Vacca, G.; Wand, H.; Nikolausz, M.; Kuschk, P.; Kästner, M. Effect of macrophytes and filter materials on bacteria removal in pilot-scale constructed wetlands. *Water Res.* **2005**, *39*, 1361–1373. [CrossRef]
19. Headley, T.; Nivala, J.; Kassa, K.; Olsson, L.; Wallace, S.; Brix, H.; Afferden, M.V.; Müller, R. Escherichia coli removal and internal dynamics in subsurface flow ecotechnologies: Effects of design and macrophytes. *Ecol. Eng.* **2013**, *61*, 564–574. [CrossRef]
20. Carranza-Diaz, O.; Schultze-Nobre, L.; Moeder, M.; Nivala, J.; Kuschk, P.; Koeser, H. Removal of selected organic micropollutants in planted and unplanted pilot-scale horizontal flow constructed wetlands under conditions of high organic load. *Ecol. Eng.* **2014**, *71*, 234–245. [CrossRef]
21. Tanner, C. Macrophytes as ecosystem engineers in subsurface-flow treatment wet-lands. *Water Sci. Technol.* **2001**, *44*, 9–17. [CrossRef]
22. Lima, M.X.; Carvalho, K.Q.; Passig, F.H.; Borges, A.C.; Filippe, T.C.; Azevedo, J.C.R.; Nagalli, A. Performance of different substrates in constructed wetlands planted with *E. crassipes* treating low-strength sewage under subtropical conditions. *Sci. Total Environ.* **2018**, *630*, 1365–1373. [CrossRef] [PubMed]
23. Calheiros, C.S.C.; Quitério, P.V.B.; Silva, G.; Crispim, L.F.C.; Brix, H.; Moura, S.C.; Castro, P.M.L. Use of constructed wetland systems with Arundo and Sarcocornia for polishing high salinity tannery wastewater. *J. Environ. Manag.* **2012**, *95*, 66–71. [CrossRef] [PubMed]
24. Carballeira, T.; Ruiz, I.; Soto, M. Effect of macrophytes and surface loading rate on the treatment efficiency of shallow subsurface constructed wetlands. *Ecol. Eng.* **2016**, *90*, 203–214. [CrossRef]
25. Maltais-Landry, G.; Maranger, R.; Brisson, J.; Chazarenc, F. Nitrogen transformations and retention in planted and artificially aerated constructed wetlands. *Water Res.* **2009**, *43*, 535–545. [CrossRef] [PubMed]
26. Leto, C.; Tuttolomondo, T.; La Bella, S.; Leone, R.; Licata, M. Effects of macrophyte species in a horizontal subsurface flow constructed wetland-phytoremediation of treated urban wastewater with *Cyperus alternifolius* L. and *Typha latifolia* L. in the west of Sicily (Italy). *Ecol. Eng.* **2013**, *61*, 282–291. [CrossRef]
27. CGWB. Central Ground Water Board Report on "Aquifer Mapping and Management Plan of Aligarh District Uttar Pradesh" Ministry of Water Resources, River Development and Ganga Rejuvenation Government of India. 2017. Available online: http://cgwb.gov.in/AQM/NAQUIM_REPORT/UP/Aligarh.pdf (accessed on 10 September 2021).
28. APHA. *Standard Methods for the Examination of Water and Wastewater*, 21st ed.; American Public Health Association: Washington, DC, USA, 2005.

29. Li, X.; Guo, R. Comparison of nitrogen removal in floating treatment wetlands constructed with *Phragmites australis* and *Acorus calamus* in a cold temperate zone. *Water Air Soil Pollut.* **2017**, *228*, 132. [CrossRef]
30. Lindner, R.C. Rapid analytical methods for some of the common inorganic constitutes of macrophyte tissues. *Macrophyte Physiol.* **1994**, *19*, 76–89. [CrossRef]
31. Fiske, C.H.; Subbarow, Y.J. The colorimetric determination of phosphorus. *Biol. Chem.* **1925**, *66*, 375–400. [CrossRef]
32. Kadlec, R.H. *Status of Treatment Wetlands North America in Proceeding Conference of the Use of Aquatic Macrophytes for Wastewater Treatment in Constructed Wetlands*; Dias, V., Vymazal, J., Eds.; ICN and INAG: Lisbon, Portugal, 2003; pp. 363–401.
33. Tanaka, N.; Jinadasa, K.B.S.N.; Werellagama, D.R.I.B.; Mowjood, M.I.M.; Ng, W.J. Constructed tropical wetlands with integrated submergent-emergent macrophytes for sustainable water quality management. *J. Environ. Sci. Health Part A* **2013**, *41*, 2221–2236. [CrossRef]
34. Nguyen, X.C.; Chang, S.W.; Nguyen, T.L.; Ngo, H.H.; Kumar, G.; Banu, J.R.; Vu, M.C.; Le, H.S.; Nguyen, D.D. A hybrid constructed wetland for organic-material and nutrient removal from sewage: Process performance and multi-kinetic models. *J. Environ. Manag.* **2018**, *222*, 378–384. [CrossRef]
35. Wu, Y.; Han, R.; Yang, X.; Zhang, Y.; Zhang, R. Long-term performance of an integrated constructed wetland for advanced treatment of mixed wastewater. *Ecol. Eng.* **2017**, *99*, 91–98. [CrossRef]
36. Katsenovich, Y.P.; Hummel-Batista, A.; Ravinet, A.J.; Miller, J.F. Performance evaluation of constructed wetlands in a tropical region. *Ecol. Eng.* **2009**, *35*, 1529–1537. [CrossRef]
37. Abdel-Shafy, H.I.; El-Khateeb, M.A.; Regelsberger, M.; El-Sheikh, R.; Shehata, M. Integrated system for the treatment of blackwater and greywater via UASB and constructed wetland in Egypt. *Desalin. Water Treat.* **2009**, *8*, 272–278. [CrossRef]
38. Zurita, F.; Belmont, M.A.; Anda, J.D.; White, J.R. Seeking a way to promote the use of constructed wetlands for domestic wastewater treatment in developing countries. *Water Sci. Technol.* **2011**, *63*, 654–659. [CrossRef]
39. Fountoulakis, M.S.; Daskalakis, G.; Papadaki, A.; Kalogerakis, N.; Manios, T. Use of Halophytes in pilot-scale horizontal flow constructed wetland treating domestic wastewater. *Environ. Sci. Pollut. Res.* **2017**, *24*, 16682–16689. [CrossRef]
40. Cakir, R.; Gidirislioglu, A.; Cebi, U. A study on the effects of different hydraulic loading rates (HLR) on pollutant removal efficiency of subsurface horizontal flow constructed wetlands used for treatment of domestic wastewaters. *J. Environ. Manag.* **2015**, *164*, 121–128. [CrossRef] [PubMed]
41. Vymazal, J. Plants used in constructed wetland with horizontal subsurface flow: A review. *Hydrobiologia* **2011**, *674*, 133–156. [CrossRef]
42. Saeed, T.; Sun, G. A review on nitrogen and organics removal mechanisms in subsurface flow constructed wetlands: Dependency on environmental parameters, operating conditions and supporting media. *J. Environ. Manag.* **2012**, *112*, 429–448. [CrossRef]
43. Vymazal, J. Horizontal subsurface flow and hybrid constructed wetland systems for wastewater treatment. *Ecol. Eng.* **2005**, *25*, 478–490. [CrossRef]
44. Aalam, T.; Khalil, N. Performance of horizontal sub-surface flow constructed wetlands with different flow patterns using dual media for low-strength municipal wastewater: A case of pilot scale experiment in a tropical climate region. *J. Environ. Sci. Health Part A* **2019**, *54*, 1245–1253. [CrossRef]
45. Li, J.; Wen, Y.; Zhou, Q.; Xingjie, Z.; Li, X.; Yang, S.; Lin, T. Influence of vegetation and substrate on the removal and transformation of dissolved organic matter in horizontal subsurface-flow constructed wetlands. *Bioresour. Technol.* **2008**, *99*, 4990–4996. [CrossRef] [PubMed]
46. Jóźwiakowski, K.; Bugajski, P.; Kurek, K.; De Carvalho, M.D.F.N.; Almeida, M.A.A.; Siwiec, T.; Borowski, G.; Czekała, W.; Dach, J.; Gajewska, M. The efficiency and technological reliability of biogenic compounds removal during long-term operation of a one-stage subsurface horizontal flow constructed wetland. *Sep. Purif. Technol.* **2018**, *202*, 216–226. [CrossRef]
47. Angassa, K.; Leta, S.; Mulat, W. Organic matter and nutrient removal performance of horizontal subsurface flow constructed wetlands planted with *Phragmites Karka* and *Vetiveria Zizanioide* for treating municipal wastewater. *Environ. Process.* **2017**, *5*, 115–130. [CrossRef]
48. Ennabili, A.; Ater, M.; Radoux, M. Biomass production and NPK retention in macrophytes from wetlands of the Tingitan Peninsula. *Aquat. Bot.* **1998**, *62*, 45–56. [CrossRef]
49. Abou-Elela, S.I.; Golinielli, G.; Abou-Taleba, E.M.; Hellal, M.S. Municipal wastewater treatment in horizontal and vertical flows constructed wetlands. *Ecol. Eng.* **2013**, *61*, 460–468. [CrossRef]
50. Vymazal, J.; Kropfelova, L. *Wastewater Treatment in Constructed Wetlands with Horizontal Sub-Surface Flow*; Springer: Dordrecht, The Netherlands, 2008; Volume 14.
51. Raboni, M.; Gavasci, R.; Urbini, G. UASB followed by sub-surface horizontal flow phytodepuration for the treatment of the sewage generated by a small rural community. *Sustainability* **2014**, *6*, 6998–7012. [CrossRef]
52. Kadlec, R.; Knight, R. *Treatment Wetlands*; Chemical Rubber Company Press: Boca Raton, FL, USA; Lewis Publishers: Boca Raton, FL, USA, 1996.
53. Vymazal, J. Removal of nutrients in various types of constructed wetlands. *Sci. Total Environ.* **2007**, *380*, 48–65. [CrossRef]
54. Zhang, W.J.; Yang, P.; Yang, X.Y.; Chen, Z.; Wang, D.S. Insights into the respective role of acidification and oxidation for enhancing anaerobic digested sludge dewatering performance with Fenton process. *Bioresour. Technol.* **2015**, *181*, 247–253. [CrossRef]
55. Vymazal, J. Constructed wetlands for treatment of industrial wastewaters: A review. *Ecol. Eng.* **2014**, *73*, 724–751. [CrossRef]

56. Williams, R.F.; Avery, L.; Winward, G.; Jeffrey, P.; Smith, C.S.; Liu, S.; Memon, F.A.; Jefferson, B. Constructed wetlands for urban greywater recycling. *Ijep* **2008**, *33*, 93–109. [CrossRef]
57. Oopkaup, K.; Truu, M.; Nolvak, H.; Ligi, T.; Preem, J.K.; Mander, U.; Truu, J. Dynamics of Bacterial Community Abundance and Structure in Horizontal Subsurface Flow Wetland. *Water* **2016**, *8*, 457. [CrossRef]
58. Avila, C.; Garfí, M.; Garcia, J. Three-stage hybrid constructed wetland system for wastewater treatment and reuse in warm climate regions. *Ecol. Eng.* **2013**, *61*, 43–49. [CrossRef]