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Artículos científicos

Degradación de contaminantes en humedales artificiales en serie con especies macrófitas del trópico húmedo

Degradation of contaminants in serially constructed wetlands with macrophyte species from the humid tropics

Degradação de poluentes em áreas úmidas artificiais em série com espécies de macrófitas dos trópicos úmidos

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Resumen

Los humedales artificiales (HA) son una tecnología de tratamiento de aguas residuales eficiente, económica y amigable con el ambiente. En este estudio se evaluó un HA en serie que opera con un gasto entre 14 y 30 m^3 $día^{-1}$ de agua residual doméstica, determinando la remoción de contaminantes y el comportamiento cinético. El tratamiento consta de pretratamiento, un humedal artificial de flujo libre (HAFL) con *Thalia geniculata*, seguidamente un humedal artificial de flujo subsuperficial (HAFS) con *Sagittaria latifolia* y un HAFL con *Eichhornia crassipes* y *Pontederia cordata*, a los cuales se les determinó en fase de arranque y estabilización las variables temperatura, pH, color, turbiedad, sólidos disueltos totales y conductividad eléctrica. El medio de soporte en los HA presentó una partícula de 19±2.36 mm y porosidad de 49.6±3.8. Los HA operaron con un tiempo de retención hidráulica de 30.74 horas. Las constantes de desempeño global en el tren de tratamiento (kGA) fue de 2.03 $días^{-1}$ para DQO, 2.03 $días^{-1}$ para NT y 1.84 $días^{-1}$ para PT, todos estos comportamientos se presentaron con temperaturas entre 26 °C y 28 °C. Finalmente, a un año de operación el sistema removió el 89.2 % de la DQO, 87.0 % de NT y 84.8 % de PT, cumpliendo con criterios de descarga de la norma NOM-001-SEMARNAT-2021 y se sabe que la mayoría de las macrófitas alcanzan su eficiencia máxima de remoción de contaminantes entre los dos y tres años de operación. *Sagittaria latifolia*, *Eichhornia crassipes* y *Pontederia cordata* son las especies de menor tamaño, pues presenta una biomasa final (base húmeda) de 73.9±9.2 kg, 42.3±3.7 kg y 73.4±2.3 kg, respectivamente, por lo que son más fáciles de manejar en su siembra, mientras que *Thalia geniculata* presentó 61.3±8.2 kg, y muestra dificultades en su manejo, pues se desenraiza fácilmente debido a su tamaño y a la acción del viento.

Palabras clave: eficiencia de remoción, *Eichhornia crassipes*, *Pontederia cordata*, *Sagittaria latifolia*, *Thalia geniculata*.

Abstract

Constructed wetlands (CW) are an efficient, economical and environmentally friendly wastewater treatment technology. In this study, a series of CW operated with a domestic wastewater flow between 14 and 30 m^3 day^{-1}. The removal efficiency of pollutants and their kinetic behavior was evaluated. The treatment consisted of pretreatment on a free flow constructed wetland (FFCW) with *Thalia geniculata*, followed by a subsurface flow constructed wetland (SSFCW) with *Sagittaria latifolia* and a FFCW with *Eichhornia crassipes* and *Pontederia cordata*. After the starting and stabilization phase, the variables temperature, pH, Color, Turbidity, Total Dissolved Solids and Electrical Conductivity were determined. The support medium in the CW presented a particle size of 19±2.36 mm and a porosity of 49.6±3.8. The CWs operated with a hydraulic retention time of 30.74 hours. The global performance constants in the treatment train (kGA) were 2.03 $days^{-1}$ for COD, 2.03 $days^{-1}$ for TN and 1.84 $days^{-1}$ for TP. In all cases, the temperature ranged from 26 to 28 °C. After a year of operation, the system removed 89.2% of COD, 87.0% of TN and 84.8% of TP, complying with the discharge criteria of the NOM-001-SEMARNAT-2021 standard. Likewise, most of the macrophytes reached their maximum removal efficiency during the second and third year of operation. *Sagittaria latifolia*, *Eichhornia crassipes* and *Pontederia cordata* were found to present the smallest species with a final biomass (moist base) of 73.9 ± 9.2 kg, 42.3 ± 3.7 kg and 73.4 ± 2.3 kg respectively, hence they can be easier to handle in their production. planting, while *Thalia geniculata* presented 61.3 ± 8.2 kg. Finally, *Thalia geniculata* showed some difficulties during the experimental runs since it was easily uprooted due to its size and the wind speed.

Key words: Removal efficiency, *Eichhornia crassipes*, *Pontederia cordata*, *Sagittaria latifolia*, *Thalia geniculata*.

Resumo

As zonas húmidas construídas (HA) são uma tecnologia de tratamento de águas residuais eficiente, económica e amiga do ambiente. Neste estudo foi avaliada uma série de HA que opera com consumo entre 14 e 30 m^3 dia^{-1} de águas residuárias domésticas, determinando a remoção de contaminantes e o comportamento cinético. O tratamento consiste em pré-tratamento, um pantanal artificial de fluxo livre (HAFL) com *Thalia geniculata*, depois um pantanal artificial de fluxo subsuperficial (HAFS) com *Sagittaria latifolia* e um HAFL com *Eichhornia crassipes* e *Pontederia cordata*, que foram determinados nas variáveis de início de fase e estabilização temperatura, pH, cor, turbidez, total de sólidos dissolvidos e condutividade elétrica. O meio suporte no HA apresentou tamanho de partícula de 19±2,36 mm e porosidade de 49,6±3,8. O HA operou com tempo de retenção hidráulica de 30,74 horas. As constantes de desempenho global no trem de tratamento (kGA) foram de 2,03 $dias^{-1}$ para DQO, 2,03 $dias^{-1}$ para NT e 1,84 $dias^{-1}$ para PT, todos esses comportamentos ocorreram com temperaturas entre 26 °C e 28 °C. Por fim, após um ano de operação, o sistema retirou 89,2% do COD, 87,0% do TN e 84,8% do TP, atendendo aos critérios de descarga da norma NOM-001-SEMARNAT-2021 e sabe-se que a maior parte do As macrófitas atingem sua eficiência máxima de remoção de contaminantes entre dois e três anos de operação. *Sagittaria latifolia*, *Eichhornia crassipes* e *Pontederia cordata* são as espécies de menor porte, pois possuem biomassa final (base úmida) de 73,9±9,2 kg, 42,3±3,7 kg e 73,4±2,3 kg, respectivamente, facilitando seu manejo em sua semeadura. , enquanto *Thalia geniculata* apresentou 61,3±8,2 kg, e apresenta dificuldades no seu manejo, pois se desenraíza facilmente devido ao seu tamanho e à ação do vento.

Palavras-chave: eficiência de remoção, *Eichhornia crassipes*, *Pontederia cordata*, *Sagittaria latifolia*, *Thalia geniculata*.

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Introduction

Starting in this new century, there has been a decrease in the quantity and quality of water due to an increase in its use and consumption that exceeds population growth (United Nations Development Program [UNDP], 2016). . Among the most significant reservoirs of water on our planet are natural wetlands, that is, vast ecosystems serve as essential reservoirs and sources for a wide variety of uses. Furthermore, they are cradles of biodiversity and primary productivity, essential for the survival of numerous species of plants and animals, which is why they are considered the most productive environments on the planet (Ramsar, 2013).

Globally, the use of artificial wetlands (HA) has been increasingly promoted as a solution for wastewater treatment, as they imitate the processes that nature uses to purify water. HA integrate physical, chemical and biological processes that involve plants, microorganisms and the atmosphere (Russo *et al* ., 2019), of which two main types can be identified according to the flow they use: surface flow (HAFL), where water moves exposed to the atmosphere, and subsurface flow (HAFS), where water flows through a support medium, generally gravel. These systems are capable of removing significant amounts of organic matter, nitrogen, phosphorus, suspended solids, bacteria and heavy metals present in wastewater, which occurs due to complex physicochemical and microbial interactions that occur within them. The effectiveness of these processes depends on factors related to the design and operation of the HA, as well as the presence of macrophytes (essential biological component in the HA) and the type of medium used (Calheiros et al., 2009; *Peña* and Lara, 2012).

Regarding the research that supports the above, we can refer to a study focused on serial artificial wetland (HA) systems that evaluated the quality of the treated water in a system composed of three subsurface flow HA (HAFS), which used plants such as rush (*Scirpus americanus*), cattail (*Typha domingensis*), and water lily (*Eichhornia crassipes*). This system was stabilized for 44 days, with a retention time of 15 days for each HAFS. The results revealed a decrease in parameters such as COD (71%), calcium (91%), chloride (77%), nitrite (82%), ammonium (99.9%), and phosphate (77%). However, the nitrate ion only showed a 36% decrease, and the electrical conductivity increased by 93% (Ramos *et al*., 2007).

In another study, the impact of artificial aeration on purification processes in HAFS was investigated. For this, six experimental units built with polyvinyl chloride were used, with dimensions of 1.25 m in length, 0.2 m in width and 0.3 m in depth each. Forced aeration was achieved by using an air compressor and aeration tube. As a result, at the end of the treatment with forced aeration, removal efficiencies of 90.1% in COD, 99.7% in N-NH4, and 51.3% in total nitrogen were obtained , with final concentrations of 184.6 mg/L, 21.7 mg/L, L and 0.27 mg/L, respectively. Furthermore, a substantial improvement in the removal of organic compounds and nitrogen was observed with the application of frontal aeration (Li *et al* ., 2014).

Likewise, a study of a hybrid HA in a wastewater treatment plant was carried out in the Czech Republic (Vymazal and Kröpfelová, 2015). This system consisted of saturated vertical flow units, vertical flow and horizontal flow in series, covering a total area of 10.1 m^2. In detail, the first saturated vertical flow wetland occupied an area of 2.54 m^2 and used *Phragmites australis* and crushed rock as a support medium with diameters varying from 4 to 32 mm. The second vertical flow wetland covered an area of 1.56 m^2 and used *Phragmites australis* along with sand as a support medium. The third wetland, with horizontal flow, was vegetated with *Phalaris arundinacea* and used crushed rock as a support medium of 4 to 8 mm in diameter. In this study, evaluations of various parameters were carried out, including BOD_5, COD, TSS, NT, NH_4-N and NO_3-N at the inlets and outlets of each of the wetlands, which allowed the performance of the system to be evaluated. at each stage. The results demonstrated that this multistage HA system was highly effective in removing organic substances, suspended solids, and nitrogen. The total removal efficiency was 92.5%, 83.8%, 96.0%, 88.8% and 79.9% for BOD_5, COD, TSS, NH_4- N and TN, respectively. During the vertical flow stage, significant nitrification was achieved with a removal rate of 4.17 g NH_4 -N m^2/d, while in the anaerobic stages (first and third) conditions for denitrification were provided with removal rates of 0.83 g NO_3–N m^2/d and 0.47 g NO_3–N m^2/d, respectively.

Now, in the southeast of Mexico, specifically in the Pantanos de Centla Biosphere Reserve, there is one of the most important reserves of aquatic plants in Mesoamerica. Despite their relevance, these species have been little evaluated regarding their potential for wastewater phytoremediation. However, there are pilot-scale studies on certain species of macrophytes that require validation in real conditions and on a larger scale in HA (Gallegos -Rodríguez *et al*., 2018; Jimenez -Lopez *et al*., 2017).

For this reason, the objective of this project was to evaluate the kinetic behavior and removal capacity of basic contaminants, such as pH, temperature, total dissolved solids, turbidity, color, COD, TN and TP, in a wastewater treatment plant. (WWTP), which was composed of artificial wetlands arranged in series (HAFL-HAFS-HAFL) and treated water coming from the DACBiol-UJAT university campus. The plant species used in this system were *Thalia geniculata* , *Sagittaria latifolia* , *Pontederia cordata* and *Eichhornia crassipes* , typical of the humid tropics.

The implementation of this treatment technology on the university campus not only contributes to the reduction of energy consumption, but also decreases the costs associated with the operation and maintenance of the WWTP. In addition, it generates valuable technical information on the startup and operation process of a full-scale HA system, facilitating the collection of essential data for compliance with regulatory requirements in terms of effluent discharge before the competent authorities.

Materials and methods

Location

The HA was installed in the Academic Division of Biological Sciences (DACBiol), campus of the Juárez Autonomous University of Tabasco (17° 59' 26” and 17° 59' 17” north latitude; 92° 58' 16” and 92° 58 ' 37” west longitude). The vegetation was collected in swampy areas of the municipality of Centla, Tabasco (18° 18.952' north latitude and 92° 32.376' west longitude).

Design and construction

The treatment system was designed and built in series, and began operations in February 2021 to December 2022 with a HA free flow (HAFL) arrangement, followed by a HA subsurface flow (HAFS) and finally with a HA free flow (HAFL), based on national and international criteria (Brown *et al*., 2011; Conagua, 2019). The treatment plant consists of a tanker sump and three HAs in series. Each HA consists of 8.33 m long, 2.5 m wide and 1.0 m high, with an operating tie of 0.6 m of ¾ inch river gravel for the HAFS and 0.2 m for the HAFL. The bed had a particle size between 19 ±2.36 mm, porosity of 49.6 ± 3.8, a density of 1,666.7 ± 119.3 kg/m ^{3} and permeability >1000. The receiving body is the soil (infiltration), for which an absorption pit was made when there is a surplus, since the treated waste is used to irrigate green areas.

Operation and maintenance

The wastewater flow comes from the DACBiol sump, which receives waste from the school's restrooms and cafeterias. This water is discharged into the HA by gravity through pipes equipped with ball valves and adapters, such as threaded unions, elbows and T-type unions, all made of PVC with a diameter of 4”. This system conducts the flow of wastewater to a distribution register with a capacity of 0.1 m^3, which is responsible for distributing the flow of wastewater to the HA.

The maintenance of this system involves the collection of dead plants and any other organic matter present inside the wetlands. This is done with the purpose of preventing the spread of pests and preventing the effluent from acquiring an unwanted color. In addition, a daily check of wetlands, pipes, valves and connections is carried out to ensure that they are in good physical condition, that is, free of obstructions that could hinder the flow of water and ensuring adequate laminar flow.

Sowing sampling and stabilization of species

Sampling and collections were carried out under criteria of preservation and care of the species. Complete young plants, including flowers and fruits, were collected for later planting and identification (Zepeda -Gómez and Lot, 2005). Specifically, the macrophyte species (*Thalia geniculata*, *Sagittaria latifolia*, *Pontederia cordata* and *E. crassipes*) existing in the Pantanos de Centla Biosphere Reserve were selected. In reactor 1 (HAFL-Tg) 34 *Thalia geniculata* plants were planted with a 0.3 m long stem, so that a part of the plant protrudes above the water surface. In rector 2 (HAFS-Sl) 34 *Sagittaria latifolia* plants were planted, placing the root 0.25 m inside the support medium and the stem 0.2 m long on the surface of the support medium. This same criterion was applied to HAFL-Mx, to which 30 *Pontederia cordata plants* and 30 *E. crassipes* were planted.

The HA was first fed with clean water, maintaining an adequate level for the species to adapt and survive. Seven days later, the wastewater from the sump was added, where suspended solids were removed, and the concentration was gradually increased until the flow was 100% wastewater in the HA (30 days) (Conagua, 2019; Crites and Tchobanoglous, 2004).

Wastewater characterization

The start of operations of the HA was on February 22, 2021. The physicochemical characterization of the wastewater was evaluated in two periods of 15 days interspersed, taking a total of eight sampling days, which were the end of start-up period (from August 13 to 29, 2022) and end of stabilization that corresponds to the year of operation (March 11 to 27, 2017).

In each period a total of 32 simple samples were taken. The determination of the basic physical contaminants—such as temperature, turbidity, color, hydrogen potential (pH) and total dissolved solids (TDS)—was done using the SM 2550, SM 2130 B, APHA 2120, SM 9040 B and SM methods. 2540, respectively.

To calculate temperature, SDT and pH, the multiparametric HANNA Waterproof Tester model HI 98129 was used; Color was estimated with a LaMotte SMART3 photometer, and turbidity with a HANNA HI 98703 turbidimeter. COD was determined by the USEPA 410.4 method. The NT was carried out in accordance with NMX-AA-029-SCFI-2001, based on the Kjeldahl method for the determination of total nitrogen, and to determine total phosphorus the stannous chloride method was used (NMX-AA-026- SCFI-2001).

The flow was estimated by the direct volume over time method (Briones and García, 2014), monitoring 10 days; Each day is made up of six capacities measured during operating hours (8:00 a.m. to 6:00 p.m.).

Experimental design

The results were recorded in a log and captured in an Excel database. A one-factor randomized design was proposed to analyze the treatment system (process units) evaluating the response variables, that is, pH, temperature, TDS, EC, color, turbidity, COD, PT and TN.

The results behaved as non-parametric because they did not meet the postulates of normality and homoscedasticity, so their analysis was determined using the Kruskal-Wallis test and the Mann-Whitney median contrast. All data were analyzed with Statgraphics *software* (version 16.1). The number of repetitions was three samples per day for each treatment (figure 1). The Kruskal-Wallis test evaluated the hypothesis of equality between medians of the response variables within each of the four treatment levels (spoiler, HAFL-Tg, HAFS-Sl, HAFL-Mx). It was found that there is a statistically significant difference with a 95.0% confidence level ($p < 0.05$) except for the SDT.

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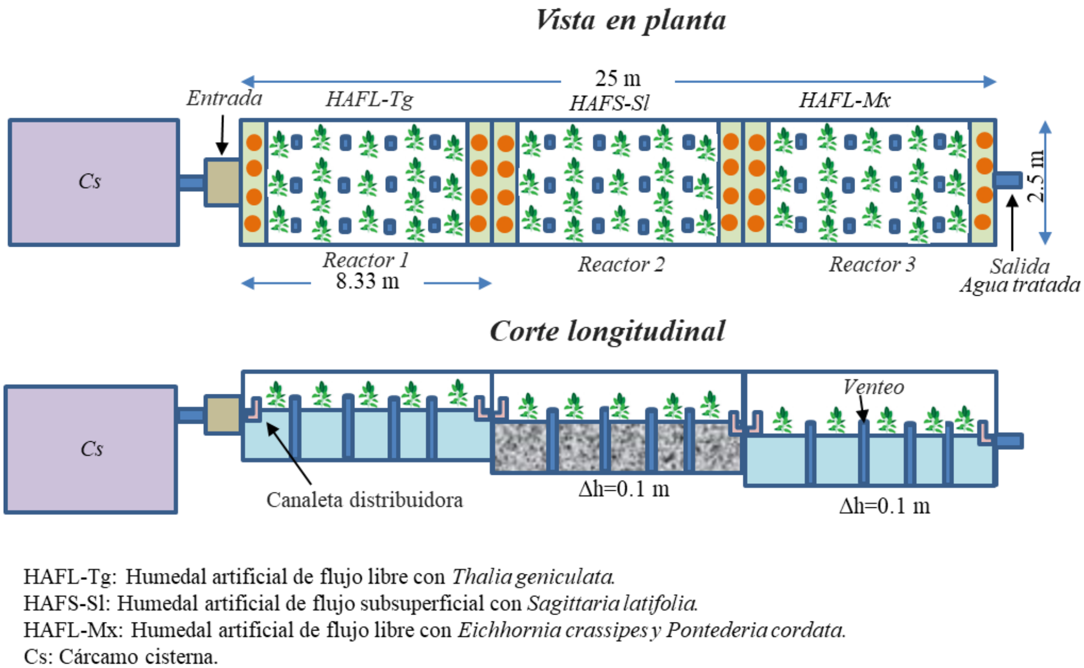
Figure 1. Elements of the DACBiol-UJAT HA treatment system



Source: self made

Retention time, removal efficiency and degradation kinetics

A mixed gravel support medium (crushed rock from the Teapa River, southern region of Tabasco) was placed in the reactor and the hydraulic retention time (HRT) was calculated with the operating flow rate of the wastewater (Conagua, 2019).

$$HRT = nd\,A/Q \qquad (1)$$

Where n is the porosity, d is the height of the support medium, A is the cross section of the reactor and Q is the water flow rate.

The contaminant removal efficiency was calculated as follows (López -Ocaña, *et al.*, 2019), where ɳ represents the removal efficiency in %, C1 the concentration of the wastewater influent and C2 the concentration of the water effluent. residuals.

$$\eta = [(C_1 - C_2) / C_1] \text{ x } 100 \qquad (2)$$

The behavior of wastewater is a first-order kinetic reaction, the degradation rate k was estimated with the following equation (Crites and Tchobanoglous, 2004; López-Ocaña *et al.*, 2019).

$$ko = -\ln(Cn / Co) / \tau \quad (3)$$

Where τ = retention time for color removal, Cn = color effluent concentration of reactor n (UC), Co = influent concentration, ko = degradation constant.

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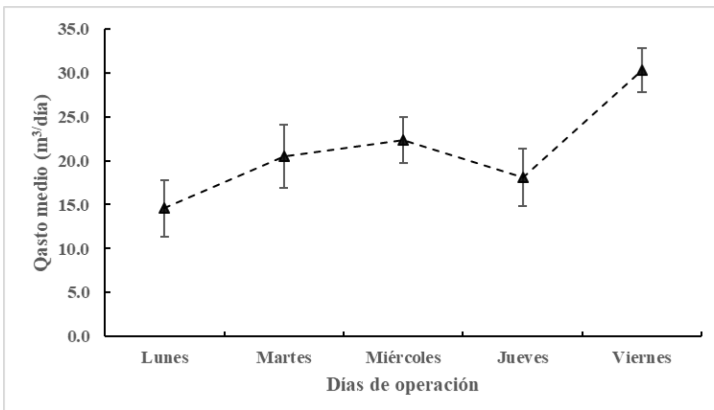
Results

Operating expenses in start-up and stabilization

During the study period, the average flow (Q_{med}) measured in the sump was 21.2±3.0 m^3/day, the minimum (Q_{min}) was 14.6±3.2 m^3/day and the maximum (Q_{max}) was 30.3 ±2.5 m^3/day. The HRT for serial HAs were estimated to be 30.74 hours. This flow varies during the year because of student mobility during school periods. The HA operates discontinuously, since on weekends the inflow tends to be below the minimum expenditure due to the low influx of students. The general behavior of the receiving station on the university campus is presented in figure 2.

Figure 2. Average values (±*SD*) of the flow in the study period (*N=60*)



Source: self made

Water quality characteristics

The results in Table 1 present the average values (±*SD*) of the effluents from the treatment stages in the start-up and stabilization stage of the DACBiol-UJAT wastewater treatment system. The quality of the inlet wastewater (sumphole) in this period presents conditions of medium to strong wastewater, since the COD concentration ranged from 250 to 500 mg/L, the TN between 30 to 65 mg/L, the TP of 5 to 10 mg/L and the SDT between 500 to 850 mg/L. These characteristics show a condition of easy degradation (Crites and Tchobanoglous, 2004; Tchobanoglous, 1996).

Table 1. Average values (±*SD*) in the effluents of the treatment units (*N=32*)

Parameters	Cárcamo		HAFL-Tg		HAFS-Sl		HAFL-Mx	
	x	±*SD*	*x*	±*SD*	*x*	±*SD*	*x*	±*SD*
pH (UpH)	7.3	0.2	7.4	0.3	7.4	0.2	7.5	0.3
Temperature (ºC)	27.6	2.1	26.7	2.8	26.4	2.4	26.0	1.6
TDS (mg/L)	620.8	51.0	610.7	41.8	609.5	35.5	586.7	50.5
Turbidity (UTN)	54.4	7.7	34.8	2.8	19.0	33.4	29.4	6.2
Color (UC)	918.5	68.4	477.3	122.8	428.0	142.0	578.0	116.0
COD (mg/L)	319.0	74.9	206.2	51.9	133.3	236.6	169.2	28.1
NT (mg/L)	50.1	14.0	32.1	7.8	12.5	22.4	26.2	2.8
PT (mg/L)	5.8	1.4	3.7	0.9	2.1	3.9	3.0	0.4

Source: self made

The results presented in Table 2 correspond to the evaluation stage after a year and a half of operation of the HA after the stabilization of the DACBIol-UJAT wastewater treatment system. In this period the water is classified as strong residual, since the COD concentration is 966 (±215) (Tchobanoglous, 1996).

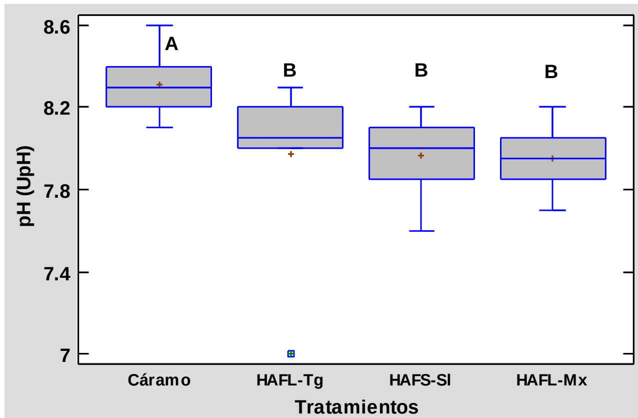
Table 2. Average values (±*SD*) in the effluents of the treatment units (*N=32*)

Parameters	Cárcamo		HAFL-Tg		HAFS-Sl		HAFL-Mx	
	X	±*SD*	*x*	±*SD*	*x*	±*SD*	*x*	±*SD*
pH (UpH)	8.3	0.2	8.0	0.4	8.0	0.2	7.9	0.2
Temperature (ºC)	27.7	0.9	27.6	1.0	27.2	0.7	26.3	1.1
TDS (mg/L)	626	117	662	127	664	126	664	123
Turbidity (UTN)	140	43	71	17	22	3	16	4
Color (UC)	1534	370	824	385	505	156	442	161
COD (mg/L)	966	215	455	155	140	36	105	60
NT (mg/L)	135	53	84	30	19	5	18	12
PT (mg/L)	17	6	9.6	2.6	2.7	0.7	2.6	1.6

Source: self made

Hydrogen potential. The Kruskal-Wallis test found that there is a statistically significant difference (95.0% confidence, $p < 0.05$), since the lowest median value corresponds to the HAFL-Mx treatment with a pH of 7.95 UpH, followed by the HAFS-Sl treatment with 7.9 UpH and the highest occurs in the sump treatment with 8.3 UpH (figure 3).

Figure 3. Median values (±Q_1 , Q_3) for the *pH variable* (UpH)

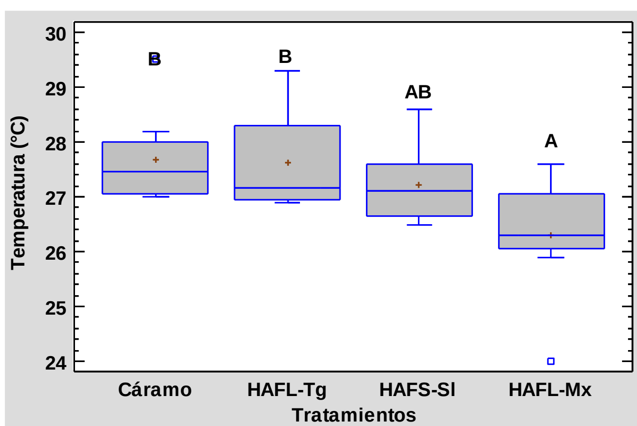


Note: Different letters indicate statistically significant differences (*N* = 32).

Source: self made

Temperature. The Kruskal-Wallis test found a statistically significant difference (95.0% confidence, $p < 0.05$), the lowest median temperature value occurs in the HAFL-Mx with 26.3 °C, followed by the HAFS-Sl treatment with 27.1 ° C and the treatment with the highest value was presented in the sump treatment with 27.45 °C (figure 4).

Figure 4. Median values (±Q_1, Q_3) for the *temperature variable* (°C)



Note: Different letters indicate statistically significant differences (*N* = 32).

Source: self made

Total dissolved solids. The Kruskal-Wallis test found a statistically significant difference between the medians (95.0% confidence, $p < 0.05$). The treatment with the lowest

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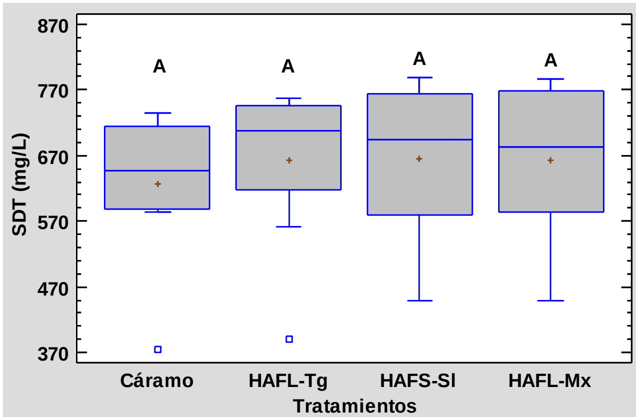
median value was presented in sump with 647.0 mg/L, followed by HAFL-Tg treatment with 683.5 mg/L and the highest value occurred in HAFL-Tg with 707.5 mg/L (figure 5).

Figure 5. Median values (±Q_1, Q_3) for the variable *total dissolved solids* (mg/L)

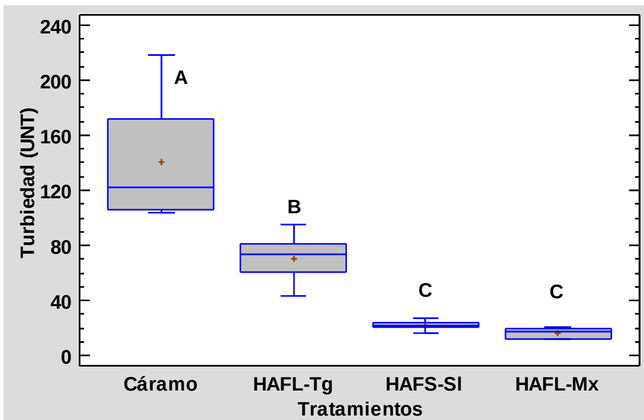


Note: Different letters indicate statistically significant differences (N = 32).

Source: self made

Turbidity. The Kruskal-Wallis test found statistically significant differences between the medians (95.0% confidence, $p < 0.05$). The lowest median value of turbidity occurs in the HAFL-Mx effluent treatment with 19 NTU, followed by HAFS-SL with 25.25 NTU and the highest turbidity value is the upstream with 95.4 NTU (figure 6).

Figure 6. Median values (±Q_1, Q_3) for the *turbidity variable* (UNT)

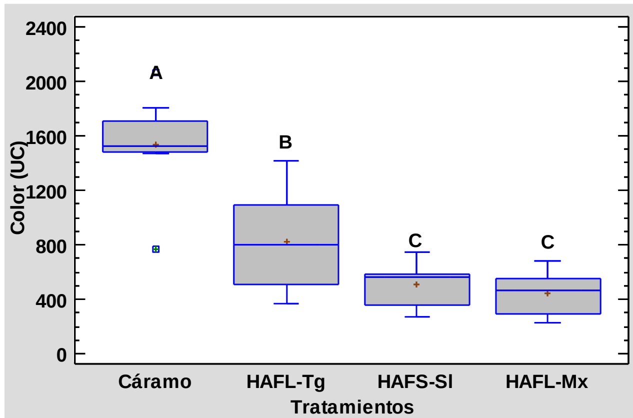


Note: Different letters indicate statistically significant differences (N = 32).

Source: self made

Color. The Kruskal-Wallis test found that there are statistically significant differences between the medians (95.0% confidence, $p < 0.05$). The treatment with the lowest color value is found in the HAFL-Mx with 468.5 UC, followed by HAFS-Sl with 563.5 UC and the highest color value is the culm with 1528.5 UC (figure 7).

Figure 7. Median values (±Q_1, Q_3) for the *color variable* (UC)

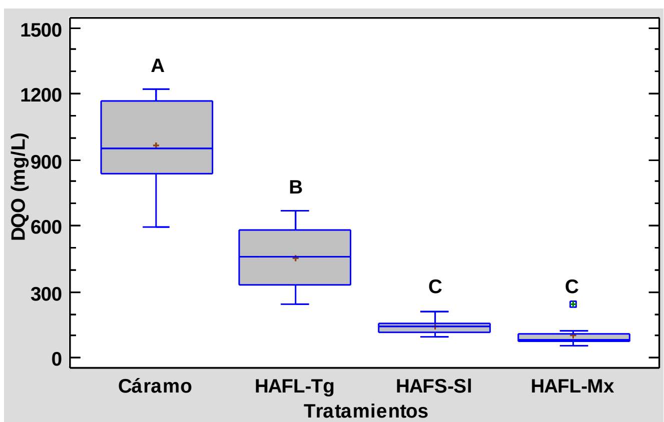


Note: Different letters indicate statistically significant differences (*N* = 32).

Source: self made

Chemical oxygen demand. The Kruskal-Wallis test found statistically significant differences between the medians (95.0% confidence, $p < 0.05$). The treatment with the lowest COD value is found in the HAFL-Mx with 80.6 mg/L, followed by HAFS-Sl with 139.9 mg/L and the treatment with the highest COD value is the cárcamo with 949.6 mg/L (figure 8).

Figure 8. Median values (±Q_1, Q_3) for the *COD variable* (mg/L)

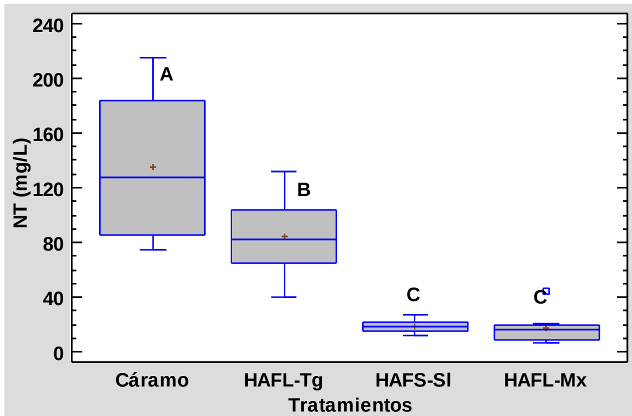


Note: Different letters indicate statistically significant differences (*N* = 32).

Source: self made

Total nitrogen. The Kruskal-Wallis test found statistically significant differences between the medians (95.0% confidence, $p < 0.05$). The treatment with the lowest NT value is found in HAFL-Mx with 16.2 mg/L, followed by HAFS-Sl with 18.6 mg/L. Finally, the treatment with the highest NT value is the sump with 127.6 mg/L (figure 9).

Figure 9. Median values (±Q_1, Q_3) for the *total nitrogen variable* (mg/L)

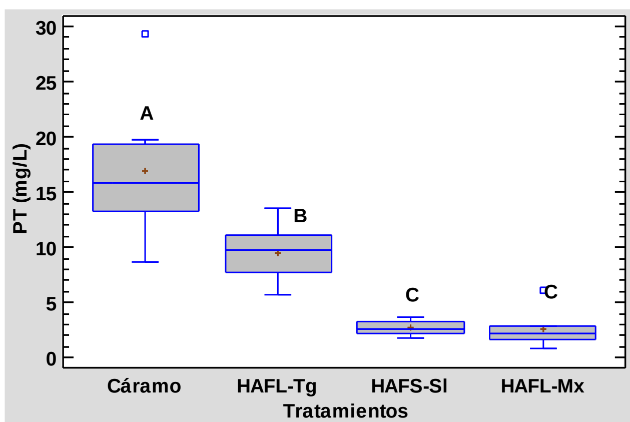


Note: Different letters indicate statistically significant differences (*N* = 32).

Source: self made

Total phosphorus. It was found that there are statistically significant differences between the medians (95.0% confidence, $p < 0.05$). The treatment with the lowest NT value is found in the HAFL-Mx with 2.25 mg/L, followed by HAFS-Sl with 2.65 mg/L and the treatment with the highest PT value is the cárcamo with 15.8 mg/L (figure 10).

Figure 10. Median values (±Q_1, Q_3) for the variable *total phosphorus* (mg(L)



Note: Different letters indicate statistically significant differences (*N* = 32).

Source: self made

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Removal efficiencies

During the start-up stage, the total efficiency of the process in turbidity presented a value of 45.91%, that is, the water came out more turbid than the input due to the adaptation process of microorganisms and vegetation in the wetlands. The color was only removed with an efficiency of 37.07% and the COD was removed at 46.06%, which is normal in the start-up and stabilization stage. The removal of the other contaminants and nutrients is presented in table 3.

In the case of the parameters that show a negative efficiency (-) in their values, it is due to the concentration of the effluent in this unit is greater than that of the influent and this phenomenon is known as short circuit (Vázquez -*González* and López- Ocaña, 2011). At the end of stabilization, turbidity removal was 88.3%, which shows an adaptation of microorganisms and vegetation in the wetlands. The color was only removed with 71.2% and the COD at 89.2%, which shows a significant adaptation of the species. The removal of the other contaminants and nutrients is presented in table 3.

Table 3. Removal efficiencies of the treatment units (*N=32*)

Parameters	HAFL-Tg (ER %)		HAFS-Sl (ER %)		HAFL-Mx (ER %)		ETP (%)	
	Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2
SDT	1.64	-5.7	0.19	126.1	3.75	0.1	5.50	-6.0
Turbidity	35.97	49.5	45.55	4.1	-55.15	10.4	45.91	88.3
Color	48.03	46.3	10.34	156.3	-35.05	12.5	37.07	71.2
COD	35.38	52.9	35.35	53.0	-26.95	14.1	46.96	89.2
N.T.	35.94	37.7	61.14	6.3	-109.83	-10.7	47.77	87.0
P.T.	35.69	43.4	42.04	0.9	-40.85	-10.3	47.50	84.8

Note: Phase 1: six months into operation. Phase 2: a year and a half into operation.

Source: self made

Kinetic coefficients in wetlands

This analysis considered that the reactors, because they are plugging flow and due to the quality of the water, behave with reaction order n = 1 (Crites and Tchobanoglous, 2004), obtaining apparent kinetic coefficients (k) for each of the wetlands and the global performance constant in the treatment train (k_{GA}), since they consider the effect of the support medium, the microorganisms and the vegetation. These behaved differently: during startup the HAFS-Sl presented the best performance, as it obtained for turbidity a k = 2.04 days $^{-1}$, for color a k = 0.36 days $^{-1}$ and for COD a k = 0.36 days $^{-1}$.

The HAFL-Tg obtained k = 0.91 days $^{-1}$ for turbidity, k = 1.33 days $^{-1}$ for color and 0.89 days $^{-1}$ for COD. Finally, the one with the lowest performance was the HAFL-Mx obtaining for turbidity a k = -0.89 days $^{-1}$, for color a k = -0.62 days $^{-1}$ and for COD a k = -0.49 days $^{-1}$. Table 4 shows the kinetic coefficients of nutrients and other parameters by period. After a year and a half of operation, the HAFS-Sl shows the best performance in the degradation of basic pollutants.

Table 4. Kinetic coefficients (k in days $^{-1}$) of the treatment units (*N=32*)

Parameters	Six months of operation				After a year and a half of operation			
	k-R1	k-R2	k-R3	k_{GA}	k-R1	k-R2	k-R3	k_{GA}
Turbidity	0.91	2.04	-0.89	0.69	1.38	3.94	0.65	1.99
Color	1.33	0.36	-0.62	0.36	1.26	1.65	0.27	1.06
COD	0.89	1.47	-0.49	0.62	1.53	3.97	0.58	2.03
N.T.	0.90	3.17	-1.50	0.86	0.96	5.00	0.11	2.03
P.T.	0.91	1.91	-0.73	0.70	1.16	4.27	0.08	1.84

Note: R1: HAFL-Tg, R2: HAFS-Sl and R3: HAFL-Mx.

Source: self made

Species characteristics

The main part of plants (photoautotrophs) are roots and rhizomes. These collect solar energy to transform inorganic carbon into organic and transfer oxygen from the atmosphere through leaves and stems to the support medium where the roots are located. Oxygen creates aerobic regions where microorganisms use the available oxygen to produce the degradation of organic matter and nitrification (Brown *et al*., 2011; Conagua, 2019).

The growth of the species can vary according to the climatic conditions of the area where the wetland is installed, that is, the growth of the species is influenced by ambient temperature, water temperature, nutrients such as N and P and the amount of organic matter available in the wastewater (Delgadillo *et al*., 2010). At the beginning of operations, the vegetation was allowed to grow for up to six months to begin regular applications of 100% residual water (Gallegos -Rodríguez *et al*., 2018).

In this study, the species that had the greatest adaptation due to their reproduction were the species *Sagittaria latifolia* with 73.9 kg of final biomass, followed by *Pontederia cordata* with 73.4 kg, this being found in the HAFS-Sl. The species *Thalia geniculata* in the HAFL-Tg achieved a total of 61.3 kg of plant biomass in its final period due to its size, root volume and density. This affected the hydrology in the wetland, as it modified the flow of water through the root and rhizome network, and blocked exposure to wind and sun (Delgadillo *et al*., 2010).

In the HAFL-Mx where *Pontederia cordata* and *Eichhiornia crassipes* caused the accumulation of vegetation remains, which increased the amount of organic matter in the wetland (Brown *et al*., 2011), since the dead leaf litter that was deposited in the HA was not extracted for the most part because it was covered by *Eichhiornia crassipes*. When it was observed that the species exceeded the contaminant retention capacity, the aerial part was cut and the wetland was eliminated to avoid the incorporation of additional organic matter into the water, among other substances (Delgadillo *et al*., 2010). The other parameters analyzed in the species are presented in table 5.

Table 5. Characteristics of the species at the end of the evaluation (*N=30*)

Parameters	*S. latifolia*		*E. crassipes*		*P. cordata*		*T. geniculata*	
	x	*±SD*	*x*	*±SD*	*x*	*±SD*	*x*	*±SD*
Weight/plant (g)	820.6	252.1	672.1	270.4	986.7	257.5	1250.0	350.0
Leaves	3.9	1.7	12.8	2.5	6.0	1.5	4.0	2.0
Long. of the leaf (m)	29.0	6.4	18.0	23	16.6	3.5	40.0	1.6
Sheet width (cm)	2.0	0.7	9.2	1.1	23.0	0.9	29.7	2.6
Long. of the root (cm)	16.3	4.3	14.7	6.1	14.7	6.4	22.0	5.3
Stem diameter (cm)	5.6	2.4	7.4	5.6	14.8	3.0	8.0	23
Long. of stem (cm)	34.0	8.0	8.0	5.0	80.0	2.8	190.0	9.8
Humidity (%)	60.0	5.0	68.0	10.0	62.0	13.0	32.0	8.0
Initial mass (kg)	27.9	6.9	20.2	23	29.6	1.3	42.5	8.4
Final biomass (kg)	73.9	9.2	42.3	3.7	73.4	23	61.3	8.2
Final dry biomass (kg)	29.5		13.5		35.2		41.7	

Source: self made

Discussion

It is important to clarify that the results of this study are based on a serial HA wastewater treatment system, in which the HA operate as secondary-tertiary treatment.

The pH enters the HA system with slightly alkaline characteristics, whose average values (±SD) in the influent were 8.3±0.2 and in the effluent 7.9±0.2 UpH. In this sense, the values allow the establishment of bacteria that favor the degradation of contaminants (Kadlec, 2009). Winanti *et al*. (2017) showed that in HAFS with *Canna sp.* The pH tends to be neutralized depending on the TRH, since the influent residual water was 8.15 ± 0.28, after one day 6.60 ± 0.21, and on the fifth day it was established at 6.80 ± 0.35. This shows that both the type of species used, and HRT will have an effect on water quality.

In the study developed by Charris and Caselles (2016), they treated water with a pH in the influent of 8.0 ± 0.92 and the effluent obtained in HAFS with *Cyperus ligularis* was 7.0 (± 0.1) and in the HAFS with *Echinochloa colonum* with 7.0 ± 0.1, operating with 3 days of HRT. Another study shows that there is an effect of the support media on the pH in wastewater treatment, since when using support media such as PIECA, CAS, EVOL and gravel materials (substitutes for the support medium in HA), The treatments presented pH of

10.98, 9.12, 5.20 and 7.69, respectively (Luna and Ramírez, 2004). The effluent discharges in the treatment train are complying with the maximum permissible limit of environmental regulations, which establishes that it can be discharged to receiving bodies of 6 to 9 pH units (NOM-001-SEMARNAT-2021).

The temperatures present in the effluents of the reactors that make up the HA in series show that the temperature values favor the growth and stabilization of mesophilic microorganisms, which can establish favorable colonies for the degradation of contaminants with temperatures of 20 °C to 45 ° C (Kadlec, 2009). Likewise, the discharge temperatures meet the maximum permissible limit in discharges of wastewater and national goods, which is less than 35 °C (NOM-001-SEMARNAT-2021).

The total permissible dissolved solids in water for public, industrial and agricultural use range between values of 500 and 850 mg/L. In this sense, high concentrations affect the organoleptic characteristics of water and increase electrical conductivity, which is related to corrosion processes and, eventually, to the toxicity of the water that contains them (Crites and Tchobanoglous, 2004; Tchobanoglous, 1996).

Taking as reference the international criterion for wastewater discharge to surface bodies of the USEPA CWA (1972), which is 400 mg/L, the values of this study do not meet the limits of this parameter. It has been shown that dissolved ions are removed at higher HRT and by the type of species implemented in the HA. A study evaluated the removal of ions (SDT) in HA with *Typha angustifolia* treating domestic wastewater with TRH for 12, 24 and 36 hours, whose inlet concentration was 477.48 mg/L, obtaining 104.04 mg/L (78.21 %), 85.47 mg/L (82.1%) and 73.25 mg/L (84.76%), respectively (Arivoli and Mohanraj, 2021).

On the other hand, turbidity indicates the quality of the treated wastewater in relation to the residual material in colloidal suspension. This parameter is not regulated in Mexican regulations, although it is based on the international criterion of the USEPA CWA, which establishes that the discharge for surface waters is 20 NTU. In this sense, the treatment train of our study meets this discharge criterion. During the start-up stage, the HA inlet concentration was 31.0±16.9 NTU and the effluent in reactor 3 was 37.7±19.1, while after one year of operation the inlet was 99.79±14.37 NTU and the effluent was 19.25± 9.03 UNT. These results were achieved with 30.74 hours of HRT.

Another study managed to obtain similar results in efficiency by using 2 HAFS with HRT of 9 d and *Typha latifolia* in one reactor and *Cyperus papyrus* in another unit to treat domestic water. As a result, they were able to remove 84.55% and 87.27%, respectively, of the initial turbidity concentration of 33.00 NTU (Bedoya *et al*., 2014).

In this evaluation period, a concentration was not obtained below the color established by the EPA CWA (*Clean Water Act*) for surface water discharge, which is 250 UC, so in the start-up stage It remained at 578.0±116.0 UC, and after one year of operation at 442.00±160.99 UC. The color removal in this investigation was lower than that obtained in the two HAFS with TRH of 9 d and *Typha latifolia* in a reactor and *Cyperus papyrus* because it managed to remove 90.9% and 92.3% of the initial concentration (583 UC). This change was probably due to the higher TRH, since in this way the colloidal solids are in contact with the support medium and bacteria for longer, so they can be adsorbed. This would decrease the color concentration, although the input concentration is almost three times higher in our study (Bedoya *et al*., 2014).

This parameter is considered important in Guatemala, where a maximum permissible limit of 750 UC is established in wastewater discharges to receiving bodies of urbanizations (AG 138-2017), and in the case of Honduras the Quality Standards for Discharge of Wastewater in Receiving Bodies (agreement no. 058) establishes that it is < 200 UC (Spanish Agency for International Development Cooperation [AECID], 2021).

COD is currently considered the main water quality criterion for discharge to receiving bodies (NOM-001-SEMARNAT-2021). In the case of DACBiol, the maximum permissible limit for its discharge is 150 mg/L in its monthly average and the average (±SD) present in the effluent is 105±60 mg/L. In the case of the NT and PT parameters, they do not apply to this receiving body time; However, the most stringent discharge criteria for NT and PT established for reservoirs, lakes and lagoons are met, which is 15 mg/L and 5 mg/L, respectively.

In Indonesia, the potential of constructed wetlands for wastewater was evaluated on a campus with *Canna sp*. species, sand with porosity of 0.42, a hydraulic conductivity of 420, HRT of 5 days and K_{20} of 1.84, which showed a decrease in pH from 8.15 to pH 7, removals of COD (85-87%), TN (91-97%) and TP (97-99%) (Winanti *et al*., 2017).

Likewise, a pilot-scale study at the National Institute of Technology, Karnataka campus, India, evaluated a HAFS and a HAFV as effective post-treatment of secondary biological treatment system effluents using Pennisetum pedicellatum and *Cyperus* rotundus. The experiments were carried out in two HRT (12 h and 24 h), obtaining a COD removal of 60% and 65% for HAFS and HAFV, respectively (Thalla *et al*., 2019).

In the present study, our serial HAs in the DACBiol operate as secondary and tertiary treatment with HRT of 30.74 hours and an apparent global K of 2.03 days $^{-1}$, achieving a removal of 89.2% of COD, 87.0% of TN and 84.8% in PT, conditions superior to those found by Thalla *et al*., (2019), lower than those of Winanti *et al*., (2017), but which allow compliance with the discharge standards in Mexico (NOM-001-SEMARNAT-2021).

Conclusion

The present study shows that in the serial HA treatment, the one that has the best performance is the HAFS-Sl during the first phase and the HAFL-Tg in the second phase, since the system reached 89.2% in COD removal, 87.0 % of NT and 84.8% of PT. It is important to clarify that this performance corresponds to the first year of operation, since after the second and third year the wetlands reach their maximum operating efficiency.

Regarding the apparent kinetic coefficients (k) for each of the wetlands, they show a better performance in HAFS-Sl during the two phases and the global performance constant in the treatment train (kGA) was 2.03 days $^{-1}$ for COD, 2.03 days $^{-1}$ for NT and 1.84 days $^{-1}$ for PT. All these behaviors occur at temperatures between 26 °C and 28 °C.

The species with the best performance and reproduction were *Sagittaria latifolia, Eichhiornia crassipes* and *Pontederia cordata*, which are smaller in size, so they are also easier to manage when planting. On the other hand, *Thalia geniculata* shows difficulties in its management, as it is easily uprooted due to its size and the action of the wind.

Future lines of research

The species presented in this study have potential for degradation of basic contaminants, as shown in this stage. In this configuration of the HA (free-subsurface-free), the study must continue to understand the removal process of heavy metals, for which the assimilation of these in water, the sediment, the support medium and in macrophyte species (stems, daughters, and roots).

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