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Treatment Wetland with *Thalia geniculata* for Wastewater Depuration in the Department of Sucre, Colombia

Vicente Vergara-Flórez [1], Jorge Mieles-Galindo [2], Graciela Nani [3], Mayerlin Sandoval-Herazo [3,*] and Luis Carlos Sandoval Herazo [3,*]

[1] Docente Facultad de Ingeniería, Universidad de Sucre, Sincelejo 700001, Colombia; viceunisucre@yahoo.com
[2] Grupo de Investigación Conservación del Recurso Hídrico y Alimentos (CRHIA), Universidad de Sucre, Sincelejo 700001, Colombia; jumieles@hotmail.com
[3] Wetlands and Environmental Sustainability Laboratory, Division of Graduate Studies and Research, Tecnológico Nacional de México/ITS de Misantla, Km 1.8 Carretera a Loma del Cojolite, Misantla 93821, Mexico; genanir@itsm.edu.mx
* Correspondence: mayerli.sandoval24@gmail.com (M.S.-H.); lcsandovalh@gmail.com (L.C.S.H.); Tel.: +52-2351115306 (L.C.S.H.)

Abstract: Municipal and industrial wastewater discharge is a longstanding environmental problem that pollutes water bodies, affecting both the landscape and human health. In the department of Sucre, Colombia, nearby urban sewage is discharged into the Arroyo Grande de la Sabana, and only Sincelejo city has a treatment system in place. Therefore, it is critical to identify effective treatment methods for removing contaminants from water. The objective of this study was to evaluate the efficiency of a constructed wetland (CW) with horizontal subsurface flow (HSSF) planted with *Thalia geniculata* for treating wastewater from the Arroyo Grande de la Sabana in Sucre, Colombia. The study investigated the effectiveness of a constructed wetland planted with *Thalia geniculata* for treating wastewater from the Arroyo Grande de la Sabana in Sucre, Colombia. Two different hydraulic retention times (HRTs) of 3 and 5 days were tested, and the plant population density was analyzed to determine the better adaptation of plants to the constructed wetlands. The results showed that on the fifth day of treatment, nitrate concentrations decreased by 33.22%, nitrite by 93.04%, and phosphate by 95.66%. Additionally, the biochemical oxygen demand (BOD_5) and chemical oxygen demand (COD) values decreased by 97.27% and 80.27%, respectively. On the third day of retention, turbidity, and total suspended solids (TSS) in the water decreased by 90.13% and 83.08%, respectively. The study concluded that the 5-day HRT was most effective in removing contaminants such as nitrites, nitrates, and phosphates, while the 3-day HRT was more efficient for TSS, turbidity, total coliforms (TCs), and fecal coliforms (FCs).

Keywords: treatment wetland; hydraulic retention time; macrophytes; removal efficiency; sewage water

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1. Introduction

Water is a vital natural resource essential for the development and well-being of any community. Unfortunately, many sources of water are being contaminated by untreated wastewater discharge, which has a significant impact on both water quality and the surrounding ecosystems. This contamination poses a significant risk to human health and the ecological dynamics of fauna and flora [1]. Untreated wastewater discharge promotes the depletion of dissolved oxygen and increases biochemical oxygen demand (BOD_5) and chemical oxygen demand (COD). The presence of total suspended solids (TSS) and nutrients such as nitrates, nitrites, and phosphates can lead to eutrophication, negatively impacting water quality and the surrounding ecosystem. These nutrients can also promote the growth of pathogens, including viruses and bacteria, and contribute to the transformation of chemicals that may cause toxicity, such as heavy metals [2].

In Colombia, the percentage of untreated wastewater is 47%. Currently, 2126 million m^3 of municipal wastewater is generated per year, which represents a worrying problem in the country [3]. The Arroyo Grande de la Sabana micro-watershed is impacted by wastewater from seven municipalities in the department, including Sincelejo, which has the largest population and therefore discharges the most pollutants [4]. This micro-watershed starts at San Antonio Hill in the municipality of Sincelejo and flows into Ciénega de Santiago Apóstol in the municipality of San Benito Abad, passing through Corozal, Morroa, Betulia, and Sincé. The main function of this water source is to receive wastewater with significant organic loads from the municipalities mentioned above. In the case of Sincelejo, the wastewater undergoes partial treatment before being discharged into the stream. However, Corozal, Los Palmitos, Morroa, Galeras, and Sincé do not have adequate treatment plants, and some have only oxidation ponds that receive little maintenance or are not in operation, leading to the direct discharge of untreated wastewater into the water source [5].

The described situation indicates contamination resulting from the discharge of untreated wastewater, leading to the deterioration of the Arroyo Grande de la Sabana. Several studies have reported high values of pollutants in this stream, such as 196.5 mg/L for BOD5, 350 mg/L for COD, and 140 mg/L for TSS, making it the most contaminated stream in the department of Sucre. Without the implementation of remediation plans, this problem will continue to worsen. Constructed wetlands are considered a viable ecological alternative for treating pollutants in wastewater, with plants playing a crucial role in the removal processes and overall system performance. Each plant used in constructed wetlands has different structural and physiological adaptations to survive in different environmental conditions. Specifically, aquatic plants have been used for treating wastewater or as bioindicators of water quality [6].

Thalia geniculata, colloquially known as peguajó or pehuajó, is a species of the genus *Thalia*, which belongs to the *Marantaceae* family, which is considered a competitive ornamental plant, as it can grow in eutrophic conditions, and its high growth rate and biomass production reflect its potentially high capacity to absorb nutrients and remove pollutants [7]. It is an herbaceous plant native to the tropics of the Americas and has a conservation status of "Least Concern" according to the IUCN Red List. This species is very common in wetlands in the Sucre department zone, located in the Caribbean Plain, Orinoquia, Pacific, Cauca Valley, and Magdalena Valley. It is easily adapted and tolerant to edaphoclimatic parameters [8]. This type of macrophyte species has been studied in phytoremediation processes with constructed wetlands. However, the potential or partial capacity of this type of species to remove specific contaminants is still unknown, particularly in tropical areas with a high diversity of natural wetlands [9]. Therefore, the objective of this work was to evaluate the pollutant removal efficiency of a constructed wetland planted with *T. geniculata* in the treatment of water from Arroyo Grande de la Sabana, with a focus on specific contaminants such as BOD_5, COD, and TSS.

2. Materials and Methods

2.1. Collection and Population Density of *T. geniculata*

The *T. geniculata* plants that were used for planting in the constructed wetland were collected from three different bodies of water located in the municipality of Sincelejo, as shown in Figure 1. The selection process involved choosing plants that were less than 1 m in height, with a healthy appearance and developed rhizomes. Sampling point 1 (P1) corresponded to El Lago (9°19′39.71″ N 75°22′49.22″ W), sampling point 2 (P2) to the SENA La Gallera headquarters (9°14′03″ N 75°24′44″ W), and sampling point 3 (P3) near the Sincelejo WWTP (9°17′34.96″ N 75°21′43.76″ W). Finally, the plants were placed in plastic baskets filled with soil and water from the collection sites for transportation and subsequent planting in the constructed wetlands. A total of 6 plants were selected for planting in the treatment wetland. Population density of the plants under natural conditions was determined at the three sampling points (P1, P2, and P3) using 1 m^2 quadrants placed every 2 m along the shoreline. The number of individuals per square meter was counted during two different climatic periods, one during the rainy season and the other during the dry season, following the methodology described in [6].

Figure 1. Location of sampling points.

2.2. Design and Construction of the Constructed Wetland

Two horizontal subsurface flow (HSSF) constructed wetlands (CWs) of rectangular shape were constructed in transparent glass material with dimensions of 0.4 m wide, 1.2 m long and 0.5 m deep. One of the wetlands served as a control experiment, that is, it did not contain vegetation, to determine its influence on the removal of pollutants. For each wetland, a 1/2″ PVC valve was installed at the opposite end of the water inlet (Figure 2). The CWs were filled to a height of 0.20 m with river gravel (boulder) of diameter less than 30 mm.

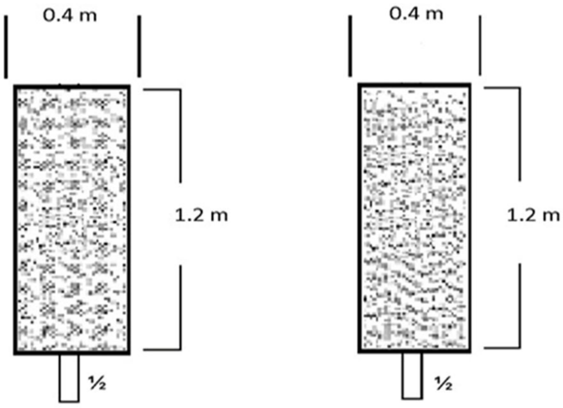

Figure 2. Constructed wetlands installation, plan view.

The CWs were manually supplied with wastewater from the Arroyo Grande de la Sabana and operated in batch mode. They were placed on a platform with a height of 0.5 m from the ground and a slope of 0.5%. Considering that boulder takes up space within the wetlands, the average approximate treatment capacity volume of each one was around 20 L.

2.3. Adaptation of T. geniculata in the CW

The plants were planted in natural filter media in one of the CWs, taking into consideration their population density in the natural environment. The filter medium was layered on top of a bed of stones. After the adaptation period of the macrophytes, 20 L of wastewater was added to each wetland per trial.

2.4. Water Sampling and Physicochemical Analysis

Samples were collected during the months of June, July, and August of 2021 in the Arroyo Grande de la Sabana, downstream of the Sincelejo WWTP, considering the climatic transition during these months known as the "Veranillo de San Juan". A combination of rainy and dry days was observed during this period [10], allowing for a more comprehensive characterization of the stream waters. It should be noted that, at the sampling point, untreated waters from the municipality were also discharged into the stream in addition to the treated waters from the aforementioned WWTP.

Once the samples were collected, the wastewater was discharged into the CWs, with inputs and outputs analyzed after three and five days of operation. Hydraulic retention times T3 and T5 were established for the vegetated treatment wetland, and C3 and C5 for the control wetland. Physicochemical analyses were conducted to monitor the CWs, including turbidity, pH, BOD5, COD, TSS, fecal coliforms (FCs), total coliforms (TCs), nitrites, nitrates, and phosphates. These analyses were performed at the Water Laboratory of the University of Córdoba, and the methods described in the Standard Methods for the Examination of Water and Wastewater [11] were followed. The analyses conducted and the corresponding methods are listed in Table 1.

Table 1. Methods for the determination of physicochemical parameters.

Parameter	Method	Unit
Turbidity	SM 2130 B	NTU
pH	SM 4500-H+ B	pH units
FCs	9222 D	NMP/100 mL
TCs	9222 B	NMP/100 mL
BOD_5	SM 5210-B; 4500 O-C	mg/L
COD	SM 5220 C	mg/L
TSS	SM 2540 D	mg/L
Nitrites	SM 4500-NO_2- B	mg/L
Nitrates	SM 4500-NO_3- B	mg/L
Phosphates	SM 4500-P E	mg/L

2.5. Organic Matter and Nutrient Removal Efficiency in CWs

The removal efficiency was determined using the following formula [12] (1):

$$E = [(So - S)/So] \times 100 \quad (1)$$

where:

E: Removal efficiency of the system, or one of its components [%]

S: Output pollutant concentration

So: Input pollutant concentration

2.6. Statistical Analysis

A statistical analysis (ANOVA) was performed on the removal variables using Infostat Software 2020 version to determine if there are significant differences between the types of wetlands (vegetated and control) and HRT.

The assumptions of normality and homogeneity of variance were tested using the Shapiro–Wilk and Levene tests, respectively, related to parametric samples. Comparisons of the results obtained regarding the efficiency of the removal of the analyzed parameters from Equation (1) were performed using a completely randomized design with a 2 × 2 factorial arrangement to evaluate significant differences. All analyses were conducted with a 95% confidence level.

3. Results and Discussion

3.1. Population Density Analysis

The samplings conducted in the three natural wetlands where *T. geniculata* was present (P1: El Lago; P2: sede SENA Gallera and P3: Near the Sincelejo WWTP) revealed an average population density of 13 plants/m^2 for this aquatic macrophyte, as presented in Table 2. Based on this result, it was possible to extrapolate and estimate the total number of plants in the CWs, considering their area of 0.48 m^2, which amounts to 6 plants.

Table 2. Population density of *T. geniculata* at sampling points.

	Density Number Plants/m^2	
	Rainy Season	Dry Season
P1	14	9
P2	15	9
P3	15	12
Average	15	10
Final average		13

It is worth noting that no scientific literature was found regarding the availability, abundance, or density of *T. geniculata* in the department of Sucre that would allow for comparison with the results obtained in this study. *T. geniculata*, a plant of the tropical dry forest, has physiological adaptations for drought tolerance. Nevertheless, during the dry season, the population density of *T. geniculata* per square meter decreased compared to the rainy season due to water loss through evaporation, high temperatures, and plant transpiration. Another study found that *T. geniculata* can reach heights of up to 3 m in areas surrounding the shoreline and edge of streams in shallow flooded sectors, although the dominant species in that area is Paspalum repens with 15% coverage, followed by *T. geniculata* with 14% coverage. In these communities, *T. geniculata* provides important shelter and food for aquatic fauna [13].

3.2. Characterization of Physicochemical Parameters

Of the physicochemical parameters evaluated prior to treatment, i.e., the characterization of the water sampled from the stream, only TSS exceeded the maximum permissible discharge limits for wastewater proposed by the Colombian Ministry of Environment and Sustainable Development, stipulated in Resolution 631 of 2015 (Table 3).

Table 3. Initial characterization of wastewater samples taken at the Bremen Bridge sampling point.

Parameter	Unit	Average Value	Standard Deviation	Maximum Permissible Values
pH	pH unit	7.24	0.22	6.00 a 9.00
Nitrates	mg/L	1.09	1.13	10
Nitritos	mg/L	0.62	0.88	2
Turbidity	NTU	87.71	82.50	-
Phosphates	mg/L	1.29	1.82	5
BOD_5	mg/L	34.22	47.69	90.00
TSS	mg/L	117.84	132.58	90.00
COD	mg/L	73.66	70.90	180.00
FCs	UFC/100 mL	2906.60	3761.56	-
TCs	UFC/100 mL	200,220.00	392,170.90	-

However, although the other physicochemical parameters analyzed comply with the established regulations, the water sampled from the stream can be considered to have a significant level of contamination in terms of organoleptic quality. It is noteworthy that, prior to the implementation of the wastewater treatment plant (WWTP) in Sincelejo, the results were different. CARSUCRE [4] reported values of up to 196.5 mg/L for BOD_5, 350 mg/L for COD, and 140 mg/L for TSS, which were used as reference values for the present study. In contrast, CARSUCRE [3] reported BOD_5 values of 84.6 mg/L and classified the water at various locations in the water body, including the sampling points for the present study, using the Scale of Classification of Water Quality Source: CONAGUA 2006, which indicates that these waters have a certain level of contamination. Based on the CONAGUA 2006 index, the average BOD_5 of 34.22 mg/L obtained in this study (Table 1) falls within range C ($30 < BOD_5 < 120$), indicating that the water is contaminated shown in Table 4.

Table 4. BOD_5 results and wáter classification by CONAGUA 2006 scale.

Station	Mar 18, 21	Classification	May 11, 21	Classification
Puente San Miguel	146.40	FC	131.70	FC
Puente Relleno Sanitario	199.95	FC	283.80	FC
Puente Vía Las Palmas	197.40	FC	59.70	C
Puente Bremen	130.50	FC	84.60	C
Puente Vía Corozal-Sincelejo	64.50	C	131.40	FC

3.3. CWs Operation Results

The average pH values measured in the wastewater prior to treatment in the constructed wetlands are 7.24 (Table 3). This coincides with the results obtained at the outlet of the wetlands, which show a basic trend. The measured results in the constructed wetland planted with *T. geniculata* at HRTs of 3 and 5 days are 7.32 and 7.39, respectively. These values are within the permitted range in Resolution 631 of 2015 from the Ministry of Environment and Sustainable Development of Colombia and are consistent with the pH value of 7.41 reported in another study that used subsurface flow-constructed wetlands planted with the same plant. [14].

In terms of nitrate removal, the treatment wetland was more efficient in removal with values of 10.52% and 33.22% for T3 and T5, respectively, compared to the control, which did not result in any removal for either HRT. When examining the relationship between treatment and retention time (p-value ≤ 0.05), the results suggest that retention time is the key factor that affects nitrate removal, considering that the value obtained on the fifth day exceeds that of the third day with a statistically significant difference (Figure 3a). The additional two days of retention time in T5 facilitated better absorption and assimilation of nitrates by the plants, which are essential for their growth. [15]. Although the nitrate results did not exceed the maximum permissible discharge limits, the removal was not considerable. These results could be due to the type of wetland, because anaerobic conditions predominate in horizontal wetlands, and nitrification is the biological conversion of NH_4^+ to NO_3^- by the action of obligate aerobic bacteria [16]. The results coincide with those of [17] who also treated wastewater in horizontal wetlands with Cyperus articulatus, reporting lower nitrate removals in contrast to other contaminants.

The same pattern as observed for nitrates was also evident for nitrites since the treatment wetland achieved removal efficiencies of values 58.19% for T3 and up to 93% for T5. Compared to the control, which obtained a removal value of 24.66% the control for T5, the differences were statistically significant (p-value ≤ 0.05). Likewise, significant differences were found in the nitrite removal efficiency for the 3- and 5-day HRTs, with significantly higher removal percentages after 5 days (Figure 3b). These results were superior to those reported by [18] who used vertical wetlands planted with Eichhornia crassipes to treat the same type of effluent. The high removal efficiency could be related to the presence of bacteria with nitrifying functions in the roots [19], which transform nitrite to nitrate through nitrification, and finally, the latter is assimilated by the plant. On the other hand, the control showed a removal of 24.67%, which can be attributed to the presence of bacteria in the wastewater that can perform removal functions, despite the absence of plant roots for bacterial attachment [20].

Regarding turbidity, there were no significant differences in the removal efficiency between the treatment wetland and the control wetland (p-value > 0.05). However, slightly higher percentages were observed in the control wetland, with removal efficiencies of 96.44% for C3 and 94.15% for C5 (Figure 3c). The high removal values observed in both wetlands could be attributed to the granular material added, which acts as a natural filter, facilitating the settling of suspended solids and particulate matter, as well as the presence of bacterial communities that play an important role in the degradation of particulate matter [19]. For the Treatment*Time interaction, the p-value is greater than the significance level $\alpha = 0.05$, indicating that there is no interaction between time and treatment and that the removal of substances or particles in the water is not more efficient over the evaluated time period.

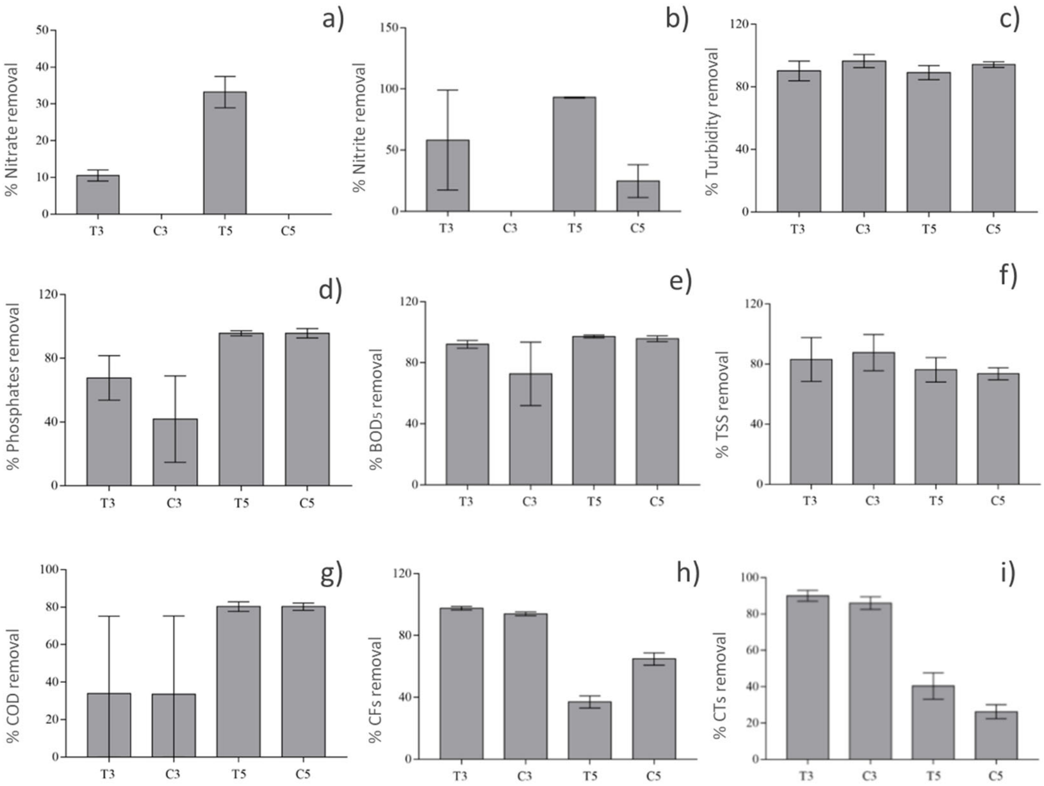

Figure 3. Removal efficiency: (**a**) Nitrates, (**b**) Nitrites, (**c**) Turbidity, (**d**) Phosphates, (**e**) BOD_5, (**f**) TSS, (**g**) COD, (**h**) FCs and (**i**) TCs.

For phosphates (Figure 3d), both wetlands achieved removal efficiencies of up to 95% at a HRT of 5 days (T3 = 67.69%; T5 = 95.66%; C3 = 41.83; C5 = 95.65%) (Figure 3d). This suggests that there is no statistically significant difference in phosphate removal between the planted wetland and the control. However, significant differences in removal efficiency were observed between the two retention times, with higher removal after 5 days. The apparently paradoxical results between the control and the treatment, are likely due to the presence of microorganisms in the process, which form biofilms and assimilate this nutrient to carry out essential processes in their growth [21]. These results indicate that both the planted wetland and the control are capable of effectively removing phosphates from the system. These results were higher than those reported by [22] who achieved a removal efficiency that varied between 15% and 49%, treating municipal wastewater in the same type of wetland using Vetiveria zizanioides as vegetation. Phosphate removal in CW is achieved through absorption by plants and transformation by microorganisms, so the superior results compared to other studies could be due to the plant species used [23].

The treatment wetland achieved a reduction of up to 90% in BOD_5. However, the ANOVA analysis (p-value > 0.05) indicated that the average removal percentages were statistically similar toe those of the control. Nevertheless, a significant difference was observed in HRT (p-value ≤ 0.05), where the removal efficiency was 92.10% and 97.27% for 3 and 5 days, respectively (Figure 3e). The reduction in BOD_5 in CWs planted with *T. geniculata* was much greater than reported in other CW systems [14], where a removal efficiency of 85.6% was obtained. The improved removal efficiency in our system can be attributed to the degradation of water compounds by bacteria attached to the plant's roots or inside the rhizome and superficial medium in an aerobic or anaerobic manner [24]. Additionally, the plants absorb nutrients from the wastewater, which promotes their growth and development [9]. It should be noted that the high availability of nutrients in the wetland resulted in significant plant growth, leading to an increase in roots and rhizomes, and ultimately promoting greater degradation and efficiency.

Both constructed wetlands were capable of reducing TSS (T3 = 83.08%; T5 = 74.19%) and (C3 = 87.60; C5 = 73.51%) (Figure 3f), with no statistically significant differences (p-value > 0.05). The removal efficiency was positive for both retention times, with the third day being the most efficient at 83.08%. However, there were no statistically significant differences in efficiency between the evaluated times (p-value > 0.05). TSS removal in the CWs occurs through physical processes of settling and filtration [25], which is confirmed by the results of the study. There were no statistically significant differences between the treatments evaluated in this research, indicating that the observed TSS removal is due to the aforementioned processes. TSS removals were not sufficient to be within the maximum permissible limits. In other studies [26] removal efficiencies greater than 90% are reported. This difference could be attributed to the filter material because the suspended matter is mainly eliminated by filtration through the granular medium [16]. On the other hand, the goal of CWs is to remove a greater amount of TSS with a longer HRT, which allows for more contact time with the substrate and plant roots. However, in this study, TSS removal on the fifth day of HRT showed a decrease in removal percentage due to an increase in the number of particles contributed by the substrate to the system. Despite this interference, the results still demonstrated the efficiency of *T. geniculata* in removing TSS, with removal values remaining within the range of up to 84% [14].

In terms of COD removal, the efficiency of the CWs did not exhibit statistically significant differences, as evidenced by the calculated ANOVA with a p-value > 0.05. However, there was a significant difference in COD removal with respect to HRT, where the elapsed time affected the average percentage of COD removed. Specifically, COD removal was 33.94% for T3 and 80.27% for T5 (Figure 3g). The removal efficiency achieved with a HRT of 5 days was similar to that reported by [27] who managed to remove 80.69% using Cyperus papyrus as vegetation in the treatment of a similar effluent. The efficiency of *T. geniculata* in removing COD is attributed to the substrate-root-microorganism complex, which facilitates the development of biofilms responsible for the biochemical reactions of contaminant transformation [9].

The test statistics for the data on CFs and CTs indicate that the presence of *T. geniculata* and HRT significantly affect the removal of these bacteria in the studied CWs. This relationship yielded a p-value < 0.05. The results demonstrate that CWs planted with *T. geniculata* can remove up to 97% of CFs and 90% of CTs present in wastewater (Figure 3h,i), which is consistent with findings from previous studies on this plant. The highest removal efficiency for CFs and CTs was observed at 3 days of HRT, possibly due to an increase in particulate matter contributed by the filtering medium used for planting, resulting in an increase in physical parameters such as turbidity and SST, which favor the growth of microorganisms. These values demonstrate the efficiency of removing or reducing the concentrations of these bacteria in wastewater. [25]. The removal of CFs achieved in this study is similar to those of [27] who reported a removal efficiency of 97%. However, the removal of CTs was higher (98%) than reported. in this studio. However, the results obtained are superior to those obtained in other studies [21], which achieved only a removal between 7% and 25%.

4. Conclusions

In conclusion, the discharge of untreated or partially treated wastewater into water resources poses a significant threat to water quality and ecosystems, leading to contamination and ecological degradation. This study has shown that HCFSSH is a viable ecological alternative for the treatment of pollutants, where *Thalia geniculata* play a crucial role in the removal of contaminants. The results of TSS and CFs/CTs were statistically significant, indicating that wetlands planted with *T. geniculata* were more effective at removing these pollutants compared to the control. On the other hand, the results of phosphates, turbidity, $BOD_{5,}$ and COD were not similar, since a better performance in pollutant removal was obtained in the wetlands planted with *T. geniculata*.

HCFSSHs planted with *T. geniculata* demonstrated that a 5-day HRT was the most effective in removing pollutants such as nitrites, nitrates, and phosphates, as well as decreasing BOD_5 concentrations.

In terms of turbidity, TSS, TCs, and FCs, a 3-day HRT was found to be the most efficient for removal in the HCFSSHs planted with *T. geniculata*.

These findings provide a basis for further research and the development of sustainable strategies for protecting and managing water resources in areas affected by contamination.

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