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Evaluation of a Wetland Constructed with *Typha domingensis* Pers., for the Recovery of Contaminated Water from Hospital Effluents

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ABSTRACT

The objective of this research project was to evaluate the implementation of a phytoremediation system for the effluents generated by the National Cancer Institute (INCAN), Central Department, Paraguay to contribute to the management of the liquid waste that it generates. The system consisted of a set of three pools, in which were made up of floating *Typha domingensis* Pers plants, at an approximate density of 10 plants per m^2, all the roots formed a filter, which was in direct contact with the effluent. The effluent was of continuous flow; the flows of entry and exit were regulated according to the generation of liquid waste by INCAN. There were 5 measurements made with an interval of 7 days for each measurement, at a point of entry and exit of the effluent, to determine the system. The parameters evaluated were: BOD_5, COD, NTK, PT, pH, temperature, conductivity, turbidity, dissolved oxygen and fecal coliforms. The results showed a considerable reduction of the pollutants generated for all the parameters evaluated, obtaining an efficiency of 67.9 to 92.4% in the evaluated parameters, indicating that it is very feasible to implement this type of systems for phytodepuration of liquid waste.

Keywords: bioremediation, *Typha domingensis*, effluent, pollutants, environment.

INTRODUCTION

Hospital effluents are residual liquids of diverse composition generated in these health centers, which come from the sewage network with a great variability of chemical substances and biological materials disposed in them. Both the solid residues and the liquid effluents disposed by these centers are a great threat to the public health of their community, even more so that in most hospitals, the effluents are not treated on site but are discharged or stored in large pits, which can lead to greater complications for the environment (Paz et al., 2004; Muñoz et al., 2014).

In case these residual waters, such as hospital effluents, are disposed into the basin without any prior treatment, it can cause the spread of diseases, causing a great impact on the environment, because certain communities use the water from the basin to satisfy their daily needs, such as irrigating their crops, which will lead to food contamination (Paz et al., 2004; Cepeda, 2018).

In hospital effluents, mixtures of chemical substances can form even more harmful substances forming a gas produced by combustion gases, or they can also form substances such as dioxins and furans. New and more dangerous chemical substances are produced. Characterization enables to identify them and determine what treatments can be applied (Cepeda, 2018).

The BOD_5/COD ratio is a fundamental indicator of the level of contamination and the characterization of the type of contaminant that could be in abundance, taking into account that if this ratio is less than 0.2, it could be determined if it is a contaminant of an industrial nature, with a low biodegradability index, in which case physicochemical treatments are convenient, and if the ratio is greater than 0.5, it could be discharge of an urban nature and somewhat more biodegradable, thus being able to be treated with models of bioremediation systems (Dovale, 2013; Suarez 2018).

Constructed wetlands are fobiopurification systems, designed in order to emulate the natural characteristics and processes (chemical, physical and biological) of a wetland, being constructed in complex and integrated ways in order to make the most of certain factors to improve its performance (Rincon and Millan, 2013; Zhou et al., 2021).

The vegetation acts as a biological filter of harmful substances, also transports oxygen from its leaves to the roots and interacts with the water; it fixes nutrients, retains certain contaminants and transforms them into the substances that are less harmful to the environment, thus achieving the removal of a large part of the unwanted organic and inorganic matter (Delgadillo et al., 2010).

These treatment systems have been used for more than fifty years in Europe and Asia as a phytoremediation therapy for wastewater in small towns; at present, they are already used on a larger scale throughout the world, being widely implemented in Latin America, benefitting from them easily owing to our tropical climate and the natural vegetation in the area (Zhou et al., 2021; Troche et al., 2021).

The objective of this research was to evaluate the efficiency of a constructed wetland to recover hospital effluents, by determining physical, chemical and microbiological parameters.

MATERIALS AND METHODS

Research location

The experiment was conducted at the National Cancer Institute (INCAN), under the Ministry of Public Health and Social Welfare, district of Capiatá, located in the Central Department, Paraguay. The proposed treatment system was built within the INCAN premises, with the coordinates of the location being Latitude 25°21’03.9” S and Longitude 57°24’55.9” W. The analyses were carried out in the Biotechnology Laboratory and, the Water Quality Laboratory of the Centro Multidisciplinario de Investigación y Tecnología (CEMIT) of the National University of Asunción (UNA).

Wetland construction

The wetland, as it can be seen in Figure 1, consisted of three Syopar® brand fiberglass pools with the following dimensions: 5 m long, 2.8 m wide and 1.3 m deep; 2 m wide, 3 m long and 1.5 m deep; 2 m wide, 2.8 m long and 1 m deep. Each pool was contiguous, the inlet and outlet flow of the effluent was continuous and the flow and load was according to the generation of hospital effluents (CONACYT, 2016).

Installation and adaptation of *Typha domingensis* plants

In the research 10 *T. domingensis* plants per square meter were used in the three pools (266 plants in total), using a flotation support system. Subsequently, the plants were left in ambient conditions and periodic monitoring was carried out to evaluate their development. The *Typha* plants were obtained from seeds, according to the propagation protocol proposed by Samudio et al. (2014).

Determination of physicochemical and bacteriological parameters

Sampling

To determine the baseline state of the effluent produced by INCAN, a sample was taken of the effluent before the implementation of the constructed wetland, and all the parameters considered for the trial were analyzed.

Subsequently, once the plants reached an optimal level of growth and adaptation to the system (approximately 30 days after the transplant), the first sampling of the constructed wetland began, considering that the plants and the system in general were already working and capturing the generated pollutants. From this first sample, successive samples were taken, with an interval of 7 days (once a week) in a period of 6 weeks, thus obtaining 6 samples of the wetland for their determination and subsequent evaluation.

As shown in Figure 1, there were two points for sampling (Point 1: effluent before entering the constructed wetland; Point 2: effluent after leaving the constructed wetland). All the samples

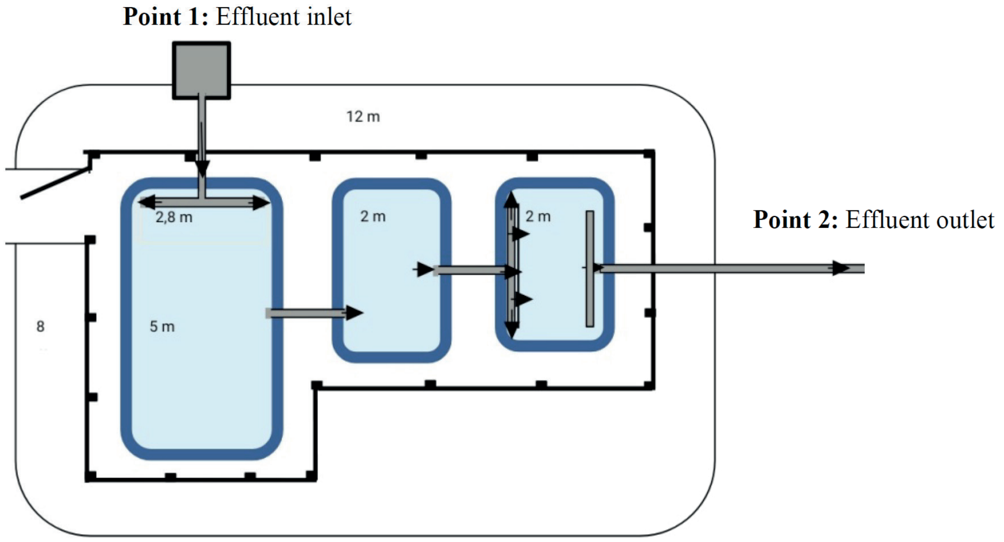

Figure 1. Wetland construction plan: a) Effluent inlet (INCAN 1); b) Treated water outlet (INCAN 2)

were placed in sterile containers, with a capacity of two liters, provided with an airtight lid, and were transported in a cooler to the CEMIT Water Quality and Microbiology laboratories to conduct the different laboratory analyses, using the techniques described by Rice et al. (2012).

For the *in situ* determinations: temperature, conductivity, oxygen and pH, there were used a container and a beaker, determined with the multiparametric field equipment that measures the four aforementioned parameters.

Variables:

1. Quality of the effluent and treated effluent: considering the physicochemical and microbiological, parameters (BOD_5, COD, NTK, PT, pH, temperature, conductivity, turbidity, dissolved oxygen and fecal coliforms), which are considered in the norm established for wastewater final disposal.
2. Wetland Efficiency: capacity of a wetland system to fulfill established functions, within the limits of contaminants concentration as established by Article 7 in accordance with the Resolution 222/02 of the SEAM.

Methods used for each study parameter:

a) Potential of Hydrogen (pH): Determination was made *in situ*, at point 1 and point 2 using the pH meter, according to the technique described by Rice et al. (2012).

b) Temperature: Determination was made in situ using a portable thermometer, as equipment. According to the techniques described in Rice et al. (2012), measurements were made directly at the points to be analyzed.

c) Turbidity: This parameter was measured by collecting the sample according to sampling techniques mentioned above, *in situ*, a turbidimeter was used as testing equipment, as described by Rice et al. (2012).

d) Conductivity: It is a parameter that must be measured *in situ*. It was measured directly at the points of the evaluated effluents, using a field conductivity meter according to the technique described by Rice et al. (2012). For certain samples, such as hospital effluents where there is a likelihood of electrode contamination, functionality was verified by frequently checking the Potassium Chloride solution.

e) Dissolved Oxygen: The measurement of this parameter was carried out *in situ*, in such a way as not to alter the basal conditions and so that it is representative. An Oximeter portable field (Multi 350i SET 1; Thermo Scientific ORIO®) was used, according to the technique described by Rice et al. (2012), verifying the calibration settings of the equipment.

f) COD: Chemical oxygen demand – Closed reflux, colorimetric method: The calibration curve was prepared with five Potassium Hydrogen Phthalate solution standards; 2 mL of the sample was measured and added to tubes filled with 3 mL of digest solution (potassium dichromate, sulfuric acid, mercury fate); it was brought to 150 °C for 2 hours. It was allowed to cool and the absorbance was read at a wavelength of 600 nm. It was calculated using the formula mg O_2/L = (Abs of the sample – Abs of the blank) × factor × dilution (Rice et al., 2012; Alberto et al., 2013).

g) BOD_5: Biochemical demand for oxygen after five days: The dilution water was bubbled (phosphate buffer, magnesium sulfate, calcium chloride, ferric chloride) for 25 minutes. Three dilutions of the samples were made, filling the hermetic flasks with dilution water to overflowing; then, the dissolved oxygen was measured with the oximeter. The flasks were incubated at 20 °C for 5 days, on the fifth day dissolved oxygen was measured again. It was calculated using the formula: mg O_2/L = (initial dissolved oxygen – final dissolved oxygen) x 300 x Dilution (Rice et al., 2012; Alberto et al., 2013).

h) Total Nitrogen: macro method – Kjeldahl: 250 mL of the sample, 0.5 mL of magnesium sulfate and 10 mL of the digest mix were added into a Kjeldahl flask; it was mixed and digested over a heating device until the mix became clear. Then, it was refilled with 250 mL of distilled water, and homogenized. A 25 mL sample was taken and transferred to an Erlenmeyer flask with lid, 25 mL of distilled water, 1 drop of ethylenediaminetetraacetic acid (EDTA) and 1 mL of citrate buffer were added, and then it was again homogenized. It was neutralized with 30% sodium hydroxide to a pH of 10. After this, 2 mL of reagent A (dried reagent containing phenol and sodium nitroferricyanide) and 2 mL of reagent B (concentrated reagent of sodium hypochlorite and sodium hydroxide) were added. The mix was homogenized and after 1 hour the calculation was made using the formula: mg/L N = (Abs of the sample – Abs of the blank) × Factor × Dilution (Rice et al., 2012; Alberto et al., 2013).

i) Total Phosphorus: automated ascorbic acid reduction method; 25 mL of the sample previously digested by the Kjeldahl method (same digestion that was used in the determination of total nitrogen). Then, 2 drops of phenolphthalein were added and neutralized with 30% sodium hydroxide until a permanent pink color was obtained. Then a 5 normal (N) sulfuric acid was added dropwise, until the mix became clear. Afterwards, 8 mL of mixed reagent (potassium and ammonium double tartrate, ammonium molybdate, ascorbic acid, sulfuric acid) were added and brought to 50 mL. After 10 minutes, the reading was made at a wavelength of 880 nm.The calculation was made using the formula: mg/L P = (Abs of the sample – Abs of the blank) × Factor × Dilution (Rice et al., 2012; Alberto et al., 2013).

Microbiologic analysis; Fecal coliforms: NMP procedure for fecal coliforms

In the presumptive phase, five tubes with liquid lauryl tryptose medium were used, 10 mL of sample were inoculated in each tube, the portions to be studied were mixed with the medium by gentle agitation. The inoculated tubes were incubated at 35 °C, after 24 hours each tube was shaken gently and gas production was observed. Since in some tubes the gas production was not observed, they were incubated again for 48 hours. Gas formation constitutes a presumed positive reaction.

In the confirmatory phase, the primary tubes in which gas was formed were taken; the fermentation tubes containing brilliant green lactose bile were inoculated with a 3mm diameter sterile loop and incubated at 35 °C for 48 hours. The production of any amount of gas at 48 hours constitutes a positive result. The presumptive tubes that have shown some amount of gas during the 48 hours of incubation in the confirmation test were studied. With a metal loop, the cultures of each fermentation tube were transferred to the *Escherichia coli* (EC) medium, the tubes were incubated with EC medium in a water bath at 44.5 °C for 24 hours; the appearance of gas in an EC medium at 24 hours or less of incubation is considered a positive reaction. The MPN values were calculated from positive tubes using Tables 9221: III, IV and V, which indicate the NMP values for different series of sowings and results (Rice et al., 2012, Alberto et al., Redondo and Arias, 2011).

Observations

The samples were refrigerated and stored until their corresponding analysis without the addition of preservatives or freezing techniques that could interfere with the results. The BOD_5 and COD determinations were made within 24 hours of taking the sample and the *in situ* determinations were carried out as accordingly in the workplace. The dissolved oxygen (DO) was measured with the WTW oxi 3310 oximeter, pH, temperature and conductance with the field Multi 350i SET 1 field equipament; Thermo Scientific ORIO®. The spectrophotometer used in the colorimetric determinations was an UV-1700 Pharma Spec®.

All methods that were used are validated by the CEMIT Water Quality Laboratory, in which they have specialized and trained personnel for said determinations and an accredited laboratory for this purpose.

Determination of contaminant removal

According to the parameters analyzed at the inlet (effluent) and outlet of the system (treated effluent), the percentage of removal of the values obtained by each sampling and determined parameter was calculated. Then, the median of the values obtained was found, obtaining a percentage of removal of each parameter. The calculation was carried out using the following formula:

$$\%R : \frac{(CE - CS)}{CE} \text{ x } 100 \quad (1)$$

where: R – removal; CE – inlet concentration; CS – outlet concentration

Comparison of the values obtained from each parameter of the effluent and treated water with those established in Article 7 of the 222/02 Resolution of the Secretariat of Environment.

The results of the effluent and treated water were determined with respect to each parameter, to compare the established limits; after that, the quality of the discharged water was established according the minimum requirements for each parameter defined by Article 7 of the Resolution 222/02 of the SEAM.

A comparative analysis was carried out between the data obtained in this research and Resolution 222/02: Water quality in national territory – Secretariat of Environment (SEAM), according to the norms established for effluents final disposal, using a Microsoft Office Excel® spread sheet to process the collected data. The results are presented in a narrative form and complemented with tables and graphs for a better understanding.

RESULTS

pH (Potential of Hydrogen)

Figure 2 shows that in most of the samples there was a decrease in pH in the effluent treated with the wetland, at the inlet of the effluent values of 6.5 to 7.5 were recorded, while the values of the effluent treated were between 6.2 and 6.9.

Dissolved oxygen

Figure 3 shows a considerable increase in the amount of oxygen dissolved in the effluent after being treated by the evaluated treatment system in all the samples. The values between 0.72 and 1.8 mg O_2/L of the untreated effluent and 1.52 to 2.32 mg O_2/L of the treated effluent were recorded.

Temperature

The temperature values did not present significant variations between the treated and untreated hospital effluents, as these values ranged between 20.5 °C and 23.3 °C.

Conductivity

The conductivity values with a significant difference and a decrease in the effluent values ranging from 597 to 786 µs, compared to the treated effluent where results were around 121 to 325 µs.

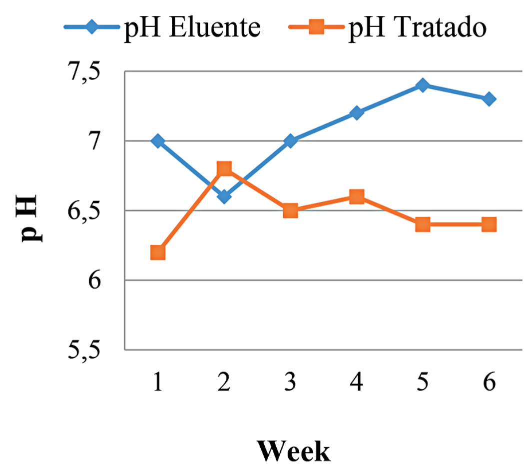

Figure 2. pH behavior in the treated and untreated effluent in 6 consecutive weeks

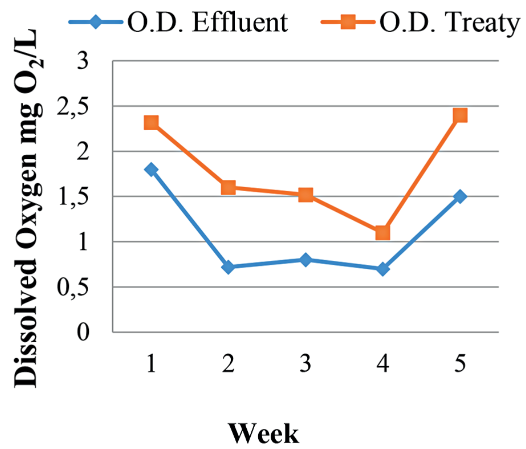

Figure 3. Dissolved oxygen behavior in the treated and untreated effluent in 6 consecutive weeks

Turbidity

Figure 4 shows the behavior of the samples from points 1 and 2 in terms of turbidity. There was a decrease in all the sampling week to week. Regarding the effluent without treatment, the values were between 10.9 and 70.3 NTU and in the treated effluent the values ranged from 5.27 to 20.7 NTU.

Biochemical demand for oxygen

Figure 5 shows that the BOD_5 values obtained during the test, which presented a decrease in all the samples after the effluent passed through the system. The values of the untreated effluent were around 185 to 436 mg O_2/L and the values of the treated effluent were around 18.8 and 37.7 mg O_2/L.

Chemical oxygen demand

The data in Figure 6 shows the behavior of COD in consecutive samplings, where there was a decrease in the values, the ones of the effluent without treatment ranged between 466 and 581 mg O_2/L, and the values of the treated water between 69.2 and 131 mg O_2/L.

In Figure 7, the total Nitrogen is observed in the course of the trial, obtaining in the results a decrease in the values in all samples for the treated effluent. The effluent values were 26.3 to 78.9 mg/L and the results obtained from the treated water ranged from 3.2 to 16.9 mg/L.

Total phosphorus

Figure 8 shows that the values obtained for total phosphorus throughout the study decreased at all points compared to hospital effluent, which gave the values between 2.93 and 6.46 mg/L, and treated water values of 0.88 to 4.14 mg/L.

Contaminant removal percentage

The percentages of removal of the contaminants under study were established, using the formula in

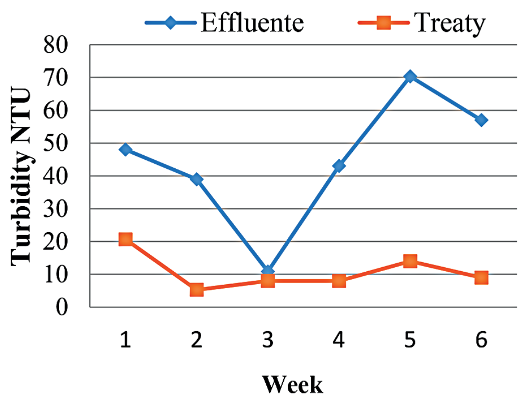

Figure 4. Turbidity behavior in the treated and untreated effluent in 6 consecutive weeks

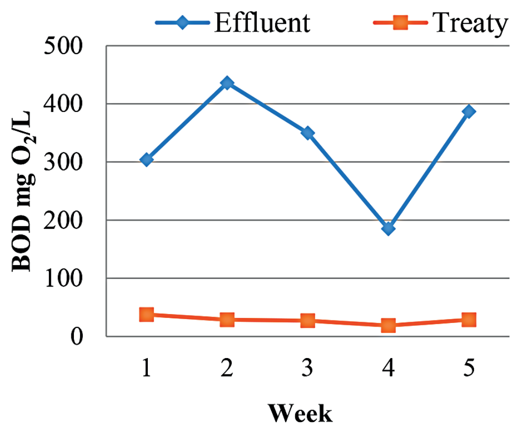

Figure 5. BOD behavior in the treated and untreated effluent in 6 consecutive weeks

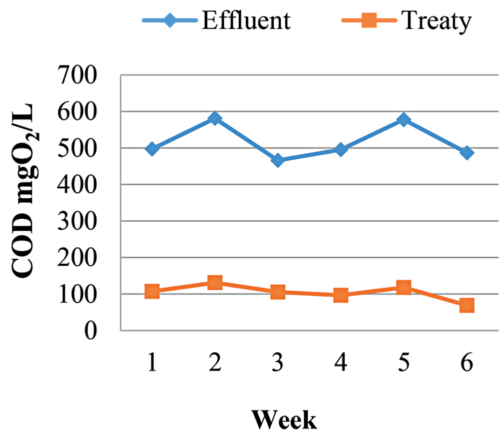

Figure 6. COD behavior in the treated and untreated effluent in 6 consecutive weeks

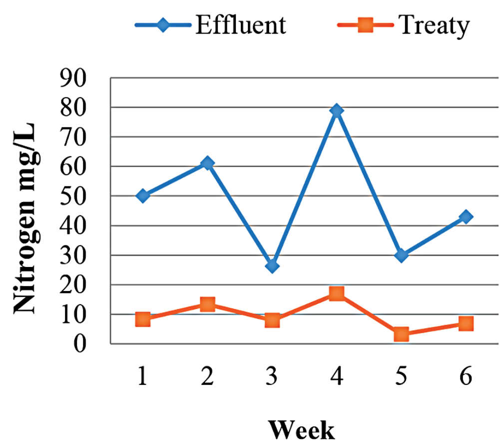

Figure 7. Nitrogen behavior in the treated and untreated effluent in 6 consecutive weeks

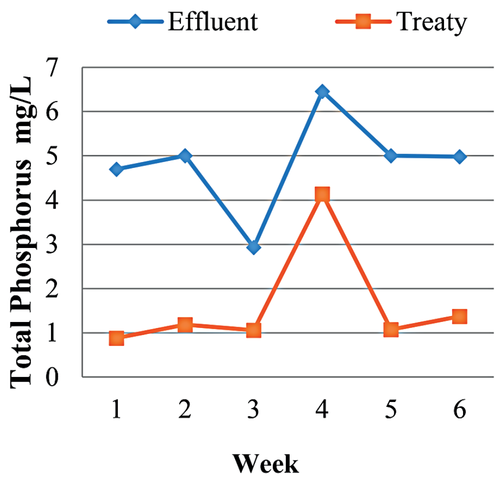

Figure 8. Total phosphorus behavior in the treated and untreated effluent in 6 consecutive weeks

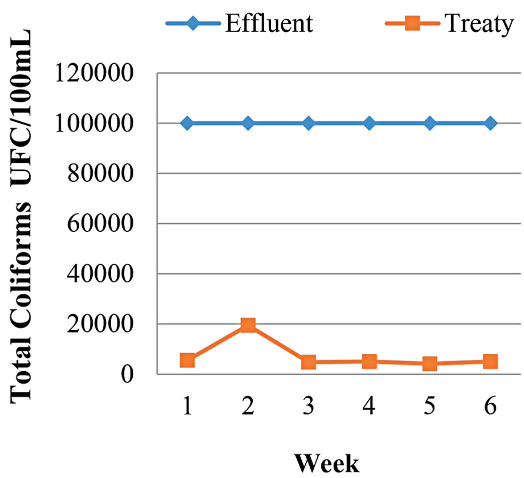

Figure 9. Total coliforms behavior in the treated and untreated effluent in 6 consecutive weeks

the methodology Eq. 1. The results are shown in Table 1 for each parameter analyzed.

The medians of the results obtained from the different parameters throughout the study are observed, in order of removal percentage, where it is seen that the greatest removal was obtained with respect to total coliforms (Figure 9) with an average of 92.46% followed by BOD_5 with 89.89%, total nitrogen with 82.24%, COD with 79.34% and finally total phosphorus with 67.99%.

DISCUSSION

According to what was established in this research, compliant with the proposed objectives and taking into account the purification parameters of *T. domingensis*, which were analyzed in the course of this research, such as pH that showed higher values in the effluents than the ones of the treated water, always around 7, this may be due to the high content of fat present in the effluents. On the other hand, Delgadillo et al. (2010) mentions that *T. domingensis* tolerates a wide pH range (4–9), thus being a fundamental factor for their good development without being affected by these pH values. Likewise, these values obtained are within the ranges allowed and established by the water quality standards of the SEAM.

Another parameter without major variation was temperature, which remained stable between 20 °C and 25 °C, demonstrating that the waste disposed in hospital effluents does not have a high industrial content, rather cleaning waste products, drug remains, of urban nature and somewhat more biodegradable. According to Delgadillo et al. (2010), the optimal temperature for the development of bacterial activity is between 25 and 35 °C; during the study, the records were close to these values, which could be a reason for bacterial development. No very high temperature values were recorded that could affect the flora and fauna of the receiving waters, owing to this, there was a good growth of *T. domingensis*, thus helping to stabilize the temperature in the middle

Table 1. Contaminant removal percentage

Week	BOD_5	COD	TN	TP	TC
1	88.95	76.96	84.3	81.2	94
2	92.98	77.45	79.2	74.84	80
3	91.25	72.75	76.3	64.51	95.6
4	94.89	84.27	78.58	35.91	94.1
5	79.62	79.35	88.88	79.4	96.7
6	91.65	85.25	86.2	72.1	94.4
% total	89.89	79.33	82.24	67.9	92.4

Note: BOD – biological oxygen demand; COD – chemical oxygen demand; TN – total nitrogen; TP – total phosphorus; TC – total coliforms.

of the wetland and counteracting the depletion of dissolved oxygen levels in them.

Regarding the dissolved oxygen (DO) values, in all the samples, an increase in the DO concentration was obtained in treated water compared to the values in the effluent. This reveals the importance and one of the most important characteristics of tourbiopurifying agent, which is the oxygenation of water, absorbing oxygen from the atmosphere and depositing it through its roots that are in contact with the environment (Rinalli and Lundholm, 2008).

Other parameters measured and no less important that were analyzed, were those of conductivity and turbidity. In both cases, significant decreases in their values were seen, making clear the effectiveness of adopted work system, since the decrease in these values could be an indirect indicator of water purification; it could be because the absorption of particles and/or contaminants dissolved in the medium. The decrease in turbidity allows the good passage of light into the medium, thus helping the good growth of the natural biota. Low conductivity values would indicate retention of organic matter that could be present as a waste substance in hospital effluents.

The BOD_5 values obtained during this study showed a considerable decrease in their values, in all the samplings, the good performance of the wetland was reflected, indicative of a high absorption of organic matter. Thus, the COD values also decreased at all points, again an indication of efficiency and retention of contaminants, in this case of the inorganic matter present in the effluent. All the values obtained for the treated water were below the minimum values required by the SEAM.

Thus, it is also important to characterize the type of effluent, taking into account the BOD_5/COD ratio, which makes it possible to determine how much COD (organic and inorganic matter contained in a sample) of a discharge is capable of being purified by microorganisms in five days (DBO_5) and therefore the character of biodegradability of the different discharges. When the BOD_5/COD ratio is less than 0.3, the discharge is considered as non-biodegradable, the values between 0.3 and 0.7 make it slightly biodegradable, while the values greater than 0.7 favor recovery conditions by biological methods, according to Ardila et al. (2012). All the obtained results had a ratio greater than 0.7 and according to all the above; thus the waste could be characterized as biodegradable.

Regarding the values of total nitrogen and total phosphorus, there was a fairly marked decrease in the treated water; all values were below the minimum required by the SEAM, making clear the retention capacity of these contaminants by our biopurifying agent and their disposal in the built system, since the roots remain completely in contact with the medium. According to Delgadillo et al. (2010), the purifying potential of wetlands varies seasonally and is directly proportional to the increase in temperature; therefore, at high temperatures, the capacity to remove phosphorus, phosphates and nitrogen is greater. It should also be noted that considerable amounts of nitrogen in the form of nitrites and ammonia nitrates are present in effluents. The decomposition and mineralization processes carried out by the microorganisms present, transform that nitrogen to nitrites or nitrates (nitrification) and finally to nitrogen (denitrification). These processes depend largely on the concentration of oxygen in the system, which is thus absorbed by the plants present in the adopted treatment system that provides everything necessary for its absorption and its transformation to enter the nitrogen cycle.

The total coliform values obtained during the research decreased in all samples after passing through the wetland, evidencing at all times the great removal of total coliforms. The values were almost around what is recommended by the SEAM. These results are related to what was stated by Kemper et al. (2009), about the concentrations of fecal coliforms present in wastewater which were efficiently reduced from the first sampling and that it can decrease the levels by one or two logarithmic orders.

Contaminant removal percentage

As for the contaminant removal percentage, when perfoming weekly calculations of each of the contaminants that vary from one another, the BOD_5 levels showed that the percentages range between 80% and 95%, and in terms of the COD values, the percentages ranged between 75% and 85%.According to what was previously found, the characterization of the effluent with a good biodegradable behavior is once again left in evidence.

Regarding Total Nitrogen, a greater retention was obtained with an average of 82% higher than the values obtained for total phosphorus, which obtained an average of 68%. This is due to the good behavior of the plant and its good participation in the nitrogen cycle. However, in both cases the values higher than 50% were found, thus achieving high percentages of removal.

With regard to fecal and total coliforms, higher percentages have been reached with respect to allother contaminants on a weekly basis, exceeding 90%, with a general average during the research time, of 92% removal of this contaminants. This is one of the most important factors for the final disposal of these effluents and even more so knowing the proximity of a watercourse to the area studied.

Taking into account all these results and high percentages of removal or retention in all the aforementioned contaminants, the use of this bioremediation system could be recommended, reaching important values and percentages to be considered.

Comparison of the mean of each parameter evaluated with respect to the limits established by the standard of water quality

Taking into account one of the assumed objectives, considering the determination of the quality of treated water according to Resolution 222/02: Water quality in national territory - SEAM, the average values obtained in terms of pH were 6.5, remaining within the required range of 5 to 9. The average temperature recorded was 21.7 °C, while what is required is <40 °C, also remaining within what is required in terms of quality standards.

The mean BOD_5 concentration was 30.6 mg O_2/L and the COD was 105.8 mg O_2/L. These two remained within the minimum required values, which are <50 mg O_2/L and <150 mg O_2/L.

The mean concentration of total phosphorus and total nitrogen were 1.62 mg/L and 8.87 mg/L, respectively, being below the established limit, which are <4 mg/L and <40 mg/L, respectively.

Total coliforms had values of 7,533 CFU/100 mL, values that are hovering around the permitted limit, which is<4,000 CFU/100 mL.

According to the comparative data between the parameters analyzed in this research and the established limit values, the water treated by the constructed wetland as a treatment system using *T. domingensis* as a biopurifying agent, meets the minimum requirements and criteria established by Article 7 of Resolution 222/02 of the SEAM.

CONCLUSIONS

A decrease has been observed in all the parameters evaluated such as total nitrogen, total phosphorus, BOD, COD and total coliforms. An efficiency of around 80% of removal or retention of contaminants was obtained, thus demonstrating that this system is feasible for the remediation of hospital effluents.

Acknowledgements

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