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Evaluation of Physicochemical and Microbiological Parameters of Wastewater Submitted to Constructed Wetland Treatment System

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ABSTRACT

The use of natural systems such as *wetlands* constructed in the treatment of wastewater has become increasingly important due the ability of these systems to remove large amounts of macronutrients and microorganisms and to promote the degradation of organic matter. Given this background, the main objective of this study was to evaluate the efficiency of an experimental constructed wetland horizontal flow system subsurface cultivated with *Zantedeschia aethiopica* filled with sand and gravel substrates. The parameters were evaluated by the characterization of physicochemical and microbiological indicators of wastewater from the septic tank of the State University of Goiás in Anápolis city, Goiás, Brazil. The reductions of macronutrients ranged from 57.9 to 94.7%, and 87.0 to 91.0% for organic matter. In addition, a significant reduction in coliform levels in wastewater was observed after wetland treatment. It was concluded that the constructed wetland system could be proposed to treat wastewater from septic tanks at both secondary and tertiary levels, due to its high percentage efficiency in the removal of organic matter and macronutrients. The results obtained demonstrated the applicability of the studied system to be used as a versatile, sustainable, and economical treatment of effluents complementary to the septic tank treatment system.

Keywords: biological filters; effluent treatment; wastewater; wetlands built; sewage.

RESUMO

O uso de sistemas naturais como *wetlands* construídos no tratamento de águas residuais, tem se tornado cada vez mais importante devido à capacidade desses sistemas em remover grandes quantidades de macronutrientes e micro-organismos, além de promover a degradação da matéria orgânica. Diante desse cenário, o objetivo principal deste trabalho foi avaliar a eficiência de um sistema experimental de fluxo horizontal de *wetland* construído em subsuperfície cultivado com *Zantedeschia aethiopica* preenchido com substratos de areia e cascalho. Os parâmetros foram avaliados por meio da caracterização de indicadores físico-químicos e microbiológicos do efluente da fossa séptica da Universidade Estadual de Goiás na cidade de Anápolis, Goiás, Brasil. A redução dos macronutrientes variou de 57,9 a 94,7% e de 87,0 a 91,0% para a matéria orgânica. Além disso, foram observadas reduções significativas de coliformes após o tratamento de águas residuais em áreas úmidas. Concluiu-se que o sistema *wetland* construído pode ser proposto para o tratamento de efluentes de fossas sépticas em



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nível secundário e terciário, devido ao seu alto percentual de eficiência na remoção de matéria orgânica e macronutrientes. Os resultados obtidos demonstraram a aplicabilidade do sistema estudado em ser utilizado como um tratamento versátil, sustentável e econômico de efluentes complementar ao sistema de tratamento de fossa séptica.

Palavras-chave: filtros biológicos; tratamento de efluentes; águas residuais; *wetlands* construídos; esgoto.

1. Introduction

Water, also called universal solvent due to its potential properties such as solubility power, is an extremely important natural resource for human life. Currently, the major problem around the world is water contamination, due to several reasons such as improper sewage treatment, industrial waste, marine dumping issues, radioactive waste material and some agricultural products. Water pollution has an adverse effect on the environment, and can also be responsible by air pollution that results in very dangerous results for human health (Yaqoob et al. 2020; Shindhal et al. 2021).

The high rate of urbanization and industrialization in recent years is generating a very large amount of wastewater. The lack of treatment of these wastewaters can lead to the release of effluents containing a large amount of organic matter causing the reduction of dissolved oxygen in the water, toxic compounds such as metals that draw attention due to their harmful effect on human metabolism and to the ecosystem as a result of their high persistence in the environment, disease-causing pathogenic microorganisms and nutrients such as phosphorus and nitrogen that can stimulate the growth of aquatic plants and the proliferation of algae, leading to the eutrophication of lakes and streams, thus deteriorating their quality (Rana & Maiti, 2020; Nahiun et al. 2021).

Wastewater treatment systems can be classified into conventional and natural systems. In conventional systems (primary, secondary and tertiary treatments), the physical and biological processes of wastewater treatment are often complex operations that require intensive energy input (for mechanical devices/equipment) and/or input of material as flocculants and oxidants (Ketema et al. 2021; Gikas et al. 2017).

Natural wastewater treatment such as built wetlands is characterized generally into three categories, namely, subsurface flow constructed wetlands (SSFCWs), surface flow constructed wetlands (SFCWs), and hybrid systems. Further, based on the flow path, SSFCWs are differentiated into vertical flow constructed wetlands (VFCWs) and horizontal flow constructed wetlands (HFCWs). According to the macrophytic growth, they are categorized into emergent, free-floating, submerged, and floating-leaved macrophytes (Ali et al. 2020; Kumar & Dutta, 2019; Raouf et al. 2019).

Constructed wetlands are being widely used for treating many classes of contaminants such as heavy metals, domestic and industrial wastewater, textile dye effluents, pesticides, petroleum hydrocarbons, explosives, radionuclides, etc. This treatment method overcomes the shortcomings of conventional wastewater treatment methods as it is a cost-effective, non-intrusive, and eco-centric technology (Rana & Maiti, 2020).

To obtain effective information for these systems to be used as sustainable alternatives (creation of green areas with landscape aspect) and economic (low costs of implantation, maintenance, and operation), this work aims to propose complementary treatment to the treatment by septic tank used in the State University of Goiás – Campus Anápolis, to improve the standards of wastewater before discharge in the water bodies.

2. Material and methods

The experiment was developed at the State University of Goiás, Campus Anápolis, Brazil, located on geographic coordinates of latitude 16°22'52"S and longitude 48°56'45.5"W. All wastewater generated at the University, from the teaching and research laboratories and sanitary units, is conducted to septic tanks units, which goes through primary treatments, and is then taken to the final distribution box.

The wastewater used in the experiment was removed employing a septic pumper truck from the last tank of a system of five septic tanks in series. The volume of wastewater was stored in a tank with a capacity of 5,000 L to supply the built wetland unit.



The horizontal and subsurface wetland system was built with a polyethylene box for agricultural storage with a surface area of 1 m² with dimensions of 1.20 x 1.00 m in length and width, respectively. The lid of the box was cut to a height of 0.90 m. The drainage system was installed at the bottom of the box with 100 mm agricultural irrigation networks piping with 10% of its perimeter with 10 mm holes distributed. Subsequently, the box was filled with a 60 cm layer of filter bed with approximately 20 cm of gravel 1, sand, and gravel 0. Above the filter, the bed was inserted a 20 cm layer of a substrate formed by 2 portions of soil, 1 portion of sand, and 1 portion of organic fertilizer, and the macrophyte species used in the system was *Zantedeschia aethiopica*. The wetland feeding system was similar to the drainage system with a surface application rate of 75.84 l/m² per day, equivalent to approximately 58.93 g/m² per day of volatile solids.

The collection of effluent samples for treatment in the wetland system was carried out in triplicate from August to November 2017 in two stages. In the first stage, the sample was removed from the 5,000 L storage tank of the septic tank to characterize the raw effluent and in the second stage, the sample was collected after a 90-day hydraulic detention period of the effluent in the system for physical-chemical characterization, and microbiological after treatment.

The wetland system (Figure 1) was constructed using agricultural storage boxes whose cells were filled with sand substrates, gravel 0, and gravel 1 and the macrophyte species used in the system for ornamentation purposes was *Zantedeschia aethiopica*.

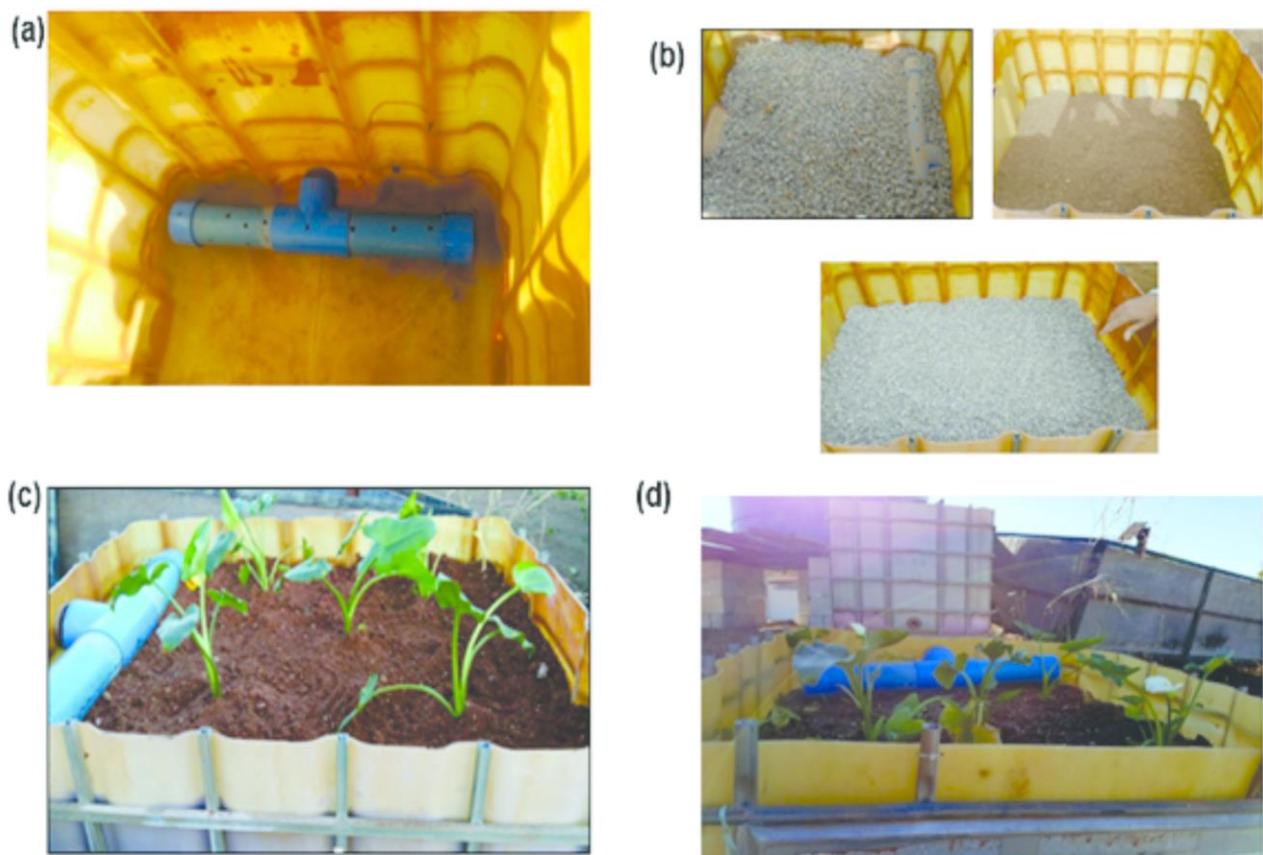


Figure 1: Construction of the built wetland system. (a) agricultural storage box with drainage pipe; (b) substrates of gravel 0, sand, and gravel 1; (c) *Zantedeschia aethiopica* seedlings and (d) built wetland system.

The results obtained from the constructed wetland system were analyzed using the calculation of efficiency of removal of physicochemical indicators in the wastewater before and after treatment using Equation 1.



$$Ef(\%) = \frac{C_e - C_s}{C_e} \times 100 \quad (1)$$

At where:

C_s = Output concentration;

C_e = Intake concentration;

$Ef(\%)$ = Percentage removal efficiency of pollutant load.

The statistical analysis of the data obtained for the wastewater without, after treatment of the wetland system constructed from horizontal subsurface flow, was performed using the paired Student t-test at 5% of probability, and the statistical significance was interpreted according to the p-value obtained. The physicochemical and microbiological parameters of the samples before and after treatment on the constructed wetland system were analyzed according to the methodologies summarized in Table 1.

Table 1. Methodologies used for physicochemical and microbiological parameters.

Parameters	Unit	Method
Conductivity	$\mu\text{S cm}^{-1}$	Thermo orion conductivity meter 3-star
Total Solids	mg L^{-1}	Thermo orion conductivity meter 3 star
pH	-	Thermo orion pH meter 3-star
Turbidity	NTU	UNT Hach 2100P turbidimeter
TOC	mg L^{-1}	Analyzer sievers 900
COD	mg L^{-1}	Hach-colorimetric COD meter
Nitrogen	mg L^{-1}	Kjeldahl-MATOS method with adaptations (Matos 2012)
Phosphorus	mg L^{-1}	UV-MATOS Spectrometer with adaptations (Matos 2012)
Calcium (Ca^{2+})	mg L^{-1}	Titrativa method of potassium permanganate- NBR13799 / 97 (ABNT 1997)
Magnesium (Mg^{2+})	mg L^{-1}	Titration method of EDTA-PAYNE and COMBS, 1968 with adaptations (Payne & Combs 1968)
Potassium (K^+)	mg L^{-1}	Flame photometer- NBR13811 / 97 with adaptations (ABNT 1997)
Chloride (Cl^-)	mg L^{-1}	Method of Mohr- NBR 5759 P MB 1056 (ABNT 1975)
Total coliforms	MPN 100 mL^{-1}	Multiple tubes technique - APHA 1998

pH - hydrogenation potential; NTU - nephelometric turbidity unit; TOC - total organic carbon; COD - chemical oxygen demand; MPN – most probable number.

2. Results and discussion

The results of the evaluated indicators before and after treatment on the constructed wetland system, and the differences between results submitted to the Student t paired test with 5% probability, are shown in Table 2.

Table 2. Physicochemical and microbiological parameters analyzed in the wastewater before and after treatment of the built wetland system.

Parameters	Untreated wastewater (Mean \pm SD)	Post-treatment wastewater (Mean \pm SD)	Removal percentage (%)	Student t test (p)
Conductivity ($\mu\text{S cm}^{-1}$)	2066.00 \pm 1.0	201.70 \pm 0.58	90.20	< 0.0001
pH	7.30 \pm 0.07	7.20 \pm 0.050	-	0.4241



Total Solids (mg L ⁻¹)	1070.0 ± 1.0	25.30 ± 0.58	97.60	< 0.0001
Turbidity (UNT)	131.00 ± 1.0	4.00 ± 0.06	96.90	< 0.0001
TOC (mg L ⁻¹)	202.00 ± 1.5	25.00 ± 1.0	87.60	< 0.0001
COD (mg L ⁻¹)	860.00 ± 0.58	77.00 ± 1.0	91.00	< 0.0001
Nitrogen (mg L ⁻¹)	37.40 ± 0.45	2.00 ± 0.21	94.70	< 0.0001
Phosphorus (mg L ⁻¹)	8.90 ± 0.08	1.40 ± 0.02	84.30	< 0.0001
K ⁺ (mg L ⁻¹)	23.00 ± 0.25	4.60 ± 0.05	80.00	< 0.0001
Ca ²⁺ (mg L ⁻¹)	0.015 ± 0.0004	0.001 ± 0.00065	93.33	0.0005
Mg ²⁺ (mg L ⁻¹)	20.90 ± 0.46	8.80 ± 0.02	57.90	0.0004
Total coliforms (NMP 100 mL ⁻¹)	> 2400	9.30	-	< 0.0001

UNT - nephelometric turbidity unit; TOC - total organic carbon; COD - chemical oxygen demand; MPN = most probable number; SD - standard deviation and p - descriptive level of paired t-test of difference of samples before treatment versus post-treatment.

3.1 Physicochemical Parameters

3.1.1 Conductivity

Significant reductions were observed in conductivity with a percentage removal of 90.20% of dissolved ionic and cationic solids. The reduction of electrical conductivity in the medium occurs mainly through ion exchange and adsorption of calcium, magnesium, potassium, chloride, and other ions by the macrophytes plants, which show the efficiency of the system.

The Brazilian National Environment Council Resolution – CONAMA 430/2011 (2005) does not establish limits of electrical conductivity for the launching of effluents in bodies receiving water, however, this factor must be evaluated, because high values of electrical conductivity indicate a large amount of dissolved solids.

Santos et al. (2016) evaluated the efficiency of a wetland treatment system built for the swine effluents and detected a reduction of 85% of the electrical conductivity value due to the nutrient consumption by macrophytes. The plants absorbed the ions present in the medium and released nutrients that were previously complexed in ionic form with stabilization of organic matter and consequent reduction of electrical conductivity.

3.1.2 Hydrogenionic Potential

The mean values of pH did not show a significant difference, remaining close to neutrality with the value of 7.30 for untreated wastewater and 7.20 post-treatment. Similar results were previously reported in a study of the treatment of sanitary sewage in constructed wetland systems that found close to neutral pH values ranging from 7.20 to 7.50 (Prata et al. 2013). These results indicated that there is not a large amount of compounds in the wastewater responsible to increase or decrease the pH, compounds like carbonates, bicarbonates, hydroxyls groups, sulfates, ammonia, and other ions that can be generated in the organic matter decomposition (Travaini-Lima, F. & Sipaúba-Tavares 2012; Amaral et al. 2021; Torrens et al. 2021)

It was found during the study period that values close to neutrality guarantee a better development of microorganisms and accelerated degradation of the organic matter. As previously mentioned, according to CONAMA Resolution No. 430/2011 (2005), the discharge pattern of wastewater treated in water receiving bodies must be in the pH range of 5.0 to 9.0. All the results obtained in the evaluation of this attribute during the monitoring period are within the standards required by the Brazilian resolution.



3.1.3 Total solids and turbidity

The total solids correspond to the settled dissolved and suspended solids, being organic and inorganic (Sperling 2003). The removal of turbidity in wastewater is directly related to the filtration process, that is, to the capacity of retention of total solids by the substrates and the microbial activity. The filtration process, in turn, is related to the porosity of the filter medium, the larger the void content in the system, the greater the total solids retention, and the lower the turbidity of the wastewater (Zanella 2008).

The percentage removal of total solids in the system was 97.60%, with concentrations of 1070 mg.L⁻¹ for the sample of untreated effluent and 25.30 mg.L⁻¹ post-treatment. A turbidity removal efficiency of 96.90% was observed demonstrating the great efficiency of the wetland system built in the retention of suspended solids contained in the wastewater. The values found for untreated water samples of 131 NTU and post-treatment of 4 NTU were considered extremely significant. The conductivity results allowed us to infer that there was a satisfactory removal of solids and organic matter from the wastewater. Dell'Osbel et al. (2020) show in your hybrid wetland system a satisfactory reduction of 99.9% in turbidity.

The significant retention of total solids and reduction of turbidity in natural wastewater treatment systems occurs mainly through substratum filtration, sedimentation, precipitation of insoluble compounds, oxidation by microorganisms, presence of roots and rhizomes developed by the macrophytes acting as a barrier to retention and assimilation by them (Amaral et al. 2021). A similar result to the present study was obtained by Dornelas (2008) who, when evaluating a subsurface horizontal wetland system with effluent after treatment by reactors, found a 91% total solids removal efficiency. Costa et al. (2018) concluded that constructed subsurface horizontal outflow systems are a wastewater treatment technology based on processes found in natural floodplain ecosystems and constitute a great alternative for post-treatment of anaerobic effluents.

3.1.4 Organic matter

The determination of carbon is an important parameter to indicate the presence of organic carbonaceous matter in the wastewater and the quality of the final effluent is therefore characterized by low biochemical oxygen demand (BOD), chemical oxygen demand (COD), and soluble and suspended total organic carbon (TOC) values (Colares et al. 2020).

This study, were detected significant reductions in total organic carbon concentration in wastewater without the treatment from 202 mg.L⁻¹ to 25 mg.L⁻¹ after treatment, with an efficiency of 87.60% of removal of organic carbonaceous matter. Our results were similar to that found by Andrade (2015) that evaluated a wetland wastewater treatment system constructed with aerated step, and found an organic matter concentration of 374 mg.L⁻¹ in terms of TOC to the untreated sewage, and final values between 41 and 50 mg.L⁻¹ for the treated effluent, detected efficiency up to 89 % in the removal of organic matter. Remotion efficiency of around 85-99% has been by Amaral et al. and 2021 Dell'Osbel, et al. (2020), and 65-75% by Schierano et al. (2020) and Torrens et al. (2021).

In some treatment processes, the reduction of carbonaceous organic matter occurs by adsorption by the substrates and by the action of the microorganisms present in the medium that promote organic matter decomposition. Microorganisms have the property of organizing themselves into structural units called flocs. The flocs represent heterogeneous structures containing adsorbed organic matter, inert material from the sewage, microbial material produced, and living and dead cells of the microorganisms (Colares et al., 2020; Dotro et al., 2017).

The flocs formed in the middle concentrate are separated from the liquid by the simple physical mechanism of sedimentation. This separation allows the final effluent to be clarified with reduced concentrations of suspended organic matter (Parde et al., 2020). The average percentage of chemical oxygen demand removal in the system was 91%. The COD obtained for the crude effluent was 860 mg.L⁻¹ and after treatment, the value decreased to 77 mg.L⁻¹. Similarly, other authors (Berté et al. 2005) found a reduction of organic matter removal in terms of COD of 94.52%. Higher values of COD are associated with the nitrification process, which decreases the ammonium presence and increases the nitrates, in this process the nitrifying bacteria use CO₂, bicarbonate, ammonia, and nitrite as an energy source, reducing these compounds in the wastewater (Travaini-Lima & Sipaúba-Tavares 2012).



3.1.5 Nitrogen

The average efficiency of total nitrogen removal in the system was 94.70%. There was a reduction in the total nitrogen concentration in the wastewater without and after treatment from 37.40 mg.L⁻¹ to 2 mg.L⁻¹. According to Greenway (2004), Dell'Osbel, et al. (2020), Schierano et al. (2020) and Torrens et al. (2021), and Rousso et al. (2019), the main microbial processes that occur in wetlands built for the removal and transformation of nitrogen are ammonification, nitrification, volatilization, adsorption, ion exchange and denitrification and all this process depend on the pH. The nitrification and denitrification process occur in pH 6.5-8.0 (Travaini-Lima & Sipaúba-Tavares 2012). Prata et al. (2013) when evaluating four wetlands systems constructed in the sanitary sewage treatment after being submitted to the primary treatment by septic tank, having the gravel 0 as support medium and the yellow lily (*Hemerocallis flava*) as macrophyte, obtained the highest average removal efficiency of total nitrogen of 52.40 % with a holding time of 3,9 days. The authors observed that the longer the detention time of the system, the greater the removal of nitrogen.

3.1.6 Phosphorus

For the analysis of total phosphorus it was observed that there was a reduction in the concentration of the constructed wetland system from 8.90 mg.L⁻¹ in the untreated wastewater to 1.40 mg.L⁻¹ post-treatment with a phosphorus removal percentage of 84.30%. The reduction of phosphorus in wetlands systems is mainly due to adsorption and precipitation processes using substrates rich in iron, aluminum, calcium, and magnesium, assimilation by the roots of macrophytes, filtration promoted by crushed sand, and removal of inorganic phosphate by the action of microorganisms by converting this ion into organic microbial biomass (Dell'Osbel, et al., 2020; Schierano et al., 2020; Torrens et al., 2021; Rousso et al., 2019).

Crushed limestones were used in the built wetland system for phosphorus adsorption. Calcium carbonate was one of the main binders that absorbs phosphorus and forms different precipitates, for example, calcium phosphate (Ca³(HPO⁴)²), and dicalcium phosphate (CaHPO⁴), tricalcium phosphate (Ca³(PO⁴)²) leading to the reduction of phosphorus in the wastewater.

Vohla et al. (2011) reviewing several filtering materials used for the removal of phosphorus from wastewater by constructed wetlands, reported that in systems using sand and gravel as substrate, the main removal mechanisms of P are adsorption reactions and precipitation with Ca, Al, and Fe, but also, as in all media, assimilation by microorganisms and plants can play a remarkable role. At pH levels above 6.0, the reactions are a combination of adsorption to iron and aluminum oxides and precipitation with soluble calcium phosphates. At lower pH levels, precipitation with iron and aluminum phosphates becomes more and more important.

3.1.7 Calcium, Magnesium, and Potassium

The macronutrients calcium, magnesium, and potassium are essential elements for the growth of macrophytes, and the development of microorganisms, and potassium, after nitrogen, is the element most required by those plants for their development. In addition, calcium and magnesium are two of the main elements responsible for the permanent hardness of the water. The highest mean efficiency of removal of these elements in the constructed wetland system was obtained for the calcium element with 93.33 %, due to its very low input concentration, followed by potassium with 80 % and the lowest for magnesium with 57.90%. The reduction of these macronutrients in the constructed wetland system occurs mainly when absorbed by plants or microorganisms as nutrients for their development, by precipitation reactions, mainly in the form of hydroxides, sulfates, and carbonates adsorption by the substrates, and by processes of cation exchange occurring in the middle. In the adsorption removal process, the positive charges of the ions bind to the negatively charged sites on the surface of the substrate present in the wastewater or in the plant itself. In the ion exchange process, an ion present in the solution is exchanged for an ion with a similar charge attached to a still solid particle. It is represented by the ability to contain cations that can be replaced by the macronutrients sodium, potassium, calcium, and magnesium. It has positive correlations with the incorporation of NH⁴⁺ and some trace elements such as copper (Cu²⁺) and lead (Pb²⁺). The selectivity of the metals at the ion exchange sites occurs in the following order: Na⁺>K⁺> Ca²⁺> Mg²⁺ (Yang et al. 2018).



3.2 Microbiological Parameters

3.2.1 Total coliforms

The overall mean value of post-treatment wastewater obtained for total coliforms was 9.30 MPN 100 mL⁻¹. It was observed a significant removal of coliforms regarding results of untreated wastewater that exceeded the MPN scale (2,400 MPN 100 mL⁻¹). Dang et al. (2020) evaluated a wetland system built with *Brachiaria mutica* vegetation in the treatment of septic effluents and obtained a percentage of total coliform removal of 90.40%. It was observed that the removal of pathogens in the system occurs mainly by sedimentation, filtration, death by predation, and competition among microorganisms in the environment, with detention time being the most important factor that causes death and inactivation of micro-organisms.

According to Dotro et al. (2017), the main mechanisms of pathogen removal in constructed wetland systems include physical, chemical, and biological factors. Physical factors include filtration and sedimentation, chemical factors include oxidation and adsorption to organic matter, and biological removal mechanisms include oxygen release and bacterial activity in the root zone (rhizosphere), as well as aggregation and retention in biofilms, natural death, predation, and competition to limit nutrients or trace elements.

4. Conclusions

In order to analyze the wastewater of the State University of Goiás and propose a complementary treatment system to the septic tank used in the Anápolis campus, it was concluded through this work the effectiveness of the system used and also in the waste management systems that are being used by university laboratories. The performance obtained for the wetlands system constructed with the subsurface horizontal flow was very satisfactory with high percentages of removal of total solids, organic matter, macronutrients, turbidity, and chlorides, being suitable as a post-treatment system of wastewater coming from treatment by septic tank providing improvements in the quality of wastewater treated. The treatment was efficient in the reduction of eutrophic nutrients with a percentage of removal of 94.70% for nitrogen and 84.30% for phosphorus. Through these results, it can be concluded that the subsurface horizontal wetlands system can be used as tertiary treatment. In terms of organic matter, removal percentages of 87.60% for TOC and 91% for COD were obtained. The high indexes obtained can be explained by the action of the microorganisms developed in the biofilm and the sand and gravel substrates that help in the process of removal of the organic matter.

It was possible to verify the reduction of accounts of total coliform number, the results showed a decrease in the value obtained, indicating the efficiency of wetland as an auxiliary system to promote the reduction of fecal contamination of the wastewater before the return to the environment (Sarma, 2020). The results obtained in this work, although preliminary, indicated that the treatment system studied is in fact a system that improves the quality of wastewater.

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6. Declaration of interest statement

No potential conflict of interest was reported by the authors.

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