



# Article Plant Biomass Production in Constructed Wetlands Treating Swine Wastewater in Tropical Climates

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Abstract: The production of both aboveground and belowground plant biomass in constructed wetlands (CW) is a poorly understood topic, although vegetation plays an important role in the process of pollutant removal from wastewater. The objective of this study was to evaluate the aboveground and belowground biomass production of Typha latifolia and Canna hybrids in a largescale constructed wetland treating swine wastewater in tropical climates. Parameters, such as temperature, DO, pH, COD, TSS, TN, TP, and TC, as well as destructive and non-destructive biomass, were evaluated. It was found that, despite the high concentrations of pollutants, the vegetation adapted easily and also grew healthily despite being exposed to high concentrations of pollutants from swine water. Although Typha latifolia (426 plants) produced fewer plants than Canna hybrids (582 plants), the higher biomass of the Typha latifolia species was slightly higher than that of Canna hybrids by 5%. On the other hand, the proximity of the water inlet to the system decreased the capacity for the development of a greater number of seedlings. As for the elimination of pollutants, after treatment in the constructed wetland, COD: 83.6  $\pm$  16.9%; TSS: 82.2  $\pm$  17.7%; TN: 94.4  $\pm$  15.8%; TP:  $82.4 \pm 23.2\%$ ; and TC:  $94.4 \pm 4.4\%$  were significantly reduced. These results show that wetlands constructed as tertiary systems for the treatment of swine wastewater produce a large amount of plant biomass that significantly helps to reduce the concentrations of pollutants present in this type of water in tropical areas. The use of these plants is recommended in future wetland designs to treat swine wastewater.

Keywords: constructed wetlands; plant biomass; swine wastewater treatment

## 1. Introduction

Constructed wetlands (CWs) are eco-treatment technologies that have gained popularity due to their low cost, easy operation, and zero energy costs in solving water pollution problems of domestic, industrial, and agricultural origin [1,2]. Their components and performance are well known and widely studied [3,4], including hydraulic retention times [5–7], substrates ranging from petrified materials to recyclable waste [8]. On the other hand, vegetation is one of the components of CWs that plays a very important role in the system [9], since it has three main functions: the first is that it absorbs pollutants and favors their elimination [10], the second is that it provides oxygen through the release in the radical zone [11], and the third is hydraulic, which is intensified in CWs with horizontal



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**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). subsurface flow [12–14]. These functions are affected or favored in the selection of plants. Some of the best-known vegetation used in subsurface flow CWs systems are Cyperus papyrus, Phragmites australis, Typha latifolia, and Scirpus spp. [15], which are typical plants of natural wetlands [16]. Ornamental plants are also used in CWs, the most common are *Canna* spp., *Iris* spp., *Heliconia* spp., and *Zantedeschia* spp., [17]. Even when plants that can be used in CWs are known, their functioning and pollutant removal capacity of these plants are under discussion, authors such as Kim et al. [18] indicate that plants (macrophytes) in CWs absorb less than 5% of total phosphorus present in wastewater. In addition, the removal of nitrogen present in wastewater by plants is 5 to 10%, which can translated into a range of 15 to 32 mg N  $g^{-1}$  [19]. Other studies have reported on plant development and biomass production in horizontal flow CWs, such as [20], which found a positive correlation between aboveground biomass production with PO<sub>4</sub>-P removal of *Typha* spp., plants, Zantedeschia aethiopica, and Alpinia purpurata, finding that Typha spp., had higher aboveground biomass development than belowground. In systems with biomass (common reed) in relation to systems without the presence of biomass, with continuous feeding, the mass quantity of ammonium removed ranged from 0.52 to 0.58 and 0.67 g N m<sup>-2</sup>, removing 26% more than systems without the presence of biomass, but the production of biomass was not quantified [21].

These data are encouraging in terms of nutrient removal in horizontal flow CWs with constant saturation, even though these have been evaluated with domestic or municipal wastewater, without taking into account waters with a higher presence of nutrients that can increase its toxicity, decreasing biomass production, and the nutrient assimilation capacity of plants in CWs under continuous saturation conditions [22]. However, to this date there are very few studies that quantify the biomass production of vegetation both in the root zone and in the aerial zone in horizontal subsurface flow CWs [23–25] and much less when wastewater of agro-industrial origin is used, as is the case of wastewater generated by the swine agroindustry that has already been evaluated in CWs at laboratory or mesocosm scale in terms of its removal of pollutants [26,27]. Therefore, this study reports for the first time the total biomass production of both above and belowground biomass from a large-scale horizontal subsurface flow constructed wetland in the vegetation of a system that treats water as a tertiary swine wastewater treatment system on a farm located in a tropical region.

## 2. Materials and Methods

# 2.1. Biomass Source

In this study, 5 randomly selected plants of *Typha latifolia* and *Canna hybrids* were evaluated (for each large-scale constructed wetland zone A, B, C, D, as shown in Figure 1), respectively, from a constructed wetland (CW) of horizontal flow 20 m long  $\times$  5 m wide with a depth of 0. 65 m (Figure 1), filled with red volcanic gravel, commonly used in Mexico as a filter in constructed wetlands [28], with diameter 2–4 cm, with a porosity of 0.68%, in which a total of 600 plants were planted. The first 10 m of the system were planted with *Typha latifolia* (300 plants of 15 cm in height) and the remaining 10 m closer to the CW outlet were planted with *Canna hybrids* (300 plants of 10 cm in height), which were obtained in their natural state in the central zone of the state of Veracruz, Mexico. The system was installed in a 5000-pig farm where the CW (tertiary treatment) was part of a treatment train that treated the wastewater produced by pig farming on the farm, which produced 3 m<sup>3</sup> of wastewater per day. The full-scale CW operated with a hydraulic retention time of 14 days. All systems operated under real environmental conditions in a warm subhumid climate with an average annual temperature of 23 °C, an average annual precipitation of 1500 mm and at 500 m above sea level [29].



Figure 1. Large-scale constructed wetland system.

The plants were planted in four different zones (Figure 1), *Typha latifolia.*, was exposed to greater toxicity since it would be planted at the beginning of the treatment in a constructed wetland with horizontal flow, in these systems, since as the wastewater advances in the system it tends to decrease the concentrations of contamination.

#### 2.2. Operation and Duration of Monitoring

The system received pre-treated water from a treatment train consisting of one anaerobic reactor and one aerobic reactor, with a capacity of 72 m<sup>3</sup> and a hydraulic retention time of 14 days reactively.

The system was evaluated for a period of 6 months. Before starting the evaluation period, the system was adapted to the new water quality conditions for 3 months; the plants were induced to an initial preparation process where they were acclimatized to the current water quality conditions. It is worth mentioning that the plants were taken from a natural wetland that receives untreated domestic wastewater, which facilitated their adaptation to the new water pollution conditions.

As for contaminant removal (CR), it was determined by Equation (1):

$$Em = ((Ci - Ce)/Ci) \times 100\%$$
 (1)

where Ci is the concentration of the pollutant in the influent (mg  $L^{-1}$ ) and Ce is the concentration of the pollutant in the effluent (mg  $L^{-1}$ ).

# 2.3. Survival to Adaptation and Survival of Plants

Plant survival was monitored visually every month, all plants were numbered to have control of their survival and the offspring were assigned a new numbering month by month, to give adequate follow-up to their plant development, as well as flower production. As a control, the same parameters were measured in 5 plants of the same age of maturity planted in soil in their natural state, planted in the same initial characteristics of size as those of CW, and the same monitoring time was used.

# 2.4. Measurement of Plant Development

Plant development was measured in the sixth month using a tape measure, recording 100% of the mature and new individuals of each plant species, in order to measure height, leaf length, number of leaves, leaf width, and these data were processed to obtain averages and establish future non-destructive biomass volumes. As a control, the same parameters were measured in 5 plants of the same age of maturity planted in soil in their natural state with the same characteristics as those of the initial CW in terms of size and monitoring time.

#### 2.5. Destructive Biomass Measurement

Five mature plants of *Typha latifolia* and *Canna hybrids*, respectively, were sacrificed, in which the area and below-ground biomass were determined by separating the plant from the root and washing it with tap water to remove the presence of solids in the root that could interfere with the biomass result. They were immediately placed in an oven at 100 °C for 72 h, in order to have a constant weight and be able to calculate the biomass [30]. The plants were weighed on a high-precision digital analytical balance (Shi-madzu AUW-220D,

SHIMADZU, Berlin, German). Subsequently, the results of both above and below ground were summed, the results of the five individuals of each plant species were averaged in order to calculate the ratio of 1 g above and below ground biomass to plant size. In the case of *Canna hybrids*, the plan growth formula was used to obtain the data in Table 1 for growth in both plant species. As a control, the same parameters were measured in 5 plants of the same maturity age planted in soil in their natural state, planted in the same characteristics of the initial CW in terms of size and monitoring time.

Parameter	Input (mg L <sup>-1</sup> )	Output (mg L <sup>-1</sup> )	Method
Water temperature (°C)	$16.4\pm4.2$	$15.2\pm2.5$	
Dissolved Oxygen (DO)	$1.7\pm0.3$	$2.4\pm0.2$	
pH	$7.1\pm0.2$	$7.8\pm0.3$	
Chemical Oxygen Demand (COD)	$789.6 \pm 134.1$	$129.8\pm53.6$	Standard
Total Suspended Solids (TSS)	$607.3 \pm 107.5$	$108\pm94.5$	Method [31]
Total Nitrogen (TN)	$294.3\pm46.6$	$16.4\pm9.1$	
Total Phosphorus (TP)	$53.4 \pm 12.4$	$9.4\pm4.6$	
Total Coliforms (TC)	$1.6 imes 10^0 \pm 0.7$	$9 imes 10^{-1}\pm 0.6$	

Table 1. Characteristics of wastewater that entered the CW tertiary treatment system.

Average  $\pm$  standard error (*n* = 24).

#### 2.6. Non-Destructive Biomass Calculation. Non-Destructive Biomass Measurements

For the calculation of the total non-destructive biomass of the CW system in all plants, the total number of plants with an average age of 6 months was considered and the total number of new seedlings was weighted by means of multivariate statistical analysis where the data of 1 g of biomass of the root zone and 1 g of biomass of the area zone (including stems and root) were obtained, data from the quantification of destructive biomass.

Finally, by the ratio of dry weight and height of the plants, the total extrapolated biomass produced both above and below ground was calculated for each of the two species planted in CW.

#### 2.7. Quantification of Total Coliforms

The quantification of fecal coliforms was performed using the Colilert method. This methodology allows the detection of water quality sanitation indicators based on the ability of total coliforms to produce the enzyme  $\beta$ -galactosidase that metabolizes the Colilert indicator nutrient, O-nitrophenyl- $\beta$ -Dgalactopyranoside (ONPG), which changes the samples to a yellow color. The Colilert method is endorsed by the US Environmental Protection Agency (EPA) for use in water and wastewater analysis, guaranteeing its efficiency [31].

#### 2.8. Data Analysis

The statistical analysis and calculation of the data was carried out in the SPSS V18.0 program (IBM, Armonk, NY, USA). Analysis of variance (ANOVA) was used to determine the differences in the growth of plants in CW and in their natural state, a Pearson correlation test with a 95% confidence interval was used, as well as a test of independence of data.

## 3. Results and Discussion

# 3.1. Monitoring Operation and Duration

The swine wastewater that entered the wetlands presented two pretreatments; Table 1 shows the characteristics of the system's input and output.

#### *3.2. Water Quality Parameters*

# 3.2.1. Temperature, pH, Dissolved Oxygen

Water temperature is an indicator that directly affects both the development of microorganisms and the presence of dissolved oxygen (DO) in constructed wetlands, and the optimum temperature in these systems for the correct development of biochemical reactions is 16–35 °C [32,33]. The average inlet and outlet temperature is shown in Table 1, with a non-significant decrease. Points out that low temperatures are associated with a decrease in organic matter (OM) removal, i.e., temperature promotes OM oxidation [34]. On the other hand, pH is a variable that like temperature has an effect on the development of organisms, the suitable range for the existence of most life is 5–9 [35]. For this parameter, an increase of 7 units was observed in the output with respect to the input of the system evaluated, which is in agreement with the range indicated by Sandoval-Herazo et al. [35].

# 3.2.2. Physicochemical Parameters and Microorganisms

COD determines the amount of oxygen required to oxidize the organic matter in a water sample. In this study, there was a clear significant difference (p = 0.05) between the output and the input (Table 1). Suspended solids are substances present in water that have a solid state when they are pure, when determining this parameter, a significant decrease (p < 0.05) was observed between the output (108.0  $\pm$  94.5 mg L<sup>-1</sup>) and the input  $(607.3 \pm 3.0 \text{ mg L}^{-1})$ . Total nitrogen is the TN of all forms present in this case, in the water samples obtained, which in this work presented a significant decrease (p < 0.05) in the output (16.4  $\pm$  9.1 mg L<sup>-1</sup>) with respect to the input (294.3  $\pm$  46.6 mg L<sup>-1</sup>). Wetlands perform the removal processes of this nutrient by adhesion, absorption, sedimentation, and microbiological processes [36]. Phosphorus (P) is another major pollutant in swine effluents, P in swine wastewater occurs in the form of organic and inorganic P. P removal in wetlands is achieved by chemical and physical adsorption, precipitation with other ions, sedimentation, and uptake by plants and microorganisms. Inorganic P is adsorbed to the substrate matrix or becomes available for uptake by plants and microorganisms [37]. In this study the TP concentration was  $9.4 \pm 4.6$  mg L<sup>-1</sup> at the end of treatment. The presence of TC in treated wastewater indicates poor quality treatment; in this study, the presence of TC at the entrance of the treatment was  $1.6 \times 10^0 \pm 0.7$  and at the exit of  $9 \times 10^{-1} \pm 0.6$ , showing a decrease in its presence.

## 3.2.3. Removal of Contaminants

Figure 2 describes the percentage of removal in 5 parameters evaluated in the system studied, the first of which is the concentration of organic matter, expressed as COD, whose evaluation presents a mean removal value of  $83.6 \pm 16.9\%$ , higher than that (79.4%) using *Typha latifolia*, [38]. An 89.1% removal rate using the same organism was obtained in the treatment of basic pollutants in wastewater [39], similar to that obtained in this work.



Figure 2. Removal of contaminants.

TSS is the residue remaining in a capsule after evaporating and drying a sample at a temperature of  $105.0 \pm 2.0$  °C. TSS removal by the CW system averaged  $82.2 \pm 17.7\%$ .

The main inorganic nutrients entering wetlands are nitrogen and phosphorus. In the wetland, nitrogen and phosphorus are removed from the surface water and transferred to the sediment, wetland plants or atmosphere in this study, total Nitrogen was removed  $94.4 \pm 15.8\%$  and total Phosphorus was removed  $82.40 \pm 23.2\%$ . Among the different nitrogen species, they greatly influence aquatic systems as they are readily available to be taken up by aquatic microorganisms instead of other organic particles [40].

Phosphorus is removed primarily through physical and chemical processes, entering a wetland in both organic and inorganic forms. The relative proportion of each form depends on the soil characteristics, vegetation, and land use of the drainage basin Phosphorus assimilation and storage in plants depends on vegetative type and growth characteristics. Leaves and stems of emergent and submerged vegetation help settle particles by slowing water and allowing the particles to fall [41].

To continuously remove phosphorus, it is necessary to "build" new soils within the wetland from remnant plant stems, leaves, root debris, and non-decomposable parts of algae, bacteria, fungi, and dead invertebrates [42].

CW have been shown to be capable of removing a wide variety of pollutants, including bacterial contamination [43]. In this study this is no exception since the removal of TC was  $94.4 \pm 4.4\%$ , indicative of efficient treatment. Giácoman-Vallejos et al. [44] conducted a study where he evaluated the removal of pathogens from domestic and swine wastewater using experimental constructed wetlands, he found that *Typha latifolia* (86%) is more efficient in removing TC than *Typha dominguensis*.

They developed and evaluated of a horizontal underground flow CWs on a pilot scale for the treatment of swine wastewater, using a *Pennisetum clandestinum* and *Pennisetum purpureum*, As a result, they reported a removal efficiency for the vegetation used of 64.9 and 66.5% for COD, 58.9 and 62.5% for TN, and up to 48.53% for TP [45]. With these results they concluded that the use of horizontal flow wetlands is feasible, as they can efficiently treat swine effluents, since it is possible to remove organic matter and nutrients.

It should be noted that retention time defines the length of time that contaminants remain in contact with plants and microorganisms to be biologically and chemically transformed [46].

During the development of this work, it was observed that *Canna hybrids* presented a greater adaptation to the environment in which they developed, since during the 6 months of evaluation there were 0 non-viable (lifeless) individuals, while the adaptation of *Typha latifolia* at the beginning of the experiment did not occur, since during the first and third month of the development of the experiment 128 and 43 individuals, respectively, ceased to be viable. This means that *Canna hybrids* adapt more easily to changes (Table 2), in addition to having a higher rate of new individuals (n = 581), which is an advantage, since the greater the number of live individuals, the higher the water quality at the exit of the treatment.

Species		Number of Plants per Month					Average Plant Height (m) at the End	
		1	2	3	4	5	6	of the Study (Month 6)
	Initial	300	172	257	253	305	379	1.72
Typha	Dead	128	0	43	0	0	0	
latifolia	New	0	85	39	52	74	47	0.74
·	Sown in natural environment	-	-	-	-	-	-	1.56
	Initial	300	274	274	386	481	521	1.61
Canna	Dead	26	0	0	0	0	0	
hybrids	New	0	0	112	95	40	61	0.54
	Sown in natural environment	-	-	-	-	-	-	0.97

Table 2. Adaptation and survival of species used.

# 3.3. Biomass

The accumulation and distribution of biomass in plants are genotypic characteristics easily affected by the environment and its interaction [47]. Thus, the proportion of biomass assigned to leaves, stems and fruits at each developmental stage depends on growth rate and distribution rate, which are governed by leaf area, climate, and nutrient availability [48]. Figure 3 shows the growth rate of the species used. Figure 4 shows that the number of leaves of *Canna hybrids* was higher at the end of the experiment (17 leaves) compared to *Typha latifolia* (8 leaves). The stem thickness of *Typha latifolia* was greater from the beginning (3.5 cm) to the end (6 cm) of the experiment (Figure 4b).



Figure 3. Plant growth in constructed wetlands.

The width of the leaves of *Canna hybrids* (Figure 4c) was greater than those of *Typha latifolia*, and conversely the length was greater in *Typha latifolia* (Figure 4c). The height of *Typha latifolia* was greater than that of *Canna hybrids*, in a study *Typha latifolia* significantly outperformed *Juncus* and *Scirpus* in both growth and effluent quality improvement [49], as shown in this work, the promised height of *Typha latifolia* was 1.72 m, which means that *Typha latifolia* is a useful organism for plant biomass production and water quality improvement in a constructed wetland.

In a constructed wetland, the presence of biomass is important, since it represents a better removal of pollutants, even though found that in the treatment of lightly loaded wastewater, plants have a greater significant effect on purification than in normal water. This suggests that harvesting could be a valid exploitation strategy in dilute water conditions, but it is also important to evaluate when it should be carried out [50]. Wetland biomass is commonly used as livestock fodder, soil conditioner, or fertilizer due to its nutrient content, but could also be harvested for bioenergy production [51]. In ornamental plants, biomass increase does not represent a problem, since pruning can be performed continuously and the plants can be marketed.

The importance of fresh weight analysis (non-destructive biomass) in crops is that it includes the quantitative determination of the water content present. On the other hand, the evaluation of dry weight biomass (destructive biomass) represents the weight without water content.



**Figure 4.** *Typha latifolia* and *Canna hybrids* development during the study period at the actual level. (a) Number of leaves, (b) Plant stem thickness, (c) Leaf width, (d) Plant height, (e) Leaf length, (f) Number of shoots. Mean  $\pm$  standard deviation.

## 3.3.1. Destructive Biomass

Figure 5 shows that there is a decrease from A to D in the dry weight of the plants evaluated in both leaves and roots. The *Canna Hybrids* at the entrance of the system developed less biomass (D). For *Typha latifolia*, a higher dry weight was obtained both in the aerial part and in the root (A,B). On the other hand, *Canna Hybrids* presented the lowest weight (p < 0.5) in the aerial part and in the root part, regarding *Typha latifolia*.



**Figure 5.** Aerial and root biomass development of *Typha latifolia* (zone A and B) and *Canna hybrids* (Zone C and D) of Constructed wetland as a tertiary swine wastewater treatment system. Mean  $\pm$  standard deviation.

#### 3.3.2. Non-Destructive Biomass

Table 3 shows the results of the non-destructive biomass analysis (root and leaf). The highest value obtained was for *Typha latifolia* in leaf (20,238.60 g) and root (12,582.80 g), which was located in position B of the system (see Figure 1) very close to the inlet, where the highest load of pollutants is found, therefore its development may be due to the fact that the nutritional load it received was used by the plant, through its large number of roots translocating the nutrients contained in the effluent to the aerial part. The fact that it had an extensive development promotes the efficiency of the system since the plants act as filters that remove, reduce, transform, mineralize, degrade, volatilize, concentrate, or stabilize pollutants (organic and inorganic) in soil, sludge, water, and sediments [52].

Plant		Zone	Total Biomass (g)
	А	Aerial	16,585.80
Typha latifolia		Root	10,482.80
	В	Aerial	20,238.60
		Root	12,582.80
Canna hybrids	С	Aerial	14,588.00
		Root	5835.20
	D	Aerial	18,500.40
		Root	7879.80
		Total	106,693.40

Table 3. Total biomass.

The second highest value was for plants located in position D (at the end of the system), the leaves had a weight of 18,500.40 g and the root had a weight of 7879.80 g. Roots play an important role since they can only absorb nutrients if they are dissolved in water, so the plants that were arranged in A and C, probably received available (dissolved) nutrients. In total, 106,693.40 g of biomass were obtained in the system.

It would be of interest to perform an experiment where the placement of the plants is inverted to evaluate the same parameters and to know the influence of the nutrient load in the system.

At least biomass production in zone A and B of the *Typha latifolia.*, it could be due to the highest levels of toxicity in which this plant was found exposed to being closer to the water inlet to the system.

## 4. Conclusions

This study demonstrated the adaptability and tolerance to high concentrations of contaminants of *Typha latifolia* and *Canna hybrids* plants, which although they have been widely used in constructed wetlands, very few studies have been evaluated in similar conditions, much less on a large scale, so their use is recommended in future designs that treat swine wastewater.

Regarding biomass production, more biomass was produced in the aerial zone in relation to the subterranean zone and more biomass was produced by *Typha latifolia*, in the constructed wetland zone "B" > "A" and in zone "D" > "C" of the *Canna hybrids*, the overall biomass production was greater in *Typha latifolia*, even though the *Canna hybrids* produced a greater number of seedlings. This may have been due to the fact that they were farther away from the entrance of water into the system where the presence of pollutants was still higher, a situation that favored their reproduction.

*Typha latifolia* is not adapting as well might be very well linked to the fact that the plant is struggling with toxic concentrations present in porcine wastewater and being in zones A and B of the CW, closest to the inlet of water to the system.

As for the elimination of pollutants, after treatment in the constructed wetland, COD:  $83.6 \pm 16.9\%$ ; TSS:  $82.2 \pm 17.7\%$ ; TN:  $94.4 \pm 15.8\%$ ; TP:  $82.4 \pm 23.2\%$ ; and TC:  $94.4 \pm 4.4\%$  were significantly reduced. These results show that wetlands constructed as secondary

systems for swine wastewater treatment produce a large amount of plant biomass that helps significantly to reduce the concentrations of pollutants present in this type of water in tropical areas, as it appears that the higher biomass production is related to a higher elimination of pollutants present in wastewater.

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