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Artículos científicos

Efecto de la poda de macrófitas sobre microorganismos adheridos al medio de soporte y la carga orgánica en humedales artificiales

Effect of Macrophyte Pruning on Microorganisms Adhered to the Support Medium and the Organic Load in Artificial Wetlands

Efeito da poda de macrófitas sobre os microrganismos aderidos ao meio de suporte e a carga orgânica em áreas úmidas artificiais

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Resumen

Esta investigación evaluó el efecto de la poda de macrófitas sobre microorganismos adheridos al medio de soporte y la remoción de DBO_5 en tres humedales artificiales de flujo subsuperficial (HAFS) experimentales con especies de *Phragmites australis* (HAFS-Carrizo), *Pontederia sagittata* (HAFS-Tule) y grava como testigo (HAFS-Grava). Se utilizaron nueve unidades (con réplicas). Los HAFS con 1.2 m de ancho, 2.5 m de largo y 0.5 m de profundidad se alimentaron con 200 L/d agua residual doméstica. Las variables estudiadas al medio de soporte fueron microorganismos adheridos, porosidad, densidad, diámetro y humedad para estimar el tiempo de retención y conductividad hidráulica. En las especies se midieron las variables de productividad después de la poda y se estimó la remoción y el coeficiente cinético de degradación k para la DBO_5. El experimento fue un diseño de un factor (tratamiento) con tres niveles (HAFS-Tule, HAFS-Carrizo y HAFS-Grava); se evaluaron durante tres meses, después del año de operación. Los tratamientos se evaluaron mediante Anova (paramétricos) y la prueba de rangos de Kruskal-Wallis (no paramétricos) entre grupos antes y después de podar ($p < 0.05$). La grava presentó en promedio ($N = 12$) 22.92 ± 1.47 % de humedad, 2.32 ± 0.15 cm de diámetro, densidad de partícula 2.71 ± 0.10 gr/cm^3 y porosidad de 36.52 ± 3.36 %, el TRH fue de 3.1 días en todos los tratamientos. El mejor tratamiento fue HAFS-Tule: antes de la poda la biomasa de microorganismos presentó valores medianos ($N = 12$) de 42931.6 mg/kg ($Q_1 = 40259.7$; $Q_3 = 54478.4$) y posterior a la poda de 33444.6 mg/kg ($Q_1 = 31210.9$; $Q_3 = 36581.8$), la biomasa vegetal retirada en la poda fue de 40.85 ± 2.58 kg, se removió 95.44 % de DBO_5 con una $k = 1.004$ $días^{-1}$ (27.6 °C), lo que permite cumplir la NOM-001-Semarnat-1996.

Palabras clave: densidad de partícula, humedal artificial de flujo subsuperficial, *Phragmites australis*, *Pontederia sagittata*, porosidad.

Abstract

This research evaluated the effect of macrophyte pruning on microorganisms adhered to the support medium and the removal of BOD_5 in three experimental subsurface flow artificial wetlands (FFSCW) with species of *Phragmites australis* (FFSCW-Carrizo), *Pontederia sagittata* (FFSCW-Tule) and gravel as a control (FFSCW-Grava), being nine units (with replicas). The FFSCW 1.2 m wide, 2.5 m long and 0.5 m deep were fed with 200 L/d domestic wastewater. The variables studied to the support medium were adhered microorganisms, porosity, density, diameter and humidity to estimate the retention time and hydraulic conductivity. In the species, the productivity variables were measured after pruning and the removal and the degradation kinetic coefficient k for the BOD_5 were estimated. The experiment was a one-factor (treatment) design with three levels (FFSCW-Tule, FFSCW-Carrizo and FFSCW-Grava); being evaluated during three months, after the year of operation. Treatments were evaluated by ANOVA (parametric) and the Kruskal-Wallis rank test (non-parametric) between groups before and after pruning ($p < 0.05$). The gravel presented on average ($N = 12$) 22.92 ± 1.47 % humidity, 2.32 ± 0.15 cm in diameter, particle density 2.71 ± 0.10 gr/cm^3 and porosity of 36.52 ± 3.36 %, the TRH was 3.1 days in all the treatments. The best treatment was FFSCW-Tule, before pruning the biomass of microorganisms presented median values ($N = 12$) of 42931.6 mg/kg ($Q_1 = 40259.7$; $Q_3 = 54478.4$) and after pruning of 33444.6 mg/kg ($Q_1 = 31210.9$; $Q_3 = 36581.8$), the vegetable biomass removed in pruning was 40.85 ± 2.58 kg, removing 95.44 % of BOD_5 with $k = 1.004$ $days^{-1}$ (27.6 °C), which allows complying with NOM-001-Semarnat-1996.

Keywords: particle density, subsurface-flow constructed wetland, *Phragmites australis*, *Pontederia sagittata*, porosity.

Resumo

Esta pesquisa avaliou o efeito da poda de macrófitas sobre microrganismos aderidos ao meio de suporte e a remoção de BOD5 em três pântanos experimentais de fluxo subsuperficial (HAFS) com espécies de Phragmites australis (HAFS-Carrizo), Pontederia sagittata (HAFS-Tule) e cascalho como controle (HAFS-Grava). Foram utilizadas nove unidades (com réplicas). Os HAFS de 1,2 m de largura, 2,5 m de comprimento e 0,5 m de profundidade foram alimentados com 200 L / d de águas residuais domésticas. As variáveis estudadas para o meio suporte foram microrganismos aderidos, porosidade, densidade, diâmetro e umidade para estimar o tempo de retenção e a condutividade hidráulica. Na espécie, as variáveis de produtividade foram medidas após a poda e estimados a remoção e o coeficiente cinético de degradação k para o DBO5. O experimento foi um projeto de um fator (tratamento) com três níveis (HAFS-Tule, HAFS-Carrizo e HAFS-Grava); eles foram avaliados por três meses, após o ano de operação. Os tratamentos foram avaliados por Anova (paramétrico) e teste de classificação Kruskal-Wallis (não paramétrico) entre os grupos antes e após a poda ($p < 0,05$). A brita apresentou em média (N = 12) 22,92 ± 1,47% de umidade, 2,32 ± 0,15 cm de diâmetro, densidade de partícula 2,71 ± 0,10 gr / cm3 e porosidade de 36,52 ± 3,36%, o TRH foi de 3,1 dias em todos os tratamentos. O melhor tratamento foi HAFS-Tule: antes da poda a biomassa dos microrganismos apresentou valores medianos (N = 12) de 42931,6 mg / kg (Q1 = 40259,7; Q3 = 54478,4) e após a poda de 33444,6 mg / kg (Q1 = 31210,9 ; Q3 = 36581,8), a biomassa vegetal removida na poda foi de 40,85 ± 2,58 kg, 95,44% do BOD5 foi removido com k = 1.004 dias-1 (27,6 ° C), o que permite cumprir a NOM-001 -Semarnat-1996.

Palavras-chave: densidade de partículas, pântano de fluxo subsuperficial artificial, Phragmites australis, Pontederia sagittata, porosidade.

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Introduction

Wastewater is typically mixtures of organic and inorganic compounds classified as basic pollutants and toxic xenobiotics, among others (Miranda, Sandoval, Calvo, Moeller and Sarracino, 2017). In recent years, efforts have been made to minimize and enhance the sludge (due to the characteristics mentioned above, hazardous waste both for the environment and for living beings) that are generated as a result of the treatment of wastewater, whose quantities have increased due to population growth and the development of large cities, for example, as a raw material for biofuels and mixtures for the formation of bricks (Araujo, Molina and Noguera, 2018; Mancipe and Triviño, 2018). Hence the need to apply technological alternatives for wastewater treatment that are low-cost (investment-operation), easy to operate and maintain, and with low energy consumption (Torres, López, Romellón, Vázquez and Comparán, 2020). One of these alternatives is artificial wetlands (HA). HA are ponds in extensions of land with support medium, residual water, microorganisms and macrophyte vegetation (Conagua, 2016).

An artificial subsurface flow wetland (HAFS) is specifically designed for the treatment of some type of wastewater and is built in the form of a bed where the gravel has a height of up to 0.6 m (Asprilla, Ramírez and Rodriguez, 2020). The treatment is based on three basic principles: the biochemical activity of microorganisms, the supply of oxygen through the macrophytes during the day and the physical support of a bed that serves as a support for microorganisms, rooting the species and as a filter material. elements that, together, remove dissolved and suspended materials in wastewater (García, López y Torres, 2019).

It has been reported that the type of wetland, the hydraulic retention times (HRT) and the species have an affinity to remove some pollutants. Castañeda and Flores (2013) evaluated experimental HA with species of natural wetlands of the Los Altos de Jalisco region such as: the common reed (Phragmites australis), the gladiolus (Gladiolus spp) and the cattail (Typha latifolia). The HA operated with HRT for three, five and seven days; Four monitoring per year were carried out, and the best efficiencies were with seven days. Removal of BOD5 with reed was 80.45%, with gladiolus 63.31% and with cattail 77.19%. For total nitrogen (NT) with reed it was 64%, with gladiolus 34% and with cattail 53%. For total phosphorus (PT) the reed removed 65%, with gladiolus 68% and with cattail 75.75%. In these results it is observed that Phragmites australis was better in the removal of BOD5 and NT, while the PT achieved better removal with Typha latifolia.

Recently, Rangel et al. (2019) used Eichhornia crassipes and Typha domingensis macrophytes to optimize the kinetics of organic matter degradation and nutrient removal in HA at a laboratory scale with TRH for two and four days. It was possible to remove 92.39% of COD, 99.28% of N and 87.78% of P with E. crassipes with four days of TRH. The kinetic study was carried out comparing three models (first order model, Stover-Kincannon model and Grau-second order model) and the best fit was obtained with the Stover-Kincannon kinetic model, with k = 0.9997 and a rate maximum removal of 2500 mg / Ld.

For their part, Cui et al. (2011) studied in China the removal of contaminants in HAFS according to the first-order kinetic model. These authors report that the effluent mass load correlates with temperature to some extent. They estimated that the coefficient for SST is

0.6293 and for COD 0.6210. The SST area rate constant increases exponentially with increasing influent mass loading when the mass loading is less than 25 mg / L, but changes greatly with a higher mass loading. They consider that Beijing's environmental conditions influence kinetic constants and are higher in autumn compared to summer and winter. In other studies it has been proposed that the treatment capacity in HA is high in tropical areas due to warm temperatures and higher rates of microbial activity, for example, in Vietnam, HAFS with Phragmites vallatoria (L.) were studied and estimated that the first-order removal rate constants based on the area (k, m $^{\text{year-1}}$) (along the wetland from inlet to outlet) in four HA presented effluents with ranges of 25-95 (BOD5) , 22-30 (COD), removing 65-83% and 57-84%, respectively (Ngo et al., 2011).

The species Carex appressa, Ficinia nodosa and a control were used in biofilters of experimental columns to know the effects of pruning on the removal of NT, PT and metals. The effluent was monitored in pruned, unpruned, and unplanted control columns for 70 days, with a monthly composite water sampling that encompasses the rinsed saturated zone water and the effluent from each column. No differences were found between treatments for PT, but pruning treatments affected NO removal at later sampling dates for F. nodosa, but not for C. appressa. The elimination of N and P ranged from 77-88% and 66-93%, respectively, by both pruned and unpruned plants. The amount of N and P removed in the pruned biomass was 2.1 to 3.5 times higher than the estimated amount removed from the tributary by regrowth of the pruned columns only during the regrowth period. Consequently, the amount of nutrients removed through pruning can significantly affect long-term removal. The effluent concentrations of cadmium, copper, lead and zinc were similar between treatments with removals greater than 95%. In general, pruning appears to affect water quality improvement, but best pruning practices that can improve long-term removal (Herzog et al., 2021).

However, the use of wetlands has gradually increased in southeastern Mexico. For 2019, in Tabasco an installed treatment capacity of 2775.9 L / s was reported, served with 69 wastewater treatment plants, of which 11 have HA (primary-secondary-tertiary), through which it is treated 26.57% (1015.3 L / s) of the wastewater generated in the state (Conagua-Semarnat, 2019). Among the main operational problems reported are clogging in the first years of operation, which reduces the removal of basic pollutants, and the most used species is Tyhpa domingensis (bulrush). The species from the moment they are sown have little maintenance (pruning or replanting), so they develop their life cycle and it is common for them to present variation in removal efficiency and high concentrations in effluent, which in turn causes the phenomenon of short circuit (Vázquez and López, 2011).

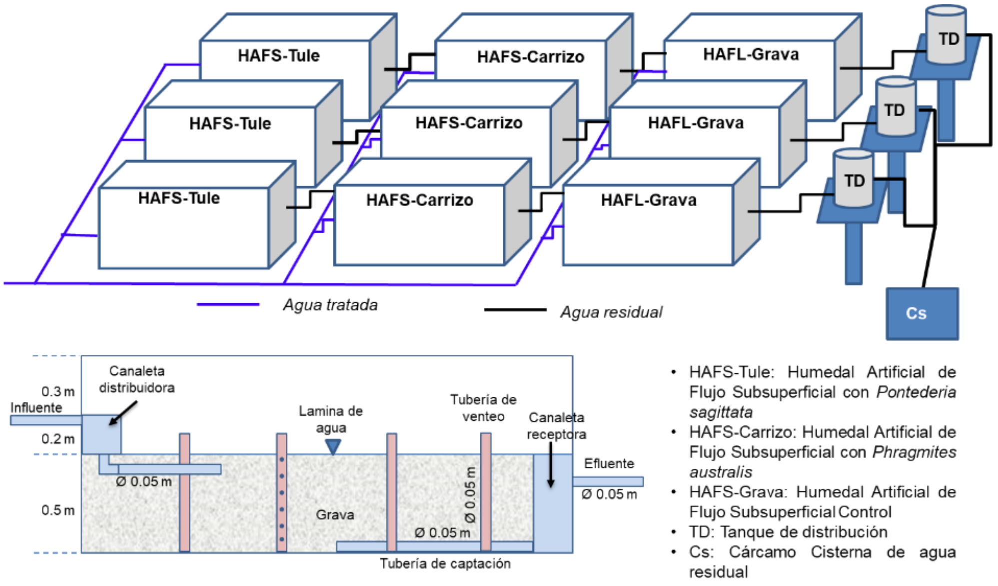
In the present study, the effect of pruning in two macrophyte species (Pontederia sagittata and Phragmites australis) on the support microorganisms and on the removal of BOD5 was evaluated. Said study was carried out after the wetlands had completed one year of operation (start-up and stabilization); They operated normally and pruning was part of the preventive maintenance. The HA were monitored for three months to identify the possible changes suffered by the support medium in terms of microorganisms and other physical variables. At the same time, the removal and kinetics of BOD5 of each species in the HAFS were evaluated. This research considers as a hypothesis that there is an effect of pruning on microorganisms and as a consequence the removal of organic matter measured as BOD5 in HAFS is altered.

Materials and methods

Experimental units

The wastewater used in the HAFS came from the concentrating station of the Autonomous University of Tabasco (UJAT), specifically from the Academic Division of Biological Sciences (DACBiol), used in sanitary services and cafeterias. Nine experimental units of HAFS were used independently designed by López et al. (2014). Two 1 HP pumps were used, connected to a 1 ”hydraulic pipe. The PVC pipe was connected to three tanks that regulate the flow (200 L). Each HAFS maintained the same flow (200 L / day), inlet velocity and organic load. In addition, each of them was composed of 10-gauge carbon steel, 2.5 m long x 1.2 m wide x 1 m high. Anti-corrosive alkyd enamel and a polyamide catalyzed epoxy were applied to protect the unit. Internally, a layer of elastomeric waterproofing was applied with layers of textile fiber, filling with mixed gravel (Ø of 2.25 cm; density of 2.65 gr / cm3; porosity of 35.81%), with a height of 0.5 m of operating tie. The operation of the experimental units begins with the collection of raw water from toilets and cafeterias in the cistern, which functions as primary treatment by fulfilling the functions of removal of urban solid waste, sedimentable solids and suspended with HRT for 45 to 250 minutes. ; then, the water is pumped to control tanks, where the flow rate and speed are regulated, and later it goes to the experimental units that are operating as secondary treatment (figure 1).

Figura 1. Diagrama de especificaciones de las unidades experimentales instaladas



Fuente: Elaboración propia

Collection of macrophyte species

The macrophyte vegetation sampling Pontederia sagittata and Phragmites australis was obtained from the Pantanos de Centla Biosphere Reserve, where they were collected and transported following the recommendations of Gallegos, López, Bautista and Torres (2018). Said collection was in August 2018. Once the plants were found in the experiment area, they were placed in a treated wastewater discharge channel. The plantation was carried out the following day for its stabilization. The distribution of species in the Pontederia sagittata plantation within the three wetlands was 15 plants for each one. While 20 rows of Phragmites australis were planted in each of the three wetlands, each row consisted of four plants. The control wetlands (HAFS-Gravel) only contained 0.5 m of gravel. The Pontederia sagittata species had rapid adaptation and acceptance of residual water, since between the first 8 and 10 days foliar growth was observed in the central part, while Phragmites australis showed adaptation between 20 and 25 days, when growth was observed. of small roots and buds at the nodes of the stem. This is a small difference compared to other species that are adapted with reconstituted water before planting. It was observed that it took approximately 40 days for stabilization to exist.

Monitoring of support medium, macrophytes and BOD $_5$

The response variables measured to the support medium and to the macrophytes were: humidity (Hum), gravel diameter (Øp) and the particle density (Dp), as established by NOM-021-RECNAT-2000, the porosity (ŋ), by the method established by Muñoz, Soler, López and Hernádez (2015), and the biomass of microorganisms, by the modified volatile matter method of López et al. (2019). And the species was determined plant biomass (kg), height (m), leaf length (cm), leaf width (cm), stem diameter (cm) and root length (cm) (Gallegos et al., 2018). These determinations were made in normal operation after one year of operation. They were measured in the month of October 15 days before pruning (October 1 to 15) and 15 days after pruning (October 15 to 30). And three samples were taken along the length and width of each HA, with an average distance of 0.35 m, 1.25 m and 2.15 m from the inlet to the outlet of the effluent; in total, 9 samples of the support medium per wetland. This to measure the immediate effect of pruning on microorganisms and species. The species pruned on October 15 were left with a 15 cm pseudostem on the support medium and weighed, taking three plants across the HA, from which the variables mentioned above were measured. The BOD5 measurement was carried out one day a week (12 composite samples) during a period of three months (September-November 2019); thus, simple samples were obtained every three hours from 8:00 a.m. to 5:00 p.m., and it was determined using the NMX-AA-028-SCFI-2001 method. It should be noted that six samples are within the period before pruning and six samples are after pruning. Pruning is recommended to be done at least twice a year (Conagua, 2016).

Experimental design and statistical analysis

A one-factor (treatment) design with three levels (HAFS-Tule, HAFS-Carrizo and HAFS-Grava) was used. The experiment was run in triplicate. The monitoring period was three months (September, October and November 2019) after one year of operation. To determine statistically significant differences in the evaluation of the HA system between the treatment groups, it was contrasted with an analysis of variance (Anova) of a classification by ranges for the data that did not comply with the Kruskal-Wallis postulates of normality and homoscedasticity, as well as a contrast of medians for independent U Mann-Whitney samples (before pruning: biomass of microorganisms, particle diameter and gravel density; after pruning: biomass of microorganisms and humidity). For the data that met the normality and homoscedasticity postulates, an Anova test was performed (before pruning: moisture and porosity; after pruning: particle diameter, gravel density and porosity).

Hydraulic retention time

Hydraulic retention time was determined for operating expenses using equation 1 (Crites y Tchobanoglous, 2000):

$$TRH = \frac{nhAs}{Q} \tag{1}$$

Where: TRH = Hydraulic retention time, n = Porosity; h = Depth of the water surface, As = Wetland area and Q = Average expenditure.

In the hydraulic test, once the volume of the reactor with the operating tie was known, the volume of gravel and the porosity, tests were carried out supplying different operating costs (50 to 350 L / day) in order to know the HRT at different expenses and thus estimate the operating expenses of the experimental units. It was established that the entire experiment would be carried out with a flow rate of 200 L / day.

Removal efficiency

The removal efficiency for BOD_5 was calculated using the following equation 2 (García *et al*., 2019):

$$ER\ (\%) = \frac{c_e - c_s}{c_e}\ x\ 100 \tag{2}$$

As: ER (%) = Removal efficiency (%), Ce = Inlet concentration (influent) and Cs = Outlet concentration (effluent).

Degradation kinetics

Because HA systems are biological reactors, their performance can be estimated using first-order kinetics (Cui et al., 2011; Environmental Protection Agency [EPA], 2000). The reaction constant k was estimated using equation 3. In the case of our study, the experiments were carried out at a temperature of 27.63 ° C on average, so it was no longer necessary to carry out the Arrhenius adjustment, as established Crites y Tchobanoglous, (2000).

$$c_e = c_o e^{-kt} \tag{3}$$

As: Ce = Concentration of the pollutant in the effluent (mg / L), Co = Concentration of the pollutant in the influent (mg / L), k = Reaction constant (d-1) and t = Hydraulic retention time (day).

Results

The results obtained from the treatments are shown below. It should be remembered that there were measurements before and after pruning the species during the experimentation period, physical parameters and bacterial biomass in the support medium. In the same way, the removal and degradation kinetics of BOD5 were studied, as well as the characteristics of the species (length and width of the leaf, height, stem diameter and root length) after pruning. The initial concentration of the water entering the experiments presented the averages of the following parameters: BOD5 408.1 mg / L, SST 310.0 mg / L, Turbidity 131.4 NTU and Color 946.1 UC, pH 7.8 and water temperature 27.6 ° C.

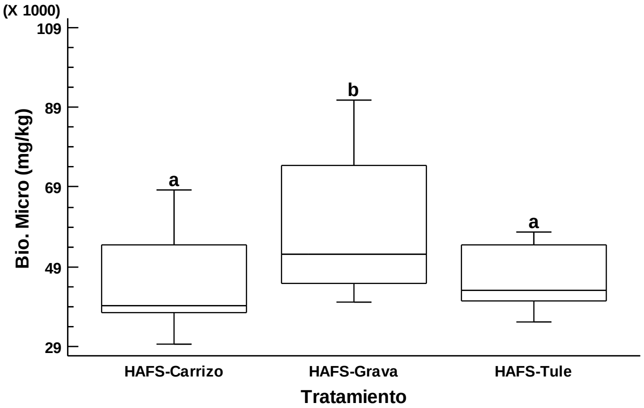
Conditions before pruning

Biomass of microorganisms

The results of Kruskal-Wallis, for the contrast of medians in the biomass of microorganisms (mg / kg) in the support medium of the different HA (HAFS-Carrizo, HAFS-Tule and HAFS-Grava) before pruning, indicate that there are statistically significant differences between the treatments ($p < 0.01$). The Mann-Whitney U test of independent samples indicated the presence of statistically significant differences in both medians of the treatments ($p < 0.05$). The lowest median value observed was in the HAFS-Carrizo, 39340.5

mg / kg with lower and upper quartiles (Q_1 = 37355.2; Q_3 = 54329.1), followed by HAFS-Tule with 42931.6 mg/kg (Q_1 = 40259.7; Q_3 = 54478.4), therefore, the HAFS-Grava obtained the highest median value with 52179.6 mg/kg (Q_1 = 44789.5; Q_3 = 74419.8) (figure 2).

Figura 2. Valores de medianas (±$Q_{1,\,3}$) para biomasa de microorganismos mg/kg

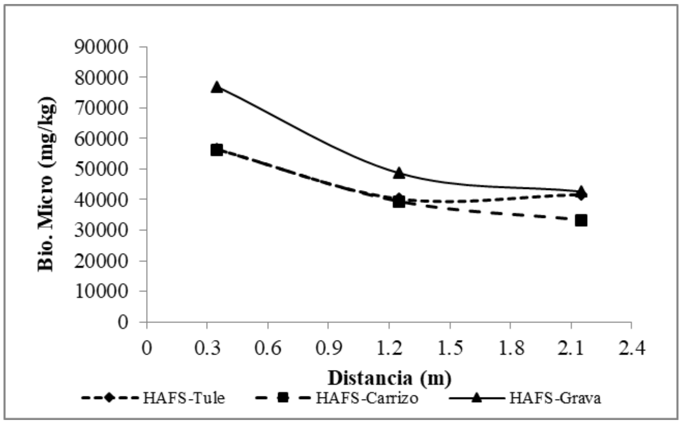


Nota: letras diferentes indican diferencias estadísticamente significativas (*N* = 12)

Fuente: Elaboración propia

In the analysis of the biomass distribution of microorganisms within the wetlands, the highest adhesion was found at the entrance of the wastewater, gradually decreasing towards the exit of the wetland (figure 3). The median biomass concentrations of microorganisms at the entrance for the HAFS-Tule at the distance of 0.35 m were 56498.08 mg / kg (Q_1 = 54927.35; Q_3 = 57650.22), decreasing at the exit by 41695.60 mg / kg (Q_1 = 39214.34 ; Q_3 = 42862.75) at the distance of 2.15 m. For the HAFS-Carrizo, a data of 56270.08 mg / kg was found (Q_1 = 54350.77; Q_3 = 60701.09), decreasing at the exit by 33295.33 mg / kg (Q1 = 30363.34; Q_3 = 36656.01). While for the HAFS-Grava the input concentration was 76917.52 mg / kg (Q1 = 74653.74; Q3 = 81902.83) decreasing at the output by 42654.63 mg / kg (Q1 = 41366.98; Q3 = 46394.18) respectively.

Figura 3. Distribución de microorganismos (mg/kg) en la sección longitudinal del HAFS

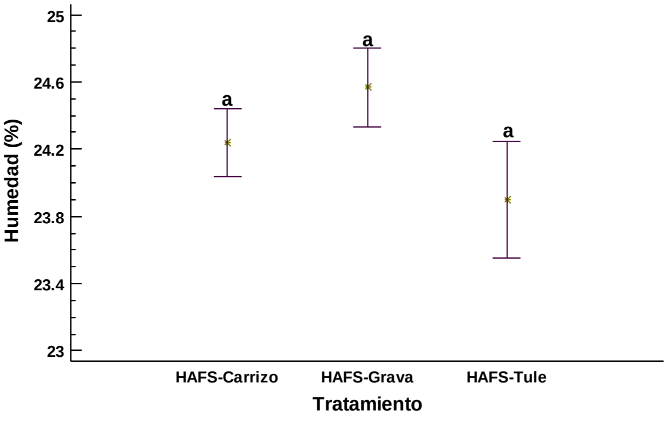


Fuente: Elaboración propia

Humidity

The results of the Anova in the comparison of the average values of humidity (%) in the support medium of the different HAs (HAFS-Tule, HAFS-Carrizo and HAFS-Grava), indicate that there are no significant statistical differences between the treatments (Anova, F2.33 = 1.55, p> 0.05). The highest average humidity value was obtained in the HAFS-Grava with 24.56 ± 0.81%, followed by the HAFS-Carrizo with 24.23 ± 0.69%, while the lowest average was for the HAFS-Tule treatment with 23.89 ± 1.20% (figure 4).

Figura 4. Valores promedios (±EE) en la humedad (%)



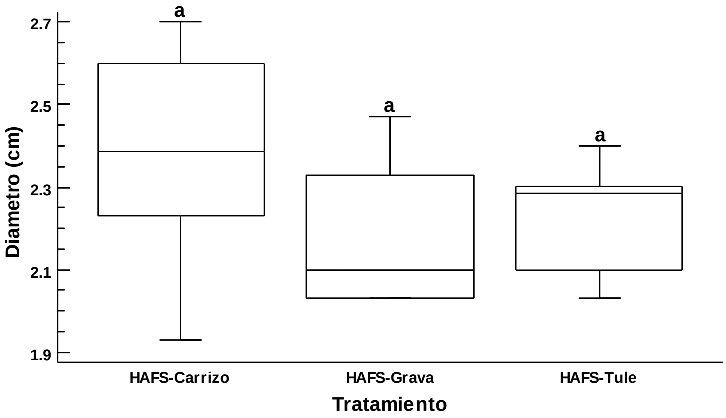
Nota: letras diferentes indican diferencias estadísticamente significativas (*N* = 12).

Fuente: Elaboración propia

Particle diameter (gravel)

The Kruskal-Wallis analysis, for the contrast of medians of the diameter (cm) measured in the support medium of the different HAs (HAFS-Tule, HAFS-Carrizo and HAFS-Grava), indicates that there are no statistically significant differences between the medians of each of the three treatment levels ($p > 0.05$). Therefore, the median value of the diameter obtained for the HAFS-Carrizo is 2.38 cm ($Q_1 = 2.23$, $Q_3 = 2.60$), the HAFS-Grava had a median of 2.10 cm ($Q_1 = 2.03$; $Q_3 = 2.33$) and the HAFS-Tule obtained a median of 2.28 cm ($Q_1 = 2.10$, $Q_3 = 2.30$) (figure 5).

Figura 5. Valores de medianas ($\pm Q_{1,\ 3}$) del diámetro (cm) del medio de soporte



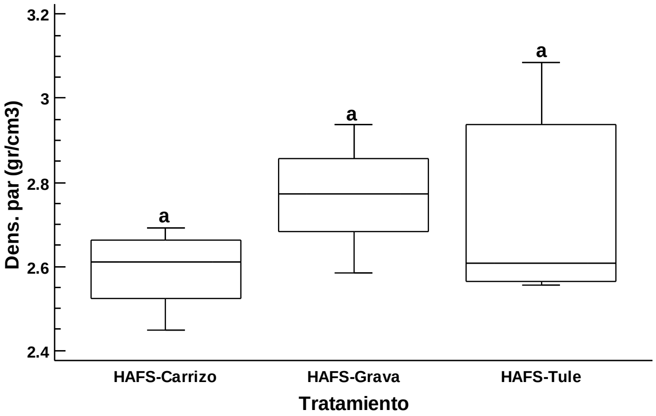
Nota: letras diferentes indican diferencias estadísticamente significativas (*N* = 12)

Fuente: Elaboración propia

Particle density (gravel)

For the density of the particle (gravel), the results of Kruskal-Wallis for the contrast of medians determined in the support medium of the different HAs (HAFS-Tule, HAFS-Carrizo and HAFS-Grava) indicate that there are no statistical differences. significant among the medians of each of the three levels of treatment ($p > 0.05$). The particle density in HAFS-Carrizo presents a median of 2.60 gr/cm^3 ($Q_1 = 2.52$; $Q_3 = 2.66$), while HAFS-Grava has a median of 2.77 gr/cm^3 ($Q_1 = 2.68$; $Q_3 = 2.85$) y el HAFS-Tule presented a median of 2.60 gr/cm^3 ($Q_1 = 2.56$; $Q_3 = 2.93$) (figure 6).

Figura 6. Valores de medianas (±$Q_{1, 3}$) en la densidad de la partícula (gr/cm^3)



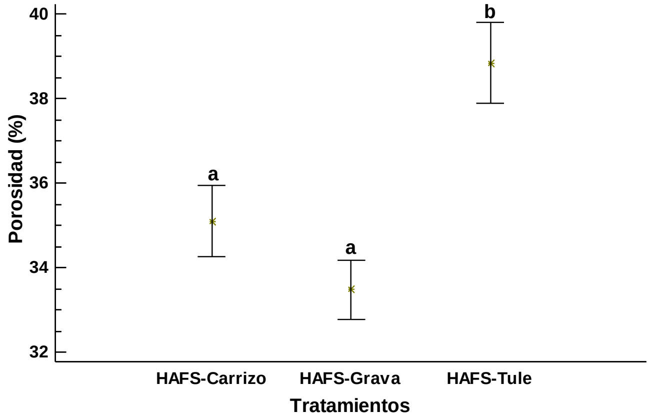
Nota: Letras diferentes indican diferencias estadísticamente significativas (*N* = 12)

Fuente: Elaboración propia

Porosity

The results of the Anova comparison of porosity for the different HAs (HAFS-Tule, HAFS-Carrizo and HAFS-Grava) indicate that there is a highly significant statistical difference between the treatments (Anova, F2.33 = 10.79, p <0.001) . Tukey's multiple contrast a posteriori test indicated the presence of statistically significant differences between the treatments (p <0.05). The highest average value of porosity was obtained in the HAFS-Tule with 38.85 ± 3.30%, followed by the HAFS-Carrizo with 35.10 ± 2.91%, while the lowest average was for the HAFS-Grava treatment with 33.48 ± 2.41% (figure 7).

Figura 7. Valores promedios (±EE) de la porosidad (%)



Nota: letras diferentes indican diferencias estadísticamente significativas (*N* = 12).

Fuente: Elaboración propia

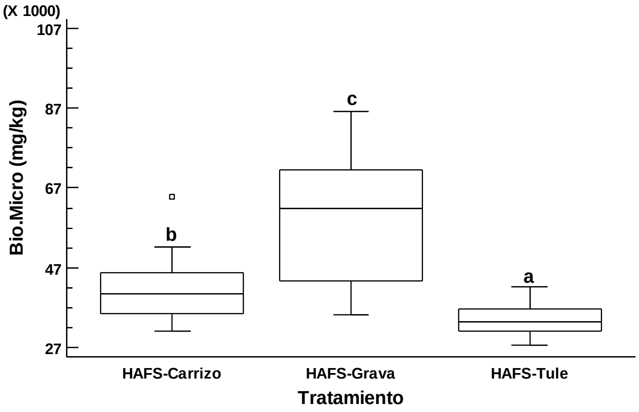
Conditions after pruning

Next, the results of the hydraulic characteristics of the different treatments (HAFS-Tule, HAFS-Carrizo and HAFS-Grava) obtained after cutting the vegetation are presented.

Biomass of microorganisms

The results of Kruskal-Wallis for the contrast of medians in the measurement of biomass of microorganisms (mg / kg) in the support medium of the different HA (HAFS-Carrizo, HAFS-Tule and HAFS-Grava) after pruning indicate that there are statistically significant differences between the treatments ($p < 0.01$). The Mann-Whitney U test of independent samples indicated the presence of statistically significant differences in both medians of the treatments ($p < 0.05$). The lowest median value observed was the HAFS-Tule 33444.6 mg/kg ($Q_1 = 31210.9$; $Q_3 = 36581.8$), followed by HAFS-Carrizo 40489.7 mg/kg ($Q_1 = 35586.4$; $Q_3 = 45830.4$), therefore, the HAFS-Grava obtained the highest median value of 61746.1 mg/kg ($Q_1 = 43753.1$; $Q_3 = 71624.1$) (figure 8).

Figura 8. Valores de medianas (±$Q_{1,\ 3}$) para biomasa de microorganismos (mg/kg)



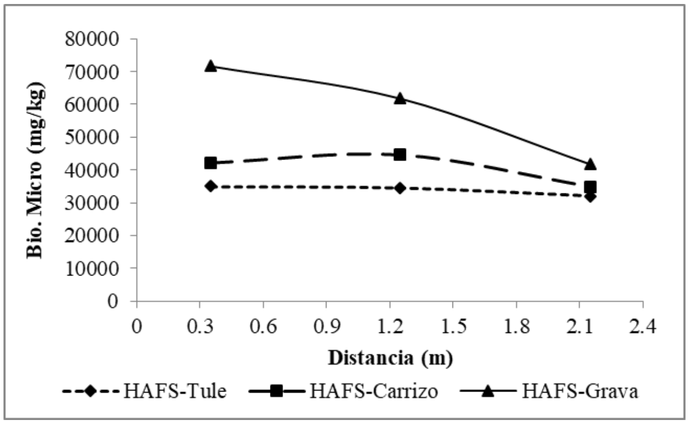
Nota: letras diferentes indican diferencias estadísticamente significativas ($N = 12$).

Fuente: Elaboración propia

The behavior shown after pruning is similar to that shown in the phase before pruning: it gradually decreases from influent to effluent (figure 9). The median values of the biomass of microorganisms determined at the entrance for the HAFS-Tule (0.35 m) were from 34971.82 mg/kg ($Q_1 = 31770.51$; $Q_3 = 38627.77$) decreasing at the exit in 32032.52 mg/kg ($Q_1 = 29910.73$; $Q_3 = 33931.94$) in the distance of 2.15 m. For HAFS-Carrizo, the initial concentration was of 42152.11 mg/kg ($Q_1 = 38487.35$; $Q_3 = 47348.75$) tapering off with

34928.23 mg/kg (Q_1 = 33643.46; Q_3 = 37087.16). While for HAFS-Grava the initial concentration was 71624.12 mg/kg (Q_1 = 66507.38; Q_3 = 79117.46) decreasing at the exit in 41717.19 mg/kg (Q_1 = 38667.33; Q_3 = 43673.42).

Figura 9. Distribución de la biomasa de microorganismos (mg/kg) en la sección longitudinal del humedal

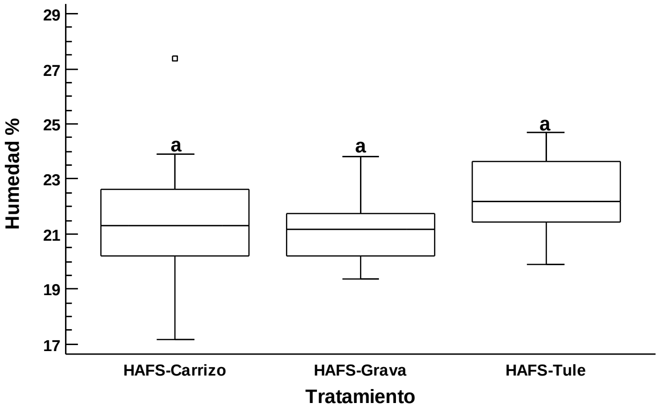


Fuente: Elaboración propia

Wetness

The Kruskal-Wallis analysis, in the humidity (%) of the support medium to the different HAs (HAFS-Tule, HAFS-Carrizo and HAFS-Grava), indicates that there are no statistically significant differences between the medians of each of the three levels of treatment ($p > 0.05$). Therefore, the median value of humidity (%) obtained for the HAFS-Carrizo is 21.32 % (Q_1 = 20.21; Q_3 = 22.63), HAFS-Grava had a median of 21.15 % (Q_1 = 20.22; Q_3 = 21.74) and the HAFS-Tule obtained a median of 22.19 % (Q_1 = 21.43; Q_3 = 23.63) (figure 10).

Figura 10. Valores de medianas ±$Q_{1, 3}$ en la humedad (%)



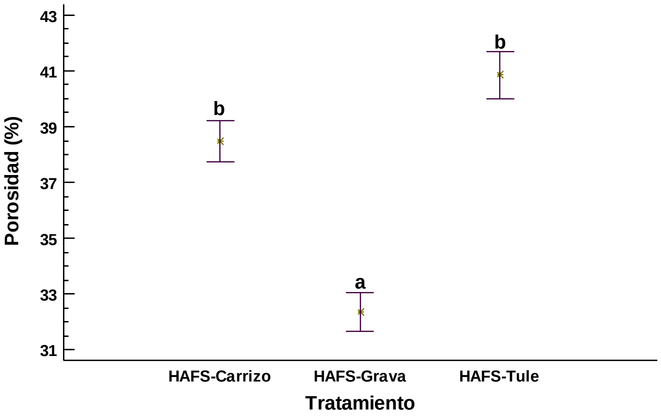
Nota: letras diferentes indican diferencias estadísticamente significativas (*N = 12*)

Fuente: Elaboración propia

Porosity

The results of the Anova indicate that there is a highly significant statistical difference between the treatments (Anova, F2.33 = 33.39, p <0.001). Tukey's multiple contrast a posteriori test indicated the presence of statistically significant differences between the treatments (p <0.05). The highest average value of porosity was obtained in the HAFS-Tule with 40.85 ± 2.90 %, followed by HAFS-Carrizo with 38.46 ± 2.53%, while the lowest mean was for the HAFS-Grava treatment with 32.38 ± 2.39% (figure 11).

Figura 11. Valores promedios (±EE) de la porosidad (%)



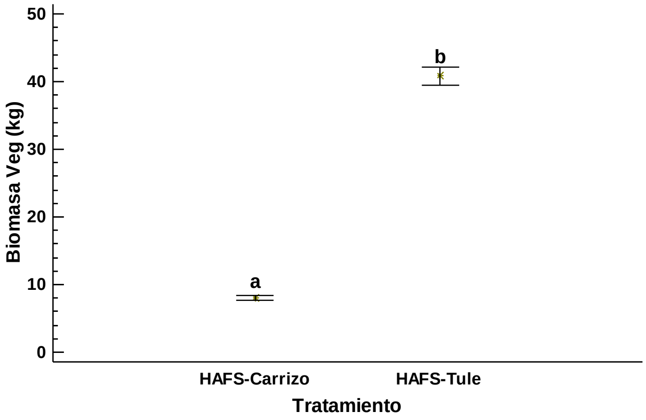
Nota: letras diferentes indican diferencias estadísticamente significativas (*N* = 12).

Fuente: Elaboración propia

Species characteristics

The results of the Student's t analysis, comparing the biomass means of Pontederia sagittata (Tule) and Phragmites australis (Carrizo) used in the HAFS-Tule and HAFS-Carrizo treatments after pruning, indicate that there are statistical differences highly significant ($p < 0.05$) (figure 12). The HAFS-Tule obtained an average vegetation biomass of 40.85 ± 2.58 kg, while for the HAFS-Carrizo it was 8 ± 0.73 kg.

Figura 12. Valores promedios (±EE) de la biomasa de las especies (kg)



Nota: letras diferentes indican diferencias estadísticamente significativas ($N = 12$).

Fuente: Elaboración propia

The characteristics of the leaves (length and width), height (m), diameter and length of their roots, of which three were measured at 0.35 m from the entrance, three at 1.25 m in the middle and three more at 2.15 m from the start, presented a final average that is summarized in Table 1.

Tabla 1. Mediciones de las especies después de la poda (*N = 9*)

Especie	Distancia (m)	Altura (m)	L. hoja (cm)	A. hoja (cm)	Ø tallo (cm)	L. raíz (cm)	Peso (kg)
Phragmites australis	0.35	1.65 ± 0.1	18.92 ± 2.4	1.57 ± 0.1	1.78 ± 0.2	8.80 ± 1. 6	0.18 ± 0.0
	1.25	1.64 ± 0.1	17.97 ± 7.9	1.65 ± 0.1	1.69 ± 0.3	9.43 ± 0.9	0.18 ± 0.8
	2.15	1.54 ± 0.2	17.62 ± 8.5	1.63 ± 0.2	1.66 ± 0.2	8.60 ± 1.6	0.18 ± 0.0
Pontederia sagittata	0.35	1.37 ± 0.1	33.53 ± 3.0	20.81 ± 2.6	5.49 ± 0.6	20.32 ± 2.4	2.92 ± 0.0
	1.25	1.29 ± 0.1	36.21 ± 0.6	22.50 ± 2.0	6.53 ± 0.8	16.05 ± 1.4	2.41 ± 0.3
	2.15	1.32 ± 0.1	35.96 ± 1.7	23.71 ± 1.0	6.58 ± 0. 7	20.00 ± 3.0	2.50 ± 0.1

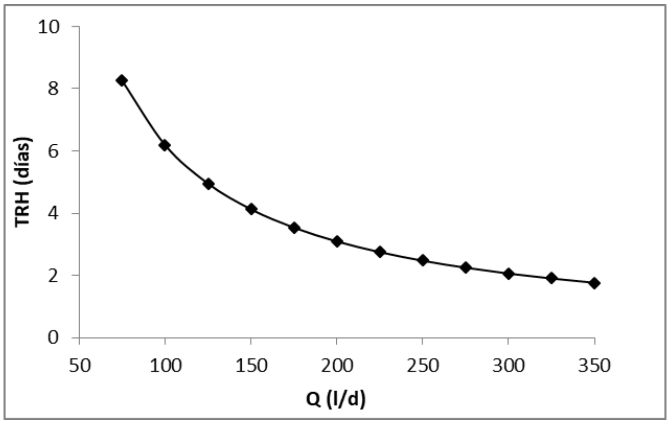
Nota: Distancia = Distancia longitudinal del HAFS, Peso = Peso de la especie.

Fuente: Elaboración propia

Hydraulic retention time and BOD removal

Once the TRH was estimated by varying the operating expenses, it was established that at 200 L / d the experiments would have 3.1 days of TRH, and it was confirmed that the higher the operating expense, the lower the TRH, which implies a deficiency in the removal of pollutants in the HAFS, since the contact of the water with the bacteria and the root system will be less in the treatments (figure 13).

Figura 13. Tiempos de retención hidráulica contra diferentes caudales



Fuente: Elaboración propia

Table 2 shows the removal and the degradation kinetic coefficient of BOD5 in each of the treatments. It is observed that the reaction rates are different for each HAFS with its species and the degradation of organic matter is related to the concentration of microorganisms in the support medium. All treatments were with 3.1 days of HRT. The

HAFS-Tule before pruning on average presented a k = 0.60 días^{-1} and removed 84.52 % of DBO$_5$; after pruning a k = 1.004 días^{-1} with 95.4 % of DBO$_5$. The HAFS-Carrizo before pruning presented a k = 0.46 días^{-1} stirring 75.73 % de DBO$_5$ and after pruning a k = 0.67 días^{-1} stirring 87.47 % of DBO$_5$. The HAFS-Grava presented before pruning a k = 0.21 días^{-1}, stirring 47.13 % of DBO$_5$, after pruning presented a k = 0.22 días^{-1}, removing 50.01% of DBO$_5$. Thus, the Carrizo and Grava treatments require higher HRT in order to achieve an efficiency similar to that of HAFS-Tule..

Tabla 2. Mediciones de las especies después de la poda (*N* = 6)

Tratamiento	Antes de la cosecha				Después de la cosecha			
	Inf.	Efl.	ER (%)	k (días^{-1})	Inf.	Efl.	ER (%)	k (días^{-1})
HAFS-Tule	415.0	63.0	84.82	0.61	393.5	23.5	94.03	0.91
	338.0	60.0	82.25	0.56	430.0	15.0	96.51	1.08
	436.0	65.0	85.09	0.61	415.0	16.0	96.14	1.05
	395.0	49.0	87.59	0.67	380.0	20.0	94.74	0.95
	410.0	61.0	85.12	0.61	440.0	15.0	96.59	1.09
	360.0	64.0	82.22	0.56	390.0	22.0	94.36	0.93
HAFS-Carrizo	415.0	98.0	76.39	0.47	393.5	59.3	84.93	0.61
	338.0	90.0	73.37	0.43	430.0	53.5	87.56	0.67
	436.0	95.0	78.21	0.49	415.0	43.7	89.47	0.73
	395.0	89.0	77.47	0.48	380.0	48.1	87.34	0.67
	410.0	91.0	77.80	0.49	440.0	44.2	89.95	0.74
	360.0	104.0	71.11	0.40	390.0	56.3	85.56	0.62
HAFS-Grava	415.0	220.5	46.87	0.20	393.5	212.0	46.12	0.20
	338.0	210.5	37.72	0.15	430.0	206.5	51.98	0.24
	436.0	195.7	55.11	0.26	415.0	196.5	52.65	0.24
	395.0	220.3	44.23	0.19	380.0	210.0	44.74	0.19
	410.0	202.2	50.68	0.23	440.0	205.0	53.41	0.25
	360.0	186.5	48.19	0.21	390.0	190.5	51.15	0.23

Nota: Inf. = Influente, Efl. = Efluente, ER (%) = Eficiencia de remoción.

Fuente: Elaboración propia

Discussion

The microorganisms before and after pruning present a higher concentration in the control with respect to the wetlands with species and in the HAFS-Tule they showed a difference of 9487 mg / kg, in the HAFS-Carrizo they presented a difference of 1696.9 mg / kg and in the case of HAFS-Grava the difference was -1105.1 mg / kg. In the latter there is an increase in microbial biomass to the support medium (fixation and adaptation), since it does not have roots or plants. The change in microorganisms in HAFS-Tule is more noticeable because it is a macrophyte plant of higher density, since the stem and root give rise to sites for a greater fixation of microorganisms and when it is pruned, a decrease in the microbial population is shown. This effect is not the same in HAFS-Carrizo because the microorganisms do not depend exclusively on the plants, in such a way that the effect on the microorganisms after pruning is less. Authors such as Li et al. (2013) establish that in P. australis, although it may present greater bacterial diversity in the root with respect to Typha angustifolia L., these are involved in the total N cycle unlike Typha bacteria, which are involved in the removal of P and organic matter. The microorganisms in the HAFS-Tule decrease after pruning and can be attributed to the fact that in the upper zone of the wetland there is oxygen released by the roots of the plants and causes its decrease due to the pruning of the species (Delgadillo, Camacho, Pérez y Andrade, 2010).

Plants, when transferring oxygen from the atmosphere through leaves and stems, release it in the roots creating aerobic regions, where microorganisms use the available oxygen for the degradation of organic matter and nitrification, so that when the species is pruned they modify aerobic microzones on the surface of roots and rhizomes (EPA, 2000; Rahman et al., 2020). Microbial populations, due to the variation of oxygen, affect the degradation of organic matter, although authors such as Herzog et al. (2021) mention that the effects that pruning has on improving water quality remain unknown for certain pollutants. In HAFS part of the organic load is concentrated at the entrance, therefore, according to Delgadillo et al. (2010), heterotrophic microorganisms require organic material as a source of energy and carbon for the synthesis of new microorganisms, which shows a greater biomass of microorganisms at the entrance of the wetland that decreases depending on the length of the wetland as a consequence of the decrease in organic matter towards the effluent.

The humidity showed decreases; the greatest change in the mean difference was presented in the HAFS-Grava (3.36%), followed by the HAFS-Carrizo (2.75%) in its medians, and the smallest difference in the mean in the HAFS-Tule (1.49%) after of pruning. These differences are attributed to the species, since the plants protect and prevent the heating of the support medium while maintaining the average humidity. Tanner and Headley (2011) evaluated the water temperature in the HA treatment and concluded that, due to the shade exerted by the plants, the temperature in the water can be lower than the ambient temperature on average from 2 to 4ºC. ºC, that is, cooler. Therefore, after pruning, solar radiation heats the gravel directly, increases the temperature and consequently evaporates the water from the upper layer, all of which leads to a decrease in humidity in the upper part of the HAFS.

The porosity of HAFS-Carrizo increased 3.4% after pruning, in HAFS-Tule the increase was 2% and in HAFS-Grava it decreased 1.1%. This porosity, although it is minimal and temporary, is due to the space left by the roots of the species after pruning, since they start their growth and stabilization again; on the other hand, there is an effect due to the harvest of those species that have completed their life cycle and have to be removed in preventive maintenance (Conagua, 2016). In the case of HAFS-Gravel, the decrease is due to the proper arrangement of the particles in the bed. It is important to mention that the porosity of the support media will be reduced as the operating time passes due to sedimentation and filtration of suspended solids, the creation of biofilms in the particles of the filtration material, chemical precipitation and the growth of roots and rhizomes of the vegetation (Vymazal, 2018), which is why it is necessary to apply an efficient primary treatment, use suitable porous materials, maintain adequate loads of organic substances and suspended solids to greatly slow down the clogging process (Conagua, 2016; Wang, Sheng and Xu, 2021).

With the parameters obtained from the support medium, it was estimated that the HAFS-Carrizo allows a water volume of 0.55 ± 0.04 m3 and 0.95 ± 0.01 m3 of gravel, a hydraulic conductivity (Ks) of 321.31 m3 / m2d and a flow velocity (v) of 0.32 m / d, while HAFS-Gravel contains 0.49 ± 0.01 m3 of water with 1.01 ± 0.00 m3 of gravel, Ks = 375.44 m3 / m2d and v = 0.38 m / d and HAFS-Tule stores 0.60 ± 0.02 m3 of water and 0.90 ± 0.01 m3 of gravel, Ks = 303.24 m3 / m2d and v = 0.30 m / d. In the three treatments, the hydraulic conductivity and the flow velocity (> 36.0 m / d for gravel) comply with the levels recommended by Rahman et al. (2020) and Conagua (2016). It is important to follow up these variables for a long time (one to two years), because, according to Hoffmann et al. (2011), in the long term these parameters change and decrease due to the development of the roots of the plants and the accumulation of non-degradable residues that remain in the support medium. In filters without plants, the soil has to be treated to recover its hydraulic conductivity (removing the surface layer of the substrate). The root system maintains the hydraulic conductivity of the coarse sand substrate, so suitable plants with a well-developed root and rhizome system play an important role in maintaining and restoring the conductivity of the filter bed. (Conagua, 2016).

The microphytes used in HA show variation in the removal of organic matter and kinetic coefficients. The first-order reaction kinetics is largely that representing the removal of organic pollutants (C, N, P) for HAFS (Cui et al., 2011; Wang, Bo and Liu, 2015). Quintero (2014), reported that BOD5 was favorably removed in HAFS with Heliconia Psittacorum, with more than 87% at TRH of 1.6 days. In our study, all treatments were with 3.1 days of HRT. The BOD5 in the influent was 408.1 mg / L with a water temperature of 27.63 ° C. And the best treatment was HAFS-Tule, which removed 95.44% of BOD5 (18.6 mg / L in the effluent) and a degradation kinetic coefficient (k) of 1,004 days-1, meeting the maximum permissible limit in NOM-001 -Semarnat-1996 of 30 mg / L in the daily average for protection of aquatic life in rivers. The HAFS-Carrizo removed 87.5% (50.85 mg / L in the effluent) with k = 0.66 days-1. Similarly, in the HAFS-Grava, 50.1% (203.44 mg / L in the effluent) was removed, with k = 0.22 days-1. The kinetic constants (k) reported by EPA (2000) and Crites and Tchobanoglous (2000) for HAFS range from 0.86 to 1.84 days-1 (20 ° C) and these also depend on the medium used, recommending for gravel k = 0.86 days-1 (20 ° C), and if these constants are used for the design, the ambient temperatures should be adjusted (Arrhenius) and evaluated under normal operating conditions (Conagua, 2016). Another study in southeastern Mexico is that of López et al. (2019), who evaluated a HAFS treating domestic wastewater (204 ± 66 L / day, 377 mg / L of BOD5) with Thalia geniculata and obtained 85.6% removal of BOD5 with k = 0.43 days-1, and although the constants are different (less than that of our study), the performance in removing BOD is similar. This efficiency is attributed to the operating temperature (27 ° C) and higher HRT (4.2 days).

Microorganisms adhering to the surface are considered responsible for biological reactions in the submerged substrate and the microbial reaction rates of HAFS can be higher than HAFL for many contaminants. Therefore, a HAFS may have a smaller surface than a HAFL for the same flow rates and water quality (Conagua, 2016; Rahman et al., 2020). HAFS, lacking oxygen, limit the removal of ammonia (nitrification), but even so the system is effective in removing BOD (95.44% for Tule and 87.5% with Carrizo), some contaminants such as metals and priority organics, given that the treatment is under aerobic and anoxic conditions (Herzog et al., 2021).

Finally, although the difference in structure and biomass recovered from the harvest of P. australis and P. sagittata is notorious, no differences are shown between the plants along each HAFS (0.35 m, 1.25 m and 2.15 m); On average, a P. australis plant has a height of 1.61 m, a leaf length of 18.17 cm, a leaf width of 1.62 cm, a diameter of 1.71 cm, a root length of 8.94 cm and a weight of 0.18 kg. While P. sagittata presented an average height of 1.33 m, leaf length of 35.23 cm, leaf width of 22.34 cm, diameter of 6.20 cm, root length of 18.79 cm and a weight of 2.71 kg. It has been reported that the removal of pollutants is reduced when a systematic pruning of the vegetation is not carried out and the pruned material is removed to sites outside the HA, so it should be done with a frequency of two to three times per year and eliminate mainly the vegetation with a yellowish appearance (older vegetation), dead and dry; in each pruning, remove between 10% and 15% of the total vegetation in the cells, preventing the nutrients from being reincorporated into the wetland (Vymazal y Kröpfelová, 2008).

Conclusion

The present results are a young wetland (one year operation) and it was found that the microorganisms in the HAFS-Tule after pruning were reduced by 22.09%. This species provides greater root density for the establishment of microorganisms. In the HAFS-Carrizo the impact is much lower, it was only reduced by 3.8%, and in the HAFS-Grava the microorganisms increased by 1.89%, possibly due to their adaptation to the substrate and to the greater tolerance of the sun.

In the three HAFS there was a gradual decrease in the entrance to the exit of the microorganisms, behaving as the organic load decreases throughout the wetland.

The most efficient treatment after pruning in the removal of BOD5 was HAFS-Tule, which removed 95.44% of BOD5 with k = 1,004 days-1, followed by HAFS-Carrizo, with 87.5% and k = 0.67 days- one. And the HAFS-Grava removed 50.1% with k = 0.22 days-1. This shows that it did increase 10.88% with respect to before and after the elimination of organic matter with Potendenria sagittata and 11.74% with P. australis.

Future lines of research

To know the potential of the effect of pruning in HA, the study in the reactors with other macrophyte species should be continued in a short term and to evaluate the hydraulic retention times to be able to remove nutrients and other xenobiotics in concentration values that allow discharge to bodies. receivers safely. Another line of research in the medium term would be to evaluate the removal efficiency with waters of medium and strong concentration with different macrophyte species, in such a way that HA can be recommended for different efficient and economic cases.

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