water

MDPI

Article

Peat as Substrate for Small-Scale Constructed Wetlands Polishing Secondary Effluents from Municipal Wastewater Treatment Plant

Meng Jin [1], Jacob Carlos [1], Rachel McConnell [1], Geof Hall [1] and Pascale Champagne [1,2,*]

1 Department of Civil Engineering, Queen's University, 58 University Ave., Kingston, ON K7L 3N6, Canada; 13mj@queensu.ca (M.J.); 12jwc@queensu.ca (J.C.); rachel.mcconnell@queensu.ca (R.M.); gh26@queensu.ca (G.H.)
2 Department of Chemical Engineering, Queen's University, 58 University Ave., Kingston, ON K7L 3N6, Canada
* Correspondence: pascale.champagne@queensu.ca; Tel.: +1-613-533-3053

Received: 2 November 2017; Accepted: 21 November 2017; Published: 28 November 2017

Abstract: With the recent development of constructed wetland technology, it has become a mainstream treatment technology for the mitigation of a variety of wastewaters. This study reports on the treatment performance and pH attenuation capacity of three different configurations of small-scale on-site surface flow constructed wetlands (SFCW): T1 (Peat + *Typha latifolia*), T2 (*T. latifolia* alone), and T3 (Peat alone) treating secondary effluent from the Amherstview Water Pollution Control Plant (WPCP) for two treatment periods (start-up period and operational period). The aim of this study was to compare the nutrients removal efficiencies between the different treatments, as well as to evaluate the effects of substrate and vegetation on the wetland system. For a hydraulic retention time of 2.5 days, the results showed that all treatment systems could attenuate the pH level during both the start-up and operational periods, while significant nutrient removal performance could only be observed during the operational period. Peat was noted to be a better SFCW substrate in promoting the removal of nitrate (NO_3-N), total nitrogen (TN), and phosphorus. The addition of *T. latifolia* further enhanced NO_3-N and TN removal efficiencies, but employing *T. latifolia* alone did not yield effluents that could meet the regulatory discharge limit (1.0 mg/L) for phosphorus.

Keywords: constructed wetland; peat; pH; *Typha latifolia*; wastewater stabilization pond

1. Introduction

Constructed wetlands are considered to be a sustainable passive wastewater treatment technology, and have been used to treat a variety of wastewaters for decades, including domestic or municipal wastewater [1,2], industrial wastewater [3,4], agricultural [5–7], acid mine drainage [8], river and lake water [9–11], groundwater [12], landfill leachate [13–15], highway [16] and airport [17] runoff. They take advantage of many of the same processes that occur in natural wetlands, but do so in a more controlled or engineered system [18]. To date, surface flow and subsurface flow wetlands are the two main categories of constructed wetland applications [2]. In recent years, subsurface flow constructed wetlands (SSFCW) have been extensively studied, as their treatment efficiency, in terms of nutrients mass removal per unit area, is typically higher than that of surface flow wetlands. Alternative influent feeding modes, such as tidal flow, step feed, and upflow mode, have also been investigated to further enhance treatment performance. Although surface flow constructed wetlands (SFCW) represent an older and less sophisticated configuration, they remain an effective treatment approach. Despite their lower treatment efficiencies compared to SSFCW, the lower construction cost and maintenance requirements associated with SFCW are desirable for smaller and more rural communities, especially when dealing with micro-polluted wastewaters or secondary effluents from wastewater treatment plants.

Substrate and vegetation are two of the three main wetland components along with hydrology [19] that need to be considered in the design of constructed wetlands, and they play an important role in SFCW function. Hence, their selections have to be made carefully in order to achieve the desire treatment objectives of the system. In general, local soils are used as the rooting medium in SFCW and was primarily used to sustain the growth of wetland vegetation. However, recent studies have shown that contribution to treatment of the overall system by these substrates has been limited. Therefore, alternative substrates have become more attractive and a number of studies have been conducted using alternative substrates in wetland applications [20–23]. These include natural, manufactured and reclaimed materials. Although manufactured materials could provide better treatment performance, natural and reclaimed products are generally preferable due to their low economic cost and geographical availability [24].

Peat is a natural and organic substrate with a structure containing mostly humic substances. Previous studies [25–28] have employed peat in constructed wetland applications, but most of these only focused on nutrient removal. As an organic substrate with relative acidic properties, peat could also be used to attenuate high pH wastewater such as alkaline mine drainage and algal-induced, elevated pH secondary effluents. One study concluded that peat could be used effectively for the attenuation of pH in synthetic wastewater, when employed in bench-scale wetland system without the presence of vegetation [29].

Typha latifolia (cattail, broad-leaved cattail) is an emergent plant which has been reported to be present on all continents except Central and South America, and it is the most widely represented vegetation species in constructed wetlands across North America [30]. It can grow aggressively and tends to out-compete other species planted in wetlands. Its relatively short growing period and advanced extensive branching horizontal rhizome system are ideal for constructed wetland applications, as they can substantially reduce nutrient concentrations through nutrient uptake and increase the rate of sedimentation by reducing current velocity.

Several comparative constructed wetland studies have been published that compare the treatment performance achieved by applying different substrates or vegetation. Most of these studies have focused on the comparison between either substrates or vegetation alone and have not compared the effects of substrates and vegetation as they act in unison [4,23,31–34]. This study fills this gap and identifies the role that substrate and vegetation play in constructed wetland applications.

The objective of this paper was to assess the effects of peat and *T. latifolia* on the attenuation of pH and treatment of a municipal secondary effluents in three small-scale on-site SFCW reactors at the Amherstview Water Pollution Control Plant (WPCP) located on the northern shore of Lake Ontario in Ontario (Canada). The performance of all treatment configurations including substrate alone, vegetation alone, and substrate and vegetation together, were monitored and compared over a period of one year. The individual treatment contributions of *T. latifolia* and peat to the treatment system were also evaluated to characterize their effects on the overall performance of the SFCW.

2. Materials and Methods

2.1. Wastewater

The Amherstview WPCP, which currently services a population over 10,000 and treats an average of 3500 m^3/d of municipal wastewater, consists of a direct activated sludge treatment process, followed by tertiary treatment and effluent polishing that takes place in three wastewater stabilization ponds operated in series (Figure 1). The wastewater applied to the small-scale on-site SFCW in this study was directly diverted from the influent of the second wastewater stabilization pond, which was characterized as a secondary effluent from the treatment plant.

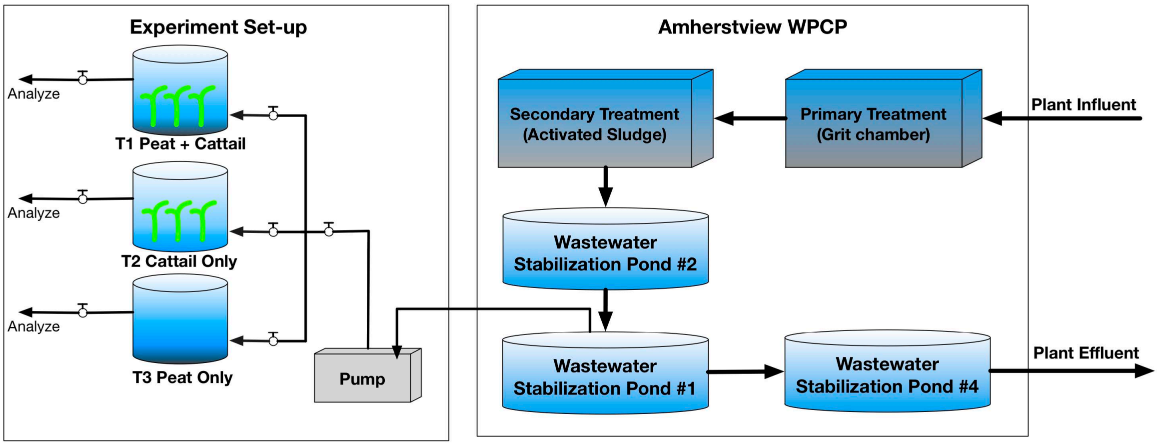


Figure 1. Schematic design of the experiment set-up and the treatment processes flow at the Amherstview Water Pollution Control Plant (WPCP).

2.2. Wetland Reactors

Three outdoor small-scale surface flow wetland reactors were built on-site at the Amherstview WPCP using open top cylindrical high-density polyethylene tanks with 6.5 m^3 volumes (diameter: 2.13 m, height: 1.82 m). Three 5 cm holes were drilled along the outer of each tank to act as inlet (200 mm height), outlet (600 mm height), and drain (50 mm height) (Figure 2a).

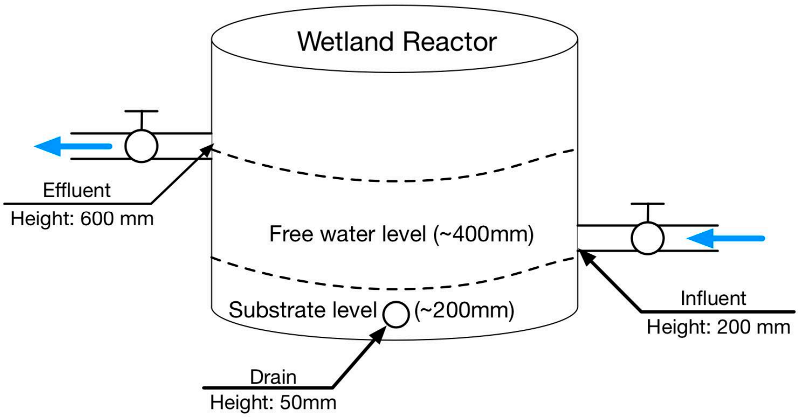
Locally available substrate and vegetation were employed in this study. Peat moss was purchased from the Home Depot in Kingston (Canada) and was applied in this experiment as substrate that could potentially attenuate high pH effluents. The properties of the peat moss used in this study are listed in Table 1. The porosity was determined by dividing the volume of water required to fill the void spaces in the substrate by the volume of the substrate. *T. latifolia* was collected from the Bayview Bog Field Site (BBFS) (44°15′20″ N, 76°38′56″ W), which is a natural wetland complex located in Loyalist Township and governed by the Cataraqui Region Conservation Authority (CRCA). The plants were harvested by Loyalist Township using a backhoe. The plants were then transported to the Amherstview WPCP by pickup trucks covered with tarpaulins to keep the plants moisturized. After receiving the plants, they were immediately transplanted to the individual wetland reactors. It is recognized that because the *T. latifolia* plants were transplanted from a natural wetland, some of the native soil was inevitably also relocated to the reactors as the goal was to minimize damage to the root zone of the plants during the transplantation process.

The wetland reactor configurations tested included: Reactor T1, peat moss and *T. latifolia*; Reactor T2, *T. latifolia* alone; Reactor T3, peat moss alone. Reactor T1 contained both a 20 cm peat moss layer, as well as approximately 20 stalks of *T. latifolia*. Reactor T2 contained *T. latifolia* alone, with approximately 20 stalks of *T. latifolia* transplanted to this reactor. The native wetland soil was used as growing media at a depth of 20 cm. Reactor T3 contained peat moss substrate alone at a depth of 20 cm. All three wetland reactors had a free water surface level of 40 cm on top of the substrate in the tank.

Table 1. Physical properties of the peat applied in this study.

Substrate	Density (kg/m^3)	Porosity *
peat	800	0.31

Note: * Porosity is from 0 to 1, where 0 indicates the lowest porosity and 1 indicates the highest porosity.



(**a**)

(**b**)

Figure 2. (**a**) Detail design and dimensions of the individual treatment wetland reactors (not to scale); and (**b**) three constructed wetland reactors with connection tubing and flow control valves in the field.

2.3. Systems Operations

Wastewater was pumped into the system from the influent of the second wastewater stabilization pond of the Amherstview WPCP using a Pentair centrifugal pump. Wastewater was distributed equally to the three wetland reactors through parallel connections. One inch polybutylene pipe was used as the main influent pipe, which was connected between the wastewater stabilization pond to the front of each treatment reactor, passing through the pump. Three quarters of an inch PVC pipe was then used to connect the one inch polybutylene pipe to the inlet of the wetland reactors using a one inch to three quarters of an inch reduction adaptor. Valves were installed to control flow to each reactor, which can only be applied to PVC tubing. One inch PVC pipes were used to connect each tank outlet to the effluent pipe. A PVC ball valve was placed before the inlet and after the outlet of each wetland reactor to control the flow.

The hydraulic retention time of each wetland reactor was determined based on the design of the pilot-scale surface flow constructed wetland at the Amherstview WPCP, and was set to a constant value of 2.5 days for the duration of the entire experiment. All wetland reactors were commissioned in the summer of 2016. A two-month acclimation period was provided to allow the substrate and vegetation to establish. After the establishment of the substrates and vegetation, the system was operated and fed with secondary effluent wastewater. The study was separated into two treatment periods, as the wetland reactors required a start-up period before normal operation. The start-up period for this experiment was from 29 September to 17 November 2016, and the operational period was from 17 May to 31 July 2017. Sample data from 17 November 2016 to 16 May 2017 were not available in that period due to freezing during the winter conditions.

2.4. Sampling and Analysis

Wastewater samples were collected on a bi-weekly basis (Mondays and Thursdays) during the start-up period, and on a weekly basis (Wednesdays) during the operational period. Weather data was collected by a meteorological station near the Kingston Airport, which is approximately 10 km away from the study site. Samples were collected from the effluent pipe of each wetland reactor as well as the second wastewater stabilization pond (influent), and were kept in a cooler at 4 °C until all laboratory testing could be completed. Samples were analyzed in duplicate. For each sample, analysis was carried out for pH, chemical oxygen demand (COD), ammonium (NH_4-N), NO_3-N, phosphate (PO_4-P), TN, and total phosphorus (TP). During the operational period, DO, alkalinity, and total suspended solids (TSS) were also measured. pH was analyzed using a Fisher Scientific Accumet XL60 benchtop meter with a Fisher Scientific pH probe manufactured by Fisher Scientific, USA. DO was measured using a YSI DO meter. Alkalinity, NH_4-N, NO_3-N and PO_4-P concentrations were analyzed using a Hach model DR 2800 spectrophotometer according to APHA Standard Methods. TN was measured using the Hach TNT Persulfate Digestion Method No. 10072. TP was measured using the Hach PhosVer® 3 with Acid Persulfate Digestion Method 8190. COD was analyzed with the same spectrophotometer according to the APHA Standard Methods. TSS was analyzed according to the APHA Standard Methods [29,35].

2.5. Statistical Analysis

To compare the performance of the different wetland reactor treatments, parametric and non-parametric statistical analyses were performed using Microsoft EXCEL. These were used to evaluate wastewater treatment performance, including removal efficiency (R, %), and removal rates (RR, g/m^2 d) as defined by Equations (1) and (2), respectively.

$$\text{R}\ (\%) = \frac{C_0 - C_t}{C_0} \times 100\% \tag{1}$$

$$\text{RR}\ (\text{g/m}^2\ \text{d}) = \frac{(C_0 - C_t) \times Q}{A} \tag{2}$$

where R is the removal efficiency (%), RR is the removal rates (g/m^2 d), C_0 is the influent concentration (mg/L), C_t is the effluent concentration (mg/L), Q is the flow rate (m^3/d), A is the area of reactors (m^2).

Normality tests were performed to verify whether the distribution of data approximated normality. Both graphical (histogram and normality plot) and statistical (skewness and kurtosis) methods were used to determine the data distribution. When data approximated normality, the data were analyzed through one-way analysis of variance (ANOVA) and illustrated the statistically significant difference (p value < 0.05) of the means across all treatments at a significant level of 0.05. When a significant difference was observed, post-hoc comparison analysis (Bonferroni test) was performed to determine the statistically significant difference between treatments at a significant level of 0.05. Paired t-tests were also used to directly compare samples between two treatment wetlands. When data that did not approximate normality were observed, a non-parametric statistical analysis, Mann-Whitney test, was performed to determine the variance between treatments.

3. Results

3.1. Overall Performance

Tables 2 and 3 provide the mean effluent nutrient concentrations and mean pollutant removal efficiencies in three wetland reactors during both the start-up (Table 2) and operational period (Table 3), respectively. The average influent hydraulic loading rate was 0.16 m^3/m^2 d (2.5 days hydraulic retention time) for each reactor.

Table 2. Mean effluent pollutant concentrations and removal efficiencies in each treatment wetland reactors for start-up period [1].

Parameter	Unit	Influent	Peat + Cattails T1		Cattails T2		Peat T3	
			Effluent Conc.	R %	Effluent Conc.	R %	Effluent Conc.	R %
pH		7.43 (0.41)	6.25 (1.09)		7.12 (0.24)		6.39 (1.27)	
COD	mg/L	14.2 (14.1)	49.2 (39.6)		15.9 (5.7)		44.0 (42.2)	
NH_4-N	mg/L	0.10 (0.08)	0.23 (0.22)		0.09 (0.04)	10%	0.22 (0.22)	
NO_3-N	mg/L	0.83 (0.32)	0.61 (0.26)	26.5%	0.52 (0.20)	37.3%	0.71 (0.31)	19.4%
TN	mg/L	8.75 (2.80)	8.21 (2.11)	6.17%	5.14 (2.58)	41.2%	8.58 (1.56)	0.8%
PO_4-P	mg/L	0.71 (0.13)	0.70 (0.28)	1.4%	0.59 (0.13)	16.9%	0.82 (0.22)	
TP	mg/L	2.90 (1.83)	3.42 (2.29)		2.27 (2.47)	18.2%	3.74 (1.95)	

Note: [1] Standard deviation of pollutant concentrations is indicated in brackets.

Table 3. Mean effluent pollutant concentrations and removal efficiencies in each treatment wetland reactors for operational period [1].

Parameter	Unit	Influent	Peat + Cattails T1		Cattails T2		Peat T3	
			Effluent Conc.	R %	Effluent Conc.	R %	Effluent Conc.	R %
pH		8.25 (0.81)	7.55 (0.96)		7.52 (0.92)		6.97 (0.37)	
DO	mg/L	6.10 (0.93)	6.44 (1.56)		5.92 (2.11)		6.32 (1.10)	
TSS	mg/L	4.1 (3.5)	9.8 (6.3)		6.4 (6.3)		17.6 (13.2)	
Alkalinity	mg/L	121.6 (15.9)	80.8 (21.0)		119.8 (37.9)		68.4 (54.9)	
COD	mg/L	12.2 (7.6)	49.0 (13.6)		24.4 (4.8)		49.8 (13.1)	
NH_4-N	mg/L	0.20 (0.32)	0.97 (0.33)		0.47 (0.36)		1.69 (0.53)	
NO_3-N	mg/L	7.93 (2.02)	1.08 (0.88)	86.9%	0.64 (0.42)	92.7%	1.43 (0.87)	81.7%
TN	mg/L	8.29 (1.62)	2.19 (1.49)	70.6%	2.04 (0.78)	75.0%	2.68 (1.43)	64.5%
PO_4-P	mg/L	0.75 (0.14)	0.31 (0.22)	58.7%	0.67 (0.23)	10.7%	0.38 (0.21)	49.3%
TP	mg/L	1.29 (0.33)	0.77 (0.32)	40.3%	1.13 (0.25)	12.4%	0.87 (0.27)	32.6%

Note: [1] Standard deviation of pollutant concentrations is indicated in brackets.

Overall, the results showed that pH could be effectively attenuated in all reactors during both treatment periods. Higher NO_3-N and TN removal efficiencies were recorded during the operational period compared to the start-up period. Phosphate and TP removal efficiencies were significantly improved in reactors where peat was present during operational period as well. However, due to the low influent COD concentrations, the introduction of peat and cattails to the system appeared to increase the effluent COD levels during both periods. The wetland reactors achieved a better overall performance across the reactors during normal operational period compared to start-up period. A more detailed comparison of each individual pollutant is presented in the following sections.

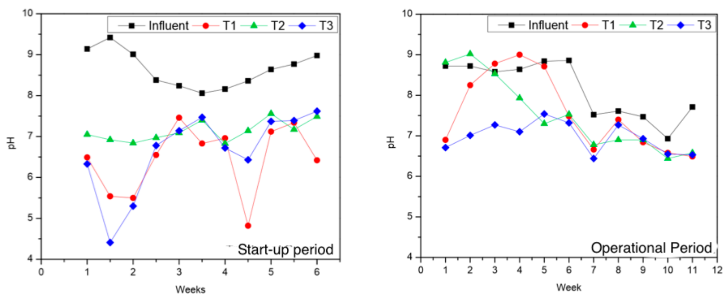
3.2. Secondary Effluent Characteristics

Tables 2 and 3 show the influent water quality (secondary effluent from the treatment plant) that entered the experimental system, and a strong difference was noted in pH, NO_3-N, and TP concentrations during start-up and operational period. The higher pH level of the influent during the summer was generally attributed the strong algal activities in the wastewater stabilization ponds that would exhaust the inorganic dissolved carbon and negatively impacted the pH balance in the water. The NO_3-N level was significantly higher during the operational period than during the start-up period, as the nitrification process was not likely as effective during the fall season due to the lower ambient temperature, which would slow down the activity of nitrifying bacteria [36]. Although the influent PO_4-P concentrations were similar between seasons, more than double the amount of TP was present during the operational period compared to the start-up period indicating other forms of phosphorus were likely present.

3.3. Attenuation of pH

Algal blooms have frequently been reported to occur in the Amherstview WPCP wastewater stabilization pond system during the summer months, and have been identified as the main reason for the elevated pH, and these pH levels have often exceeded the regulatory discharge limit allowed for this system in the past [37]. The introduction of a SFCW was identified as a potential solution to resolve this pH issue. In this study, all treatment wetland reactors were noted to reduce the pH to a level below the regulatory discharge limit for the entire experimental period. However, the overall pH level was found to increase during the operational period, mainly due to the increased algal activity in the wastewater stabilization pond, as a result of the elevated ambient temperatures and strong sunlight irradiation [38]. The peat substrate played an important role in reducing the mean pH level during the start-up period, as T1 and T3 significantly reduced the pH level in the first season from 7.43 to 6.25 and 6.39, respectively. Peat moss mainly consists of humic and fulvic acids, which can be quickly released under alkaline conditions in aqueous environments and can contribute to the reduction in pH levels. This instant impact was also demonstrated in a previous bench-scale study [29]. During the operational period, T3 was found to maintain effluent pH levels below 7, whereas the effluent pH level for T1 was higher (7.55). This could be due to the presence of vegetation, as the operational period experiences a more complete growth of *T. latifolia*, and its growth might have altered the water chemistry.

The pH trends during both treatment periods over time are shown in Figure 3. The pH level was reduced in T1 and T3 during the first two weeks of the start-up period. The short-term effect was likely due to the initial release of acidic compounds from the peat, which quickly decreased the pH level below 5.5. After the initial decrease in pH, the pH level in T1 and T3 were generally maintained between 6.5 and 7.5 throughout start-up period. The pH level in T2 was relatively consistent and remained between 7 and 7.5 with less fluctuation compared to T1 and T3 during the start-up period. This was to be expected as there was no external source of acidic compounds entering the system. The starting pH levels of T1 and T3 were similar at the beginning of operational period (pH ~6.8). T3 was found to maintain a relatively stable pH level throughout the operational period, whereas T1 experienced a pH increase up to approximately 9 during Weeks 2–4, but eventually the pH was observed to decrease again to approximately 7. T2 had a similar pH level as the influent at the beginning of operational period. However, the pH was gradually noted to decrease from ~9 to 6.5 towards the end of operational period. It was noted that precipitation greatly affected the influent pH levels, where after a heavy rainfall event, stormwater runoff, which is typically pH neutral, would generally enter the stabilization pond and mitigate the high pH effluents. Several heavy rainfall events occurred after Week 6 during the operational period, which is reflected in the pH trend observed.



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

Figure 3. (**a**) pH variation over start-up experimental period; and (**b**) pH variation over operational experimental period.

3.4. Nitrogen Removal

Due to the nature of the shallow wastewater stabilization pond, in which aerobic conditions typically prevail, NH_4-N can be easily oxidized and converted to NO_3-N, resulting in a low effluent NH_4-N concentrations. The elevated pH in the stabilization pond could also encourage the volatilization of NH_3 to the atmosphere, which further lower the NH_4-N level. Therefore, the NH_4-N concentration from the secondary effluent was consistently low during the entire experimental period. As a result, further removal of NH_4-N in the following treatment process was not anticipated. In this experiment, the influent NH_4-N concentrations were 0.10 mg/L and 0.20 mg/L for the start-up and operational periods, respectively. Only T2 could further remove the NH_4-N in start-up period, and only a 10% removal efficiency was observed. Effluent NH_4-N concentrations were increased in all other scenarios during both treatment periods. Low NH_4-N removal efficiencies were also reported in other studies where influent concentrations were low. A surface flow constructed wetland study showed that only a 11.8% of NH_4-N removal efficiency was achieved with an influent concentration of 0.88 mg/L [39]. Another study also observed no positive removal of NH_4-N, when the influent NH_4-N level was below 0.41 mg/L [40]. The increase in NH_4-N concentration might be due to dissimilatory nitrate reduction to ammonia, which is a reduction process that could reduce NO_2-N and NO_3-N back to NH_4-N under anoxic/reduced environments [41]. In this study, the increase in COD concentrations and high COD/NO_3-N ratio could favor the production of NH_4-N through dissimilatory nitrate reduction. The introduction of peat might also contribute to the high NH_4-N effluent concentrations due to the nitrogen released by peat. Despite the limited ability to further remove NH_4-N from the secondary effluent, all treatments removed NO_3-N and TN during both treatment periods. Maximum NO_3-N and TN removal efficiencies of 37.3% and 41.2% were achieved during the start-up period, and 92.7% and 75.0% during the operational period in T2. During both treatment periods, NO_3-N accounted for most of TN removal in all reactors.

In a typical constructed wetland reactor, nitrification-denitrification, anaerobic ammonium oxidation (ANAMMOX) and plant uptake are the three main pathways to remove NO_3-N and TN [42]. However, the low NH_4-N input and the aerobic conditions present in all reactors promoted by a shallow reactor depth and the vegetative oxygen diffusion, indicated that ANAMMOX would not likely to be the primary route for nitrogen removal. As no vegetation was employed in T3, most of the TN was expected to be removed through the nitrification and denitrification route and a 64.5% of TN removal efficiency indicated that peat could provide adequate TN removal efficiency. However, the introduction of vegetation produced TN removal efficiencies of 41.2% and 75.0% during the start-up and operational periods, respectively. The vegetation not only provided another TN removal route, but most likely also stimulated the biological activity in the reactors. The TN removal efficiency of T1 was always between that of T3 and T2 during both periods. However, T1 had higher NH_4-N concentrations compared to T2, which would suggest the presence of bacteria performing dissimilatory nitrate reduction processes. These bacteria may have competed with denitrifier (heterotrophic bacteria), which in turn would have reduced the overall TN removal efficiency.

3.5. Phosphorus Removal

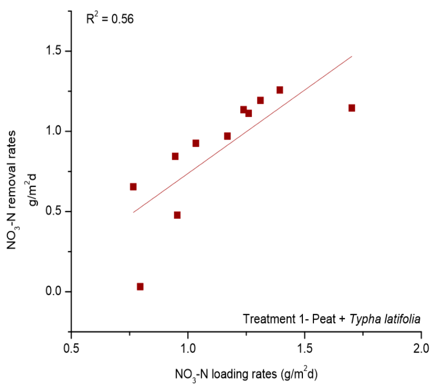
The overall phosphorus removal efficiency was low during the start-up period, and only T2 could reduce both PO_4-P and TP concentrations. T1 was only able to decrease PO_4-P concentrations by 1.4%, while T3 exhibited a higher effluent concentration than the influent. The introduction of peat could be responsible for the increase in phosphorus during the start-up period, as the mobile phosphorus could easily have been detached from the peat and released to the water column, which in turn could have contributed to a high phosphorus concentration in the effluent. Decaying plant material was another source of phosphorus that could have contributed to the effluent, but this would be expected to have a lower impact on the reactors than peat, as T2 could reduce both PO_4-P and TP from the reactor during the start-up period. During the operational period, T1 and T3 both outperformed T2, as both PO_4-P and TP concentrations reductions were observed with maximum PO_4-P and TP removal efficiencies of 58.7% and 40.3%, respectively. Substrate plays an important role in the overall retention of phosphorus in constructed wetlands, and it is the main component of phosphorus storage. Some researchers have reported that substrates accounted for more than 50% of the TP removal compared to other components, including water, periphyton, and macrophytes [43]. Peat is considered to be an excellent phosphorus sink, and it has a strong phosphorus retention ability [29]. PO_4-P, which is also known as reactive phosphorus, is the form commonly used by bacteria, plants and algae as a vital nutrient in surface waters. The removal of PO_4-P accounted for more than 80% of the TP removal. One study found that constructed wetlands are effective at removing PO_4-P compared to other forms of phosphorus, including organic phosphates, and particulate phosphorus [44].

3.6. Organics Removal

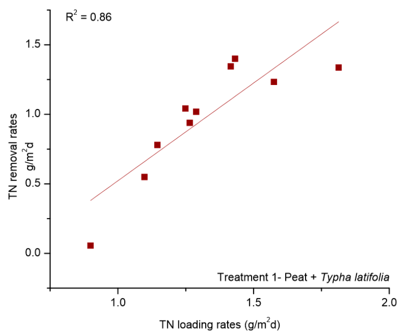
The influent secondary wastewater effluent had a relatively low COD concentration, and all treatment wetland reactors were unable to further reduce COD concentrations. An increase in COD was noted in T2 during the start-up period, whereas T1 and T3 significantly increased the COD concentrations to 49.2 mg/L and 44.0 mg/L, respectively. The COD level was well maintained during the operational period, and only a slightly increase was noted in T3. Although the properties of peat vary based on its location or origin, typically peat has a high organic and carbon content resulting from its formation. Therefore, leaching of carbon will inevitably occur when peat and most of other organic substrates are applied to wetland reactors. This was also reported in other studies when organic substrates were applied to mitigate micro-polluted wastewater [23]. Another explanation could be that color-producing organics within peat may transform into intermediate non-colored recalcitrant organic materials. These materials strongly resist further degradation, which would contribute to elevated COD levels [45]. The low COD removals by peat was also found in other studies that employed a peat-filled substrate filter to treat landfill leachate and municipal wastewater, and only a 17% of COD removal efficiency was recorded when influent COD concentrations were well above 500 mg/L [46].

3.7. Loading Rates and Removal Rates

Figure 4 shows the correlation plots (indicated by statistical parameter coefficient of determination, R^2) of NO_3-N and TN loading vs. removal rates (g/m^2 d), as wastewater passed through all wetland reactors. As observed in Figure 4, both NO_3-N and TN removal rates increased with increases in loading. With the exception of NO_3-N removal in T1, all other treatments had a R^2 value between 0.75 and 0.87. However, the NO_3-N removal rates did not increase when the loading rates of NO_3-N were beyond 1.5 g/m^2 d for all wetland reactors. The TN removal rates exhibited similar trends as NO_3-N. A comparison of NO_3-N and TN removal rates also indicated that NO_3-N removal accounted for almost all of the TN removal.



(**a**)



(**b**)

Figure 4. *Cont.*

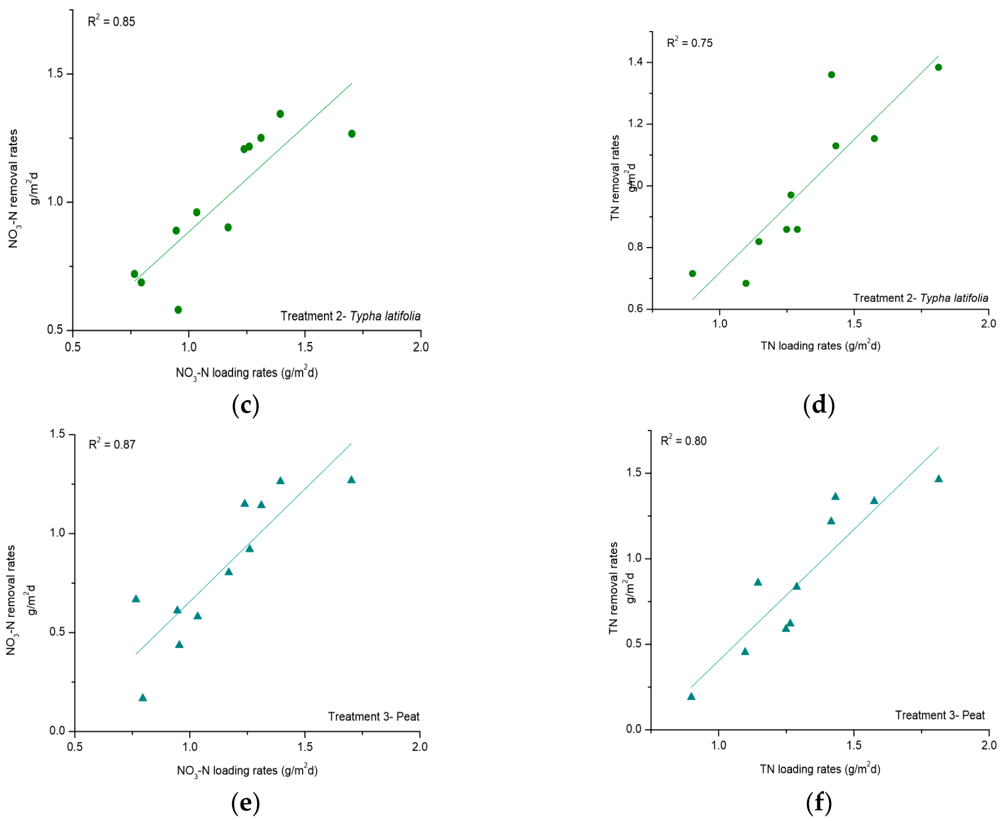


Figure 4. Correlation plots of: T1-NO3-N (**a**); T1-TN (**b**); T2-NO3-N (**c**); T2-TN (**d**); T3-NO3-N (**e**); and T3-TN (**f**) loading rates and removal rates in T1, T2 and T3 over the entire experimental period.

3.8. Effects of Substrate and Vegetation

Table 4 indicates the statistical comparison of selected nitrogen and phosphorus removal efficiencies and removal rates (g/m^2 d) between T1 and T2, T1 and T3, and T2 and T3, to evaluate their individual effect on the wetland reactors. Only results from the operational period were employed for analysis using paired *t*-tests. As observed in Table 4, no significant statistical differences were found between T1 and T3 in terms of removing nitrogen or phosphorus compounds, despite T1 having higher nitrogen removal efficiencies than T3, and vice versa in terms of phosphorus removal efficiencies.

However, the introduction of peat to the wetland system with *T. latifolia* (T1 and T2) had a much more impact than the introduction of *T. latifolia* to a wetland system with peat (T1 and T3), as most of the significant statistical differences ($p < 0.05$) were observed between T1 and T2. Reactors with peat strongly outcompeted the reactors without peat in terms of phosphorus removal efficiencies and removal rates

Table 4. Statistical comparison (*p* values) between removal efficiencies and removal rates across the treatment wetland reactors [1].

Parameter	T1 (Peat + Cattails) and T2 (Cattails)		T1 (Peat + Cattails) and T3 (Peat)		T2 (Cattails) and T3 (Peat)	
	Removal Efficiency	Removal Rates	Removal Efficiency	Removal Rates	Removal Efficiency	Removal Rates
NO_3-N	**0.0003**	**0.0001**	0.2348	0.2359	**0.0366**	**0.0291**
TN	0.6020	0.7752	0.2632	0.2529	0.1718	0.2238
PO_4-P	**0.0431**	**0.0407**	0.4117	0.2846	**0.0451**	**0.0429**
TP	**0.0459**	0.0992	0.4289	0.3544	0.0578	0.0691

Note: [1] The significant statistical difference ($p < 0.05$) is indicated in bold numbers.

3.9. Effects of Seasons

Figure 5 illustrates the temperature and precipitation variation at the Amherstview WPCP, and Table 5 summarizes the data during the entire experimental period. Previous studies have demonstrated that the performance of constructed wetlands tends to be reduced under cold climate conditions, especially removal processes involving microorganisms and bacteria. Low nitrogen removal efficiencies by constructed wetlands in cold climates have been reported in other studies, and the results from this study mirror their results [47]. Both NO_3-N and TN had significantly higher removal efficiencies during the operational period than during the start-up period across all reactors. During the start-up period, most of the transplanted *T. latifolia* began to decay due to the climate conditions. Consequently, decaying plant material accumulated in the reactors could release nutrient back into the water column, thereby reducing the removal efficiency. Most of the growth of *T. latifolia* occurred during the operational period which meant the plants required more nutrients to support their growth.

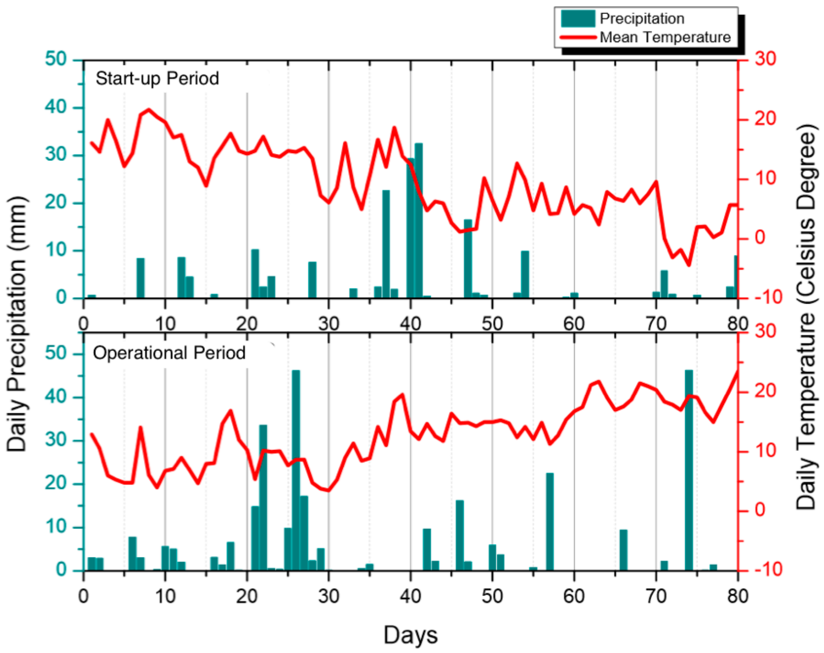


Figure 5. Changes in temperature and precipitation at the Amherstview WPCP over the entire experimental period.

Table 5. Summary of temperature and precipitation data during the entire experimental period.

Experiment Period	Temperature			Precipitation		
	Min.	Max.	Average	Min.	Max.	Average
Start-up	−4.4 °C	21.7 °C	9.0 °C	0 mm	32.5 mm	2.9 mm
Operational	3.5 °C	23.5 °C	15.3 °C	0 mm	46.3 mm	2.7 mm

3.10. Regulation Consideration

This small-scale on-site experiment was designed as a preliminary test for an upcoming pilot-scale SFCW at the Amherstview WPCP. The intention of the construction of a pilot-scale SFCW is to not only increase their rated treatment capacity, but also to further improve the performance of the treatment plant in order to meet increasingly stringent regulatory discharge guidelines. In this experiment, all treatments failed to meet the TP regulatory discharge limits during the startup period. T1 and T3 could meet all regulatory discharge limits during operational period, but T2 was still unable to meet the TP discharge limit. As the secondary biological treatment at the Amherstview WPCP was not designed to remove excess nutrients (phosphorus), the TP concentration in the secondary effluent was still high enough to promote excessive algal growth. The results indicated that employing vegetation alone in a SFCW was not sufficient to reduce phosphorus concentrations to below regulatory discharge limits due to its lack of ability to retain phosphorus. An appropriate substrate in the wetland reactor is required to enhance the phosphorus removal efficiency and reduce phosphorus concentrations below the regulatory discharge limit.

4. Conclusions

In this study, T1 and T3 could treat secondary effluent to meet the municipal wastewater regulatory discharge guidelines during the operational period at the Amherstview WPCP. More importantly, they could maintain the pH level below 7.5 throughout the entire experimental period. Effective NO_3-N, TN, and TP removal efficiencies were observed. T2 was not able to meet the phosphorus discharge limit and was only able to reduce the TP level to 1.13 mg/L with a 12.4% of removal efficiency. Nutrient leaching could potentially contribute to the low performance observed during the start-up period. NO_3-N and TN removal efficiencies were more effective under warmer conditions, and the growing season for vegetation enhanced the efficiency through the plant uptake.

When comparing their individual effects on the wetland system, the introduction of peat had a larger impact than the *T. latifolia*. Peat could effectively reduce the elevated pH level and greatly enhanced the phosphorus removal efficiency due to its own physical-chemical properties. However, it also reduced the ability of *T. latifolia* to remove nitrogenous compounds. On the other hand, the presence of *T. latifolia* was found to improve the nitrogen removal efficiency by providing an additional removal route, although in this study, no significant difference was found in either nutrient removal efficiency or removal rate. In the future, a longer acclimation period would be suggested as a requirement to allow the wetland system to acclimatize and reach maximum function.

Acknowledgments: The authors wish to acknowledge the staff at the Amherstview WPCP for their assistance in sample collection; and Marie-Josée Merritt, Rami Maassarani, Jenna Campbell, and Lorie McFarland at Loyalist Township for their assistance with technical background, and site information. The authors would also like to acknowledge the financial support of the Queen's University, Natural Sciences and Engineering Research Council (NSERC), Collaborative Research and Training Experience Program (CREATE) in civil engineering STEWARD-System Training and Education in Water Assets Research and Development, NSERC Collaborative Research and Development (CRD), Canada Research Chairs program and the Beaty Water Research Center.

Author Contributions: Meng Jin conceived and designed the experiments; Meng Jin, Jacob Carlos, and Rachel McConnell performed the experiments and analyzed the data; and Meng Jin, Jacob Carlos, Rachel McConnell, Geof Hall, and Pascale Champagne wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Jácome, J.A.; Molina, J.; Suárez, J.; Mosqueira, G.; Torres, D. Performance of constructed wetland applied for domestic wastewater treatment: Case study at Boimorto (Galicia, Spain). *Ecol. Eng.* **2016**, *95*, 324–329. [CrossRef]
2. Pandey, M.K.; Jenssen, P.D.; Krogstad, T.; Jonasson, S. Comparison of vertical and horizontal flow planted and unplanted subsurface flow wetlands treating municipal wastewater. *Water Sci. Technol.* **2013**, *68*, 117–123. [CrossRef] [PubMed]
3. Paing, J.; Serdobbel, V.; Welschbillig, M.; Calvez, M.; Gagnon, V.; Chazarenc, F. Treatment of high organic content wastewater from food-processing industry with the French vertical flow constructed wetland system. *Water Sci. Technol.* **2015**, *72*, 70–76. [CrossRef] [PubMed]
4. Saeed, T.; Sun, G. A lab-scale study of constructed wetlands with sugarcane bagasse and sand media for the treatment of textile wastewater. *Bioresour. Technol.* **2013**, *128*, 438–447. [CrossRef] [PubMed]
5. Giácoman-Vallejos, G.; Ponce-Caballero, C.; Champagne, P. Pathogen removal from domestic and swine wastewater by experimental constructed wetlands. *Water Sci. Technol.* **2015**, *71*, 1263. [CrossRef] [PubMed]

6. Speer, S.; Champagne, P.; Crolla, A.; Kinsley, C. Hydraulic performance of a mature wetland treating milkhouse wastewater and agricultural runoff. *Water Sci. Technol.* **2009**, *59*, 2455. [CrossRef] [PubMed]
7. Gottschall, N.; Boutin, C.; Crolla, A.; Kinsley, C.; Champagne, P. The role of plants in the removal of nutrients at a constructed wetland treating agricultural (dairy) wastewater, Ontario, Canada. *Ecol. Eng.* **2007**, *29*, 154–163. [CrossRef]
8. Clyde, E.J.; Champagne, P.; Jamieson, H.E.; Gorman, C.; Sourial, J. The use of a passive treatment system for the mitigation of acid mine drainage at the Williams Brothers Mine (California): Pilot-scale study. *J. Clean. Prod.* **2016**, *130*, 116–125. [CrossRef]
9. Saeed, T.; Paul, B.; Afrin, R.; Al-Muyeed, A.; Sun, G. Floating constructed wetland for the treatment of polluted river water: A pilot scale study on seasonal variation and shock load. *Chem. Eng. J.* **2016**, *287*, 62–73. [CrossRef]
10. Mawuli, D.; Xiaochang, W.; Yucong, Z.; Yuan, G.; Jiaqing, X.; Yaqian, Z. Characteristics of nitrogen and phosphorus removal by a surface-flow constructed wetland for polluted river water treatment. *Water Sci. Technol.* **2015**, *71*, 904–912.
11. Martín, M.; Oliver, N.; Hernández-Crespo, C.; Gargallo, S.; Regidor, M.C. The use of free water surface constructed wetland to treat the eutrophicated waters of lake L'Albufera de Valencia (Spain). *Ecol. Eng.* **2013**, *50*, 52–61. [CrossRef]
12. Stefanakis, A.I.; Seeger, E.; Dorer, C.; Sinke, A.; Thullner, M. Performance of pilot-scale horizontal subsurface flow constructed wetlands treating groundwater contaminated with phenols and petroleum derivatives. *Ecol. Eng.* **2016**, *95*, 514–526. [CrossRef]
13. Sim, C.H.; Quek, B.S.; Shutes, R.B.; Goh, K.H. Management and treatment of landfill leachate by a system of constructed wetlands and ponds in Singapore. *Water Sci. Technol.* **2013**, *68*, 1114–1122. [CrossRef] [PubMed]
14. Speer, S.; Champagne, P.; Anderson, B. Pilot-scale comparison of two hybrid-passive landfill leachate treatment systems operated in a cold climate. *Bioresour. Technol.* **2012**, *104*, 119–126. [CrossRef] [PubMed]
15. Wallace, J.; Champagne, P.; Monnier, A.-C. Performance evaluation of a hybrid-passive landfill leachate treatment system using multivariate statistical techniques. *Waste Manag.* **2015**, *35*, 159–169. [CrossRef] [PubMed]
16. Zhao, J.; Zhao, Y.; Xu, Z.; Doherty, L.; Liu, R. Highway runoff treatment by hybrid adsorptive media-baffled subsurface flow constructed wetland. *Ecol. Eng.* **2016**, *91*, 231–239. [CrossRef]
17. Murphy, C.; Wallace, S.; Knight, R.; Cooper, D.; Sellers, T. Treatment performance of an aerated constructed wetland treating glycol from de-icing operations at a UK airport. *Ecol. Eng.* **2015**, *80*, 117–124. [CrossRef]
18. Kadlec, R.H.; Wallace, S. *Treatment Wetlands*, 2nd ed.; CRC Press: Boca Raton, FL, USA, 2008.
19. Vymazal, J. Plants in constructed, restored and created wetlands. *Ecol. Eng.* **2013**, *61*, 501–504. [CrossRef]
20. Herrera-Melián, J.A.; González-Bordón, A.; Martín-González, M.A.; García-Jiménez, P.; Carrasco, M.; Araña, J. Palm Tree Mulch as Substrate for Primary Treatment Wetlands Processing High Strength Urban Wastewater. *J. Environ. Manag.* **2014**, *139*, 22–31. [CrossRef] [PubMed]
21. Liu, M.; Wu, S.; Chen, L.; Dong, R. How Substrate Influences Nitrogen Transformations in Tidal Flow Constructed Wetlands Treating High Ammonium Wastewater? *Ecol. Eng.* **2014**, *73*, 478–486. [CrossRef]
22. Mateus, D.M.R.; Vaz, M.M.N.; Pinho, H.J.O. Fragmented Limestone Wastes as a Constructed Wetland Substrate for Phosphorus Removal. *Ecol. Eng.* **2012**, *41*, 65–69. [CrossRef]
23. Saeed, T.; Sun, G. A comparative study on the removal of nutrients and organic matter in wetland reactors employing organic media. *Chem. Eng. J.* **2011**, *171*, 439–447. [CrossRef]
24. Yin, H.; Yan, X.; Gu, X. Evaluation of thermally-modified calcium-rich attapulgite as a low-cost substrate for rapid phosphorus removal in constructed wetlands. *Water Res.* **2017**, *115*, 329–338. [PubMed]
25. Gunes, K. Restaurant Wastewater Treatment by Constructed Wetlands. *Clean-Soil Air Water* **2007**, *35*, 571–575. [CrossRef]
26. Saeed, T.; Afrin, R.; Muyeed, A.A.; Sun, G. Treatment of tannery wastewater in a pilot-scale hybrid constructed wetland system in Bangladesh. *Chemosphere* **2012**, *88*, 1065–1073. [CrossRef] [PubMed]
27. Kasak, K.; Mander, Ü.; Truu, J.; Truu, M.; Järveoja, J.; Maddison, M.; Teemusk, A. Alternative filter material removes phosphorus and mitigates greenhouse gas emission in horizontal subsurface flow filters for wastewater treatment. *Ecol. Eng.* **2015**, *77*, 242–249. [CrossRef]
28. Lizama Allende, K.; Fletcher, T.D.; Sun, G. The effect of substrate media on the removal of arsenic, boron and iron from an acidic wastewater in planted column reactors. *Chem. Eng. J.* **2012**, *179*, 119–130. [CrossRef]

29. Jin, M.; Champagne, P.; Hall, G. Effects of different substrates in the mitigation of algae-induced high pH wastewaters in a pilot-scale free water surface wetland system. *Water Sci. Technol.* **2017**, *75*, 1–10. [CrossRef] [PubMed]
30. Vymazal, J. Emergent plants used in free water surface constructed wetlands: A review. *Ecol. Eng.* **2013**, *61 Pt B*, 582–592. [CrossRef]
31. Calheiros, C.S.C.; Rangel, A.O.S.S.; Castro, P.M.L. Treatment of industrial wastewater with two-stage constructed wetlands planted with Typha latifolia and Phragmites australis. *Bioresour. Technol.* **2009**, *100*, 3205–3213. [CrossRef] [PubMed]
32. Schierano, M.C.; Maine, M.A.; Panigatti, M.C. Dairy farm wastewater treatment using horizontal subsurface flow wetlands with Typha domingensis and different substrates. *Environ. Technol.* **2017**, *38*, 192–198. [CrossRef] [PubMed]
33. Toscano, A.; Marzo, A.; Milani, M.; Cirelli, G.L.; Barbagallo, S. Comparison of removal efficiencies in Mediterranean pilot constructed wetlands vegetated with different plant species. *Ecol. Eng.* **2015**, *75*, 155–160. [CrossRef]
34. Sehar, S.; Naeem, S.; Perveen, I.; Ali, N.; Ahmed, S. A comparative study of macrophytes influence on wastewater treatment through subsurface flow hybrid constructed wetland. *Ecol. Eng.* **2015**, *81*, 62–69. [CrossRef]
35. American Public Health Association (APHA). *Standard Methods for the Examination of Water and Wastewater*; American Public Health Association: Washington, DC, USA, 2012.
36. Champagne, P.; Liu, L.; Howell, M. Aerobic Treatment in Cold-Climate Countries A2. In *Current Developments in Biotechnology and Bioengineering*; Elsevier: Amsterdam, The Netherlands, 2017; pp. 161–201.
37. Wallace, J.; Champagne, P.; Hall, G. Multivariate statistical analysis of water chemistry conditions in three wastewater stabilization ponds with algae blooms and pH fluctuations. *Water Res.* **2016**, *96*, 155–165. [CrossRef] [PubMed]
38. Liu, L.; Hall, G.; Champagne, P. Effects of environmental factors on the disinfection performance of a wastewater stabilization pond operated in a temperate climate. *Water* **2016**, *8*, 5. [CrossRef]
39. Maine, M.A.; Hadad, H.R.; Sánchez, G.C.; Di Luca, G.A.; Mufarrege, M.M.; Caffaratti, S.E.; Pedro, M.C. Long-term performance of two free-water surface wetlands for metallurgical effluent treatment. *Ecol. Eng.* **2017**, *98*, 372–377. [CrossRef]
40. Hernández-Crespo, C.; Gargallo, S.; Benedito-Durá, V.; Nácher-Rodríguez, B.; Rodrigo-Alacreu, M.A.; Martín, M. Performance of surface and subsurface flow constructed wetlands treating eutrophic waters. *Sci. Total Environ.* **2017**, *595*, 584–593. [CrossRef] [PubMed]
41. Behrendt, A.; Tarre, S.; Beliavski, M.; Green, M.; Klatt, J.; de Beer, D.; Stief, P. Effect of high electron donor supply on dissimilatory nitrate reduction pathways in a bioreactor for nitrate removal. *Bioresour. Technol.* **2014**, *171*, 291–297. [CrossRef] [PubMed]
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] [PubMed]
43. Lai, D.Y.F. Phosphorus fractions and fluxes in the soils of a free surface flow constructed wetland in Hong Kong. *Ecol. Eng.* **2014**, *73*, 73–79. [CrossRef]
44. Ciria, M.P.; Solano, M.L.; Soriano, P. Role of Macrophyte Typha latifolia in a Constructed Wetland for Wastewater Treatment and Assessment of Its Potential as a Biomass Fuel. *Biosyst. Eng.* **2005**, *92*, 535–544. [CrossRef]
45. Ong, S.-A.; Uchiyama, K.; Inadama, D.; Yamagiwa, K. Simultaneous removal of color, organic compounds and nutrients in azo dye-containing wastewater using up-flow constructed wetland. *J. Hazard. Mater.* **2009**, *165*, 696–703. [CrossRef] [PubMed]
46. Kõiv, M.; Vohla, C.; Mõtlep, R.; Liira, M.; Kirsimäe, K.; Mander, Ü. The performance of peat-filled subsurface flow filters treating landfill leachate and municipal wastewater. *Ecol. Eng.* **2009**, *35*, 204–212. [CrossRef]
47. Fan, J.; Zhang, J.; Ngo, H.H.; Guo, W.; Yin, X. Improving low-temperature performance of surface flow constructed wetlands using *Potamogeton crispus* L. plant. *Bioresour. Technol.* **2016**, *218*, 1257–1260. [CrossRef] [PubMed]