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Development of *Heliconia latispatha* in constructed wetlands, for the treatment of swine/domestic wastewater in tropical climates, with PET as a substitute for the filter medium

Desarrollo de la *Heliconia latispatha* en humedales construidos, para el tratamiento de aguas residuales porcinas/domésticas en climas tropicales, con PET como sustituto del medio filtrante

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Abstract

The aim of this study was to evaluate the development and effect of *Heliconia latispatha* in pilot-scale constructed wetlands (CWs) for the treatment of pig wastewater mixed with domestic wastewater, using PET waste as a substitute for the filter medium, in a tropical climate. Six cells (1.6 m long, 0.40 m wide and 0.65 m high) filled with rough recycled PET waste were used; three operated with vegetation and three without vegetation and the study lasted eight months. The results showed an excellent development of *H. latispatha* under the flooded conditions in the CWs, reaching a level of development similar to the level of development in commercial soil crops. The good development of the plant was reflected in the remarkable increase in plant height (74.3%), stem thickness (48.6 %), number of plants (350%) and inflorescences (250%). In addition, the presence of *H. latispatha* significantly influenced the removal of contaminants ($p<0.05$) such as COD (chemical oxygen demand), TN (total nitrogen), TP (total phosphorus) and TC (total coliforms), so that in the planted cells, higher removals were achieved by 7.8%, 8.5%, 18% and 13.7% respectively, compared to systems without vegetation. The production of *H. latispatha* flowers and the good removal of pollutants in the subsurface flow CWs make the system a viable alternative for the production and commercialization of *H. latispatha* under tropical climates while at the same time the wastewater is treated.

Keywords: Total Nitrogen, Total Phosphorus, Ornamental plants, Wastewater Treatment, Tropical Zones.

Resumen

El objetivo de este estudio fue evaluar el desarrollo y efecto de *Heliconia latispatha* en humedales construidos (HC) a escala piloto para el tratamiento de aguas residuales porcinas mezcladas con aguas residuales domésticas, utilizando residuos de PET como sustituto del medio filtrante, en clima tropical. Se utilizaron seis celdas (1.6 m de largo, 0.40 m de ancho y 0.65 m de alto) rellenas de residuos rugosos de PET reciclado; tres operaron con vegetación y tres sin vegetación y el estudio duró 8 meses. Los resultados demostraron un desarrollo excelente de la H. latisphata bajo las condiciones de inundación de los HC, alcanzando un nivel de desarrollo similar al nivel de desarrollo en cultivos comerciales de suelo. El buen desarrollo de la planta se reflejó en el notable aumento de la altura de la planta (74.3%), grosor del tallo (48.6 %), número de plantas (350 %) e inflorescencias (250 %). Además, la presencia de H. latisphata influyó significativamente en la remoción de contaminantes ($p<0.05$) como DQO, NT, PT y CT, de modo que en las celdas plantadas se alcanzaron remociones superiores en un 7.8%, 8.5%, 18% y 13.7% respectivamente, en comparación con los sistemas sin vegetación. La producción de flores de *H. latispatha* y la buena remoción de contaminantes en los HC de flujo subsuperficial transforman el sistema en una alternativa viable para la producción y comercialización de *H. latispatha* bajo climas tropicales mientras que al mismo tiempo las aguas residuales son tratadas.

Palabras clave: Nitrógeno Total, Fósforo Total, Plantas Ornamentales, Tratamiento de Aguas Residuales, Zonas Tropicales.

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1 Introduction

In Mexico, the treatment of domestic wastewater in rural areas is very low in comparison to urban areas. For example, 9 out of 10 municipalities with more than 500 000 inhabitants have at least one wastewater treatment plant in operation, while only 1833 rural communities (of 47,233 localities) with a population of 100 to 2,499 inhabitants have a system of wastewater treatment (Zurita *et al*., 2012; Zamora *et al*., 2019; Cáñez-Cota, 2022). In addition, in rural areas is common the generation of agro industrial effluents that are not treated. A traditional practice is backyard farming for the raising or fattening of pigs (Sandoval Herazo *et al.* 2021a) where polluted water is generated and it is commonly mixed with domestic wastewater. This activity is also carried out in micro-enterprises that are not legally registered with the corresponding environmental authorities. In this way, these activities cause micro-pollution that affects aquatic ecosystems, soils and puts the stability of ecosystems at risk due to the high content of nutrients and pathogens in the effluents they generate (Hernández-Salazar *et al*., 2018). Pérez-Espejo (2002) determined that the raising or fattening pigs, specifically in Mexico generates contamination of surface water and subsoil by nitrogen and phosphorus contained in excreta, contamination by heavy metals, mostly copper and zinc, because the pig only absorbs between 5 and 15%, excreting the rest. In addition, Castrillon *et al.* (2004) reported that the generation of waste is different in each stage of growth, being pregnant females and reproductive males the ones that generate less waste, that is, on average, 3.00 and 2.81 kg of excrement daily for each 100 kg weight, respectively. While those that generate the most are the lactating piglet and the lactating female, with 8.02 kg and 7.72 kg of daily excrement for each 100 kg of weight. On the other hand, in rural areas of Mexico, the backyard pig farm is a very common activity, contributing to the 1.3% of pork national consumption (Cortés-Tinoco *et al*., 2012).

Generally, owners of larger-scale pig farms usually implement manure and wastewater treatment systems produced in the rearing process (Sandoval-Herazo *et al*, 2020), but small producers have limited resources and knowledge for the implementation and operation of these systems. Therefore, treatment systems for these smallholder facilities must be inexpensive, easy to operate and maintain. Some systems evaluated and in use to treat this type or similar agro-industrial effluents that have high pollutant loads include aerobic and anaerobic systems with different configurations (Hernandez-Fydrych *et al*., 2021; Galván-Arzola *et al*., 2022) and some ecological systems such as constructed wetlands (CWs) (Hu *et al*., 2020).

The natural purification processes carried out in CWs have vegetation, filter medium and microorganisms as crucial components. These systems have the advantage that the vegetation can be ornamental of commercial interest and filter media that can be waste products (Rodríguez-Domínguez *et al*., 2020). CWs have proven to be efficient in removing high loads of pollutants and pathogens present in wastewater (Luo *et al*., 2018a; Luo *et al*., 2018b; Amorim *et al*., 2019; Li *et al*., 2019; Mateo *et al*., 2019). In this sense, the choice of vegetation and substrate is crucial due to the role they play in the elimination of contaminants (Sandoval *et al*., 2019; Maucieri *et al*., 2020), so properly determining and selecting these elements is vital in the design of CWs.

Recommendations for the appropriate use of ornamental vegetation in CW are still under development, particularly the use of tropical ornamental plants, since in tropical zones there is a variety of species with potential to be used in CWs. Knowledge is limited and the contribution to the purification process in CW is unknown, particularly for effluents with high concentrations of pollutants such as those present in pig effluents or pig effluents mixed with domestic wastewater.

A very attractive genus is *Heliconia spp.* which is an ornamental plant that is the largest and only genus of the *Musaceae* family with around 250 species worldwide (Andersson, 1981; Jerez, 2007). *Heliconia* have a high commercial value and although their flowers can be harvested and commercialized, as a plant they take up a lot of space and their agronomic management can be complicated (Jácome-Chacón *et al* 2018). The color of the bracts of *Heliconia spp.* and its long vase life has caused an increase in its local demand in the state of Veracruz, Mexico, according to Rodríguez-Orozco (2017) who conducted a survey among the florist sector in the municipality of Acayucan, Veracruz. In addition, its different parts are usable for various purposes, for example, the leaves are used to wrap cheese and tamales (a Mexican food) (Colegio de postgraduados, 2019) and its rhizomes that allow them to reproduce as garden plants, can be a source of food and alternative medicine (Linares-Gabriel, 2020). However, its inflorescences represent the most appreciated commercial product (Jacome-Chacon *et al*. 2018). This species was already evaluated as polyculture with other species and monoculture in CWs for the treatment of the same effluent by this research group with very good development and significant effect on the removal of contaminants by using tezontle as a filter medium (Sandoval-Herazo *et al*., 2021a). Therefore, the economic potential of this species can be exploited simultaneously with the treatment of wastewater in CWs, particularly those of agro-industrial origins, such as those generated in pig farms, due to the fact that they contain high concentrations of organic matter, nitrogen, phosphorus and others microelements (Arias Martínez, *et al*., 2010; Garzón-Zuñiga & Buelna, 2014; Trejo Lizama *et al*., 2014) that could contribute significantly to the development of plants.

Although CWs are a known low-cost technology, in rural communities in Mexico (as well as in other developing countries), the cheaper a wastewater technology, the easier it is to accept. In addition, most of the cost of a subsurface flow CW is concentrated in the filter media, so it is convenient to look for alternative and inexpensive filter media. Sandoval-Herazo *et al*. (2018), found that an alternative filter medium is plastic waste, specifically Polyethylene Terephthalate (PET), which is low cost and easy to obtain. Additionally, its use in CWs could contribute to the mitigation of pollution generated by this urban solid waste. However, the studies reported in the literature where such residue is used as substrate in CWs are very limited (Saraiva *et al*. 2018; Maroneze *et al* 2014; Chen *et al* 2021). Therefore, it is necessary to investigate the possible generation of microplastics and the release of phenols, in the short, medium and long term in HC that use PET as a substrate, to ensure that the use of PET is environmentally safe (or failing that, discard its use).

Due to the aforementioned, this work aimed to evaluate the development and effect of *H. latispatha* as an ornamental emergent plan in pilot-scale CWs for the treatment of wastewater from backyard pigs mixed with domestic wastewater, using PET waste as a substitute of the filter medium; under tropical climates.

2 Materials and methods

2.1 Study site

The study was carried out at the Superior Technological Institute of Misantla, Veracruz, Mexico, which is located in the central mountainous area of the State of Veracruz, Mexico, at coordinates 19° 56' north latitude and 96° 51' west longitude; at an altitude of 300 meters above sea level. The climate in this area is warm-humid with an average temperature of 22.7° C. The average annual rainfall is 2,036.4 mm (INEGI, 2020).

2.2 Description of constructed wetland systems

Six cells, built of masonry, functioned as horizontal subsurface flow wetlands (Fig. 1). Their dimensions were 1.6 m long, 0.4 m wide and 0.65 m in height.

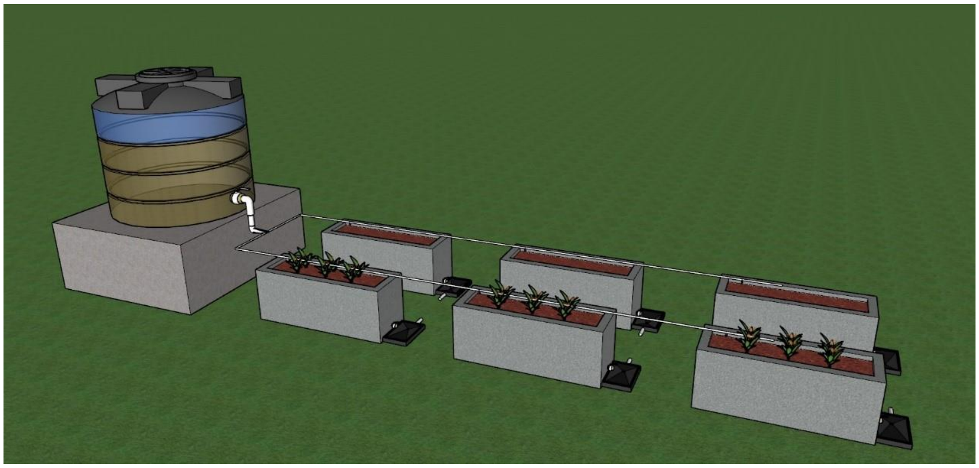

Figure 1. Experimental setup.

The cells were filled with rough and crushed waste from plastic bottles (PET) of soft drinks and water (with a porosity of 68%) generated in the cafeteria of the Superior Technological Institute of Misantla. Each cell was filled with 55 cm of PET (from the bottom) with a size between 1.5 and 2.5 cm (length and width); the remaining 10 cm were filled with tezontle with an average diameter of 2 to 2.5 cm to prevent PET from floating and the generation of vectors. The water level was maintained 10 cm below the surface, so that the layer of tezontle did not intervene in the treatment. Three cells were planted with six individuals of *H. latispatha* with a height of 15±1.2 cm on average, separated by 40 cm along each planted cell (Fig. 1), this to give enough space for the plant development throughout the study. The plants were transplanted from their natural state in an area close to the experimental site. The size of the plants was selected to be able to evaluate their growth rate in the CWs until flower production in the CWs. The other three cells were used as control treatments, without vegetation, in order to analyze the effect of the plant and PET as a substrate in the removal of contaminants present in the pig/domestic wastewater used for the study. The systems were installed outdoors and exposed to ambient conditions covering three seasons (autumn, winter and early spring).

2.3 *Vegetation development*

Measurements of plant height and stem diameter, as well as the number of plants and number of inflorescences were made to assess the plant growth from October 18, 2019 (day = 0) to April 17, 2020 (day = 240) after the plants were adapted in the CWs. The first two parameters were measured with an UltraTech® brand digital caliper; while the other two were obtained simply by counting. These measurements were made every 30 days.

2.4 *Monitoring of the systems for pollutant removal*

The systems were in operation for 8 months, from August 15, 2019 to April 17, 2020. The monitoring of the parameters to measure the removal of contaminants was carried out during the last six months every 15 days, since the first two months were for the system stabilization. During the stabilization period there was no need to replace the plants of *Heliconia*. This species is well adapted to the local climate since it grows naturally in a nearby area. Swine wastewater mixed with domestic wastewater in a 1:1 ratio was used to simulate wastewater generated in backyard pig farming in rural areas of Mexico, which is often mixed with the domestic wastewater generated in the homes where this activity is practiced. The swine wastewater came from a rearing of backyard pigs that produce between 45 to 60 liters/day of wastewater, collected at the outlet of the rearing area. Raw domestic wastewater was collected at a drainage point that discharges into the Misantla River. The two effluents were mixed and stored in a 1100 L tank to be fed to the wetland systems that operated with a hydraulic retention time of 5 days, based on previous studies in tropical areas (Cortes-Esquivel *et al*., 2012). The mixed wastewater was prepared every three days and allowed to settle for 24 hours (as a pretreatment stage) before feeding the systems. The parameters measured in the influent and effluents were Chemical Oxygen Demand, Total Nitrogen, Total Suspended Solids, Total Phosphorus, Total Coliforms, as well as pH, dissolved oxygen and water temperature, all of them according to standard methods (APHA, AWWA, WEF, 2005)

2.5 *Statistical analysis*

A completely randomized experimental design was used. The data obtained were subjected to a two-way analysis of variance and Tukey's comparison of means test ($p \leq 0.05$) using the statistical computer program Minitab 17 Statistical Software (2010).

3 Results and discussion

3.1 *Heliconia latisphata development*

Figure 2 shows the growth of *H. latispatha* plants using pig + domestic wastewater as the only source of nutrients. In this regard, it is observed that plant growth maintained a constant and statistically significant growth ($p \leq 0.05$) during the evaluation period. In general, the increase in plant height throughout the evaluated period was 74.3%, with an average daily growth rate of 0.41 cm.

On the other hand, a pattern was found in the growth of the plant, which was possible to group into three stages that were significantly different ($p \leq 0.05$) from each other (Fig. 2). In the first, slow growth was observed during the first 30 days (October 15-November 15), possibly due to the

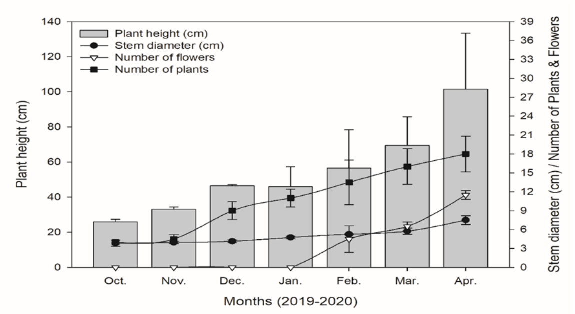

Figure 2. Growth of *Heliconia latispatha* in subsurface flow wetlands fed with wastewater from pig backyard livestock + domestic wastewater.

adaptation needs of the plant after transplanting. Then, between November 16 and January 15, a significant increase in plant height was recorded and the plants maintained this growth rate until March 15. Finally, between March and April, a sudden and statistically significant increase occurred again, registering a 31.4% increase in plant height. This last change represented an approximate growth rate of 1.06 cm per day throughout these months. The comparatively slow growth rate between November 16 and March 15 was probably due to the fact that at this stage, the plant entered the flowering period, and therefore probably diverted its nutritional reserves and/or concentrated the greatest investment of energy (ATP) in the development of the inflorescences, rather than in the growth itself (Malhotra *et al*., 2018).

The number of plants also revealed a highly significant increase ($p \leq 0.001$). Similar to plant height, during the first 30 days the increase in the number of plants was slow, probably due to the period of adaptation after transplantation. According to Loges *et al*. (2011), various species of the *Heliconia* genus show symptoms of susceptibility to transplant which causes high mortality rates, low shoot production and prolonged times for the production of new shoots. Subsequently, between 30 and 60 days after transplantation, the number of plants suddenly increased by 125% with respect to the initial number of transplanted plants. From that moment on, the increase in the following months was progressive until reaching a difference of 350% towards the end of the experiment. These results correspond to a propagation rate of 1.77±1.05 new plant shoots on average per month, reaching the maximum rate of increase between the first and second month (from 30 to 60 days), with a value of 2.5±0.7 new plants. This increase in the number of shoots may correspond to the moment in which the species managed to overcome the adaptability phase after transplantation. In this stage, the plant with the help of nitrifying bacteria that carry out the transformation, metabolizes the greatest amount of NO_2^- and NO_3^- for protein synthesis (Pelissari *et al*., 2017) and simultaneously consumes phosphorus to meet the high demand energy (in the form of ATP and phosphated sugars) that this process requires (Yadav, *et al*., 2017).

Regarding the diameter of the stem, the changes observed during the study can be considered as in two stages. This is, during the first five months, the increase in stem thickness was slow but constant. However, for the last two months, the increase was highly significant ($p \leq 0.001$) with an average increase rate of 1.6 and 5.8 mm per day, during March and April 2020, respectively. On average, stem diameter increased at an average rate of 2.01 mm per month with a total increase of 48.6%.

Finally, the number of inflorescences had an increased rate of 250% along the period of experimentation and the first flowers appeared after 120 days. These values are consistent with what was reported by Kannan *et al*. (2019), who pointed out that the average number of days required to reach the first flowering in various genotypes of the genus *Heliconia* is 124.79 days. This indicates that in CWs fed with pig + domestic wastewater, *Heliconia* develops similarly to how it behaves under commercial culture conditions. On the other hand, the same authors report that in commercial crops the plants reach their first flowering between 155 and 175 d (depending on the genotype). Therefore, the flowering of *H. latispatha* plants planted in CWs fed with pig + domestic wastewater was between 35 and 55 days earlier than that reported by (Kannan, *et al*., 2019). This earlier flowering of *Heliconia latispatha* and the sudden increase in the number of inflorescences during the last three months of evaluation (Fig. 2) can be attributed to the amount of available phosphorus in pig + domestic wastewater, due to the fact that phosphorus plays a crucial role in the reproductive phases of plants stimulating their vegetative growth that are reflected in high number of leaves and inflorescences (Shaukat *et al*., 2012). Additionally, many studies have shown that phosphorus stimulate flower production in flowering plants (Naz *et al*., 2012). In a study by Shaukat *et al*. (2012) on the production of *Gladiolus grandifloras* in soils with different doses of phosphorus application, an earlier flowering start and greater flower production were found with high doses of phosphorus (100-160 kg/ha) compared to low doses (40kg/ha).Throughout this study, an average of 11.5±0.7 inflorescences per plant was found. On soils, phosphorus tends to be adsorbed and fixed, so its availability to plant roots is rarely sufficient to promote optimal growth and development of plants (Malhotra, *et al*., 2018). However, within the CWs, the roots of the plants remain in contact with continuous inputs of phosphorus from wastewaters, for long hydraulic retention times, which can favor the retention and assimilation of the nutrient within the plant (Baldovi *et al*., 2021). Phosphorus removal by plant uptake was corroborated when analyzing phosphorus removal as will be seen later in section 3.3.

3.2 Dissolved oxygen, pH, and water temperature

Table 1 shows the measured values of dissolved oxygen, pH, and temperature in the influent and effluents. As expected, the DO value was low in the influent, but increased by 2.3 units in the planted system and only 0.7 units on average in the unvegetated system. This performance of the planted systems is similar to the behavior of the large-scale CW treating swine wastewater in tropical climates using the ornamental macrophytes *Typha latifolia* and *Canna* hybrid reported by Sandoval-Herazo *et al*. (2021b), where DO increased by 0.7 units, demonstrating the positive effect of using macrophytes in CWs. Also, the pH values increased from 6.6 to 7.7 units in the wastewater when passing through the systems. Such increase is very similar to what was reported by Almeida-Naranjo *et al.* (2020) in tropical areas of Ecuador who found a pH between 6.9 and 7.7 in the effluents when using *Heliconia stricta* in CWs to treat domestic wastewater. In CWs, the increase in pH in the effluents is frequently due to the generation of OH- during the denitrification reaction (Del Toro Farías and Zurita Martínez, 2021). Regarding water temperature, the result was contrary to that of the aforementioned parameters, since in both types of systems -planted and unvegetated systems -, the values decreased by an average of 5.5°C and 4.6 ºC, respectively. This performance coincides with the pilot-scale horizontal CW reported by Shruthi & Shivashankara, (2022), where the wastewater temperature decreased in 1.2 °C after passing through the system.

3.3 Efficiency of the systems for COD, TSS, TN and P removal

Average concentrations in the systems and removal percentages throughout the study are shown in Table 2. Furthermore, in Figure 3, the average concentrations and removal percentages per month are shown. According to the results of the analysis of variance and Tukey's comparison of the means test, the systems planted with *H. latispatha* were significantly more efficient ($p \leq 0.001$) for the reduction of COD, TN, P and TC. Regarding the SST, the systems without vegetation showed to be statistically similar to the planted systems. In general, the systems planted with *H. latispatha* presented higher efficiencies, eliminating between 34 and 60% of the contaminants evaluated, while the systems without vegetation maintained a general removal within the range of 23 to 48%. It is important to highlight that the TSS was the only one that did not show a significant difference, which indicates that the presence of *H. latispatha* does not influence the elimination of TSS in the system.

Specifically for COD, in this study, removal efficiencies between 55-70% were obtained in those systems with vegetation in comparison to 47-60% of COD in systems without vegetation. In this regard, Udom *et al.* (2018) found lower efficiencies (41.6 - 44.85%) for COD when using *Pennisetum clandestinum* and *Pennisetum purpureum* in CWs as tertiary treatment for pig effluents in Nigeria.

Table 1. Average values of dissolved oxygen, pH and water temperature in the systems along the monitoring period (six months) (Average ± Stand. Dev.) Average number of plants in the wetlands = 23.

Parameter	Influent	Constructed Wetland (Effluent)	
		Heliconia latispatha	Control
DO (mg·L^{-1})	1.1 ± 0.6	3.4 ± 0.6	1.8 ± 0.2
pH	6.6 ± 0.4	7.7 ± 0.3	7.3 ± 0.4
Water temperature (°C)	22.7 ± 1.5	17.2 ± 0.7	18.1 ± 1.1

The higher efficiencies found in this study, even in non-planted systems, were probably due to the higher COD concentration in the swine/domestic wastewater that was only sedimented (as pretreatment) in contrast to the mentioned study. Organic matter has been shown to become more recalcitrant and difficult to degrade after many treatment steps (Soares *et al*., 2022) and therefore more difficult to remove.

Regarding TN removal, there was a significant difference between vegetated and non-vegetated systems ($p<0.05$) and the removal efficiencies ranged from 18-47% and 13-33%, respectively. These results are similar to what was found by Sandoval *et al* (2021a), in the sense that the planted systems were more effective and reaffirm that the presence of *H. latispatha* contributed to TN removal by direct uptake and by its root system that allowed the development of microorganisms that mediate the different mechanisms for TN elimination such as nitrification, denitrification, ANAMMOX (anaerobic ammonium oxidation), etc. (Del Toro *et al*., 2019). However, the results obtained in this study were lower than the removals achieved for the same type of wastewater by Sandoval-Herazo *et al*. (2021a) in CWs with polyculture and monoculture of ornamental plants, who found removals between 68.1 and 74.9 % with an influent concentration of 171.9 ±74.3 mg/L of TN. The lower efficiencies in this study were probably due to the much higher concentration of TN and COD (Table 2) and the use of PET instead of tezontle. The tezontle probably contributed to the removal of TN through the adsorption of ammonium. Furthermore, at high concentrations of TN and organic matter, a larger population of microorganism is needed to mediate the reactions for nitrogen transformation and removal.

With regard to TSS, the removal efficiency in the planted systems (50-70%) was slightly higher than in non-planted systems (47-60%), without significant difference. This last result was opposite to what was found when this species was evaluated in CWs with tezontle as filter medium (the vegetated systems were significantly more effective) (Sandoval, *et al*., 2021a). These results suggest that probably, with PET as filter medium (compared to tezontle), the development of the root system is poorer and this affected the processes that lead to TSS removal, such as sedimentation, filtration, interception, etc. Additionally, the removals reached in this study were lower than the 78% for TSS reported by Benvenuti *et al*. (2018), who used *Typha domingensis* for the treatment of domestic water in CWs with a hydraulic retention time (HRT) of 11.5 days, that is, more than double the HRT in our systems.

Table 2. Average concentrations and removals of contaminants in the planted and non-planted systems during the study period.

Parameter	Units	Influent	Control	*Heliconia latispatha*
COD	Concentration ($mg \cdot L^{-1}$)**	1556.5±370.4	685.6^a	573.9^b
	Removal (%)**		47.8^b	55.6^a
TN	Concentration ($mg \cdot L^{-1}$)**	851.4±106.6	622.1^a	547.2^b
	Removal (%)**		26.4^b	34.9^a
TSS	Concentration ($mg \cdot L^{-1}$)*	931.1±114.9	434^a	413.2^a
	Removal (%)*		53.2^a	55.5^a
P	Concentration ($mg \cdot L^{-1}$)**	94.6±14.9	71.5^a	54.8^b
	Removal (%)**		23.2^b	41.2^a
TC	Concentration (MPN/100 mL)**	24933±6	496^a	120^b
	Log units	4.4±0.8	2.7^a	2.1^b
	Removal (%)**		45.7^b	59.4^a

*Means with the same superscript letters in the same row do not have statistically significant differences ($P \leq 0.05$) according to Tukey's mean comparison test.

**Means with different letters in the same row show highly significant statistical differences ($P \leq 0.05$) according to Tukey's mean comparison test.

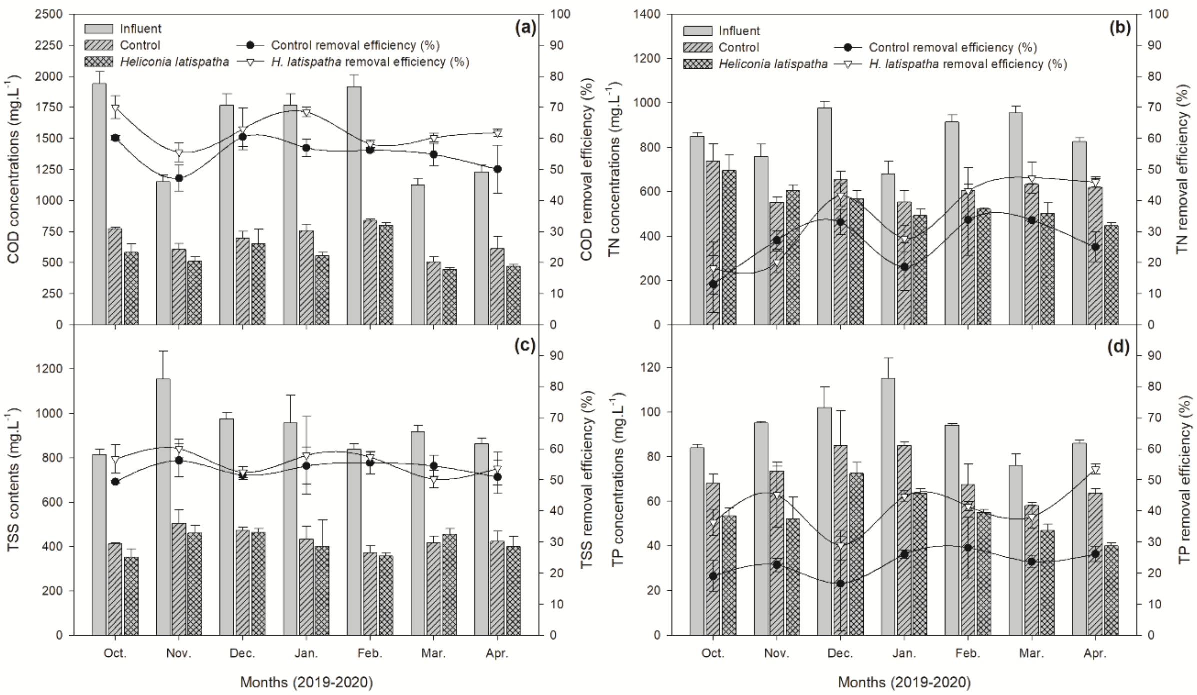

Figure 3. Contaminant removal in horizontal flow CWs using *Heliconia latispatha* as emergent vegetation and PET waste as filter medium.

With respect to the phosphorus, removal efficiencies in planted and unvegetated systems were in a range between 28-53% and 16-28%, respectively. In general, the removal of phosphorus in CWs is mainly due to adsorption in the filter medium, as well as chemical precipitation and, to a lesser extent, by the plant and microorganism uptake (Almeida-Naranjo *et al*., 2020); therefore, phosphorus removal tends to be low with conventional filter media. For example, Fountoulakis *et al*. (2017) obtained a final average removal efficiency of 13% in CWS with gravel (planted with *Tamarix parviflora*, *Juncus acutus*, *Perrenis*, *Limoniastrum monopetalum*), when treating domestic wastewater with an influent total phosphorus concentration between 3 to 87 mg/L. Furthermore, Almeida-Naranjo *et al*. (2020) found an average phosphorus removal of 23.4% in horizontal subsurface flow CWs (planted with *Heliconia Stricta* Huber) as the second stage of hybrid CWS for the treatment of domestic wastewater. Interestingly, the results on phosphorus removal are very low in comparison to the 44.3 to 63.4% obtained by Sandoval-Herazo *et al*. (2021a) with the same wastewater but using tezontle as filter medium. These results corroborate the role of tezontle in the removal of phosphorus, which due to its structural and textural properties (Tejeda *et al*., 2017) not only allow direct interaction with phosphorus by chemical and physical adsorption, but also probably favor the development of increased populations of microorganisms that contribute to phosphorus uptake and retention; these removal mechanisms are not possible (or occur to a lesser extent) when PET is used as substrate and therefore phosphorus removal is lower in this study. In addition, the higher removal in vegetated systems indicates that flower harvesting could contribute to long-term phosphorus removal.

3.4 Efficiency of the systems for fecal coliforms removal

Figure 4 shows the comparison of removals between planted and non-planted systems for TC removal. A greater removal (in 13.7%) was found in the planted systems (Table 2). In a similar way, Giácoman-Vallejos *et al*. (2015) found higher removal efficiencies in systems with vegetation (70-83%) and systems without vegetation (67-78%), using *Typha latifolia* in horizontal flow CWs as tertiary treatment after anaerobic digesters for swine effluents. The significant difference between planted and non-planted systems, as well as the capacity of *H. latispatha* to contribute

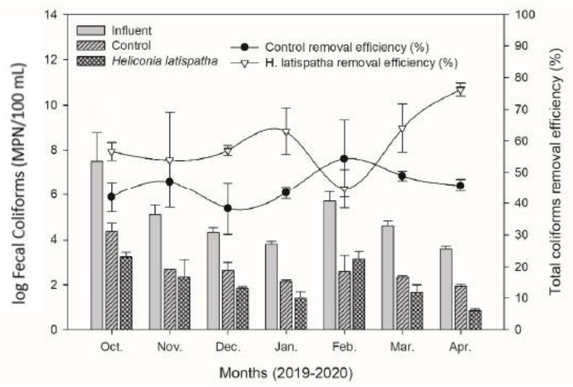

Figure 4. Total coliforms removal efficiency in horizontal flow CWs using *Heliconia latispatha* and non-planted CWs.

to TC was already found by Sandoval-Herazo *et al*. (2021a) for the same swine effluent mixed with domestic wastewater. The influence of vegetation on the inactivation of pathogens rely on factors such as the release of radical oxygen, which provides aerobic conditions to the rhizophore zone (Sánchez-Olivares *et al*., 2019) that negatively affect the growth of pathogens (Morató *et al*. (2014) as well as the release of antibiotics in the root zone which depends on the particular species of the plant (Zurita and Carreon-Álvarez, 2015); in this sense, ornamental plants seem very suitable. In this study, the radical development of *H. latispatha* was very extensive, occupying the entire bottom surface of the systems (which partly explains its contribution to TC removal).

It is important to highlight that the experiment was carried out under different environmental conditions since, as mentioned before, the study period covered three seasons, autumn, winter and early spring. In general, this situation strengthens the results in the sense that they show that this species is appropriate for this type of climate, since the change in environmental conditions did not affect its plant development or the removal of pollutants. On the other hand, the decrease in the efficiency for the removal of CT in February, expressed as a percentage, is probably due to the increase in the concentration of CT (in more than 2 logarithmic units with respect to January) as a result of the presence of visitors by the festivities in the municipality of Misantla. However, it is important to mention that the decrease in logarithmic units was actually similar to the month of January when the concentration of TC was lower.

Conclusions

In the last 5 years, research has shown a diversity of macrophytes that can be used in CW for wastewater treatment in tropical climates, within which *H. latispatha* has not been reported when PET is used as filter medium. Therefore, this study fills that gap and demonstrates the excellent development of *H. latispatha* under subsurface flow CW conditions. The development achieved by this ornamental species in the evaluated systems was similar to the level of development in commercial soil crops. The good development of the plant was reflected in the noticeable increase in plant height, stem thickness, number of plants and inflorescences. In addition, the presence of *H. latispatha* significantly influenced the removal of contaminants, such as COD, TN, TP and TC, but not SST. Therefore, the use of this ornamental plant is recommended to be implemented in larger-scale systems for the treatment of pig, domestic and other agricultural wastewater. The production of flowers of *H. latispatha* and the good removal of pollutants in subsurface flow CWs transforms the system into a viable alternative for the production and commercialization of *H. latispatha* under tropical climates while at the same time the wastewater is treated in some degree. It is important to highlight that an entire treatment train is necessary to reduce the concentrations of contaminants to the permissible limits established in Mexican standards. It is also important to note that PET by itself also contributed to the removal of these contaminants by anchoring the roots of the plants. The use of this waste would support the mitigation of contamination by urban solid waste with the reuse of PET as a substrate in CW in a larger scale.

On the other hand, future studies are required to deepen on the use of PET in CWs, including aspects such as the level of rooting it provides with respect to traditional substrates, the influence of the size of the PET residues to facilitate the rooting of the macrophyte, the evaluation of the maximum volume of PET per square meter and the number of times it can be used, as well as an economic analysis of the use of PET as a filter medium.

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