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*Scientific articles*

**Remoción de nutrientes de aguas residuales domésticas con  
*Sagittaria latifolia* y *Sagittaria lancifolia* en humedales artificiales  
de flujo libre**

***Nutrient removal from domestic wastewater with *Sagittaria latifolia* and  
*Sagittaria lancifolia* in free-flowing constructed wetlands***

***Remoção de nutrientes de águas residuais domésticas com *Sagittaria  
latifolia* e *Sagittaria lancifolia* em pântanos construídos de fluxo livre***

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## Resumen

Los humedales artificiales (HA) constituyen una solución viable para el tratamiento de aguas residuales desde una perspectiva económica, social y ambiental. La presente investigación evaluó la eficiencia de remoción del fósforo (PT), nitrógeno (NT) y demanda química de oxígeno (DQO) en aguas residuales domésticas utilizando las macrófitas acuáticas *Sagittaria latifolia* y *Sagittaria lancifolia* en humedales artificiales de flujo libre. El sistema de tratamiento incluye un tanque de distribución de aguas residuales domésticas y nueve humedales artificiales de flujo libre (HAFL): tres con *S. latifolia*, tres con *S. lancifolia* y tres sin vegetación (control). Los parámetros evaluados fueron la temperatura del agua cruda ( $26 \pm 1.6$  °C), HA con *S. latifolia* ( $26,24 \pm 0,94$  °C), HA con *S. lancifolia* ( $27,25 \pm 0,47$  °C) y el control ( $25,97 \pm 0,82$  °C); pH del agua cruda ( $7,8 \pm 0,4$  UpH), HA *S. latifolia* ( $7,72 \pm 0,06$  UpH), HA con *S. lancifolia* ( $7,53 \pm 0,11$  UpH) y el control ( $7,97 \pm 0,22$  UpH); fósforo total (PT) de agua cruda ( $14,37 \pm 1,7$  mg/L), HA *S. latifolia* ( $0,50 \pm 0,22$  mg/L), HA con *S. lancifolia* ( $0,89 \pm 0,10$  mg/L) y el control ( $3,88 \pm 0,63$  mg/L); nitrógeno total (NT) del agua cruda ( $82,94 \pm 11,4$  mg/L), HA *S. latifolia* ( $2,91 \pm 1,3$  mg/L), HA con *S. lancifolia* ( $5,4 \pm 0,9$  mg/L) y el control ( $30,28 \pm 2,5$  mg/L). DQO del agua cruda ( $970,84 \pm 115$  mg/L), HA *S. latifolia* ( $29,55 \pm 10,81$  mg/L), HA con *S. lancifolia* ( $59,62 \pm 5,88$  mg/L) y el control ( $218,92 \pm 35,43$  mg/L). En los experimentos con *S. latifolia*, se lograron eficiencias de remoción del 97% para NT, 93% para PT y 96% para DQO, mientras para la *S. lancifolia* las eficiencias de remoción de NT, PT y DQO fueron de 97%, 94% y 93%.

**Palabras clave:** DQO, Eficiencia de remoción, fósforo, macrófitas y nitrógeno.



## Abstract

Constructed wetlands (CW) represent an economically, socially, and environmentally viable solution for wastewater treatment. The present investigation evaluates the removal efficiency of phosphorus (TP), nitrogen (TN) and chemical oxygen demand (COD) in domestic wastewater using aquatic macrophytes *Sagittaria latifolia* and *Sagittaria lancifolia* in free flow constructed wetlands. The treatment system is composed of a domestic wastewater distribution tank and nine free flow constructed wetlands (FFCW) of which three contain *S. latifolia*, three *S. lancifolia* and three without vegetation (control). The parameters evaluated were raw water temperature ( $26 \pm 1.6$  °C), CW with *S. latifolia* ( $26.24 \pm 0.94$  °C), CW with *S. lancifolia* ( $27.25 \pm 0.47$  °C) and the control ( $25.97 \pm 0.82$  °C); pH of raw water ( $7.8 \pm 0.4$  UpH), CW *S. latifolia* ( $7.72 \pm 0.06$  UpH), CW with *S. lancifolia* ( $7.53 \pm 0.11$  UpH) and control ( $7.97 \pm 0.22$  UpH); total phosphorus (TP), of raw water ( $14.37 \pm 1.7$  mg/L), CW *S. latifolia* ( $0.50 \pm 0.22$  mg/L), CW with *S. lancifolia* ( $0.89 \pm 0.10$  mg/L) and the control ( $3.88 \pm 0.63$  mg/L); total nitrogen (TN), from raw water ( $82.94 \pm 11.4$  mg/L), CW *S. latifolia* ( $2.91 \pm 1.3$  mg/L), CW with *S. lancifolia* ( $5.4 \pm 0.9$  mg/L) and the control ( $30.28 \pm 2.5$  mg/L). COD from raw water ( $970.84 \pm 115$  mg/L), CW *S. latifolia* ( $29.55 \pm 10.81$  mg/L), CW with *S. lancifolia* ( $59.62 \pm 5.88$  mg/L) and the control ( $218.92 \pm 35.43$  mg/L); For the experiments using *S. latifolia*, here we show that removal efficiencies for TN, TP and COD were 97%, 93% and 96% respectively; with TN, TP, and COD removal efficiencies of 97%, 94%, and 93% observed for *S. lancifolia*.

**Keywords:** COD, Removal efficiency, phosphorus, macrophytes and nitrogen.

## Resumo

Zonas úmidas construídas (AW) são uma solução viável para o tratamento de águas residuais de uma perspectiva econômica, social e ambiental. A presente investigação avaliou a eficiência de remoção de fósforo (TP), nitrogênio (TN) e demanda química de oxigênio (DQO) em águas residuais domésticas usando as macrófitas aquáticas *Sagittaria latifolia* e *Sagittaria lancifolia* em pântanos construídos de fluxo livre. O sistema de tratamento inclui um tanque de distribuição de águas residuais domésticas e nove zonas úmidas construídas (FFWs) de fluxo livre: três com *S. latifolia*, três com *S. lancifolia* e três sem vegetação (controle). Os parâmetros avaliados foram temperatura da água bruta ( $26 \pm 1,6$  °C), HA com *S. latifolia* ( $26,24 \pm 0,94$  °C), HA com *S. lancifolia* ( $27,25 \pm 0,47$  °C) e o controle ( $25,97 \pm$



0,82 °C). ; pH da água bruta ( $7,8 \pm 0,4$  UpH), HA *S. latifolia* ( $7,72 \pm 0,06$  UpH), HA com *S. lancifolia* ( $7,53 \pm 0,11$  UpH) e o controle ( $7,97 \pm 0,22$  UpH); Fósforo total (PT) da água bruta ( $14,37 \pm 1,7$  mg/L), HA *S. latifolia* ( $0,50 \pm 0,22$  mg/L), HA com *S. lancifolia* ( $0,89 \pm 0,10$  mg/L) e o controle ( $3,88 \pm 0,63$  mg/L); nitrogênio total (NT) da água bruta ( $82,94 \pm 11,4$  mg/L), HA *S. latifolia* ( $2,91 \pm 1,3$  mg/L), HA com *S. lancifolia* ( $5,4 \pm 0,9$  mg/L) e o controle ( $30,28 \pm 2,5$  mg/L). DQO da água bruta ( $970,84 \pm 115$  mg/L), HA *S. latifolia* ( $29,55 \pm 10,81$  mg/L), HA com *S. lancifolia* ( $59,62 \pm 5,88$  mg/L) e o controle ( $218,92 \pm 35,43$  mg/L). Nos experimentos com *S. latifolia*, foram alcançadas eficiências de remoção de 97%. % para NT, 93% para PT e 96% para DQO, enquanto para *S. lancifolia* as eficiências de remoção NT, PT e COD foram 97%, 94% e 93%.

**Palavras-chave:** DQO, Eficiência de remoção, fósforo, macrófitas e nitrogênio.

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## Introduction

Water is essential for public health and economic growth, which underscores the importance of conserving this resource to ensure environmental sustainability, since around 0.01% of the planet's water is drinkable (UNEP, 2021) and after use it is contaminated and discharged as wastewater to receiving bodies, without treatment or with some previous treatment that does not comply with the established standards for its discharge as established in Mexico by NOM-001-SEMARNAT-2021. Domestic wastewater effluents generally contain large amounts of organic and inorganic load (chemical oxygen demand, COD), metals and nutrients such as nitrogen (N) and phosphorus (P) among other pollutants (Osorio et al. 2021). Nutrients (N and P) are directly related to the eutrophication of surface waters that affect aquatic systems (Muciño et al. 2017). When these waters are discharged into receiving bodies, the contaminants are diluted in the best of cases and are deposited forming septic bodies (Correa et al. 2023). Given the need to preserve and recover the precious liquid, the implementation of an efficient, low-cost remediation system is required, without affecting ecosystems and constructed wetlands (CW) are a viable alternative, as they have some advantages over conventional treatments, such as being easy to operate, requiring less energy and not using chemicals in their purification process (Quevedo, 2021). Despite the high levels of nutrients existing in the tributary, CW have mechanisms that degrade and remove contaminants, improving water quality. In addition, it allows compliance with established

quality standards and nutrients such as phosphorus and nitrogen can be recovered in the harvest of aquatic vegetation (Cisterna and Pérez, 2019).

HAs are designed and built at a depth of less than one meter, with macrophytic aquatic vegetation, which are classified as submerged, emergent and floating, where the roots grow directly in the water (Del Valle et al. 2022). According to the type of flow, they are classified as free-flow constructed wetlands (FFCW) when the water is exposed to the atmosphere or subsurface flow (FFSS) when the water is below the vegetation support (Camacho et al. 2018). Their main pollutant removal mechanisms are sedimentation, filtration, chemical precipitation, absorption by plants and microbial transformations (Castañeda, 2022). One of the most outstanding characteristics of CW is phytoremediation, where various physical, chemical and biological processes are combined between the substrate, plant species, microorganisms and the atmosphere, to remove, reduce, transform, mineralize, degrade, volatilize or stabilize contaminants and nutrients in wastewater (Larriva and González, 2017).

There are a large number of macrophyte plants that have been used in CW, such as water reeds (*Cyperus* sp., *Scirpus* sp., *Juncus* sp.), cattails or bulrushes (*Typha* sp.), sparganiums (*Sparganium* sp.) and reed (*Phragmites* sp.) (EPA, 2000), which have the capacity to accumulate and degrade more than one pollutant such as COD, TSS, TN and TP (Viramontes et al. 2020). Recently, ornamental terrestrial plants such as *Canna have been studied hybrids*, *Heliconia* sp. and *Spathiphyllum wallisii* in wastewater treatment with this technology (López-Alba et al. 2022).

Tabasco is a region where wetlands predominate because it is one of the wettest places in Mexico. Wetlands occupy 28% of the state's surface area with more than 600,000 ha and the marsh type is the most widely distributed (López-Jiménez, 2019). Among the plants present in the state's wetlands are *Sagittaria lancifolia* (Linnaeus, 1759) and *Sagittaria latifolia* (Willdenow, Carl Ludwig von , 1805) that belong to the *Alismataceae* family . Both are aquatic herbaceous plants, rooted or rarely submerged, have white flowers all year round, are found in swamps, dams, canals and lake edges (Olivo et al. 2018). There is little information available on the use of these species in domestic wastewater treatment systems in Mexico (CONAGUA, 2019) and particularly there are not enough reports on the removal of nutrients by CW, the level of treatment (primary, secondary or tertiary) therefore it is

important to generate basic information on its capacity to assimilate or eliminate pollutants and nutrients.

Therefore, this research evaluates the removal efficiency and kinetic coefficient of degradation of COD, TN and TP in FFCW, using the aquatic macrophytes *Sagittaria lancifolia* and *Sagittaria latifolia* in a pilot system.

## Materials and methods

### Location

The research was carried out at the Water Technology Laboratory of the Academic Division of Biological Sciences (DACBiol) of the Universidad Juárez Autónoma de Tabasco (UJAT), located at latitude 17°59'28.06" N and longitude 92°58'26.39" W.

### Design and construction

Nine FFCW made of resistant metal plates (10 gauge) with a volume of 3 m<sup>3</sup>, lined with alkyd paint on the outside and protected by a waterproof system and textile membrane were used. The CW contained 0.1 m of gravel with a size between 2.36 mm and 19 mm to function as a support for the vegetation. For the distribution of wastewater, 1" PVC hydraulic installations were made to move the flow from the distribution tanks with a capacity of 200 L to the wetlands. The design flow per pond was 0.2 m<sup>3</sup>/day, with a hydraulic retention time of 7.5 days, in order to achieve 92% removal of BOD<sub>5</sub> (López et al. 2014). No species were placed in three experimental ponds to function as a control; later, *S. lancifolia* was installed in three and *S. latifolia* in three.

### Operation and maintenance

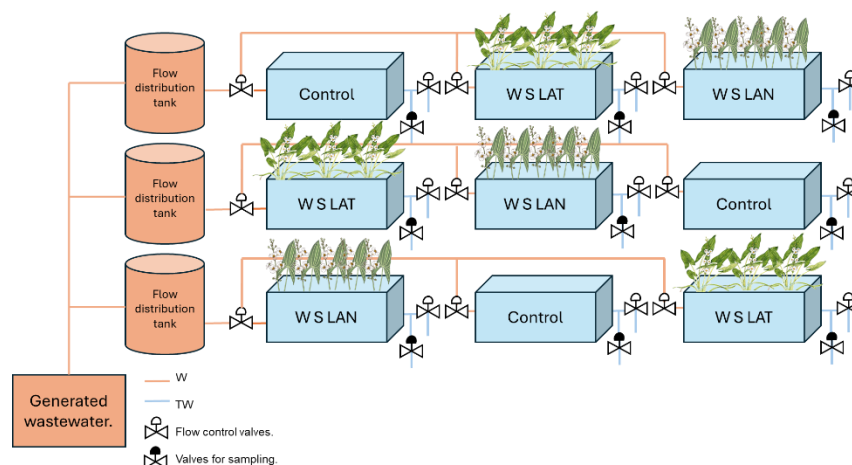
The effluent was obtained from a storage tank or domestic wastewater sump that also performs the pretreatment function (removal of sand and voluminous solids), feeding the treatment system with a 1 HP pump, connected to PVC hydraulic pipe, which directs the flow to the distribution tanks (200 L) distributing the expense to three CW (figure 1). The average flow rate ( $Q_{avg}$ ) of operation is 0.23 m<sup>3</sup>/day, the FFCW presented a hydraulic retention time (HTR) of 6.52 days, with a support medium height of 0.1 m and a porosity of  $n = 49.99\%$ . Subsequently, the effluent was discharged into a natural wetland located at the school.



## Sampling, sowing and stabilization of the species

The species used in the CW were obtained from different water bodies in the region. *S. lancifolia* was extracted from natural wetlands with the presence of wastewater discharge from the Villahermosa - Centla highway (18° 20.361'N and 92° 29.467'W) and *S. latifolia* was taken from a wastewater channel in the city of Villahermosa, Tabasco (17°59.219' N and 92°57.588'W). It is worth mentioning that the specimens of each species were extracted completely and not just the flowers or fruits (Lot et al. 2015). Young individuals were collected, removing sludge and materials adhered to the root system by washing, then they were placed in 20 L containers, with water from the medium in which the plants were removed, in order to avoid stressing the plants during transport. The collected plants were transplanted to a wastewater channel for acclimatization. After 21 days the species began to grow, and were subsequently transplanted to the CW, distributing them in six CW, three with *S. lancifolia* and three with *S. latifolia* (Figure 1). Twenty-four plants were planted per reactor, forming five rows of three plants each. The adaptation time in the experimental units was 42 days, during which time the water table was kept at 0.3 m, favoring adaptation and propagation. After this period, the wetland stabilization phase began, which lasted approximately six months, and the evaluation of the system was carried out after completing one year of its stabilization period.

**Figure 1.** Schematic of the wastewater treatment system uses free-flowing constructed wetlands, with different types of vegetation: *S. latifolia* (WS LAT), *S. lancifolia* (WS LAN) and the control wetlands (Control) with their replicas.



Source: Own elaboration

## Wastewater characterization

Sampling was conducted over a period of 12 weeks, from September to November 2023. Sampling was carried out for four days a week (48 samples). Ten sampling points were monitored, one from the distribution tank and nine from the CW, with a total of 480 simple samples, for which control parameters such as temperature, pH and electrical conductivity (EC) were determined, measured with a multiparameter (HI 9828, Hanna InstrumentMR , USA), in addition to turbidity and color determined by a LaMotteMR turbidimeter (Model TC3000we, EPA methods 180.1 and SM 2120B, Romania). In the case of TP and TN, they were determined once a week, analyzing a total of 120 samples. To determine the TN, it was carried out under PROY-NMX-AA-026-SCFI-2021, based on the Kjeldahl method for determining TN. To determine TP, the stannous chloride method (NMX-AA-026-SCFI-2001) was used, the COD was determined using the USEPA 410.4 method and was measured with the Hanna® HI839800-01 equipment.

## Experimental design

Since the data do not meet the postulates of normality and homoscedasticity, the Kruskal-Wallis test followed by Bonferroni was used to identify significant differences between sampling points. The tests and graphs were performed using STATGRAPHICS CENTURION® v19.0 software, using a significance level of  $\alpha = 0.05$ . Considering the three treatments from a factor of three CW Control without species, three WS LAN (*Sagittaria lancifolia*) and the last three WS LAT (*Sagittaria latifolia*).

## Removal efficiency

The pollutant removal efficiency was calculated as follows, where  $\eta$  represents the removal efficiency in %, C1 the concentration of the wastewater influent and C2 the concentration of the wastewater effluent. (Lin X et al. 2003),

$$\eta = (C1 - C2) / C1 \cdot 100 \quad (1)$$

## Degradation kinetics

To determine the kinetic coefficient, it was found that the behavior of wastewater is a first-order kinetic reaction, the degradation rate k (COD, TN and TP) was estimated with the following equation (Crites and Tchobanoglous, 2000; López-Ocaña et al. 2019).





$$k_o = -\ln(C_n/C_o) / \tau \quad (2)$$

Where  $\tau$ = retention time,  $C_n$ = effluent concentration,  $C_o$ = influent concentration,  $k_o$  = degradation constant. Once the concentrations have been evaluated, the coefficient graphs are created in Excel.

## Results

### Water quality characteristics

The raw wastewater that feeds the wetlands presents characteristics of a strong wastewater in the variables TP ( $82.94 \pm 11.4$  mg/L), TN ( $14.37 \pm 1.7$  mg/L) and COD ( $970.84 \pm 115.0$  mg/L) (Metcalf and Eddy, 2003). Table 1 shows the average values with standard deviation of each of the treatments performed.

**Table 1.** Average values ( $\pm SD$ ) in the effluents of the treatment units ( $N=48$ ).

Parameters	Wastewater		Control		WS LAT		WS LAN	
	X	$\pm FRO$ M	x	$\pm FRO$ M	x	$\pm FRO$ M	x	$\pm FRO$ M
pH (UpH)	8.06	0.38	7.98	0.22	7.72	0.06	7.53	0.11
Temperature (°C)	28.07	1.56	25.98	0.82	26,24	0.94	27.25	0.47
EC (mS/cm)	2.26	0.54	2.33	0.34	2.73	0.30	2.27	0.26
Color (UC)	1027.6	86.61	741.38	80.98	207.89	31.04	27.0	3.30
Turbidity (NTU)	125.50	15,21	103.41	7.89	3.89	1.15	7.68	0.58
TP (mg/L)	14.37	1.72	3.88	0.63	0.50	0.22	0.89	0.10
TN (mg/L)	82.94	11.38	30.28	2.47	2.91	1.31	5.40	0.89
COD (mg/L)	970.84	115.0	218.92	35.43	29.55	10.81	59.62	5.88

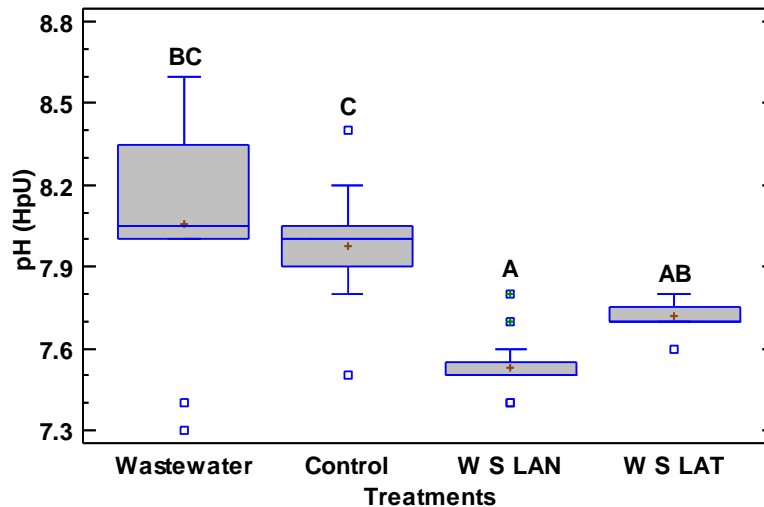
Source: Own elaboration.

Hydrogen potential (pH). The Kruskal-Wallis test showed significant differences ( $p < 0.05$ ) between the medians of the treatments evaluated with a 95.0% confidence level. The



lowest median pH value was found in the WS LAN treatment with  $7.5 \pm (Q_1 = 7.4, Q_3 = 7.6)$  UpH, followed by the WS LAT with  $7.7 \pm (Q_1 = 7.7, Q_3 = 7.8)$  UpH, for the Control it was  $8.0 \pm (Q_1 = 7.8, Q_3 = 8.1)$  UpH and finally the highest median value was reported in the Raw Water treatment with  $8.05 \pm (Q_1 = 7.4, Q_3 = 8.4)$  (Figure 2).

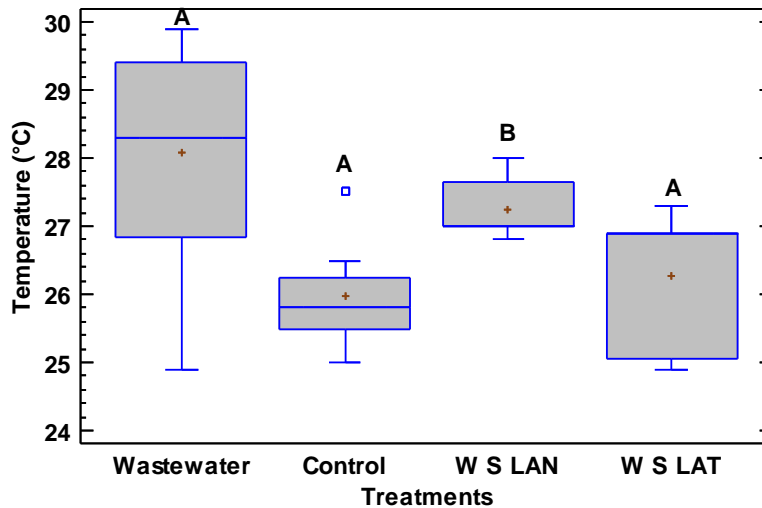
**Figure 2.** Median values ( $\pm$ RI) for the pH variable (UpH) ( $N = 48$ ).



Source: Own elaboration

Temperature ( $T$  °C). The Kruskal-Wallis test showed significant differences ( $p < 0.006$ ) between the medians of the treatments evaluated with a 95.0% confidence level. The lowest median value was found in the Control treatment with  $25.8 \pm (Q_1 = 25.03, Q_3 = 27.39)$  °C, followed by WS LAT with  $26.9 \pm (Q_1 = 25, Q_3 = 26.9)$  °C, for WS LAN it is  $27.0 \pm (Q_1 = 26.91, Q_3 = 28)$  and finally the highest median value was reported in the Raw Water treatment with  $28.3 \pm (Q_1 = 26.53, Q_3 = 29.88)$  (Figure 3).

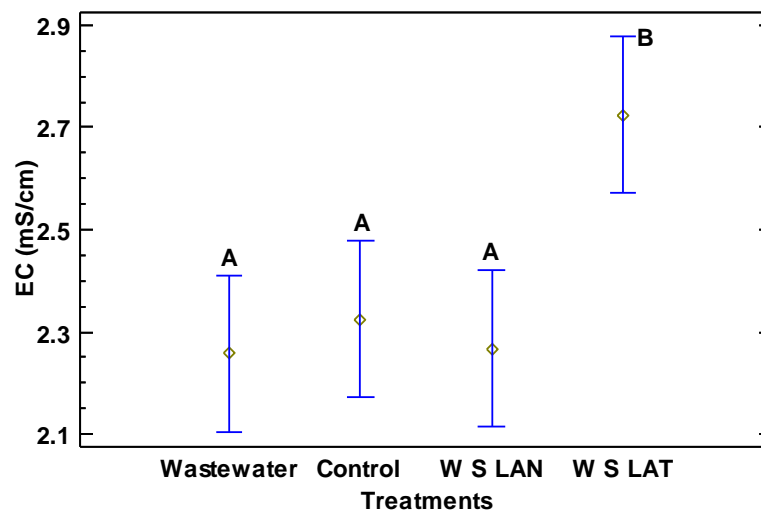
**Figure 3.** Median values ( $\pm$ RI) for the temperature variable ( $^{\circ}$ C) ( $N = 48$ ).



Source: Own elaboration.

Electrical conductivity (EC). Statistically significant differences ( $p < 0.009$ ) were found in the EC. The influent or raw water presented the average value with the lowest standard deviation with  $2.25 \pm 0.53$  (mS /cm), followed by the WS LAN treatment with  $2.27 \pm 0.26$  (mS /cm), the CW control presented  $2.32 \pm 0.33$  (mS /cm) and the highest average value was observed in the WS LAT of  $2.7 \pm 0.3$  (mS /cm). (Figure 4).

**Figure 4.** Mean values ( $\pm$ SD) of the electrical conductivity variable (mS /cm) ( $N = 48$ ).

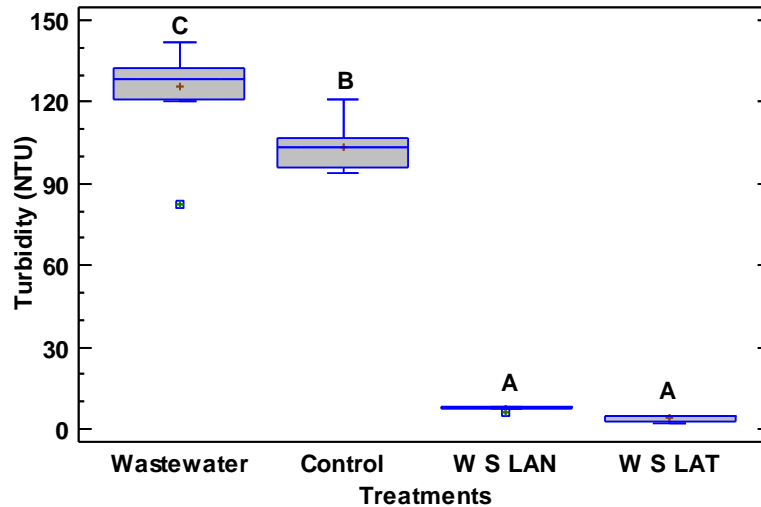


Source: Own elaboration.

Turbidity. The Kruskal-Wallis test showed significant differences ( $p < 0.05$ ) between the medians of the treatments evaluated with a 95.0% confidence level between the treatments (Figure 5). The lowest median value was presented in the WS LAT with  $4.7 \pm (Q_1$

= 2.5,  $Q_3 = 4.9$ ) NTU followed by the WS LAN with  $7.85 \pm (Q_1 = 7.6, Q_3 = 8)$  NTU, for the Control values of  $103.05 \pm (Q_1 = 95.05, Q_3 = 111.51)$  NTU were obtained, finally, the highest median value was reported in Raw water with  $128.4 \pm (Q_1 = 120, Q_3 = 139.2)$  NTU. The removal efficiency with respect to turbidity in WS LAN was 94% and HS LAT 96%.

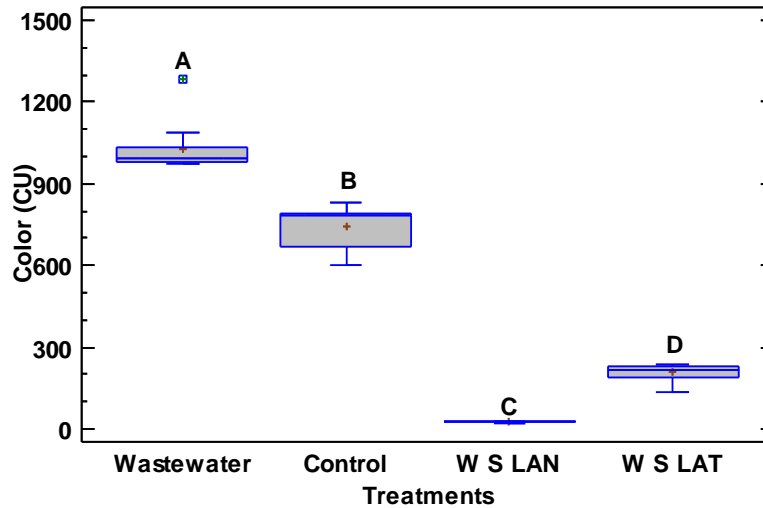
**Figure 5.** Median values ( $\pm$ RI) for the turbidity variable (NTU) ( $N = 48$ ).



Source: Own elaboration

Color. The Kruskal-Wallis test showed significant differences ( $p < 0.05$ ) between the medians of the treatments evaluated with a 95.0% confidence level. The lowest median value was presented in the WS LAN with  $28.5 \pm (Q_1 = 22.3, Q_3 = 30)$  UC followed by the WS LAT with  $216.6 \pm (Q_1 = 174.6, Q_3 = 234.7)$  UC, for the Control values of  $780.9 \pm (Q_1 = 613.2, Q_3 = 804.8)$  UC were obtained, finally, the highest median value was reported in Raw water with  $992 \pm (Q_1 = 978.2, Q_3 = 1\ 085.35)$  UC (Figure 6).

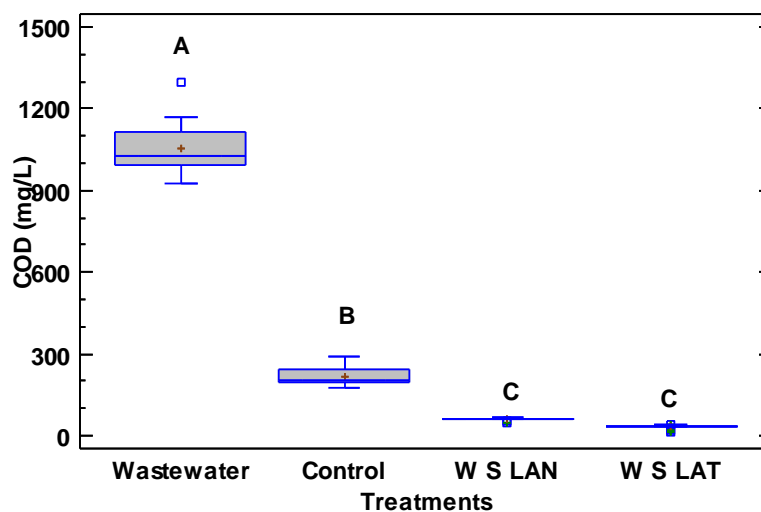
**Figure 6.** Median values ( $\pm$ RI) for the color variable (UC) ( $N = 48$ ).



Source: Own elaboration.

Chemical oxygen demand (COD). The Kruskal-Wallis test showed significant differences ( $p < 0.05$ ) between the medians of the treatments evaluated with a confidence level of 95.0%. The lowest median value was presented in WS LAT with  $36.8 \pm (Q_1 = 22.3, Q_3 = 39.67)$  mg/L followed by WS LAN with  $60.93 \pm (Q_1 = 53.2, Q_3 = 65.4)$  mg/L, for the Control values of  $205 \pm (Q_1 = 188.6, Q_3 = 267.8)$  mg/L were obtained, finally, the highest median value was reported in Raw water with  $1\ 027.15 \pm (Q_1 = 943.1, Q_3 = 1\ 166.28)$  mg/L (Figure 7).

**Figure 7.** Median values ( $\pm$ RI) for the COD variable (mg/L) ( $N = 48$ ).

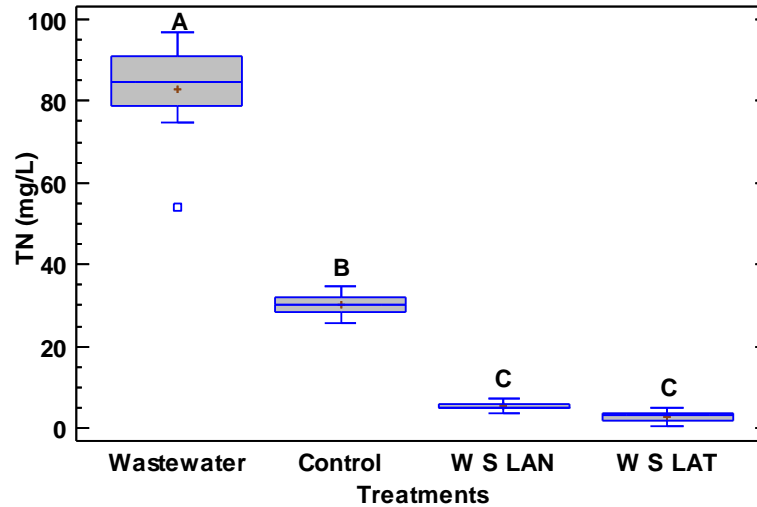


Source: Own elaboration.

Total nitrogen (TN). The Kruskal-Wallis test showed significant differences ( $p < 0.05$ ) between the medians of the treatments evaluated with a confidence level of 95.0%. The

lowest median value of TN was presented in the WS LAT with  $3.1 \pm (Q_1 = 1.4, Q_3 = 4.4)$  mg / L followed by the WS LAN with  $5.2 \pm (Q_1 = 4.5, Q_3 = 6.3)$  mg / L, for the Control values of  $30.3 \pm (Q_1 = 27.8, Q_3 = 33.1)$  mg / L were obtained, finally, the highest median value was reported in the Raw water with  $84.5 \pm (Q_1 = 75.1, Q_3 = 93.8)$  mg / L (Figure 8).

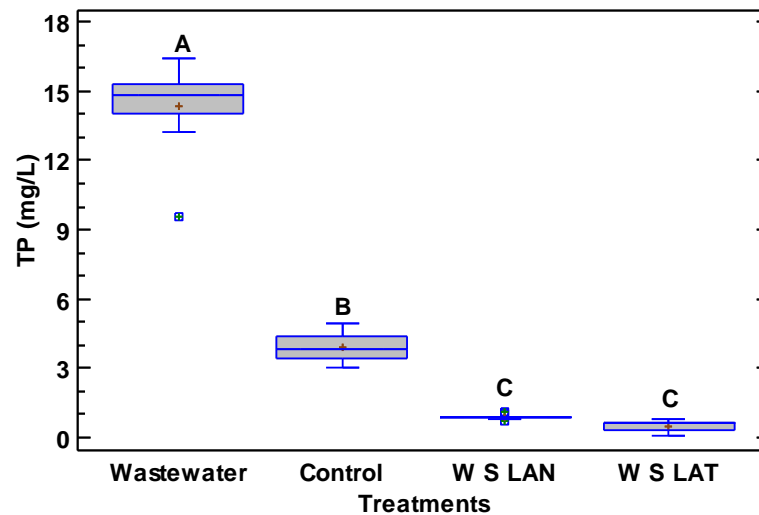
**Figure 8.** Median values ( $\pm$ RI) for the variable total nitrogen (mg/L) ( $N = 48$ ).



Source: Own elaboration.

Total phosphorus (TP). The TP was analyzed using the Kruskal-Wallis test which showed significant differences ( $p < 0.05$ ) between the medians of the treatments evaluated with a confidence level of 95.0%. The lowest median value was presented in the WS LAT with  $0.6 \pm (Q_1 = 0.2, Q_3 = 0.7)$  mg / L followed by the WS LAN with  $0.9 \pm (Q_1 = 0.8, Q_3 = 0.9)$  mg / L, for the Control values of  $3.8 \pm (Q_1 = 3.2, Q_3 = 4.7)$  mg / L were obtained, finally, the highest median value was reported in Raw water with  $14.8 \pm (Q_1 = 13.27, Q_3 = 15.4)$  mg / L. CW treatments are within the maximum permissible limits established by NOM-001-SEMARNAT-2021 with 10 mg/L (Figure 9).

**Figure 9.** Median values ( $\pm$ RI) for the variable total phosphorus (mg/L) ( $N = 48$ ).



Source: Own elaboration.

All temperature and pH treatments are within the maximum permissible limits established by NOM-001-SEMARNAR-2021, which for pH is 6 to 9 HpU and for maximum temperature 35 °C, in CW treatments the maximum permissible limits established for TP are met with values less than 150 mg/L and less than 25 mg/L in TN.

### Removal efficiency

Table 2 shows the removal efficiencies of the evaluated parameters. CW with *S. latifolia* achieved removal efficiencies of 96.49% for nitrogen, 96.50% for phosphorus and 96.72% for COD, while CW with *S. lancifolia* achieved efficiencies of 93.48%, 93.79% and 94.34%, respectively. In negative efficiencies, the phenomenon called short circuit occurs (Vázquez and López, 2011).

**Table 2.** Removal percentages between each of the treatments ( $N=48$ ).

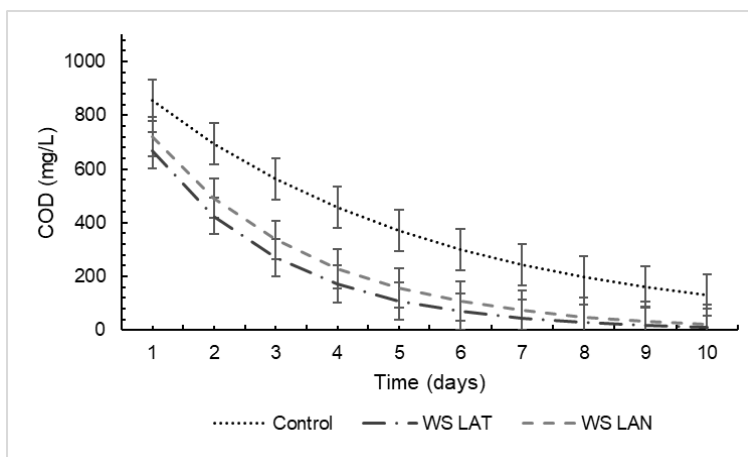
Parameters	Control (ER%)	WS LAT (ER%)	WS LAN (ER%)
EC	-2.82	-20.51	-0.35
Color	27.85	79.76	97.37
Turbidity	17.59	96.90	93.87
TP	72.96	96.50	93.79
TN	63.48	96.49	93.48
COD	79.23	96.72	94.34

Source: Own elaboration

## Degradation kinetics

The kinetic coefficients ( $k$ ) for COD, TN, TP, Turbidity and Color were estimated with a HRT of 6.52 days. The average water temperature was 26 °C in the CW Control, 26.24 °C in the WS LAT and 27.25 °C in the WS LAN. A constant ( $k$ ) for COD of 0.24 days<sup>-1</sup>, 0.52 days<sup>-1</sup> and 0.44 days<sup>-1</sup> respectively was found (Figure 10), as for TN  $k$  of 0.15 days<sup>-1</sup>, 0.51 days<sup>-1</sup> and 0.42 days<sup>-1</sup> were obtained (Figure 11); for TP  $k$  averages of 0.20 days<sup>-1</sup>, 0.51 days<sup>-1</sup> and 0.43 days<sup>-1</sup> were obtained (Figure 12), in turbidity the  $k$  were 0.03 days<sup>-1</sup>, 0.53 days<sup>-1</sup> and 0.43 days<sup>-1</sup> (Figure 13) finally, the Color  $k$  of 0.05 days<sup>-1</sup>, 0.25 days<sup>-1</sup>, 0.56 days<sup>-1</sup> (Figure 14) respectively. Based on the mentioned results, the WS LAN presents better contaminant degradation rates, compared to the WS LAT.

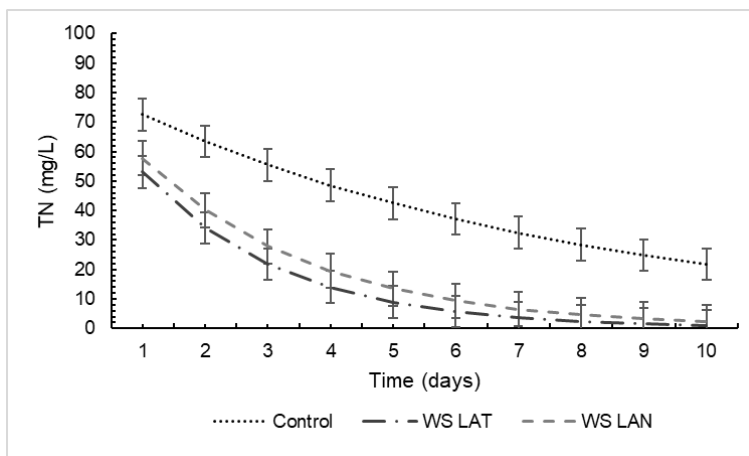
**Figure 10.** Degradation kinetics  $k$  (days<sup>-1</sup>) for COD in each of the treatments, Control with  $k= 0.24$ , WS LAT with  $k= 0.52$  and WS LAN with  $k= 0.44$ .



Source: Own elaboration

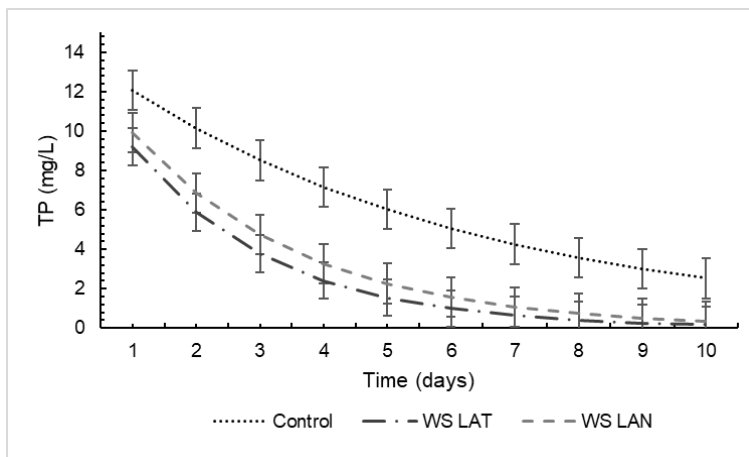


**Figure 11.** Degradation kinetics  $k$  ( $\text{days}^{-1}$ ) for TN in each of the treatments, Control with  $k=0.15$ , WS LAT with  $k=0.51$  and WS LAN with  $k=0.42$ .



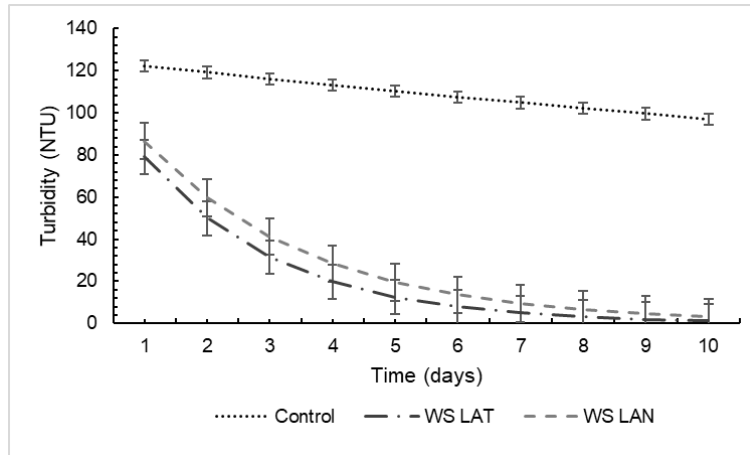
Source: Own elaboration

**Figure 11.** Degradation kinetics  $k$  ( $\text{days}^{-1}$ ) for TP in each of the treatments, Control with  $k=0.20$ , WS LAT with  $k=0.51$  and WS LAN with  $k=0.43$ .



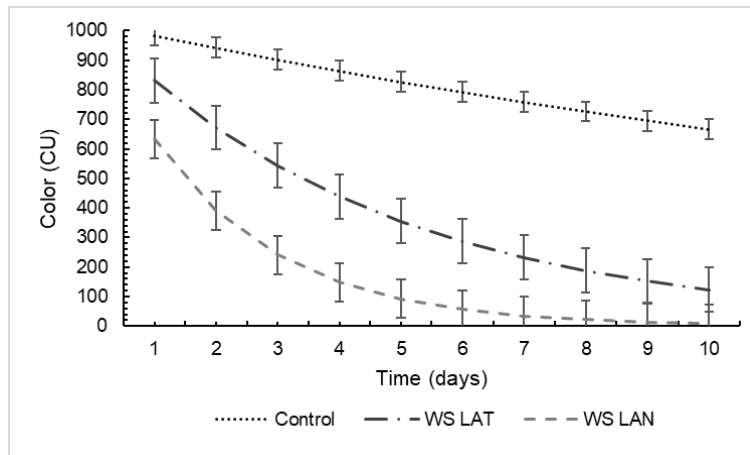
Source: Own elaboration

**Figure 11.** Degradation kinetics  $k$  (days<sup>-1</sup>) for Turbidity in each of the treatments, Control with  $k= 0.03$ , WS LAT with  $k= 0.53$  and WS LAN with  $k= 0.43$ .



Source: Own elaboration

**Figure 11.** Estimated values of kinetic degradation coefficients  $k$  (days<sup>-1</sup>) for Color in each of the treatments, Control with  $k= 0.05$ , WS LAT with  $k= 0.25$  and WS LAN with  $k= 0.56$ .



Source: Own elaboration

### Species characteristics

Table 3 shows that the growth of the species *S. latifolia* and *S. lancifolia* tends to be affected by the environmental conditions of the region where the research is being carried out. One of the main parameters that influence growth is the ambient temperature and the amount of organic matter and nutrients available (Delgadillo et al. 2010). The propagation of the species is linked to the amount of water available, which in this case was provided by the CW, and this guaranteed the success of the species.

**Table 3.** Species growth from September to December 2023.

Parameter	<i>Sagittaria lancifolia</i>		<i>Sagittaria latifolia</i>	
	Initial stage	Final stage	Initial stage	Final stage
No. plants	24	93	24	63
Plant mass (kg)	2.2 ± 1.7	4.8 ± 1.5	0.70 ± 0.35	2.90 ± 0.36
Stem length (cm)	93.3 ± 20.5	156.1 ± 24.4	0.41 ± 0.46	114.5 ± 0.8
Stem diameter (cm)	7.8 ± 2.1	12.1 ± 0.6	10.86 ± 3.1	14.3 ± 3.8
Root length (cm)	26.4 ± 12.6	42.4 ± 9.6	10.86 ± 3.18	22.12 ± 4.8
No. of sheets	1.3 ± 1.7	11.9 ± 1.4	6 ± 2.73	9.6 ± 3
Blade width (cm)	23.6 ± 11.0	44.8 ± 14.8	12 ± 5.3	20.67 ± 2.3
Blade length (cm)	23.8 ± 11.3	64.8 ± 13.5	14.4 ± 3.6	23.10 ± 8.4
Total biomass (kg)	31.5 ± 0.5	79 ± 1	24.8 ± 1.6	56 ± 1.5
Humidity (%)		69 ± 3		61 ± 1.3

Source: own creation

## Discussions

The temperature in the evaluated CWs was within the maximum permissible limits of NOM-001-SEMARNAT-2021, with values less than 35 °C. This physical parameter was maintained with little variation between treatments (25.98 to 27.25 °C), being vital for the proliferation of mesophilic bacteria, favoring the assimilation of nitrogen and phosphorus by plant roots, in addition to contributing to fauna, aquatic flora and the rate of degradation reaction in wetlands (De La Mora et al. 2020). The temperatures recorded in the treatments with species are optimal for the removal of contaminants, since they remain away from 50 °C, the threshold where aerobic digestion and bacterial nitrification stop (Sánchez et al. 2021).

On the other hand, the average pH of the LAN WS (7.53 ± 0.11) and LAT WS (7.72 ± 0.06) were slightly alkaline compared to the control CW (8 ± 0.2) and the raw wastewater (7.9 ± 0.4) since they presented a more alkaline value; which indicates that this macrophyte system allows maintaining the wastewater in conditions close to neutrality, complying with the provisions of NOM-001-SEMARNAT-2021 (5 to 9 UpH). According to Olivo et al. (2018) the acceptable pH for the proliferation of species is between 6.5 and 8.5, when the water tends to be very alkaline (> 8.5 UpH) the ammonium is converted into a toxic form which causes mortality of organisms, on the contrary, acidic water (< 5 UpH) leaches metals

from rocks and sediments, thus having an adverse effect on the metabolic rate and can be fatal.

The EC in CW represents the concentration of soluble salts in the wastewater, related to the number of dissolved ions (<sup>++</sup>) increasing the EC and TDS values. Salts and other substances affect the quality of the wastewater and the aquatic biota, since each organism can tolerate certain EC values, and at low concentrations (< 800  $\mu\text{S}/\text{cm}$ ) it is not phytotoxic to the species (Solís et al. 2018).

The percentage of color and turbidity removal achieved with *S. latifolia* and *S. lancifolia* are like the results recorded with *Typha dominguensis* Persoon, with removal efficiencies of 96.6% for turbidity and 76.8% for color in FFCW at pilot scale, under similar operating conditions (Solís et al. 2016). In another research carried out by Morales et al. (2019) they evaluated two types of species in symbiosis, in a free-flow artificial wetland the species were *Zantedeschia aethiopica* and *Eichhornia crassipes* achieving turbidity removal efficiencies of 96% and 34% color.

CWs have been reported as systems with high nitrogen and phosphorus removal rates; in the case of TN, removals of 59.4% and 57.7% have been achieved (Torres, 2017). Likewise, FFCW at pilot scale with emergent vegetation such as *Phragmites australis*, *T. latifolia* and *Iris pseudacorus* Linneo have removed up to 76% of the TN (Marín, 2017). The removal percentages vary according to the vegetation used in each system; therefore, high TN removals would be expected with rooted or emergent species (García et al. 2021). In this context, the pilot system proposed in this experiment during the first year of operation presented removal percentages of up to 93% in the CW with *S. lancifolia* and up to 97% in the CW with *S. latifolia*, presenting concentration values in its discharge less than 15 mg / L (NOM-001-SEMARNAT-2021). Soler et al. (2018) consider that there is a significant effect on the removal of TN by the type of species used. In addition, water temperature conditions and retention times greater than 6.5 days favor nitrogen degradation kinetics.

On the other hand, Arteaga et al. (2019) report that due to the rhizome and species in general, the water moves slowly generating a laminar flow, helping the adsorption of sediments, being the main route for the elimination of phosphorus. This is found in aquatic systems and is accumulated especially in the sediment in organic or inorganic form, but Quiroz et al. (2018) describe that the easy accumulation of phosphorus is due to the absence and presence of macrophytes, which provide organic matter with biodegradation facilities, although the low adsorption of the sediment and the inhibitory causes of microorganisms and

plant species at low temperatures usually present obstacles that prevent the elimination of P in CW (Arteaga et al. 2019). In another study carried out by Padrón et al. (2017) found that these treatment systems can achieve 35 to 49% TP removal, because much of this nutrient is absorbed by the plant. In the present investigation, the vegetation *S. lancifolia* and *S. latifolia* managed to remove TP up to 94% and 97% respectively, demonstrating that its discharge is less than 5 mg / L as established by NOM-001-SEMARNAT-2021, but this behavior was during the first year of operation, since Padrón et al. (2017) have reported that the removal of phosphorus in CW is efficient in a short period until the medium is saturated, in the long term the processes are more limited and assimilation by plants and biomass is reduced. Regarding the COD, it is considered that the permissible limit established by NOM-001-SEMARNAT-2021 for residual effluents to rivers, streams, canals and drains is 150 mg / L, in the HA there were concentrations in the effluent of 29.55 mg / L and 59.72 mg / L, respectively, complying with the established. An important part of the research was to verify the decrease in pollutants (COD, TN, P, Turbidity and Color) based on the degradation constant (k). Larriva et al. (2017) evaluated a free-flowing wetland with *Scirpus californicus*, a second free-flowing wetland with *Phragmites australis* and a wetland without species, using 18 to 25 mm gravel with a porosity of 38% and a volume of 0.60 m<sup>3</sup>/day resulting in a k of 0.64 day<sup>-1</sup> for the COD parameter for the wetland with *Scirpus californicus*, a k of 0.85 day<sup>-1</sup> for the wetland with *Phragmites australis* and a k of 0.64 day<sup>-1</sup> for the wetland without species, compared to what was reported in the present investigation, it presents higher k, this could be due to the species used. Gajewska et al. (2020) reported a degradation kinetics for TN (k = 2.03 days<sup>-1</sup>) and TP (k = 1.84 days<sup>-1</sup>) in a FFCW with *Phragmites australis* and a flow rate of 0.24 m<sup>3</sup>/day, compared to the ongoing research where lower k of TN of 0.42 day<sup>-1</sup> and 0.43 day<sup>-1</sup> are presented for TP. Romellón et al. (2021) evaluated two treatment trains, with species *Pontederia cordata*, *Thalia geniculata* and *Sagittaria lancifolia*, having as results in train 1 a k of 0.52 days<sup>-1</sup> for turbidity, 0.32 days<sup>-1</sup> for color and in train 2 a k of -0.47 days<sup>-1</sup> for turbidity, -0.24 days<sup>-1</sup> for color, in this case with *S. lancifolia* a slightly low k of 0.43 days<sup>-1</sup> is reported in turbidity and for color a higher k of 0.56 days<sup>-1</sup>, there being no significant differences in both investigations.

## Conclusions

The study showed that FFCW with *S. lancifolia* and *S. latifolia* have a high removal potential of TN, TP and COD. The TN presents a removal of 96.49% in WS LAT and 93.48% in WS LAN, the TP was removed in 96.50% with WS LAT and 93.79% with WS LAN, the COD was removed in 96.72% in WS LAT and 94.34% in WS LAN. Even though both have high removal percentages, the species *S. latifolia* compared to *S. lancifolia*, do not present significant statistical differences. *S. latifolia* shows a better performance, however, the best adaptation and reproduction has the species *S. lancifolia*, which contributes to a greater capture of atmospheric carbon in its plant biomass. In the case of degradation kinetics, WS LAN presents better rates, with a constant k for COD of 0.44 days<sup>-1</sup>, the constant k for TN with 0.42 days<sup>-1</sup>, for TP a constant k of 0.43 days<sup>-1</sup>, for turbidity a k of 0.43 days<sup>-1</sup> and, finally, a k for Color of 0.56 days<sup>-1</sup>.

## Future lines of research

With the results obtained in the removal of basic pollutants, nutrients and COD from domestic effluents, it is important to continue investigating whether the species have a high potential for degradation and assimilation of pollutants in CW of subsurface flow. Likewise, it is important to know the phytoremediation potential of the species in the removal of heavy metals and agricultural pollutants (assimilation in leaves, stems, roots, support medium and sediments). Finally, it would be necessary to evaluate other regional support media.

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