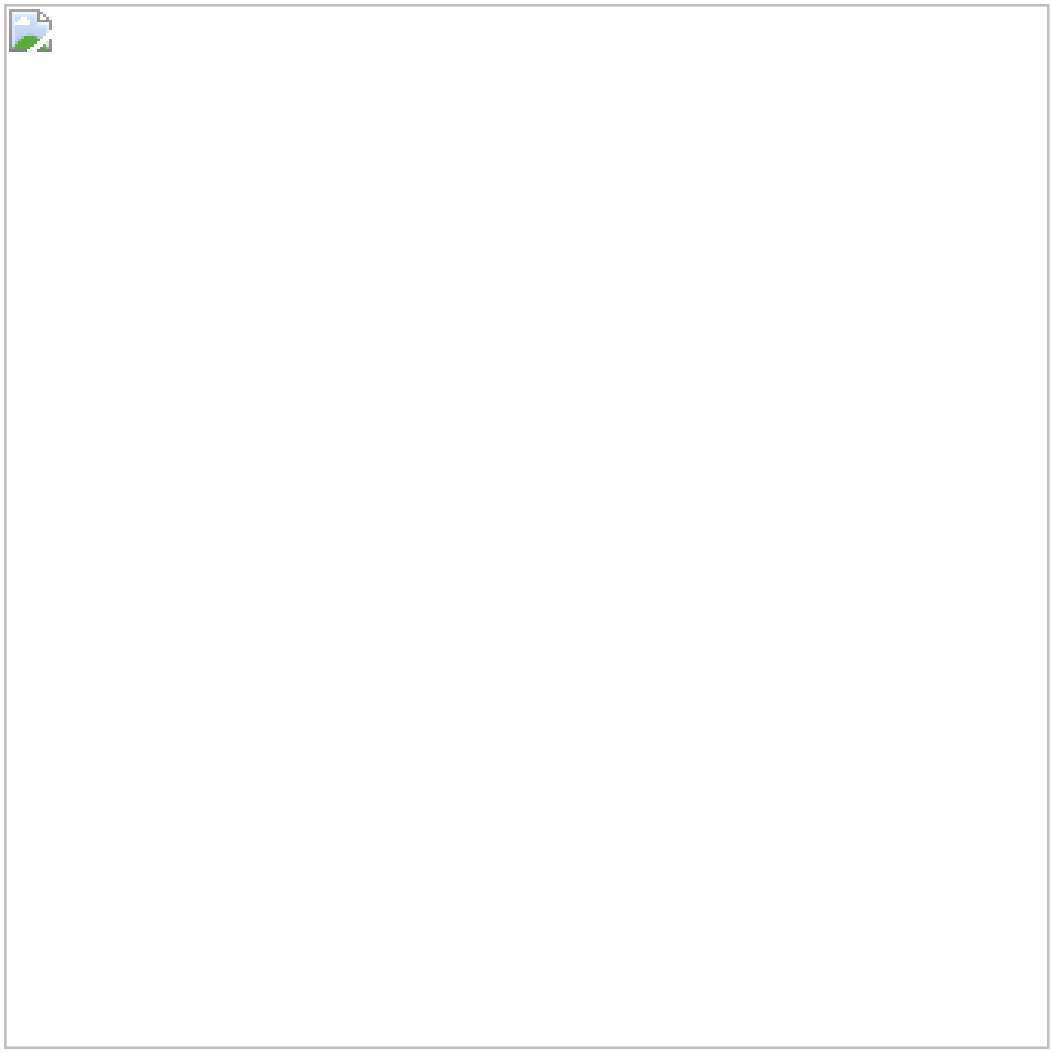

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Reuse of Treated Domestic Wastewater by Employing Artificial Wetlands in Panama

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ABSTRACT: Reuse of treated wastewater in irrigation is an alternative to achieve greater water availability and benefits to the soil due to its nutrient content. It represents a solution to challenges in water management, climate change and water scarcity in dry seasons. In Panama's Dry Arch, the lack of water is critical during dry season months, which makes it essential to look for sustainable alternatives as water source. This paper describes the use of artificial wetlands with horizontal subsurface flow, and we are going to use partially treated domestic wastewater. The aquatic plant types employed were *Echinochloa polystachya* (German grass) and *Brachiaria arrecta* (Tanner grass) with the objective of improve the quality of the effluent from the Wastewater Treatment Plant (WWTP) in Chitre for the irrigation of forages. This study was carried out from August to December 2019. Fine Gravel was used in this study as a substrate. The parameters analyzed were pH, Total dissolved solids, Electrical Conductivity, Chemical Oxygen Demand, Turbidity, Chlorides, Sulfates, Iron, Chromium+6, Copper; nutrients such as Total nitrogen and Total phosphorus. Fecal coliforms were also analyzed. Results showed that treated wastewater is a viable alternative for irrigation due to its high nutrient content, but it must be managed safely so as not to generate risks to public health.

KEYWORDS: Treated domestic wastewater, artificial wetlands, water reuse, agriculture irrigation, macro and micro minerals

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Introduction

Water availability and adequate supply are the basis for social and economic development throughout history. Therefore, water plays an important role for the sustainability of different communities on earth (Álvaro, 2010).

The Intergovernmental Panel on Climate Change (IPCC) states that climate change significantly impacts the availability and quality of water resources (IPCC, 2018), with the greatest consequences occurring in the tropics, where a large part of the developing countries are located. Tropical nations are expected to have 50% of the world's population by 2050 (Wilkinson, 2014). This situation affects basic water supply and sanitation needs for this part of the world. Negative effects related to water availability also include impacts on public health, food, and energy production (Sadoff & Muller, 2010).

In periods of drought, the reuse of treated wastewater is presented as an alternative to provide water availability. The traditional use for irrigation in agriculture has reusable substances complementary to fertilizers such as nitrogen and phosphorus. As a solution to sustainable water treatment, effluent from wastewater treatment plants can be used according to the needs and conditions of each region. The processes of using the treated wastewater for vegetable irrigation are analytically verified, depending on the nutrient needs of plants or vegetation (Pérez, 2012).

In 2011, agriculture represented 68% of all freshwater withdrawals in Latin America and the Caribbean, while those for industrial and domestic use represented 11% and 21% respectively. Agriculture is a strategic sector for rural development and poverty reduction, which plays a key role in overcoming local and global food insecurity (UNEP, 2016).

In Panama, La Villa river watershed suffers from deforestation, with scrub and agricultural activities covering 82% of the land and primary and secondary forests accounting for another 13% of the land use. The rest of the watershed consisted in urban areas, water, salt mines, and mangrove (CATHALAC and SENACYT, 2018). The results of the water balance at Los Santos weather station indicate a water deficit and little rainfall during the dry season (January–April) affecting the agricultural production (ETESA, 2019).

Generally, domestic wastewater does not contain emerging pollutants such as heavy metals; however, they can contain a high number of infectious and pathogenic agents. Also, they contain high concentrations of ammonium and nitrogen due to excreta, basing their treatment by employing various biological processes (Romero Rojas, 2010).

Artificial wetlands are natural treatment systems for different types of polluted water. Treatment wetlands are designed to optimize processes and considered sustainable and environmentally friendly options. Purification is attributed to the joint action of the soil, microorganisms, and vegetation. They are characterized by requiring low energy consumption and fewer operation personnel than with other treatment options. Therefore, their operation cost are lower; however, these systems require a larger land area. This is because vegetation uses solar energy and provides the necessary oxygen for the growth of microorganisms that degrade a large part of the organic matter (Dotro et al., 2017)

Figure 1. Study site location.

Treatment of wastewater with constructed wetlands does not represent a new environmental technology, several studies have already been carried out in developing countries and the subsequent recycling of the effluent for various purposes has been implemented. For example, a horizontal surface flow constructed wetland treatment system situated in Karachi (NED University of Engineering & Technology) was used for treating wastewater containing domestic sewage and low flows from laboratories of various university departments aiming to assess the application of constructed wetlands for reuse. Results showed that the average reductions in BOD and COD were 50% and 44 %, respectively. About 48% of effluent BOD concentrations were below the threshold of 30 mg/L. The suspended solids removal efficiency ranged from 73% to 86% with an average reduction of 78%. Roughly 38% of effluent SS concentrations were below the threshold of 30 mg/L (Almuktar et al., 2018).

Artificial wetlands are becoming a viable option for wastewater treatment and are currently being recognized as attractive alternatives to conventional wastewater treatment methods because they represent a sustainable wastewater treatment system (Zhang et al., 2015). The opportunities to harness wastewater as a valuable resource are enormous. Wastewater management is an affordable and sustainable source of water, energy, nutrients, and other recoverable materials. This research is aimed to evaluate the viability of artificial wetlands, to improve the quality of the effluent from Wastewater Treatment Plant (WWTP) in Chitre for irrigation purposes.

Material and Methods

Study area

This research was carried out in the La Villa river basin, located in the dry arch of Panama about 250 km southwest of Panama City and descends to the Pacific Ocean. The coordinates of the study area are 7°58′36″N and 80°24′5″W (Figure 1). The basin covers 56.1% of the territory of the province of Herrera and 43.9% of the province of Los Santos. It has a drainage area of 1,295.45 km^2 and an average elevation of 135 m above sea level (Opolenko, 2014). The panamanian Dry Arch represents an area of 6,293 km^2 (approximately 9% of the territory of the Republic) and includes the eastern plains and hills of the Los Santos, Herrera, and Cocle provinces. Dry arch is the territory with the lowest rainfall in Panama.

This work focused on the effluent from the Wastewater Treatment Plant (WWTP) located in Chitre. It designs include the collection and treatment of wastewater from domestic origin of 5.6 MGD for the year 2020 and an increase in its capacity of 7.0 MGD for the year 2025 (Constructora RODSA, 2012). WWTP is made up of eight sewage pumping stations covering eight sectors, it has four aeration systems, four sedimentation tanks (clarifier), two thickeners, a sludge drying bed (20 tanks in 2 sections), and finally the chlorine mixing system. There is currently no initiative to reuse the wastewater that is treated at the WWTP located Chitre. However, given the existing problems regarding the availability of water resources in the dry season, it is necessary to evaluate sustainable alternatives to meet the

Table 1. Results of the Analysis of Physical and Chemical Parameters Carried Out at the Chitre's WWTP in 2019 and Their Comparison With the Maximum Limits Established by the Technical Regulation DGNTI-COPANIT 24-1999 (Reuse Standard) and DGNTI-COPANIT 35-2019 (Discharge Standard) From the Republic of Panama.

PARAMETERS	MARCH	APRIL	MAY	JUNE	SEPT.	COPANIT 35-2000/35-2019	COPANIT 24-1999*
Aluminum (mg/L)	0.087	0.01	0.044	0.041	0.042	5	5
Boron (mg/L)	0.144	0.14	0.156	-	0.134	0.75/NA***	0.75
Cadmium (mg/L)	–0.098	–0.08	–0.47	-	-	0.01	0.01
Residual chlorine (mg/L)	1.9	1.15	0.98	0.5	0.8	1.5	1.0–2.0**
Copper (mg/L)	–0.055	0.02	–0.02	0.002	0	1	0.02
Chromium^{+6} (mg/L)	0.02	0.01	0.031	0.006	0.12	0.05	0.1
COD (mgO_2/L)	72.5	96	104.5	89.67	105	100	NA
Fluorine (mg/L)	0.04	0.52	0.44	0.26	0.16	1.5/NA	1
Total Phosphorus (mg/L)	8.86	10.07	9.25	9.59	2.58	5/10	NA
Total Iron (mg/L)	0.095	0.08	0.08	0.1	0.08	5	5
Manganese (mg/L)	0.182	0.1	0.148	0.123	0.128	0.3/0.5	0.2
Nickel (mg/L)	0.299	0.26	0.272	-	0.259	0.2	0.2
Nitrates (mg/L)	3.65	3.45	2.75	2.62	1.1	6/10	NA***
Ammonia Nitrogen (mg/L)	3.5	3.5	3.5	-	-	3	NA
pH	7.18	7.4	7.2	7.2	7.7	5.5–9.0/5.5–8.5	6.0–9.0
Total Suspended Solids (mg/L)	167	36.5	52.5	42.2	37	35	50
Sulfates (mg/L)	43.5	56	53	58.7	63	1,000	350
Sulfides (mg/L)	0.111	0.05	0.072	0.061	0.05	1	NA
Turbidity (NTU)	74.5	28.86	27.58	48.1	23.5	30	NA
Zinc (mg/L)	0.02	0.03	0.025	0.02	0.01	3	2

*Surface irrigation of forage crops, and inedible crops. **It is considered a maximum permissible limit of 2 mg/L to avoid the formation of chlorinated compounds. ***Does not apply. Opolenko (2020).

needs of the population during times of scarcity, based on the fact that wastewater is a resource always available.

Baseline

Previous water quality analysis from WWTP were carried out to evaluate its quality based on local standards regarding reuse of treated wastewater in irrigation, specifically forages and inedible crops established by Technical Regulation DGNTI-COPANIT 24-99, and DGNTI COPANIT 35-19. The latter regulates the discharge of liquid effluents in continental and marine water masses (MICI Panama, 1999, 2019). It is added because in Panama in the standards for reuse (DGNTI 24-99) does not define some important parameters (Table 1). Different regions and governmental agencies have adopted a variety of standards for use of reclaimed water for irrigation. These rules and regulations have been developed primarily to protect public health and water resources (USEPA, 2012). The standards that have been adopted in Panama are based on guidelines for water reuse from Environmental Protected Agency, and too Guidelines for the safe use of wastewater and excreta in agriculture and aquaculture from World Health Organization.

The baseline results indicate that the pollutant concentration of the outlet effluent is lower compared to the inlet flow. However, there are some parameters that exceed standard limits, such as: the total phosphorus content, nickel, nitrogen ammonia, total suspended solids, chemical oxygen demand and turbidity, which fluctuates in the analyses (CATHALAC and SENACYT, 2018).

In this sense, it was necessary to apply a treatment focused on reducing the parameters, and not exceeding the reuse standards. For this reason, the experimental unit of artificial wetlands is implemented with the objective of reducing and evaluating the quality of the effluent and its reuse possibilities.

Construction of artificial wetlands

Artificial wetlands are a natural alternative to technical methods of wastewater treatment (Stottmeister et al., 2003). This natural process involves three important components: wetland

Figure 2. Artificial wetlands with horizontal subsurface flow using German grass (HV2/HV2-R) and Tanner grass (HV3/HV3-R).

vegetation, soils, and their associated microbial to assist in treating wastewater for the primary purpose of contaminant or pollution removal from wastewater (Vymazal & Kröpfelová, 2008). Subsurface systems are designed with horizontal or vertical subsurface flow through a permeable medium (typically sand, gravel, or crushed rock). The most common artificial wetlands are designed with an horizontal subsurface flow (HSSF) configuration. In HSSF systems (with the wastewater level below the soil surface), the wastewater flows horizontally through the granular media contacting a network of aerobic, anoxic, and anaerobic zones in the subsurface. The aerobic zones occur around plant roots and rhizomes that introduce oxygen into the substrate (Zhang et al., 2015).

The type of vegetation used are plants from natural wetlands, including *Echinochloa polystachya* (German grass), and *Brachiaria arrecta* (Tanner grass), which have been chosen due to their positive effects on treatment efficiency for nutrient and organic compounds and their great capacity for absorption and purification of wastewater (Sandoval et al., 2019). In Panama, such species are typical in rural zones. Therefore, their seeds are easily collected from livestock production fields in the region.

A system of Artificial Wetlands with horizontal subsurface flow (Figure 2) was installed as an alternative treatment for residual water coming from the sedimentation process. It did not go through disinfection, to limit the effect of chlorinated water on wetland development. Additionally, a 750-L storage tank was installed to distribute wastewater to the artificial wetlands. A hydraulic retention time of approximately 24 hours was employed (CATHALAC and SENACYT, 2018).

Three artificial wetlands and their replicas were built (Miller Gil & Fábrega Duque, 2021):

Control Wetland 1 (HB1) and its replica (HB1-R): Includes only the substrate, which is made up of fine gravel without vegetation.

Artificial Wetland 2 (HV2) and its replica (HV2-R): A type of plant was implemented, the German Grass (*Echynochloa polystachya*).

Artificial Wetland 3 (HV3) and its replica (HV3-R): Another type of plant is sown, the Tanner Grass (*Brachiaria arrecta*).

The substrate of each wetland is composed of gravel and fine gravel 5 to 6 mm in diameter and its depth is 0.55 m. It has a slope of 2° toward the discharge area. The water level is 5 cm below the surface of the substrate. In the water distribution system of artificial wetland were constructed 5 cm diameter drainage pipes with 10 mm diameter perforations, spaced every 5 cm (Miller Gil & Fábrega Duque, 2021).

Field measurements

The frequency of the samplings was approximately every 2 weeks. For the analyzes, the parameters described in Table 2 were taken into consideration.

All residual water quality tests completed in the laboratory or the field was carried out with duly calibrated instrumentation and the samples analysis were carried out in the WWTP laboratory, except for fecal coliforms. These were analyzed in Toth Research & Lab located in Panama City and accredited by the National Accreditation Council LE-053 with the DGNI-COPANIT ISO IEC/17025-2006 Standard. Resolution No. 5 of March 6, 2017. For fecal coliforms, samples were preserved in coolers with ice and transported on the same day of sampling to the external laboratory already mentioned.

For the analysis of the results, an average value was calculated between each wetland and its replica. The results were compared with the maximum permissible limits for each parameter, in accordance with the technical regulations already mentioned. In the Table 2, the laboratory methods employed for the analysis of water quality are presented and it's based on Standard Methods for the examination of water and wastewater.

Table 2. Descriptive Parameters, Methods, and Number of Samples.

PARAMETERS	METHODS	NUMBER OF TESTS
pH	Multiparameter, Model Hanna HI 98194.	10
TDS (mg/L)	Multiparameter, Model Hanna HI 98194.	10
Conductivity (µS/cm)	Multiparameter, Model Hanna HI 98194.	10
Chemical oxygen demand (mg/L)	Colorimetry, Reactor Digital Block 200 Hach and Colorimeter DR 900 Hach.	7
Turbidity (NTU)	Turbidimeter, Model HI 98713.	3
Chlorides (mg/L)	Mohr's method.	3
Sulfates (mg/L)	Colorimetry, Colorimeter DR 900 Hach.	3
Total Nitrogen (mg/L)	Colorimetry Reactor Digital Block 200 Hach and Colorimeter DR 900 Hach.	7
Total Phosphorus (mg/L)	Colorimetry, Reactor Digital Block 200 Hach and Colorimeter DR 900 Hach.	3
Iron (mg/L)	Colorimetry, Colorimeter DR 900 Hach.	9
Chromium (mg/L)	Colorimetry, Colorimeter DR 900 Hach.	5
Copper (mg/L)	Colorimetry, Colorimeter DR 900 Hach.	8
Fecal Coliforms MPN/100 mL	Colilert, Quanti-Tray/2000 System, IDEXX.	2

Yield analysis of plant types

The plant tissue (foliar analysis) of tanner grass and German grass was analyzed in two stages. In the first one, an analysis of plant tissue to characterize macrominerals (nitrogen, phosphorus, potassium, calcium, and magnesium) and microminerals (manganese, iron, zinc, and copper) were done. Every 45 days plant growth samples were taken, for three sampling campaigns. This frequency was chosen because 45 days is the maximum stage of production, taking into consideration the samples for the foliar analysis in the last two cuts. Foliar analysis employing a chorophylmeter were made in the Agrobiotechnology Laboratory of the Institute of Agricultural Innovation (IDIAP in Spanish) located in Divisa, Panama.

Limitation of this study

The baseline analysis was dependent on analysis carried out by the WWTP laboratory. Therefore, some important parameters were not measured. In this study, the hydraulic retention time employed was 1 day. This time was probably not the adequate for microbial activity to perform its functions correctly. Finally, it is recommended to carry out a more detailed analysis of the biological characteristics of the treated wastewater. In this sense, it would have been ideal to consider indicators such as helminth eggs and others.

Results

Wastewater characteristics and effluent quality parameters

Table 3 shows the descriptive statistics for physicochemical parameters measured from artificial wetland.

Results indicated a decrease in the effluent from the artificial wetlands, compared to the influent values (Figure 3), evidencing a well performing wetland system. Electric conductivity, pH, Total Dissolved Solids, Temperature, Chemical oxygen demand, Chlorides, Sulfates, Iron, Chromium^{+6}, Copper, Total Phosphorus, and Total Nitrogen comply with the regulations for the reuse of treated wastewater for surface irrigation of forages and inedible crops. Turbidity was the only parameter exceed the maximum permissible limit according to COPANIT 35-19. Tanner grass represents greater removal for turbidity concentrations than German grass. This indicator is related to solids and organic matter, therefore, controlling this variable will directly improve turbidity.

Regarding nutrients, the water total nitrogen from German grass (63.28%) had a higher removal percentage than Tanner grass (53.07%). Similarly, the water total phosphorus from German grass (38.82%) obtained greater removal compared to Tanner grass (34.89%). Youssef (1988) reports that grasses can grow in the same soil and under the same environmental conditions but with notable differences in mineral content and removal percentage. Variations in mineral concentrations of grasses reflect differences in mineral uptake. The concentration of all minerals in forage plants depends on the interaction of several factors including soil, plant species, stages of maturity, yield, pasture management, and climate.

Fecal coliforms concentrations from artificial wetlands effluent are higher than effluent from WWTP. In this study, the constructed wetlands were not efficient in removing coliforms. This result was most likely due to the short retention time employed. For instance, Delgadillo et al. (2010) indicates that the hydraulic retention time for the design of subsurface flow wetlands has a range of 4 to 15 days with a usual value of 7 days. Also, subsurface

Table 3. Descriptive Statistics for Water Quality Analysis of the Effluent From the Artificial Wetlands and Comparison With the COPANIT 24-99 (Reuse Standard) and COPANIT 35-19 (Discharge Standard) From Republic of Panama.

PARAMETERS	INFLUENT TO WETLAND[F]	HB1	HV2	HV3	COPANIT 24-99	COPANIT 35-19
pH	7.47	7.56	7.05	7.14	6.00 a 9.00	5.50–8.50
EC[a] (µS/cm)	824.00	728.90	766.80	744.35	3000.00	2,000.00
TDS[b] (mg/L)	412.56	363.35	383.45	391.30	NA[d]	500.00
Temperature (°C)	29.71	27.69	27.65	28.13	NA[d]	±3°C TN[e]
COD[c] (mg/L)	71.86	39.50	42.07	37.64	NA[d]	100.00
Turbidity (NTU)	88.47	38.12	51.52	32.23	NA[d]	30.00
Chlorides (mg/L)	106.67	116.67	113.33	113.33	200.00	400.00
Sulfates (mg/L)	65.67	68.50	47.50	45.17	350.00	1,000.00
Iron (mg/L)	0.159	0.017	0.038	0.034	5.00	5.00
Chromium+6 (mg/L)	0.004	0.021	0.018	0.014	0.10	0.05
Copper (mg/L)	0.016	0.006	0.015	0.011	0.02	1.00
Total Phosphorus (mg/L)	11.03	8.63	6.75	7.18	NA	10.00
Total Nitrogen (mg/L)	24.57	10.64	9.02	11.53	NA	15.00

[a]Electric conductivity, [b]Total Dissolved Solids, [c]Chemical oxygen demand, [d]Does not apply, [e]Natural water temperature, [f]This is the effluent of the WWTP after the sedimentation unit and the influent to the wetland system.

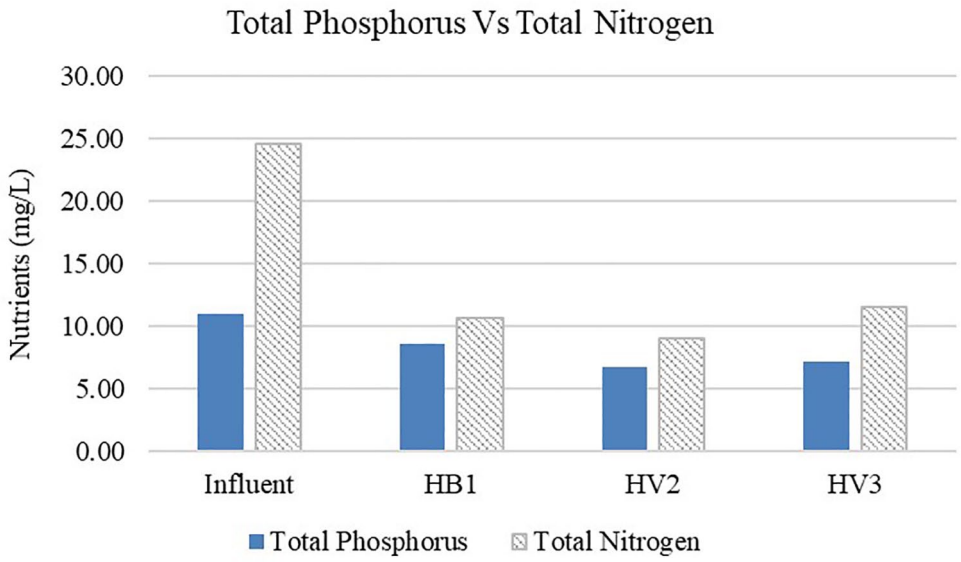

Figure 3. Mean content of total phosphorus and total nitrogen from influent and effluent of artificial wetlands. Control wetlands represent (HB1), German grass (HV2), and Tanner grass (HV3).

flow wetlands with long enough retention times can reduce fecal coliforms by an order of magnitude in systems designed to produce secondary or advanced treatment effluents (Environmental Protection, 2000). However, as the wastewater influent of artificial wetlands comes from the clarifiers, it has a higher concentration of fecal coliforms (Table 4). Control wetlands and their replica (HB1 and HB1-R) and German grass wetlands and their replica (HV2 and HV2-R) indicated a high concentration of fecal coliforms with values greater than 2419.6 MPN/100 mL. However, Tanner grass wetland and its replica (HV2 and HV2-R) show a decreased through the treatment process.

Plants growth from artificial wetlands

The data shown in Figure 4 was obtained by measuring from the base (roots) to highest part of the plant, with five intakes

Table 4. Fecal Coliform Results (MPN/100 mL).

DATE	INFLUENT WETLAND	HB1	HB1-R	HV2	HV2-R	HV3	HV3-R	WWTP EFFLUENT
01/8/20	>2,419.6	>2,419.6	>2,419.6	>2,419.6	>2,419.6	517.2	1,119.9	< 1.0

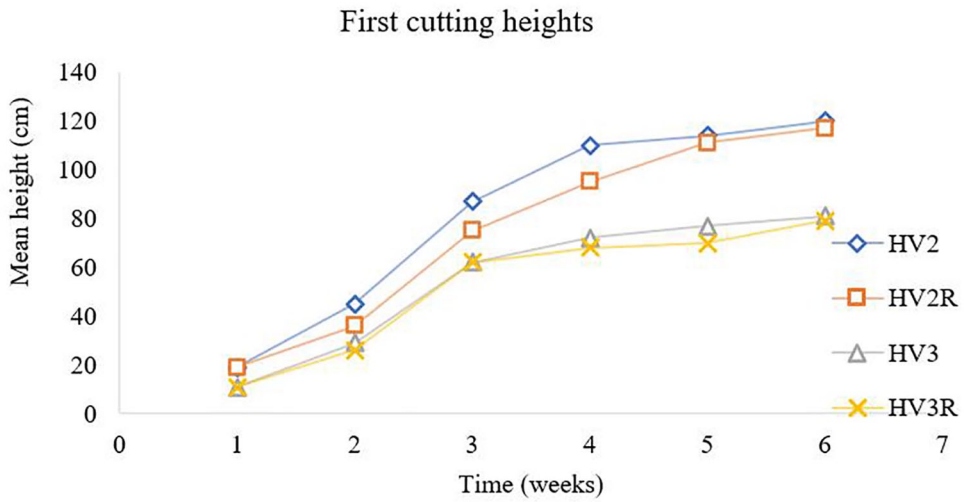

Figure 4. Grasses growth of artificial wetlands during first cutting heights. German grass represents (HV2) and their replica (HV2R). Tanner grass represent (HV3) and their replica (HV3R).

for each wetland, then an average value is calculated. The first growth cut of the grasses employed was made 6 weeks after sowing, approximately 45 days. In this period, growth was evaluated. During the first 4 weeks German grass and its replica increased linearly, after the fourth week the growth slowed down at a constant rate. In the wetland composed of Tanner grass, a more rapid growth was obtained in the first 4 weeks. Its behavior is then similar to German grass, with the difference that their average maximum height was lower.

In general, German grass had a higher growth in the first cut with a maximum sixth week height of 120 cm, compared to 80 cm height of Tanner grass for the same period. The difference in higher growth between the two grass species is due to the average maximum height of German grass is 200 cm while tanner grass is 100 cm (Florindo et al., 2014; Morison et al., 2000).

Study of wetland plant tissue

Results from plant tissue analysis (Foliar analysis) show the macrominerals and microminerals present in German grass (HV2) and Tanner grass (HV3). The foliar analysis represents an indispensable reference to evaluate the nutritional status of the plants.

Macrominerals. Figure 5 shows the variation in concentration of macrominerals considered during the first and second evaluation cut-offs.

Changes in mineral concentrations depend on the interaction of several factors, such as: soil, pasture species, age of maturation and time of analysis (dry or wet season). This is due to the season influences in the properties and conditions of the soil affecting the use of minerals by plants (Serra et al., 1996; Youssef, 1988). The macronutrients did not present noticeable variations between HV2 and HV3, except for N, which obtained a higher concentration in HV2. Regarding the decrease in the concentrations of N, P, and K, in the second period of analysis, lower water availability in the wetlands during this period of time seems to be the reason for this situation.

Tanner grass (HV3) can accumulate high levels of nitrate in its tissue. This happens under certain conditions like a high nitrate content in the soil, droughts, and low light intensity (Kumble, 1996). In this sense, HV3 obtained a higher concentration of N compared to HV2, throughout the study period. It is important to mention that N is directly related to Ca, since a high concentration of N can affect Ca absorption. In this case, N had a decrease in the second analysis period, which caused a slight increase in Ca.

In general, acceptable macronutrients values were obtained, except for the low levels found in Calcium. These results agree with Fonseca et al. (1988), who pointed out that forages in the southern zone of Costa Rica have low levels of Ca, both in the rainy season and in the dry season, at 95.45% and 83.33%, respectively. Low level of Ca are usually related to a high availability of N. Also, Ca deficiencies can be attributed to low pH and high concentrations of Potassium (K) in the soil (West Análitica, 2018).

Microminerals. Figure 6 show the variation of microminerals concentration in German grass wetlands (HV2) and Tanner

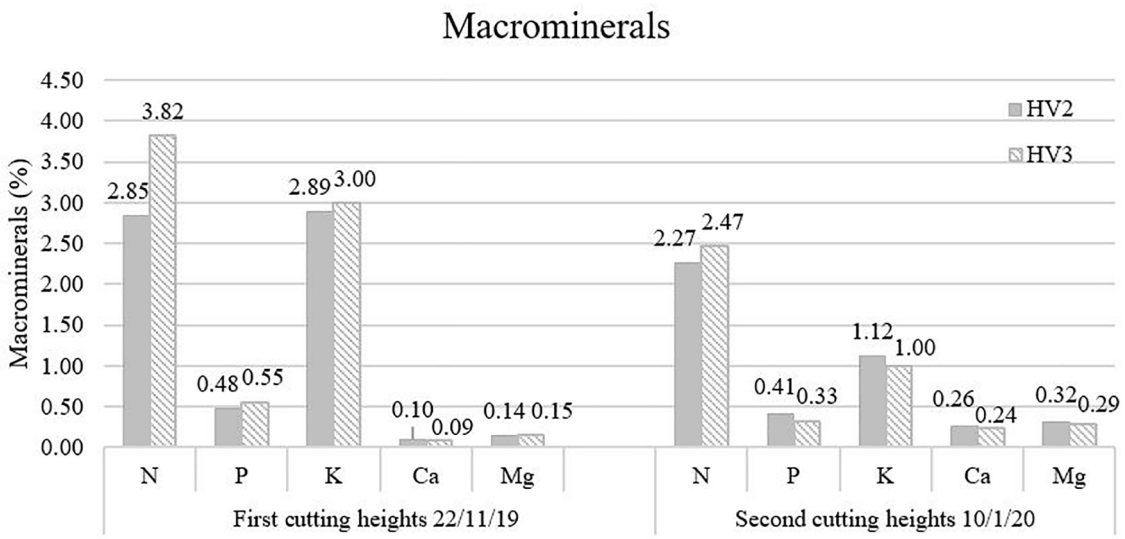

Figure 5. Mean content of Macro minerals in German grass (HV2) and Tanner grass (HV3) cut at first and second cutting heights (%).

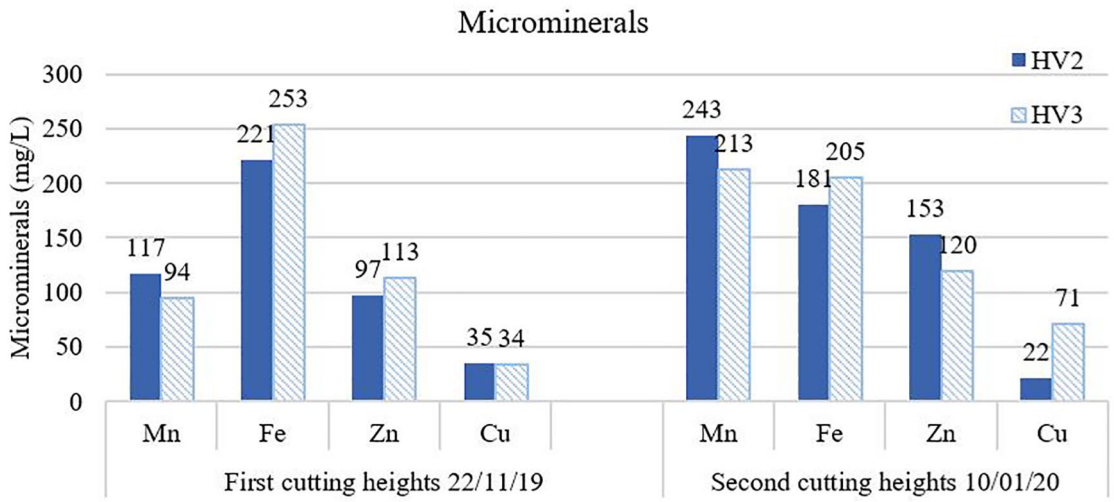

Figure 6. Mean content of Micro minerals in German grass (HV2) and Tanner grass (HV3) cut at first and second cutting heights (mg/L).

grass wetlands (HV3) during the first and second evaluation cut offs.

It is interesting to observe within this figure, an increase in Manganese (Mn) in the second cut, possibly as a result of a decrease of Nitrogen. This relation between the concentration of these elements has been mentioned already (Múnera Vélez, 2012).

Iron concentrations were obtained at acceptable levels. Since some tropical soils are rich in Fe, this element is also high in grasses (Youssef, 1988). Fonseca et al. (1988) found this element abundant in forages from the southern zone of Costa Rica (245 mg/L) and apparently its content varies little between seasons and the age of the plant.

Zn concentrations from HV2 in the first and second cut obtained average values of 97 and 153 mg/L. Regarding HV3, the results were 113 and 120 mg/L, in the first and second cut, respectively. The results are very high, compared to other reported in tropical areas like the northern (20 mg/L) and southern (39 mg/L) zones of Costa Rica (Fonseca et al., 1988, 1989). Another study carried out by Youssef (1988), reported an average value of 32 mg/L Zn concentrations in plant *Brachiaria*. In this sense, the results in HV2 and HV3 showed high levels of Zn in plant tissue.

The results in concentrations of Cu in HV2 during the first and second cut, had average values of 35 and 22 mg/L, respectively. Average values for HV3 of 34 mg/L were obtained in the first cut and 71 mg/L in the second cut. Again, these results are high in comparison with studies in Costa Rica, where relatively low Cu concentrations in five grass types: *Brachiaria* (3.4 mg/L), *Digitaria* (5.7 mg/L), *Paspalum* (5.7 mg/L), and *Pennisetum* (3.9 mg/L) (Youssef, 1988). Fonseca et al., (1989) indicate optimal ranges of 10 to 20 mg/L for pastures in Costa Rica (Cabalceta, 1999).

In general, the results obtained in this study show high levels of micronutrients, compared to those reported in Costa Rica (Fonseca et al., 1988, 1989) A reason for this behavior might be the use of soil irrigated with treated wastewater.

Discussion

Reuse of treated wastewater in agriculture has been implemented by various countries worldwide. Wastewater has valuable qualities for irrigation due to its high content of nutrients and organic matter. These characteristics are beneficial for soil improvement and increased harvests, as well as a water source in times of drought. In general, irrigation is applied to crops not intended for direct human consumption, such as pastures or forages (Lorenzo et al., 2009; Silva et al., 2008).

Results recorded for physicochemical parameters of water such as pH, EC, TDS, temperature, COD, turbidity, chlorides, sulfates, iron, Chromium+6, and Copper showed acceptable levels of removal in their concentrations. In general, these parameters comply with local standards for the wastewater reuse for the irrigation of forages and inedible crops (COPANIT 24-99). It was possible to obtain a good percentage removal of turbidity in the effluent from the wetland with German grass (41.77%) and Tanner grass (63.56%). Although it does not comply with the provisions of the COPANIT-35-19 standard, it is an important value in wastewater treatment.

Ali et al. (2018) indicate that hybrid constructed wetland system (HCWS) treated water was well below irrigation standards and therefore recommended for a safer crop production in regions with water scarcity. Bedoya Pérez et al. (2014) showed removal levels for residual water from wetland containing *T. latifolia* species of 70.4% (COD), 96.7% (BOD5), and 81.4% (TSS). However, this study also concluded that with none of the macrophytes used, it was possible to obtain an effluent that met the maximum removal levels established by Colombian regulations for total nitrogen and ammoniacal nitrogen for wastewater discharge to surface waters or sewerage systems.

Regarding nutrients, the water total nitrogen from German grass (63.28%) had a higher removal percentage than Tanner grass (53.07%). Similarly, the water total phosphorus from German grass (38.82%) obtained greater removal percentage compared to Tanner grass (34.89%). A study carried out by Romero Aguilar et al. (2009) confirms that the horizontal flow artificial wetland system is a feasible option for the removal of organic and nutrient load, with low operation and maintenance costs.

Similarly, on experimental stations, removal percentages above 90% have been recorded, given by adsorption on the substrate particles as well as by the effect of the soil on pathogenic organisms, exerted by the antibiotics produced by the roots of the plants and by the action predator of bacteria and protozoa (Herrera & Rodríguez, 2011). The grasses used in the treatments are a means to reduce the organic load of domestic wastewater, thus providing multipurpose wetlands for the management of domestic wastewater and animal feed.

Other studies have evaluated the risk to animal health from the consumption of forage maize and Tanner grass irrigated with treated domestic wastewater. The crops showed high levels of surface contamination with E. coli (10^4–10^7 25 g^{-1}) and salmonellae (up to 1.6×10^4 25 g^{-1}) but none of the animals indicated signs of disease or infection (Bevilacqua et al., 2014). The microbiological quality of animal products always complied with European Union and Brazilian standards for food safety (Bevilacqua et al., 2014). The World Health organization reference values for restricted irrigation ($\leqslant 10^4$ *E. coli* 100 mL^{-1} and $\leqslant 1$ helminth egg L^{-1}), which were developed to protect the health of agricultural workers who irrigate with wastewater under the exposure scenario, it would also protect the health of animals fed crops irrigated with sewage, and humans who consume products from such animals (Bevilacqua et al., 2014).

In this study, results indicated that wastewater treatment in artificial wetlands with horizontal subsurface flow using macrophytes such as German grass and Tanner grass are useful for the removal of different physicochemical contaminants such as pH, Electric conductivity, Total Dissolved Solids, Temperature, Chemical oxygen demand, Chlorides, Sulfates, Iron, Chromium+6, Copper, Total Phosphorus, and Total Nitrogen. Specially, Tanner Grass species (*Brachiaria arrecta*) showed a better performance in removal than German grass species in terms of the quality of water obtained in the effluent at the end of the treatment. Similar results obtained by Prado and Velasco (2013) show that highest effectiveness on removal percent was registered with artificial wetlands using *Brachiaria mutica* and *Penisetum purpureum*.

One of the most important characteristics of artificial wetlands is the role plants play in the production of root and rhizomes for to provide substrates for attached bacteria and oxygenation of areas adjacent to the root and absorb pollutants from water. Phosphorus (P), Nitrogen (N) and other nutrients are mainly taken up by wetland plants through the epidermis and vascular bundles of the roots and are further transported upward to the stem and leaves (Sandoval et al., 2019).

Conclusions

Artificial wetlands show potential for the removal of physicochemical parameters such as pH, EC, TDS, COD, Turbidity, Sulfates, Fe, and nutrients such as nitrogen and total phosphorus, but it does not meet the standards of COPANIT 24-99 because it did not demonstrate a reduction in fecal coliforms at acceptable levels. In addition, it provides evidence to use them for the decontamination of water with nutrients.

The highest levels of removal percentage of COD (47.61%), turbidity (63.56%), sulfates (31.22%), and iron (78.74%) present in the wastewater treated by the constructed wetlands, were obtained in the wetland that contained the Tanner grass. While German grass obtained levels of removal percentage of COD (41.45%), turbidity (41.77%), sulfates (27.66%), and iron (75.98%). On the other hand, the German grass obtained higher levels of removal in total nitrogen (63.28%) and total phosphorus (38.82%) than the tanner grass with values of (53.07%) and (34.89%) respectively.

Biological parameters are essential to evaluate the feasibility of reusing the treated effluent. For surface irrigation of forages and inedible crops is recommended a water quality with fecal coliform values lower than 1,000 MPN/100 mL. As for fecal coliforms higher values were obtained the maximum permissible limits set by standards. The design of constructed wetlands must be modified or optimized to reduce fecal coliform concentrations.

Treated wastewater does not intervene in the development of German grass and Tanner grass used in the constructed wetlands. The grasses obtain good growth during the study period and absorbed the macro and micro minerals present in the soil that are essential for their growth.

Finally, the reuse of treated wastewater for irrigation can present risks, which is why it is necessary to adopt all effective measures for sanitary control and thus minimize possible risks to public health.

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