

Article

Treatment of Domestic Wastewater in Colombia Using Constructed Wetlands with *Canna Hybrids* and Oil Palm Fruit Endocarp

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Abstract: Untreated domestic wastewater from rural areas poses significant risks to ecosystems and human health. Constructed wetlands (CWs) are a viable alternative for this wastewater treatment, enhancing nitrogen removal using substrates as carbon sources. This process is particularly beneficial for wastewater with low carbon-to-nitrogen (C/N) ratios, making the treated water suitable for agricultural irrigation. In this study, a Horizontal Subsurface Flow CW (HSF-CW) was evaluated using *Canna hybrids* and a mixed substrate of gravel and endocarp from oil palm fruit (EOP) as a carbon source to leverage its abundance in the region. It was also determined that the effluent complies with the permissible limits set by Resolution 1207 of 2014 from the Colombian Ministry of Environment and Sustainable Development, which establishes environmental standards for wastewater treatment to ensure environmental protection and enable safe reuse in agricultural irrigation. The key parameters analyzed included organic contaminants, heavy metals, nutrients, and microbiological indicators. Removal efficiencies of up to 91%, 94%, 98%, 52%, 73%, 78%, and 75% were achieved for BOD, TSS, total phosphorus, nitrates, nitrites, ammonium, and total nitrogen, respectively, demonstrating the CW's strong performance in contaminant removal and meeting most standards for agricultural irrigation. Although the carbon source was not highly efficient, the overall system performance supports its viability for improving water quality and promoting sustainable agricultural practices in rural areas.

Keywords: constructed wetland; ornamental plants; domestic wastewater; organic carbon addition



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1. Introduction

Global demand for water is on the rise due to population growth, urban expansion, industrialization, and agricultural development, placing significant pressure on water resources [1]. Furthermore, contamination from many sources, including the discharge of untreated wastewater, industrial effluents, agricultural runoff containing pesticides and fertilizers, and improper disposal of hazardous waste, negatively impacts water quality and surrounding ecosystems [2]. Considering the UN Sustainable Development Goals (SDGs), many countries are intensifying their efforts to address the overexploitation of water sources and the effects of climate change that prolonged periods of drought [3]. Some measures include the management of unconventional water resources, such as the use of desalinated water from brackish or saline sources, as well as treated wastewater [1]. The

latter can be applied in agriculture through planned and safe irrigation practices, which involve the careful design and management of irrigation systems to optimize water use and minimize risks. Although wastewater reuse is considered a promising solution for meeting agricultural water needs, it is still inadequate in arid and semi-arid regions. This inadequacy is often due to misconceptions about the availability of water resources and the lack of necessary infrastructure and investments. [4]. A sustainable measure involves using domestic wastewater treated through constructed wetlands (CWs), which are nature-based engineering systems that optimize natural processes to improve water quality [5]. These systems are affordable, reliable, and can treat various contaminated waters, including municipal, domestic, agricultural, or industrial. Furthermore, they stand out for their low installation costs, robustness, ease of operation and maintenance, and significant potential for implementation in developing countries, especially in rural communities [6].

However, domestic wastewater poses a particular challenge due to its complex composition and low carbon/nitrogen (C/N) ratio, which is influenced by the significant presence of organic nitrogen and ammonia compared to the amount of organic carbon available in these wastewaters [7]. Various studies have found average values of total nitrogen (TN) and ammonium nitrogen ($\text{NH}_4\text{-N}$) to be 119.3 mg/L and 106.3 mg/L, respectively, with C/N ratios ranging between 2.1 and 4.4 [8,9]. These ratios are considered low, as Sun et al. [8] mention that values below 8 indicate a low C/N ratio. The low availability of carbon in domestic wastewater poses considerable obstacles to nitrogen removal efficiency, especially in environments such as horizontal flow subsurface CWs (HSF-CW), where the denitrification process is affected due to insufficient carbon sources for the microbial reduction of nitrate to nitrogen gas. Authors have suggested a C/N ratio of at least 5 to achieve complete denitrification [10]. In this biological process, heterotrophic microorganisms use nitrates ($\text{NO}_3\text{-N}$) as electron acceptors to reduce them to gaseous forms of nitrogen (N_2) and require an adequate C/N ratio to carry out the process efficiently [10,11].

A potential solution to treat low C/N ratio domestic wastewater is the addition of carbon sources that balance the C/N ratio and optimize biological processes, especially denitrification. Among the various options for carbon sources, the endocarp of the oil palm fruit (EOP) emerges as a sustainable alternative due to its abundance in the growing regions of the Department of Sucre, Colombia. EOP represents a low-cost, renewable resource that reduces waste from the palm oil industry by converting it into a valuable resource for CWs. This country stands as the fourth largest palm oil producer in the world and the leading one in Latin America, yielding an average of 3.8 tons of crude oil per hectare. Consequently, it generates a significant amount of waste, which has primarily been utilized as biomass for fuel, in road construction, or for gasification processes [12,13]. This palm oil residue contains considerable amounts of carbon, ranging from 43 to 51% by weight (%wt), making it potentially suitable as a carbon-rich substrate for denitrifying microorganisms [12,14]. Solid carbon sources are usually more effective than liquid sources since they do not require strictly controlled dosage and uniform distribution so as not to overload the system with organic content [15,16]. By using this carbon source in an HSF-CW, organisms degrade the organic material, generating electrons as byproducts. These electrons complement nitrification–denitrification, thus improving the performance of the treatment wetland [17,18]. In addition, the endocarp has a porous structure that allows water circulation and provides a habitat for biofilm generation.

To date, there is limited information available in the literature regarding the application of this type of substrate as a carbon source in HSF-CWs for contaminant removal during real-world domestic wastewater treatment, specifically, the effectiveness of EOP as a sustainable alternative for enhancing nitrogen removal in HSF-CWs treating domestic wastewater and its potential for producing effluent suitable for agricultural irrigation remains unexplored. This study aimed to evaluate the performance of an HSF-CW for the treatment of domestic wastewater in San Marcos, Sucre Department, Colombia. The HSF-CW utilized *Canna hybrids* as vegetation and a mixed substrate of gravel and EOP as a carbon source. Specifically, this research sought to

- Assess the effectiveness of the HSF-CW in removing contaminants, particularly organic matter and nitrogen, from domestic wastewater;
- Investigate the potential of EOP, an abundant regional waste product, as a sustainable carbon source for enhancing nitrogen removal in the HSF-CW;
- Determine if the treated effluent meets the permissible limits established by Colombian regulations for safe reuse in agricultural irrigation.

2. Materials and Methods

2.1. Study Site and Design of CWs

The study was conducted in the Colombian Caribbean, specifically in the municipality of San Marcos, located in the department of Sucre at coordinates 75°8'38.23" W longitude and 8°40'11.56" N latitude. The area primarily consists of floodable terrain within the Momposina Depression, ranging in elevation from 13 to 25 m above sea level. The climate is characterized as warm and humid, with significant rainfall occurring from April to November and a dry season prevailing from December to March, with temperatures occasionally exceeding 32 °C [19]. The treatment wetland was evaluated over a period of 120 days during its start-up and adaptation phase.

The HSF-CW was designed and constructed taking into consideration the parameters of the model for the design of horizontal CWs established by Peña-Varón et al. [20], as shown in Equation (1):

$$S_A = DFR \times \frac{(L_n C_o - L_n C_e)}{K_T \times d \times n}, \quad (1)$$

where

S_A = Superficial Area (m²);

DFR = Design flow rates (m³/d);

C_o = BOD concentration of the influent (mg/L);

C_e = BOD concentration of the effluent (mg/L);

L = Length (m);

K_T = First-order reaction kinetic constant (1/days);

d = Wetland depth (m);

n = Filter porous-medium.

2.2. Type of Substrate, Vegetation and System Configuration

Gravel was selected as the filter material due to its widespread use in similar applications, offering good porosity for microbial colonization and effective filtration of contaminants from wastewater. Additionally, gravel is readily available at a low cost and is chemically inert, ensuring no adverse reactions or leaching of toxic substances into the treated water [21]. The other substrate utilized was EOP, obtained from a palm oil processing plant located in the vicinity of San Marcos municipality, Colombia. Oil palm fruit is a significant source of edible vegetable oil; however, the oil extraction process generates a considerable amount of waste, which, if not managed appropriately, can have a negative environmental impact. The endocarp is the hard shell that covers the seeds and represents around 30–40% of the fruit's weight. This material has high density and high carbon content, with 29% cellulose, 47.4% holocellulose, and 53.4% lignin [22–24]. Another advantage of utilizing this waste as a substrate is that the associated costs with collection and logistics are low, as this residue is readily available in the region due to large-scale oil palm cultivation throughout the year.

For this study, *Canna hybrids* vegetation was chosen based on three fundamental pillars: its attractive ornamental qualities, optimal adaptation to warm climates, and the ecological advantages it brings to the system. This species is native to tropical and subtropical regions, making it well-suited to the experimental site. Its extensive root system promotes water filtration and purification. Other factors, such as species availability in the area, survival, tolerance, and productivity under flooded conditions, were also considered [5].

The wastewater treatment system (Figure 1) was located taking into account that the distance between the house and the Imhoff tank should be at least 1.5 m, that the subway water well should be at least 15 m away from the system, and that it should not be located in a place with vehicle, animal or human traffic. The wetland was constructed with a width of 2 m, a length of 6 m, a depth of 0.6 m, and a 1% bottom penetration, as shown in Figure 1a. Since the wetland location is susceptible to flooding due to the effects of climate change, a small dike was formed around the wetland with the material resulting from the excavation. Before adding them to the system, both filtering materials were washed and sieved, obtaining two sizes of aggregate for the gravel. The first size was 1 inch or larger, which was used for the first layer. For the third layer, gravel with an average diameter smaller than ½ inch was used. EOP was added in the middle layer (Figure 1b). This configuration was chosen for efficient water flow in the wetland, with uniform distribution, and to benefit from denitrifying microorganisms without interfering with the nitrification process in the upper aerobic zones. A geomembrane was employed to waterproof the soil, which was then filled with two layers of rocky filtering material and an intermediate layer of organic material.

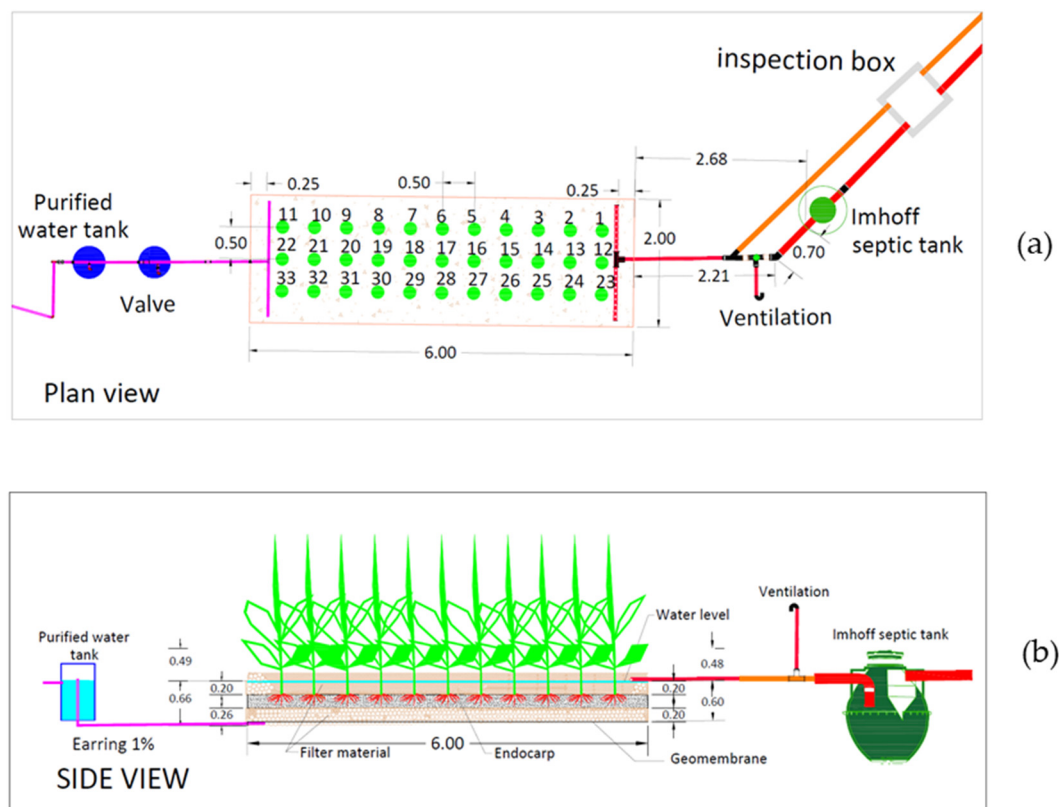


Figure 1. Schematic diagram of the CW: (a) plan view, (b) sectional view.

A notable feature implemented is the hermetically sealed Imhoff tank with a capacity of 1000 L, which minimizes the ingress of sedimentable or particulate material into the system. Furthermore, a gas exhaust pipe was installed.

The Equation (2) was used to calculate the number of plants (N_p) to be established in the wetland:

$$N_p = \left[\left(\frac{L}{dm - m} \right) \left(\frac{W}{dm - m} \right) \right] - 1 \quad (2)$$

where

$dm - m$ = Distance between floors (m);

L = Total length (m);

W = Minimum width (m).

Considering that the wetland design showed a length of 6 m, a width of 2 m, and the distance between plants was 0.5 m, the number of planted plants was 33, as shown in Figure 1a.

2.3. Physical–Chemical Analysis of Samples

Once the system was established, four specific and composite water samples were taken in the influent and four in the effluent, with an average period between samples of 7 days. Chemical oxygen demand (COD), Biochemical Oxygen Demand (BOD), total suspended solids (TSS), fats and oils (F&O), total phosphorus (TP), nitrites (NO₂-N), nitrates, ammoniacal nitrogen, and total nitrogen were determined for each sample. These parameters were measured using a portable multiparameter Hanna model HI98121 (HANNA® instruments, Woonsocket, RI, USA).

To evaluate the use of treated water in agricultural irrigation, a specific and, at the same time, composite sample was taken at the exit of the wetland following the IDEAM methodology.

2.4. Evaluation of Plant Development

In order to evaluate the adaptation and development of the wetland plants during the bioremediation process, the number of shoots, number of flowers, leaf length, stem thickness, and plant height at 0, 15, 30, 49, 70, and 84 days after treatment were counted [25]. Taken from the field data, the relative growth rate of each plant was calculated by taking the difference in plant height and dividing it by its respective time period. The results of the plant measurements were grouped into three sectors with an equal number of individuals each; sector 1 was composed of the plants closest to the entrance of the wetland, the second sector was composed of the plants in the middle zone, and sector 3 was composed of the plants closest to the exit.

2.5. Statical Analysis

For the statistical analysis of the results, Student's t-test using Statgraphics Centurion XVI was employed. The null hypothesis (H_0) stated that there are no significant differences in the values of the physicochemical parameters measured at the inlet and outlet of the CW, while the alternative hypothesis (H_1) posited that significant differences exist between these measurements. With 4 data points per sample ($n = 4$), a degree of freedom of 3, and a significance level of 5%,

$$H_0 : \mu_0 = 0 \text{ vs. } H_1 : \mu_0 < 0, p \text{ value} > 5\% H_0 \text{ is accepted, } p \text{ value} < 5\% H_0 \text{ is rejected}$$

3. Results

3.1. Domestic Wastewater Characterization

Samples were taken from the Imhoff tank to perform the characterization of domestic wastewater before feeding it into the treatment wetland. The values are shown in Table 1.

Table 1. Physicochemical characterization of domestic wastewater before CW feeding.

Parameters	Unit	Results
pH	pH unit	7.17
COD	mg/L	245 ± 14.7
BOD	mg/L	134 ± 7.28
TSS	mg/L	54.4 ± 2.82
SedS	mg/L	1.5 ± 0.59
F&O	mg/L	14.13 ± 4.72
NO ₂ -N	mg/L	0.026 ± 0.03
NO ₃ -N	mg/L	5.18 ± 0.37
NH ₄ -N	mg/L	74.35 ± 4.29
NT	mg/L	161.59 ± 26.34
TP	mg/L	4.02 ± 0.34

The values at the inlet of the Imhoff tank were 347.72 ± 7.53 mg/L for COD, 92.20 ± 4.33 for TS, and 4.69 ± 0.78 for SedS. These were removed at a rate of 29.54%, 41.10%, and 68%, respectively, during the Imhoff tank pretreatment.

The results of the physicochemical characterization showed that the concentration of contaminants present in the domestic wastewater studied falls within typical ranges [26]. Therefore, treatment is required to remove the contaminants before they can be used for agricultural irrigation or discharged into water bodies.

As observed, although the values of COD and BOD indicate a certain degree of organic matter contamination, they are low compared to the TN, resulting in an average C/N ratio of 1.5. The observed low C/N ratio of 1.5 underscores the critical need for supplemental carbon in the HSF-CW to facilitate effective denitrification. Without sufficient carbon, the microbial community responsible for converting nitrate to nitrogen gas would be limited, potentially hindering the overall nitrogen removal performance of the system.

The pH remained neutral at the inlet of the treatment wetland, with an average value of 7.17. Therefore, no adjustments were necessary, as this neutral pH provided suitable conditions in the environment for microbial development. Regarding the SedS, samples were collected without any mixing within the Imhoff tank, resulting in an average value of only 1.5 mL/L. This suggests that before entering the wetland, this tank had fulfilled its sedimentation function.

3.2. Performance Evaluation of HSF-CW

3.2.1. pH Values in the CW

Figure 2 shows the input and output pH values. Overall, the pH remained neutral throughout the operating period, with average inlet values of 7.1 in the first three months and 6.85 in the last month, slightly lower but still within the neutral range. At the outlet, the pH decreased to 5.78 in the third month.

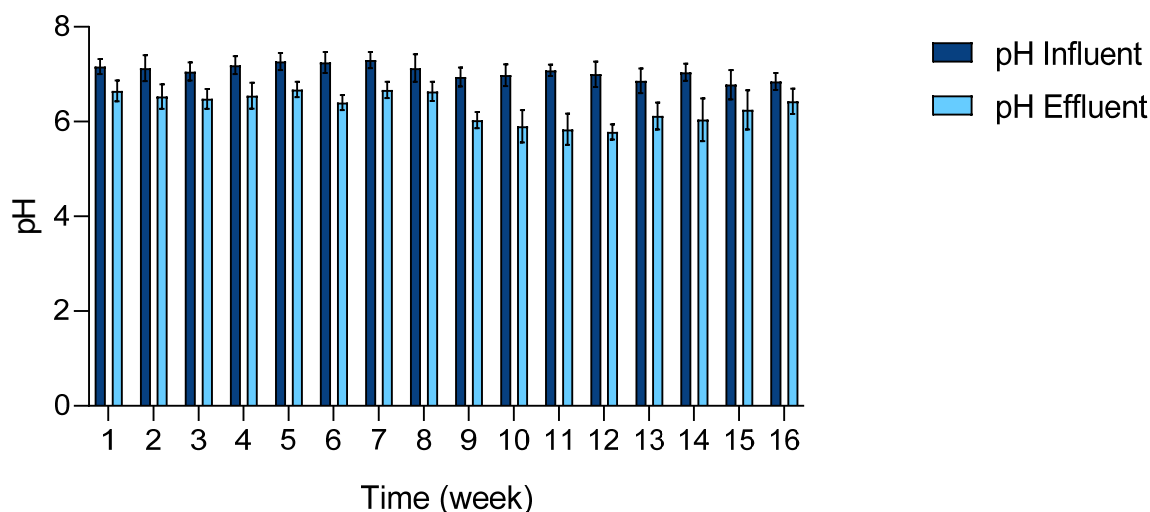


Figure 2. pH values in the CW.

The temporary dip in effluent pH to 5.78 in this month could be attributed to a surge in organic loading, potentially due to increased wastewater inflow or changes in its composition. This organic matter influx likely stimulated microbial activity, leading to the production of organic acids and a subsequent decrease in pH. However, the system's resilience is evident in the pH recovery to 6.43 in the fourth month, suggesting that the microbial community adapted to the increased organic load and restored the pH balance.

In general, the pH values of the inlet and outlet wastewater were conducive to the metabolism of microorganisms, ensuring microbial activity in the CW system and facilitating improved COD and nitrogen removal rates [27].

3.2.2. BOD and TSS Concentrations in the CW

The influent and effluent concentrations, as well as the removal efficiencies of organic matter and TSS, are shown in Figure 3.

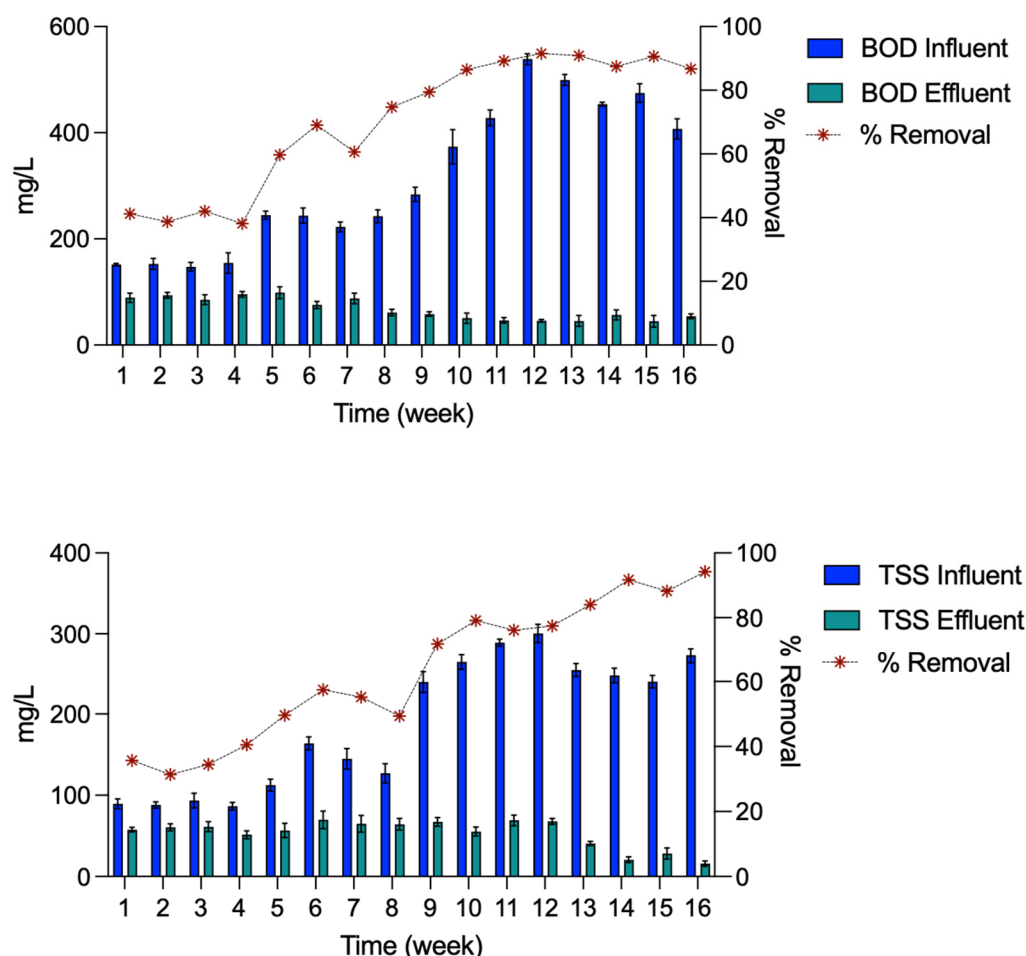


Figure 3. BOD and TSS values in the operation of the CW.

Regarding the organic matter removal efficiency represented as BOD, the average concentrations in the influent and effluent were 333.9 mg/L and 71.4 mg/L, respectively, with a statistically significant difference (p -value = 0.03). During the first two months of operation, both BOD and TSS values were low compared to the final weeks. In the second month, the average BOD value was 242.53 mg/L, increasing to 548 mg/L by week 12. This increase in organic concentration was reflected in the TSS, which rose from 127 mg/L in week 8 to 300.58 mg/L in week 12. This behavior can be attributed to the variable composition of domestic wastewater, which can change due to seasons, habits, or special activities. Notably, this increase between months 2 and 3 coincides with the December holiday period, during which various festive activities could have altered the typical influent wastewater concentration. Increased food consumption, social gatherings, and potentially higher use of detergents or cleaning products could have contributed to the elevated organic load in domestic wastewater [28].

With the BOD values and the EOP substrate as an additional carbon source, the organic carbon values in the wastewater varied, with C/N ratios of 0.7 in month 1, 1.3 in month 2, 2.8 in month 3, and 1.6 in month 4. However, even with the increase in organic concentration, the C/N ratio in the system remained below 5. Conversely, the effluent BOD values decreased in the final weeks, suggesting that despite the added carbon source, there was no increase in the outlet BOD. Removal efficiencies of up to 91.51% for BOD and 94.17% for TSS were achieved. These results are similar to those obtained by Jong and Tang [29],

who used Palm Kernel Shell as a substrate material in a Vertical Flow CW, achieving BOD removals above 90% without increasing organic matter at the system outlet. It is suggested that the available organic matter was utilized by both anaerobic microorganisms in deeper zones and denitrifying bacteria for the denitrification process. The removal of BOD and TSS increased over time due to microbial adaptation, as the heterogeneous surface and porous structure of this type of substrate can provide more favorable habitats for microbial enrichment, enhancing organic matter degradation [30]. During the first few weeks, it is common for biological treatment systems, such as constructed wetlands, to undergo a stabilization period in which the microbial community adapts to the reactor environment and a robust biofilm develops on the substrate. The surface heterogeneity and porous structure of the EOP not only provided a favorable physical environment for microbial colonization but also facilitated the retention of organic particles, increasing the efficiency of organic matter degradation as the biofilm matured. Other factors contributing to BOD removal included filtration resulting from bacterial fixation in the filter material and plant roots, sedimentation due to the 4-day hydraulic retention time, and the presence of roots that reduced water velocity, allowing organic matter particles to precipitate [31]. The layered design of the system with gravel and EOP allowed the creation of redox gradients that optimized the sequential degradation of organic matter. The upper zone, more aerobic, favored the oxidation of organic compounds, while the deeper, anaerobic zones facilitated processes such as fermentation and methanogenesis. This stratification was key to preventing system collapse due to organic matter accumulation, ensuring continuity in BOD and TSS removal. Additionally, the action of anaerobic and aerobic microorganisms around the roots played an important role [32]. Overall, the average removal efficiency was 71%, consistent with the 60–90% BOD removal efficiency reported by Sandoval et al. [33] and Vergara et al. [34] in treatment wetlands.

3.2.3. Removal of Nitrogen, TP, and F&O, and the Influence of Carbon Source

In Figure 4, the behavior of nitrogen components in the inputs and outputs of the CWs is observed. The removal efficiency of ammonium and nitrites reached a notable 78% and 72.99%, respectively, during the first month, attributable to the establishment of nitrification processes in the wetland. With the maturation of the microbial community, ammonium was efficiently converted into nitrites and nitrates. However, as a horizontal flow wetland, the conditions of saturation and depth favor an anaerobic environment with oxygen depletion, limiting nitrification due to its redox sensitivity [35,36]. For this reason, from the second month onwards, a decrease in ammonium removal efficiency was observed, with values of 41.32% and 31.37% for the second and third months, respectively, suggesting a temporary imbalance in the nitrification process. The high initial ammonium removal efficiencies seem to be primarily due to nitrification, facilitated by the availability of DO in the incoming wastewater, as well as adsorption by substrates and ion exchange. This behavior is similar to that reported by Yuan et al. [27], who evaluated woodchips as a source of sustained-release carbon in a subsurface flow CW, observing average removals of 20% in the initial days, followed by a slight decrease and subsequent increase.

A key factor in oxygen depletion by the end of the first month of experimentation was the release of organic matter from the carbon source and the activity of heterotrophic microorganisms. Dissolved oxygen (DO) could be consumed during the oxidative decomposition of COD, limiting its availability for nitrification. In these cases, an increase in the COD/N ratio may reduce the removal efficiency of $\text{NH}_4\text{-N}$ and $\text{NO}_2\text{-N}$ [37–39]. This behavior coincides with the increase in organic matter at the system inlet from week 5 onwards, with a significant rise in BOD in the third month, where the oxidation of organic matter competed for DO with bacteria responsible for ammonium degradation. Chen et al. [40] explored the influence of carbon addition (glucose and sodium acetate), finding that the removal efficiency of ammonium and nitrite decreased significantly with carbon supply, even with higher values in the effluent.

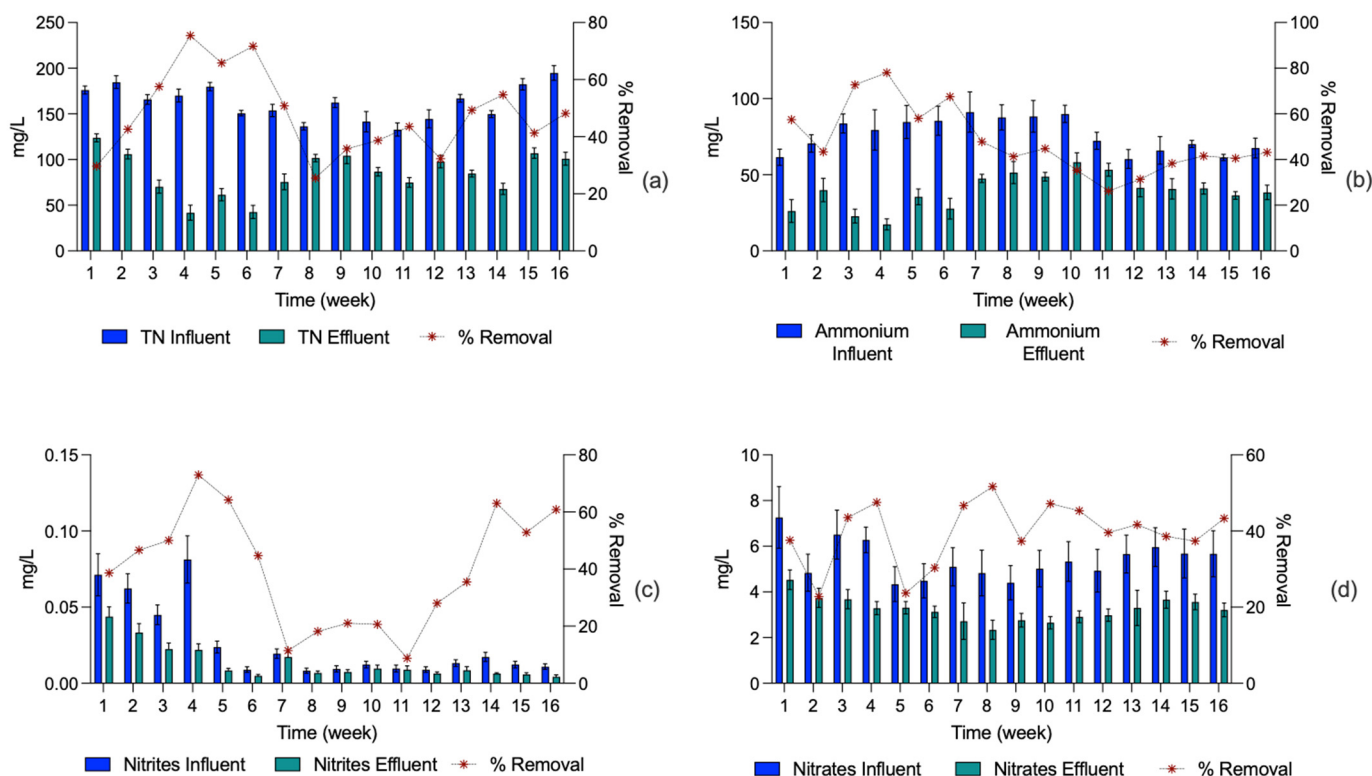


Figure 4. Values of nitrogen components in influent and effluent: (a) TN, (b) ammonium, (c) nitrites, and (d) nitrates.

Other investigations have also observed a reduction in the $\text{NH}_4\text{-N}$ removal rate in the presence of external carbon sources, attributable to the incremental oxygen consumption under inadequate DO conditions, such as in horizontal flow systems. The variation could be due to the diverse microenvironments within CWs, influenced by the concentration of pollutants and the operating conditions [41,42].

Dissimilatory nitrate reduction to ammonium (DNRA) is another factor that can affect ammonium removal efficiency in the presence of external organic carbon. This is a process where nitrate is used as an electron acceptor and reduced to ammonium under anaerobic conditions. While DNRA can contribute to nitrogen removal, it can also lead to increased ammonium levels in the effluent, potentially offsetting the benefits of other nitrogen removal processes like nitrification and denitrification. Shen et al. [43] evaluated starch/PCL mixtures as a solid carbon source in a CW, observing ammonium accumulation in the effluent despite a good denitrification rate due to certain bacteria utilizing nitrate as an electron acceptor under anaerobic conditions and with available organic carbon. Zhong et al. [36] explain that ammonification can also reduce the removal efficiency of $\text{NH}_4\text{-N}$ and nitrites, as when using microalgae to treat wastewater with a low C/N ratio in their study, the ammonium values in the effluent were higher compared to wetlands without added carbon, due to the release of organic nitrogen compounds from the carbon source.

In our study, while the potential for DNRA cannot be entirely ruled out, the ammonium concentrations in the effluent did not exceed those in the influent. This observation suggests that DNRA, if present, was not the dominant process affecting ammonium removal in our system. The absence of excessive ammonium accumulation indicates that other processes, like nitrification, denitrification, and potentially anammox (anaerobic ammonium oxidation), were more influential in shaping the nitrogen dynamics of the HSF-CW. The partial recovery of ammonium removal efficiency in the fourth month (43.16%) suggests system resilience. In the first weeks of operation, the microbial community may have experienced changes in its composition and activity while adapting to the new environment and pollutant loads, partially restoring the nitrification processes. The recovery from week

12 could be due to other anaerobic processes, such as anammox, since, at this point, the BOD input was higher, as was its removal efficiency, suggesting lower DO in the system. Martínez et al. [17] used corn stover as a carbon source in a CW, highlighting that anammox played an important role in ammonium removal by promoting suitable anaerobic zones for these microorganisms due to the lower oxygen availability. Huang et al. [44] varied the COD/N ratio using glucose and sodium acetate in a subsurface flow CW, finding that the addition of carbon may have promoted ammonium removal efficiency through the anammox process. Overall, despite the low removals, the system showed recovery trends in ammonium degradation, possibly due to the active microbial growth, reproduction, and adaptation with this carbon source, which strengthened microbial assimilation from month 4 onwards.

The efficiency in nitrate removal remained moderate and stable throughout the four-month study, fluctuating between 43.34% and 51.68%, with averages of 37.85%, 38.09%, 42.35%, and 40.25% for months 1, 2, 3, and 4, respectively. Despite fluctuations in influent and effluent concentrations and the low values observed in the nitrification process during the initial weeks of operation, the results suggest that the denitrification process was generally effective over the 16-week period. It can be observed that in week 4, a nitrate removal efficiency of 47.53% was achieved; however, it decreased to 23.70% in week 5. This decline was likely due to the adaptation of microorganisms to the medium during these initial periods and, more importantly, because the organic concentration was still low, with BOD values below 200 mg/L and a C/N ratio of approximately 0.7 during this period, which was low even with the addition of EOP. However, following a decrease in week 5, nitrate removal efficiency recovered, coinciding with an increase in BOD above 400 mg/L in week 3 and reaching values above 200 mg/L during month 2, peaking at 538.65 mg/L in week 12, with C/N ratios of up to 2.8. Along with the EOP carbon source, the system provided sufficient organic carbon to achieve more stable denitrification during the later weeks. However, as anticipated, nitrate removal did not exceed these values because the C/N ratio remained below 5, resulting in nitrate removal efficiencies below 60%. Compared to other studies, the addition of external carbon sources typically enhances nitrate removal. For instance, Yu et al. [10] used rice husk as a carbon source and achieved nitrate removal efficiencies of up to 96% in short periods, with C/N ratios of 1, 3, and 5 in wastewater. However, Wang et al. [39] only managed to remove 65% of nitrates with a C/N ratio of 3, while Chen et al. [40] found nitrite accumulation and nitrate residue with a COD/N ratio of 4, indicating that the carbon source (glucose) was insufficient for denitrification at these concentrations. These results indicate that both the quantity and quality of carbon supply significantly influence the degree of denitrification.

Another relevant factor is the position and height of the carbon source [43]. Yuan et al. [27] observed that the closer the filling position of the woodchips is to the inlet, the higher the denitrification efficiency of the system. Additionally, in systems with solid carbon sources, the denitrification rate largely depends on the biodegradability of these sources. Greater biodegradability translates into a higher denitrification rate. The biodegradability of solid carbon sources depends on their characteristics, and significantly higher nitrate removal rates have been reported for labile carbon media (corn cobs, wheat straw, and green waste) compared to softwood and hardwood media [43,45]. The EOP, considered a hard lignocellulosic material, is more resistant to microbial degradation due to its lignin and cellulose-rich composition compared to more labile materials [29]. This characteristic may have influenced the denitrification values obtained.

The removal efficiency of TN showed similar behavior to that of ammonium and nitrites during the first weeks of operation, reaching removal values between 65.83% and 75.42% between weeks 4 and 6. This reflects effective removal through nitrification and denitrification processes during this period. However, after this period, the TN removal efficiency significantly dropped in the second month (25%), which correlates with the low removal efficiency of ammonium and nitrites during this time. In this week, BOD removal was 74.72%, and although the C/N ratio was still low at 1.33, intense microbial activity was

evident in the removal of available organic matter and the consequent consumption of DO. Nevertheless, TN removal partially recovered in the third and fourth months, achieving removals between 48.18% and 54.69%, likely due to the treatment system's adaptation to the new anaerobic conditions, as mentioned previously. The removal values in the first weeks are related to the availability of a carbon source, as suggested by Yu et al. [10], who achieved TN removals of 78% in the initial evaluation stage using rice husk as a carbon source with a C/N ratio of 4, attributing these removal values mainly to the sufficient carbon source to sustain the denitrification process. The low removal in week 8 is attributed to the lack of abundance of denitrifying bacteria, possibly due to the low organic carbon content during this period [10,46], as other studies have found that the release of the carbon source begins to decline over time; thus, an important factor to consider is the rate of release of biodegradable carbon [38,40]. As a horizontal flow system, TN removal also depends on the amount of DO available in the medium, and the addition of a carbon source can alter these concentrations, as mentioned by Wang et al. [39], where spray aeration was used to improve TN removal (78%) and compensate for the decrease in DO due to the additional carbon source. TN removals can decrease by up to 40% due to the inability to provide both aerobic and anaerobic conditions for nitrification and denitrification [39,47]. Generally, TN removal will also depend on different pathways, including volatilization, ammonification, nitrification/denitrification, plant uptake, and adsorption in the matrix [48].

Overall, regarding the addition of EOP as a carbon source in the treatment wetland to enhance denitrification, the results did not meet expectations, as indicated by non-statistically significant differences in influent and effluent concentrations with values of $p = 0.162$ for ammonium and $p = 0.376$ for nitrate. However, using EOP in treatment wetlands remains an environmentally viable alternative. EOPs are abundant agro-industrial wastes generated in Colombia, and if improperly managed, they can contribute to pollution.

Figure 5 displays the input and output values of TP, as well as the removal rates achieved in the CWs. The average removal efficiency of total phosphorus was 75% with a p -value = 0.029, reflecting statistically significant differences between influents and effluents. These results agree with those reported by Rodriguez-Dominguez et al. [49], who reviewed 169 documents from 20 countries, finding average efficiency for total phosphorus between 30% and 84%. Phosphorus removal in treatment wetlands is primarily due to plant uptake through roots and retention by filter media. As mentioned in various studies, this TP removal is not always consistent with nitrogen removal [31,50]. The results also show that when the phosphorus concentration in the influent decreases, the removal efficiency also decreases. In all cases of the study, the concentration in the effluent was less than 5 mg/L, which indicates that the subsurface horizontal flow wetland evaluated is a viable alternative for the removal of TP.

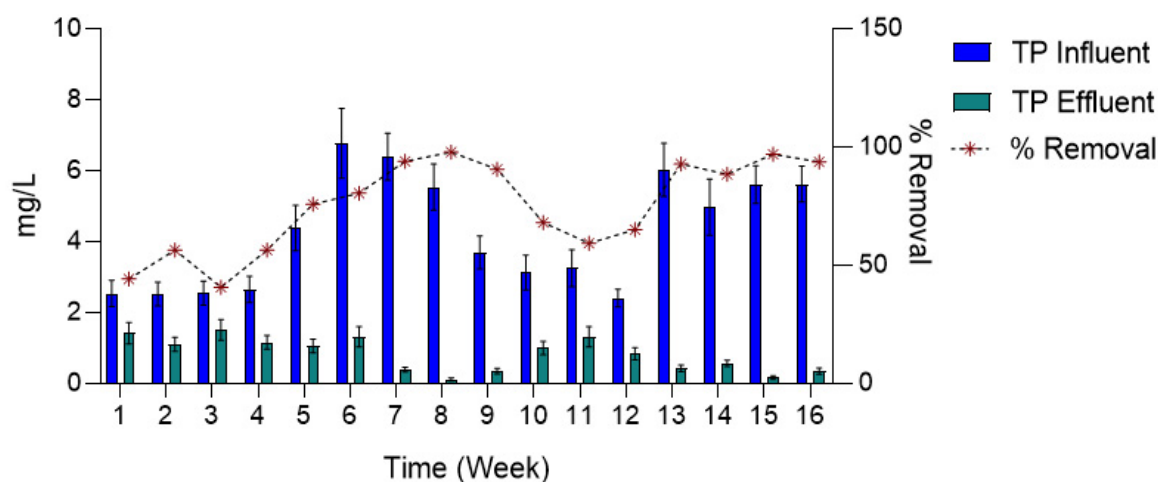


Figure 5. TP concentrations and removal.

Regarding the removal of F&O (Figure 6), an average removal of 50% was obtained, reaching removals of up to 75.47% in the third month of operation. The physicochemical nature of fats and oils affects their availability for biodegradation. They are consistently present in domestic wastewater due to the use of butter and vegetable oils in kitchens. Emulsions of fats and oils, which are more water-soluble, may be more accessible to microorganisms. The removal of F&O is enhanced by the formation of biofilm on the substrate surface, providing an ideal environment for lipolytic bacteria. These factors could have influenced F&O removal; however, more time is required for the wetland to adapt and mature to achieve higher removal efficiencies. In other studies on CWs treating domestic wastewater, removal efficiencies of up to 91% have been reported [51,52].

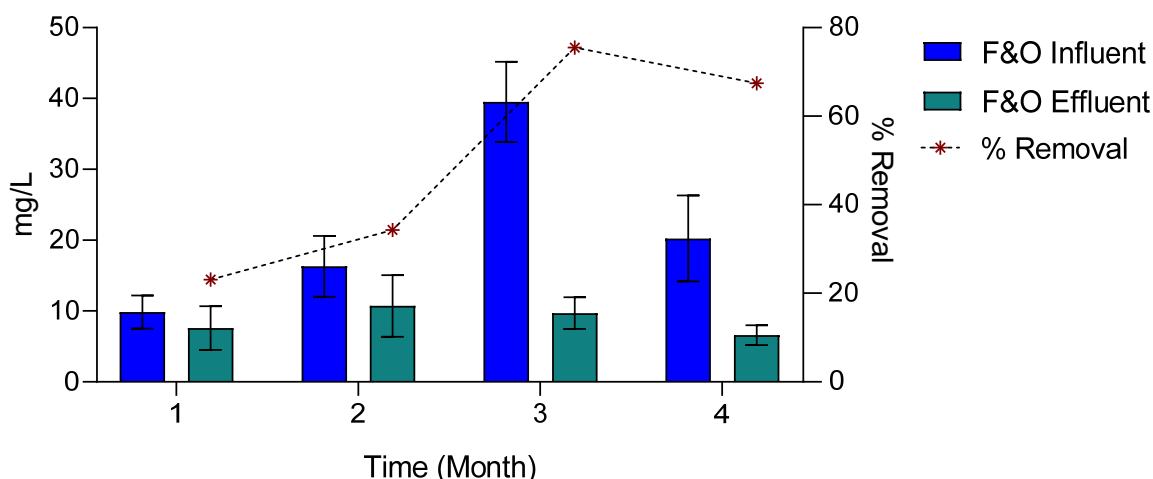


Figure 6. F&O concentrations and removal.

3.3. Plant Development

The plants exhibited significant adaptation and development over time, with biomass gains observed in all cases. This increase was most notable in the middle zone of the wetland (sector 2), where a higher number of shoots, leaves, and flowers were recorded, along with greater overall plant heights and stem lengths. The plants in the middle zone displayed increased competition for sunlight, resulting in a tendency to allocate more resources to increasing stem length rather than thickness [53], as evidenced by the data presented in Table 2. Moreover, statistically significant differences were observed in the number of shoots per plant, plant heights, stem lengths, and stem thicknesses, with p values less than 0.05 in the analysis of variance. Conversely, no statistically significant differences were found in the number of leaves and flowers per shoot, as indicated by p -values greater than 0.05.

Table 2. Average plant growth rate by sector in the CW.

	Number of Shots per Plant	Shoot Height	Number of Sheets	Length of Stems	Stem Thickness	Number of Flowers
Sector 1	1.8	0.8	2.8	0.4	0.1	0.1
Sector 2	5.4	1.4	3.7	0.8	0.1	1.2
Sector 3	3.3	1.1	3.2	0.6	0.3	0.6
p -value	0.002	0.014	0.096	0.048	0.47	0.056

The superior biomass production observed in plants of sector 2 may be attributed to the purification process within the CW. As wastewater flows, its pollutant load decreases. Consequently, plants located closer to the inlet are exposed to higher pollutant concentrations, potentially hindering certain physiological processes. Conversely, plants near the outlet encounter improved water quality but lower nutrient concentrations essential for

growth. Therefore, the intermediate sector presents an optimal balance of nutrients and reduced toxic substances, fostering enhanced plant development. These findings align partially with those of López et al. [54], who observed similar growth patterns among *Canna hybrids* in CWs, with individuals closer to the outlet exhibiting marginally greater growth than those near the inlet. In addition to their aesthetic and ecological function, *Canna hybrids* played a crucial role in contaminant removal. The roots of this plant not only served as physical support for biofilm formation but also released oxygen into the rhizosphere, creating oxygenated microzones that promoted the activity of aerobic bacteria responsible for the initial degradation of organic matter. This release of oxygen in the root zone may also have facilitated the conversion of organic compounds into forms more easily degradable by anaerobic microorganisms in the lower layers of the substrate.

3.4. Treated Wastewater for Irrigation

The removal results in the treatment wetland with the use of EOP were good in general terms and the effluents meet most of the parameters to be used as agricultural irrigation according to Colombian regulations, as seen in Table 3.

Table 3. Results of parameters for the use of treated wastewater in irrigation according to Resolution 1256 of 2021 of the Ministry of Environment and Sustainable Development, Colombia.

Parameter	Unit	Results	Quantified Limit	Allowed Value	Accordance
Total phenols	mg/L	<0.002	0.002	1.5	FULFILLS
Total hydrocarbons	mg/L	0.9	0.20	1.0	FULFILLS
Free cyanide	mg CN-/L	<0.02	0.020	0.2	FULFILLS
Chlorides	mg Cl-/L	53.2	19.9	300	FULFILLS
Fluorides	mg F-/L	<0.161	0.161	1.0	FULFILLS
Sulfates	mgSO ₄ /L	9.58	8.9	500	FULFILLS
Mercury	mg Hg-/L	0.0016	0.0005	0.002	FULFILLS
Sodium	mg Na-/L	161	0.25	200	FULFILLS
Zinc	mg Zn/L	<0.050	0.05	3	FULFILLS
Total residual chlorine	mg Cl ₂ -/L	<0.047	0.047	<1.0	FULFILLS
Nitrate (NO ₃ -N)	mg/L	2.5	0.400	5.0	FULFILLS
Aluminum	mg Al-/L	<0.046	0.046	5.0	FULFILLS
Arsenic	mg Ar-/L	0.008	0.005	0.1	FULFILLS
Beryllium	mg Be-/L	<0.05	0.05	0.1	FULFILLS
Cadmium	mg Cd-/L	<0.001	0.001	0.01	FULFILLS
Nickel	mg Ni-/L	<0.005	0.005	0.2	FULFILLS
Cobalt	mg Co-/L	<0.05	0.05	0.05	FULFILLS
Copper	mg Cu-/L	<0.02	0.02	1.0	FULFILLS
Chrome	mg Cr-/L	<0.04	0.04	0.1	FULFILLS
Iron	mg Fe-/L	22.2	0.131	5.0	FAILS
Lithium	mg Li-/L	<0.10	0.1	2.5	FULFILLS
Manganese	mg Mn-/L	0.120	0.05	0.2	FULFILLS
Molybdenum	mg Mo-/L	<0.05	0.05	0.07	FULFILLS
Lead	mg Pb-/L	<0.00680	0.0068	5.0	FULFILLS
Selenium	mg Se-/L	0.1	0.0100	0.02	FAILS
Vanadium	mg V-/L	<0.02	0.02	0.1	FULFILLS
Boron	mg Br-/L	<0.25	0.25	0.4	FULFILLS
Thermotolerant Coliforms (Fecal)	NMP/100 mL	120	1.8	100,000	FULFILLS
<i>Salmonella</i>	NMP/100 mL	<1.8	1.8	100	FULFILLS
Fecal <i>Enterococcus</i> sp./ <i>Streptococcus</i>	NMP/100 mL	<1.8	1.8	100	FULFILLS
Helminth Eggs	Eggs and larvae/L	0	0	1	FULFILLS

The effluent derived from treatment in the HSF-CW using EOP produced results that mostly comply with Resolution 1256 of 2021 from the Ministry of Environment and Sustainable Development of Colombia. Organic components (total phenols, total hydrocarbons),

inorganic components (free cyanide), and major ions (chlorides, fluorides, sulfates) showed values well below the permitted limits, except for total hydrocarbons, which showed values close to the permissible limit. These results indicate that our system effectively removed various organic and inorganic compounds present in domestic wastewater, aligning with the findings of previous studies on the removal of such compounds [55]. Regarding heavy metals, most comply with the permitted values except for iron and selenium, which were present in low concentrations but above the limits. Domestic wastewater may contain elevated levels of iron due to pipe corrosion and the use of cleaning products containing this metal. Selenium, on the other hand, can be present due to certain chemicals and food additives. These concentrations can be reduced in the CW by improving redox conditions, as anoxic conditions can favor the solubilization of iron and selenium, making their removal more difficult [56]. Additionally, further studies on the EOP regarding its quantity and position can be conducted to improve the absorption and retention of these metals.

The results for Thermotolerant coliforms (Fecal coliforms), *Salmonella*, Fecal *Enterococcus* sp./*Streptococcus*, and helminth eggs in the effluent of the wetland are well below the required limits. This compliance is due to the efficient removal of organic load carried out by the studied CW. The reduction of organic matter is crucial as it feeds and sustains microorganisms. As organic matter decreases, so does the number of Fecal coliforms. This phenomenon has been corroborated by previous studies, such as Vergara et al. [34], which highlight the direct relationship between organic load and microbial proliferation.

For Thermotolerant coliforms, the levels observed in the effluent were 120 MPN/100 mL, well below the limit of 100,000 MPN/100 mL established by the regulations. Similarly, the absence of *Salmonella*, Fecal *Enterococcus* sp./*Streptococcus*, and helminth eggs in the analyses reflects not only the system's efficiency in reducing pathogenic microorganisms but also its potential to produce a safe effluent for reuse in agricultural irrigation. The elimination of these microbiological contaminants can be attributed to several factors, including the combined action of the EOP substrate, *Canna hybrids* plants, and the physical, chemical, and biological processes occurring within the system. The prolonged retention of water and the interaction with plant roots facilitate the removal of pathogenic microorganisms and the degradation of organic matter [8,57]. To summarize, our results demonstrate significant compliance with Resolution 1256 of 2021, indicating the system's capacity to treat domestic wastewater effectively. By adhering to these regulatory standards, the treatment system ensures the safety and sustainability of effluent reuse, particularly for agricultural irrigation.

4. Conclusions

The HSF-CW, planted with *Canna hybrids* and supplemented with EOP, was effective in the treatment of domestic wastewater from a single-family home, achieving maximum removal efficiencies of 91% for COD, 94% for TSS, 98% for TP, 52% for nitrates, 73% for nitrites, 78% for ammonium, and 75% for TN. However, difficulties arose in removing ammonium and nitrite between the second and third months. This was attributed to variations in wastewater loading and persistently low C/N values, even with the use of EOP. Despite these challenges, removal efficiency did recover in the final weeks. Therefore, it is necessary to further investigate the use of EOP as a carbon source with a focus on determining optimal EOP quantity, quality parameters (such as C/N ratio and biodegradability), and substrate configurations to improve nutrient removal. Despite these challenges, the use of EOP as a substrate in CW is a viable option as it utilizes a waste product that is abundant in the region. Of the 31 parameters analyzed, only selenium and iron did not meet the maximum permissible values established in the Colombian standard. In that sense, the subsurface horizontal flow wetland allows obtaining water that is 93.5% suitable for agricultural irrigation. It was observed that the plants presented excellent adaptation during the treatment of wastewater. Notably, those in the middle sector, facing greater competition for sunlight, developed taller stems and reduced thickness. The observed differences in plant growth between sectors of the CW did not significantly impact overall

system efficiency but it highlights the importance of considering light competition and plant selection in future CW studies with this carbon source.

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