Efficiency of horizontal subsurface flow-constructed wetlands considering different support materials and the cultivation positions of plant species

**Suymara Toledo Miranda[1] ; Antonio Teixeira de Matos[2]* ;
Mateus Pimentel Matos[3] ; Claudéty Saraiva[4]**

[1]Departamento de Engenharia Agrícola. Universidade Federal de Viçosa (UFV), Campus Universitário, S/N, CEP: 36570-900, Viçosa, MG, Brazil. E-mail: suymara-miranda@hotmail.com
[2]Departamento de Engenharia Sanitária e Ambiental. Universidade Federal de Minas Gerais (UFMG), Avenida Pres. Antônio Carlos, n° 6627, CEP: 31270-901, Belo Horizonte, MG, Brazil.
[3]Departamento de Recursos Hídricos e Saneamento. Universidade Federal de Lavras (UFLA), Avenida Doutor Sylvio Menicucci, S/N, CEP: 37200-000, Lavras, MG, Brazil. E-mail: mateus.matos@deg.ufla.br
[4]Departamento de Pesquisa. Instituto de Laticínios Cândido Tostes. Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG), Rua Tenente Luiz de Freitas, n°116, CEP: 36576-000, Juiz de Fora, MG, Brazil.
E-mail: claudety@epamig.br
*Corresponding author. E-mail: atmatos@desa.ufmg.br

ABSTRACT

The present work evaluated the influence of filling substrate material (crushed PET bottles or fine gravel) on the efficiency of pollutant removal in horizontal subsurface flow constructed wetlands (HSSF-CWs). They were cultivated with a consortium of elephant grass cv. Napier (*Pennisetum purpureum* Schum) and Tifton 85 (*Cynodon* spp.) to treat wastewater from a common milk cooling tank (WWMT). For this, six HSSF-CWs were used which had dimensions of 0.6 m tall x 1.0 m wide x 2.5 m long. In order to investigate possible efficiency loss in the removal of pollutants from the system, operation was divided into two periods: Period I (from April to December 2015) and Period II (April to December 2016). Thus, the removal efficiencies of BOD_5, solids and total nitrogen (TN), total phosphorus (TP), potassium (K) and sodium (Na) from WWMT were statistically compared. Results indicated that the efficiency of the HSSF-CWs for removing pollutants increased or remained similar after one year and nine months of their operation; and PET bottles were a viable alternative substrate in HSSF-CWs based on the efficient removal of pollutants from WWMT during the one year and nine months of monitoring. Crushed PET bottles constitute a viable substrate for filling HSSF-CWs. Altering the cultivation positions of the plant species did not change pollutant removal efficiencies, but indicates the importance of species arrangement to maximize system performance.

Keywords: alternative substrates, plant consortium, wastewater.

Eficiência de sistemas alagados construídos de escoamento subsuperficial horizontal considerando diferentes materiais suporte e posição de cultivo das espécies vegetais

RESUMO

No presente trabalho, avaliou-se a influência do tipo de substrato (garrafas PET amassadas ou brita gnáissica # 0) de preenchimento na eficiência de remoção de poluentes em sistemas alagados construídos de escoamento horizontal subsuperficial (HSSF-CWs) cultivados, de forma consorciada, com capim-elefante cv. Napier (*Pennisetum purpureum* Schum) e capim-tifton 85 (*Cynodon* spp.), para o tratamento de água residuária de tanque comunitário de resfriamento de leite (WWMT). Para isso, foram utilizados seis HSSF-CWs, nas dimensões de 0,6 m de altura x 1,0 m de largura x 2,5 m de comprimento. Com o intuito de investigar a possível perda de eficiência na remoção de poluentes pelo sistema, a operação do sistema foi dividida em dois períodos: Período I (de abril a dezembro de 2015) e Período II (abril a dezembro de 2016). Assim, compararam-se estatisticamente, as eficiências na remoção de DBO$_5$, série de sólidos e nitrogênio total (TN), fósforo total (TP), potássio (K) e sódio (Na) da WWMT. Os resultados indicaram que a eficiência dos HSSF-CWs na remoção de poluentes aumentou ou permaneceu semelhante após um ano e nove meses de sua operação e que Garrafas PET amassadas podem constituir alternativa viável de substrato em HSSF-CWs, tendo em vista que propiciaram eficiente remoção de poluentes da WWMT, ao longo de um ano e nove meses de monitoramento. Garrafas PET amassadas são opção viável se substrato para enchimento de HSFF-CWs. Alteração na posição de cultivo das espécies vegetais não alterou as eficiências de remoção de poluentes, mas indicou a importância da forma de arranjo das espécies no desempenho desse sistema.

Palavras-chave: água residuária, consórcio de cultivares, substratos alternativos.

1. INTRODUCTION

There is an increasing demand for alternative technologies to treat polluted waters and wastewater that require minimal economic resources, both to implement and operate the treatment system. The use of constructed wetlands (CWs) has shown to be a sustainable option, which is why they have been used for decades in the treatment of wastewater (Wang *et al*., 2017).

In treatment with HSSF-CWs, the pollutant removal efficiency varies considerably (Zhang *et al*., 2014) in function of a complex combination of physical, chemical and biological processes for the removal of contaminants. These are further dependent on many variables, including water application rate, type of wastewater, organic application rate, hydraulic retention time, operating mode (intermittent or continuous), variation of environmental conditions such as temperature, presence and types of vegetation, substrate, biofilm formation and others (Brix, 1997; Kadlec and Wallace, 2009; Ávila *et al*., 2013).

HSSF-CWs that utilize traditional substrate materials, such as gravel, have satisfactory efficiencies for reducing biochemical oxygen demand (BOD$_5$), ranging from 78.5 to 96.3% (Matos *et al*., 2010). With the use of plastic beads, a high porosity medium (0.8 m³ m^{-3}), Sklarz *et al.* (2009) obtained an average efficiency of 95%, while Costa *et al.* (2013) found 69 to 72% when using blast furnace slag as a substrate.

On the other hand, traditional filter media often clog quickly due to either the size of their pores or by wear during the operational period (Pedescoll *et al*., 2009). Thus, the use of materials that are inert and easy to acquire may be an interesting solution in terms of the associated costs (purchase and replacement) (Saraiva *et al*., 2018). In this context, alternative

materials have been evaluated as substrates in different types of systems used for the treatment of wastewater. PET bottles (polyethylene terephthalate), whose disposal has increasingly become an environmental liability, requiring recycling and reuse, have the potential to serve as an alternative support media for HSSF-CWs (Saraiva *et al.*, 2019).

Dallas and Ho (2005) studied the efficiency of HSSF-CWs filled with two different substrates: uncrushed PET bottles cut into two or three parts (depending on the size of the bottle) which presented 94% drainable porosity, and gravel which had 40% drainable porosity. According to these authors, in addition to the substrate of cut PET bottles presenting better results in terms of nutrient/pollutant removal, there was a 50% reduction in cost of constructing the HSSF-CWs compared to those filled with gravel. Furthermore, this material is less subject to wear resulting from acid attack in the HSSF-CWs, which permits for longer system operational times without indication of clogging problems in the porous media. Saraiva *et al.* (2018) observed BOD_5 removal efficiencies ranging from 82 to 87% and from 67 to 70% for total solids removal when using crushed PET bottles for the treatment of wastewater from a milk cooling tank (WWMT).

Because plant species have different requirements with regards to nutrients and environmental development conditions, intercropping can be another important variable to increase the efficiency of these systems. In order to positively contribute to the performance of the CWs, it is necessary that macrophytes cultivated in these systems have a set of characteristics that include adaptation to the excessively reducing, acidic or alkaline environment, the presence of toxic components, and generally high salinity (Stottmeister *et al.*, 2003). The plants must have a well-developed and deep root system (Cheng et al.; 2003), and thus increase the ability of the HSSF-CWs to remove pollutants (Wu *et al.*, 2005).

The root system of plants grown in HSSF-CWs can be stoloniferous or rhizomatous, axial or fasciculated. According to Chen *et al.* (2007), the rhizomatous root system is generally larger and more superficial and presents greater longevity, while the fasciculate root system is more voluminous, formed by thin roots that grow faster, have larger surface area and greater pollutant removal capacity. According to Chen *et al.* (2004), plants with fasciculated roots present less tolerance to the anoxic conditions of the medium than those of rhizomatous roots which present a greater capacity for pollutant removal from the waste water being treated in the HSSF-CWs.

Because the physiological and productive characteristics of plant species are factors which influence the operational conditions and efficiency of the HSSF-CWs with respect to removal of pollutants, it is important to evaluate reactor performance and influence of plant species with fasciculated and deep root growth, such as elephant grass, and species which propagate by means of stolons and rhizomes, such as Tifton 85 grass. Finally, intercropping should be evaluated, with altering cultivation positions in the HSSF-CWs.

Miranda et al. (2019) observed that the sequence of plant species cultivated also influenced the hydrodynamic conditions in HSSF-CWs, which indicates the importance of analyzing the characteristics of the substrate and the effect of pore space expansion by the roots on system pollutant removal efficiency.

Little is known about the use of PET bottles as a substrate and the influence of the cultivation position of plant species with different root systems cultivated in consortium on EFFS-CW efficiency. The objective of this study therefore was to evaluate the performance of HSSF-CWs cultivated with elephant grass cv. Napier and Tifton 85 grass in consortium, using crushed PET bottles or fine gravel as a substrate, for the treatment of wastewater from a common milk cooling tank (WWMT).

2. MATERIALS AND METHODS

The experiment consisted of six HSSF-CWs, constructed of masonry, with dimensions of 0.6 m tall x 1.0 m wide x 2.5 m long, positioned on the ground with the bottom (level) and sides

IPABH

made impermeable with a 0.5 mm thick PVC tarp. They were mounted in parallel and delimited by masonry walls. The units had been in operation since April 2015 for the treatment of wastewater from a common milk tank (WWMT).

The HSSF-CWs were filled with gneiss gravel (D60 = 9.1 mm, coefficient of uniformity - CU = D60/D10 = 3.1 and initial porosity, n = 0.398 m^3 m^{-3}), and capped and crushed PET bottles (250 and 500 mL) (n = 0.642 m^3 m^{-3}). The gravel had an average diameter of 12.5 mm and the crushed PET bottles an average volume of 125 cm^3.

In the HSSF-CWs filled with capped and crushed PET bottles, a plastic screen (13 mm mesh) was placed on top of the substrate along with a 0.10 m layer of fine gravel in order to prevent PET bottles from floating in the system.

The plant species cultivated in the HSSF-CW were elephant grass cv. Napier (*Pennisetum purpureum* Schum) and Tifton 85 (*Cynodon* spp.). Each occupied half the surface of these systems in a specific cultivation order as part of the established treatment.

In order to investigate possible efficiency loss of pollutants' removal from the system, the operation was divided into periods: Period I (data generated from April to December 2015 in the experiment conducted by Saraiva *et al.* (2018)) and Period II (monitoring of the system from March 11, 2016 until December 15, 2016), with the application of WWMT. Each of these phases was performed by a different researcher, with different research objectives. During the two monitoring periods the units were fed continuously for 24 h at the inlet of the HSSF-CW bed. Wastewater generated in the WWMT sanitization process was collected in a 1000 L reservoir, from which it was sent to individual feed containers (200 L volume) of each HSSF-CW (Figure 1a). Influent distribution occurred at a central point at the inlet to each HSSF-CW through a ½ inch plastic tap at which the influent flow was controlled. Depth of the WWMT in all HSSF-CWs was maintained at 0.35 m, providing a nominal or theoretical hydraulic retention time (HRTn) of 3.0 and 1.9 d, respectively, in the HSSF-CWs filled with crushed PET bottles and gneiss gravel. Figure 1b shows the frontal and longitudinal sections of the HSSF-CWs, where it is possible to observe in all sections that the substrate is filled to a depth of 0.45 m. In the HSSF-CW filled with crushed PET bottles, a 0.10 m layer of #3 gneiss gravel was placed on the 0.35 m layer of crushed PET bottles. It should be noted that because the water level was maintained at 0.10 m below the surface of both HSSF-CWs, the liquid height in both systems was 0.35 m.

The experimental treatments were as follows: HSSF-CWs filled with the crushed PET bottles substrate, without plant cultivation (CW-P), cultivated in its first half with Tifton 85 grass and in its second half with elephant grass (CW-PTE), and cultivated in its first half with elephant grass and in its second half with Tifton 85 grass (CW-PET); HSSF-CWs filled with the fine gravel substrate, without plant cultivation (CW-B), cultivated in its first half with Tifton 85 grass and in its second half with elephant grass (CW-BTE), and cultivated in its first half with elephant grass and second half with Tifton 85 grass (CW-BET). The experiment was in operation for one year and nine months.

Each bed was fed at a flow rate of 0.18 m^3 d^{-1} and a mean organic loading rate (OLR) of 33.7 g m^{-2} d^{-1} of BOD_5, with a hydraulic retention time of 1.90 days in the CWs filled with gravel (CW-B) and 3.10 days in CWs with PET (CW-P).

Influent and effluent samples from the HSSF-CWs were collected every 30 days, and a total of eight samples were collected during the system monitoring period from April to December 2016 (Period II). During the experimental period, the aerial portions of the plants were cut 6 times, every 45 days.

To understand the variation in efficiency of the systems over time, physical, chemical and biochemical analyses of samples from the influent and effluents were performed in the Laboratory of Water Quality at the Department of Agricultural Engineering of the UFV. The

following variables were analyzed: biochemical oxygen demand (BOD$_5$), total solids (TS), total suspended solids (TSS), total nitrogen (TN), total phosphorus (TP), potassium (K) and sodium (Na) according to recommendations and methods of the Standard Methods for the Examination of Water and Wastewater (APHA *et al.*, 2012) and Matos (2015).
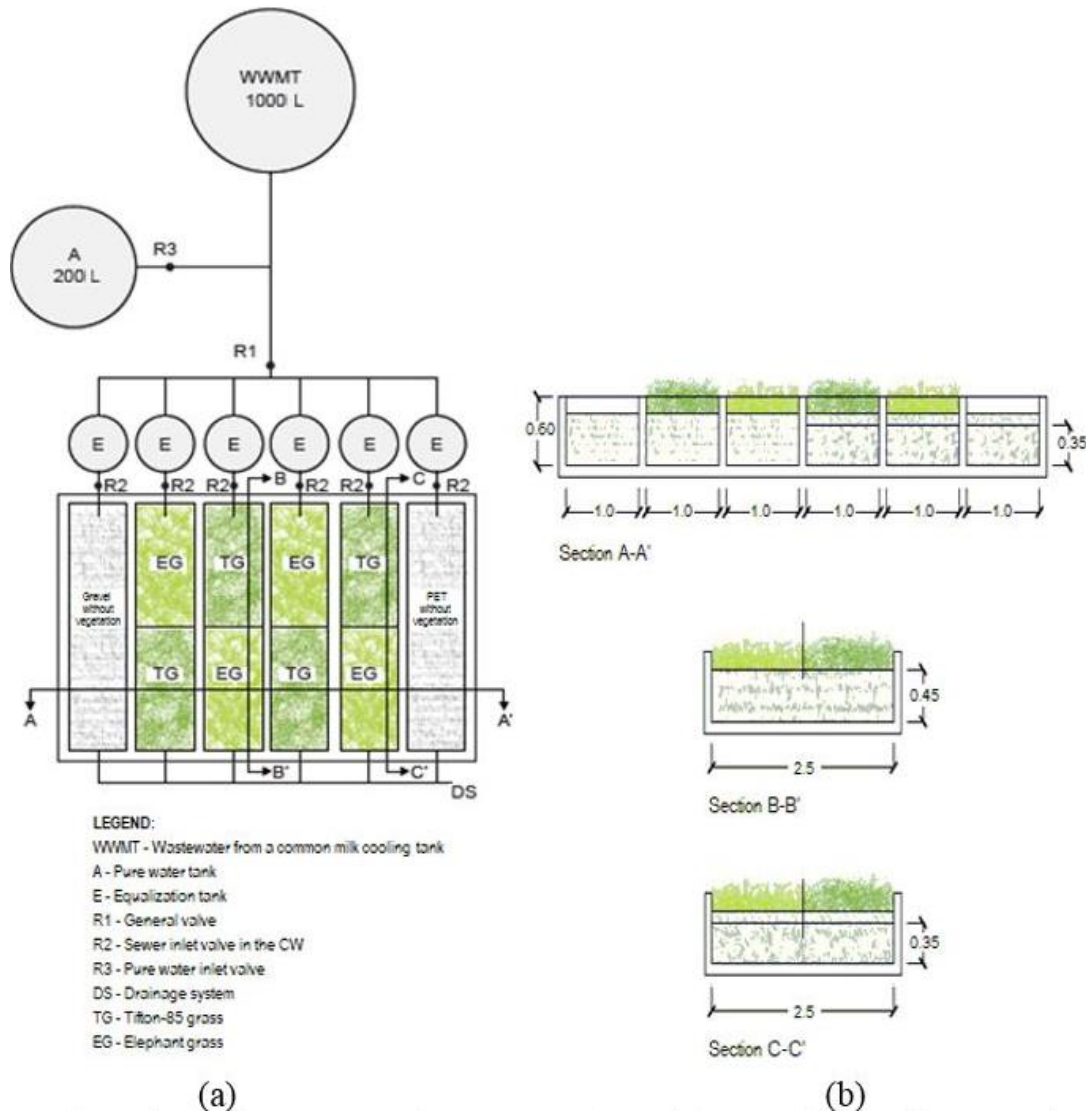

Figure 1. Schematic design representing an overview of the experiment (a); Frontal section of the HSSF-CWs (A-A') and cross sections of a HSSF-CW filled with gneiss gravel (B-B') and the other filled with crushed PET bottles (C-C') (b).

Direct influent and effluent flow measurements were performed weekly, using a stopwatch and graduated cylinder. While conducting the experiment, the water losses by evaporation and evapotranspiration were evaluated. To do this, once a month at one-hour intervals, during 10 hours a day (9:00 a.m. to 7:00 p.m.), the influent and effluent volumes to/from the system were quantified using a 250 mL graduated cylinder.

The quantifying evaporation/evapotranspiration is important because of its role in calculating the actual removal efficiencies of pollutants/nutrients, calculated as presented in Equation 1.

$$EF = \frac{[(QAf \cdot CAf) - (QAf - Ev) \cdot CEf)]}{(QAf \cdot CAf)} \tag{1}$$

Where: EF = Efficiency for removal of the pollutant (%); C_{Af} = inlet concentration (variable unit); Q_{Af} = influent flow (m³ d^{-1}); C_{Ef} = effluent flow (variable unit); Ev = evaporation/evapotranspiration in the HSSF-CWs (m^3 d^{-1}).

The removal efficiencies of the studied variables were analyzed statistically in a 2 x 3 factorial scheme, for a total of 6 treatments. The first factor, substrate, contained two levels (fine gravel and crushed PET bottles), and the second factor, combination of plants, had three levels (without vegetation - SV, elephant grass followed by Tifton 85 - ET and Tifton 85 followed by elephant grass - TE). The experiment was set up in a randomized complete block design (RCBD), where the number of blocks for each variable was a function of the number of samples.

For verification of the assumptions of normality and homogeneity of variance, the Kolmogorov-Smirnov test and the Cochran Bartlett test were applied, respectively. The data that did not meet the normality and homogeneity assumptions was analyzed by non-parametric tests, Kruskal-Wallis for comparison of groupings and that of Wilcoxon for paired data, always at a significance level of 5%. Statistica Version 13.3 was used for data processing and statistical analysis.

The means were submitted to Analysis of Variance (ANOVA, p = 0.05), and when significant the Tukey Test (p = 0.05) was applied between the means; when there was a significant interaction between the factors, unfolding was performed.

To compare the removal efficiencies between Period I and Period II, the "t" test was used for independent data at 5% significance.

3. RESULTS AND DISCUSSION

Table 1 shows the mean values of the surface application rates during experimental monitoring of the systems, Period I (April to December 2015) (Saraiva *et al.*, 2018) and Period II, the period of this study (April to December 2016). As observed, only the surface loading rate of TP differed significantly according to the t-test.

Table 1. Mean values of the surface loading rate of organic matter (BOD$_5$), total solids (TS), total nitrogen (TN), total phosphorus (TP), potassium (K) and sodium (Na) applied during the operational periods I (April to December 2015) and II (April to December 2016).

	Surface loading rates (g m^{-2} d^{-1})					
	BOD$_5$	TS	TN	TP	*K	Na
Period I	32.4 a	42.1 a	1.9 a	0.8 b	0.7 a	0.8 a
Period II	33.7 a	47.4 a	2.1 a	0.2 a	0.6 a	0.8 a

*T test for the non-parametric data
*Means followed by the same lower-case letter, in the column, do not differ according to the t-test at 5% significance.

Table 2 presents mean values for the removal efficiencies of BOD$_5$, TS, TSS, TN, TP, K and Na in the HFFV-CWs, during Periods I and II.

According to the results presented in Table 2, it was found that there was a significant difference in the removal of BOD$_5$ only in the CW-P (system filled with crushed PET bottles and without plants), where the removal efficiency in Period I (87%) was higher than in Period II (75.0%). However, this result should not be considered, since it is very close to that obtained in other treatments and may be due to the normal variation of this variable.

Table 2. Mean removal efficiencies of pollutants/nutrients from the HSSF-CWs during Periods I and II.

HSSF-CWs	Period I						
	Removal efficiency (%)						
	BOD$_5$	TS	TSS	TN	TP	K	Na
CW-P	87 b	69 a	94 a	21 a	27 a	7 a	6 a
CW-PTE	82 a	67 a	91 a	37 a	23 a	16 a	-3 a
CW-PET	87 a	70 a	96 a	43 a	29 a	23 a	8 a
CW-BTE	85 a	52 a	82 a	38 a	22 a	11 a	-12 a
CW-BET	87 a	57 a	90 a	50 a	27 a	23 a	-14 a
CW-B	87 a	58 a	91 a	30 a	20 a	15 a	6 a

HSSF-CWs	Period II						
	Removal efficiency (%)						
	BOD$_5$	TS	TSS	TN	TP	K	Na
CW-P	75 a	54 a	97 a	30 a	17 a	19 a	-5 a
CW-PTE	89 a	66 a	95 b	45 a	30 a	23 a	-2 a
CW-PET	88 a	70 a	98 a	51 b	35 a	38 a	17 a
CW-BTE	88 a	63 a	96 b	56 b	38 b	36 a	-1 a
CW-BET	90 a	54 a	97 a	57 a	39 a	41 a	2 a
CW-B	82 a	51 a	96 a	41 a	26 a	26 a	-5 a

Means followed by the same letter in the column do not differ significantly from each other at 5% significance, according to the t-test.

CW P (medium filled with crushed PET bottles, no plants); CW-PTE (medium filled with crushed PET bottles, cultivated in the first half with Tifton 85 grass and in the second half with elephant grass); CW-PET (medium filled with crushed PET bottles, cultivated in the first half with elephant grass and in the second half with Tifton 85 grass); CW-BTE (medium filled with gravel, cultivated in the first half with Tifton 85 grass and in the second half with elephant grass); CW-BET (medium filled with gravel, cultivated in the first half with elephant grass and in the second half with Tifton grass 85); CW-B (medium filled with gravel, no plants).

In relation to the TSS only CW-PET and CW-BET differed significantly, where greater efficiencies were obtained in Period II. As the bed matures, there is an increase in retention of larger particles, which could explain the increase from one period to the other, and apparently elephant grass cultivation in the first half of HFFS-CW results in greater SS removal.

The TN removal efficiencies differed statistically in the CW-PET and CW-BET, which may have occurred due to the greater stabilization in development and growth of the plants and a microbiota adapted to the WWMT treatment. In both Periods I and II a large variation in the concentration of K and Na in the effluent of the HSSF-CWs was observed; however, there was no statistical differentiation.

Results of the statistical analysis for average removal efficiencies of the variables studied during Period II, in relation to the type of support material and the combination of plants in the HSSF-CWs, are presented in Table 3.

According to the data presented in Table 3, there was no significant difference in the removal efficiency of BOD$_5$ when comparing the different substrates (crushed PET bottles and gravel). Therefore, it can be inferred that use of the porous medium composed of crushed PET bottles is a viable option based on the high removal of BOD$_5$ that it provides, and the more delayed pore clogging, given the greater porosity of this medium (0.642 m^3 m^{-3}) compared to gravel (0.398 m^3 m^{-3}).

Table 3. Mean efficiencies for the removal of BOD$_5$, TS, TSS, TP, TN, K and Na considering the different support materials and combination of plants.

*Factor	Factor level	Removal efficiency (%)			
		BOD$_5$	TS	TSS	TP
Support material	GRAVEL	84 a	56 a	97 a	36 b
	PET	87 a	63 b	97 b	27 a
Combination of plants	NP	77 a	53 a	97 a	21 a
	ET	89 b	62 b	98 a	38 b
	TE	88 b	64 b	96 a	35 b
		TN	K	Na	
Support material	GRAVEL	51 b	34 a	-1 a	
	PET	42 a	26 a	4 a	
Combination of plants	NP	35 a	22 a	5 a	
	ET	54 b	37 a	13 a	
	TE	51 b	36 a	4 a	

*Gravel (# 0 gravel), PET (crushed PET bottles), NP (no cultivated plants), ET (cultivated in the first half with elephant grass and in the second half with Tifton 85 grass), TE (cultivated in the first half with Tifton 85 grass and in the second half with elephant grass).
*Means followed by the same lower-case letter, per factor, do not differ by the Tukey test at 5% significance.

Regarding the cultivation factor, it was observed that the HSSF-CWs differed significantly at 5% significance, i.e.; there was a greater efficiency of BOD$_5$ removal in the cultivated HSSF-CWs than in those with no plants. According to Caselles-Osorio and García (2007), the presence of plants has a significant impact on the removal efficiency of organic material, due to the convective transport of oxygen, or indirectly by evapotranspiration, which increases the fluctuations of water levels and therefore creates a more aerobic environment. Mendonça *et al.* (2015) evaluated the treatment efficiency of dairy effluents in HSSF-CWs using different substrates (# 0 gravel and a mixture of sand + gravel) and plant species (without vegetation, cattail (*Typha latifolia* L) and marshland lily (*Hedychium coronarium*)) and obtained BOD$_5$ removal efficiencies that varied from 79 to 95.2% in cultivated HSSF-CWs and 77.8 to 94.9% in uncultivated HSSF-CWs, but the results were not significantly different. Matos *et al.* (2012) also verified that there was no significant difference between the cultivated and uncultivated CWs regarding the removal of BOD$_5$.

The mean TS removal efficiencies showed significant differences for the two substrates evaluated, but not between the different combinations of plant cultivation. A possible cause of the lower TS removal in the filter media composed of crushed stone may be related to the fact that it is subject to wear over time, releasing solids to the wastewater being treated, which does not occur in the case of the crushed PET bottles. Mean efficiencies of TSS removal did not show significant differences for different substrates and combinations of plant cultivation. In the work conducted by Lee *et al.* (2004), it was observed that 100% of the TSS removal occurred by physical mechanisms, with no contribution of plants or microbiological mechanisms. Thomas *et al.* (1995) and Tanner and Sukias (1995) also reported similar removal efficiencies between systems with and without plants, contrary to that found by Karathanasis *et al.* (2003) who obtained higher TSS removals in a planted unit (88%) compared to an uncultivated CW (46%). Matos *et al.* (2010) also observed slightly higher mean TSS removal efficiencies in uncultivated HSSF-CWs compared to those obtained in units cultivated with

cattail (86%) and Tifton 85 grass (90%).

The mean efficiencies of TN removal showed significant differences for different substrates and combinations of plant cultivation in the HSSF-CW beds. According to Wu *et al.* (2014), different substrates influence establishment of the biofilm and microbial community structure, as well as the treatment efficiency. A porous medium, such as expanded clay, provides a larger surface area for contact with wastewater, favoring biofilm growth. Similar to expanded clay, gravels have larger exposed areas for growth and adherence of microorganisms than beds filled with crushed PET bottles, thus favoring greater TN removal, which primarily occurs by microorganisms (nitrification/denitrification and microbial assimilation).

Better performance of cultivated versus non-cultivated HSSF-CWs for N removal may be due to the absorption of this nutrient by plants. In addition, the plant roots provide favorable conditions for the development of microorganisms capable of transforming the chemical forms of nitrogen (Liu *et al.*, 2012). According to Chen *et al.* (2016), plants release exudates from the roots that affect the density and diversity of the root microbiota, which in turn increase the rates of nutrient removal.

Macrophytes provide an increase in nutrient removal from the wastewater under treatment due to their accumulation in the biomass, fixation of inorganic and organic particulates, and if ammonium is present, its transformation into nitrate in the oxidizing rhizosphere (Brix, 1994). Zhang *et al.* (2016) stated that the presence of plants in CWs can improve the efficiency of wastewater treatment, since they verified that when compared to the control treatment (without plant cultivation), CWs cultivated with *M. Aquaticum* and *A. philoxeroides* increased the rates of total nitrogen removal by 50.9% and 36.3%, respectively.

The mean efficiencies of TP removal showed significant differences for the different substrates and combinations in plant cultivation, where greater efficiencies of TP removal were obtained in cultivated HSSF-CWs. Amorim *et al.* (2015) evaluated HSSF-CWs cultivated with Tifton 85 grass for the treatment of swine wastewater and obtained lower efficiency for the removal of TP, which varied between 20 and 30%.

Different from the results encountered in the present study, Fia *et al.* (2017) observed no significant difference in TP removal in cultivated and uncultured CWs. Mendonça *et al.* (2012) also obtained no significant difference between cultivated and uncultivated HSSF-CWs for the removal of phosphorus, finding mean removal efficiencies of 33.6% (HSSF-CW filled with gravel and cultivated with cattail), 28.8% (HSSF-CW filled with gravel and cultivated with marsh lily), 34.3% (HSSF-CW filled with a mixture of sand + gravel and cultured with cattail), 34.2% (HSSF-CW filled with a mixture of sand + gravel and cultivated with marsh lily) and 18.6% (HSSF-CW filled with a mixture of sand + gravel and without vegetation).

There was a greater efficiency of TP removal in HSSF-CWs filled with gravel compared to those filled with crushed PET bottles, which was already expected since crushed PET bottles are, in principle, chemically inert. Mendonça *et al.* (2012) corroborated with this argument by explaining that phosphorus removal occurs by adsorption processes and that phosphate ions can chemically interact with aluminum or iron present in the gravel used as substrate, thereby increasing the efficiency of TP removal in gravel-filled CWs.

Table 3 shows that there was no difference in K removal between cultivated and uncultured HSSF-CWs. Fia *et al.* (2017) evaluated the influence of vegetation on HSSF-CWs for the removal of pollutants from swine wastewater (SWW). The authors found no significant difference in potassium removal efficiency, with values of 27% in the three HSSF-CWs evaluated (uncultivated, cultivated with cattail and cultivated with Tifton 85 grass). Matos *et al.* (2010), when treating SWW in uncultivated HSSF-CWs and those cultivated with alternanthera (*Alternanthera philoxeroides* (Mart.) Griseb.), cattail and Tifton 85 grass, also found no differences in potassium removal; however, efficiencies were higher than those found in the present study (between 29 and 46%).

It is also observed in Table 3 that there was no difference in Na removal between the cultivated and uncultivated CWs, as well as when considering the different substrates. The average Na removal efficiencies were low, even presenting negative efficiencies. According to Brasil *et al.* (2005) this can be associated with the great solubility of this chemical element, its low absorption by the plants and its low association with organic material, which is strongly retained by physical processes in the HSSF-CW.

In general, the results indicated that nutrient (N, P and K) removal is more associated with the available substrate surface for the development of adhered biofilm than the HRTn of the HFSS-CWs. To increase the surface area of the crushed PET bottles, it is recommended that they have their caps removed and holes drilled in the bottom to allow for flow through the interior, and also biofilm formation on the inner wall. The use of crushed PET bottles as a support medium has shown promising, and that the selection and arrangement of plant species can also provide gain in pollutant-removal efficiency.

4. CONCLUSIONS

The pollutant removal efficiency in HSSF-CWs improved or remained similar over one year and nine months of operation, with no difference between the gravel and the capped and crushed PET bottle substrates.

HSSF-CWs filled with capped and crushed PET bottles were more efficient in TS and TSS removal than those filled with the gravel substrate; however the gravel substrate provided greater TN and TP removals from WWMT. There were higher mean removal efficiencies of BOD, TS, TP and TN in the cultivated HSSF-CWs when compared to the uncultivated units.

Based on the results obtained, the use of PET bottles as a support medium showed to be promising, and that the use of plant species can provide gains in removal efficiency. Cultivation positions of the plant species did not change the pollutant removal efficiencies, but indicates the importance of species arrangement for system performance.

5. ACKNOWLEDGEMENTS

6. REFERENCES

APHA; AWWA; WEF. **Standard Methods for the examination of water and wastewater**. 22nd ed. Washington, 2012. 1496 p.

AMORIM, F.; FIA, R.; SILVA, J. R.; CHAVES, C.; PASQUALIN, P. Unidades combinadas RAFA-SAC para tratamento de água residuária de suinocultura: Parte II Nutrientes. **Engenharia Agrícola**, v. 35, n. 5, p. 931-940, 2015. https://doi.org/10.1590/1809-4430-Eng.Agric.v35n5p931-940/2015

ÁVILA, C.; REYER, C.; BAYONA, J. M.; GARCÍA, J. Emerging organic contaminant removal depending on primary treatment and operational strategy in horizontal subsurface flow constructed wetlands: Influence of redox. **Water Research**, v. 47, p. 315-325, 2013. https://doi.org/10.1016/j.watres.2012.10.005

BRASIL, M. S.; MATOS, A. T.; SOARES, A. A.; FERREIRA, P. A. Qualidade do efluente de sistemas alagados construídos, utilizados no tratamento de esgoto doméstico. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v. 9, p. 133-137, 2005.

Rev. Ambient. Água vol. 15 n. 2, e2476 - Taubaté 2020

IPABHi

BRIX, H. Functions of macrophytes in constructed wetlands. **Water Science & Technology**, v. 29, n. 4, p. 71-78, 1994. https://doi.org/10.2166/wst.1994.0160

BRIX, H. Do macrophytes play a role in constructed treatment wetlands? **Water Science and Technology**, v. 35, n. 5, p. 11-17, 1997. https://doi.org/10.1016/S0273-1223(97)00047-4

CASELLES-OSORIO, A.; GARCÍA, J. Effect of physic-chemical pretreatment on the removal efficiency of horizontal subsurface-flow constructed wetlands. **Environment Pollution,** v. 146, p. 55-63, 2007. https://doi.org/10.1016/j.envpol.2006.06.022

CHENG, S. P.; WU, Z. B.; XIA, Y. C. Review on gas exchange and transportation in macrophytes. **Acta Hydrobiologica Sinica,** v. 27, n. 4, p. 413-417, 2003.

CHEN, W.; CHEN, Z.; HE, Q.; WANG, X.; WANG, C.; CHEN, D.; LAI, Z. Root growth of wetland plants with different root types. **Acta Hydrobiologica Sinica,** v. 27, p. 450-458, 2007. https://doi.org/10.1016/S1872-2032(07)60017-1

CHEN, Z. H.; CHEN, F.; CHENG, X. Y. Research on macrophyte roots in the constructed wetlands (A review). **Current Tropic in Plant Biology,** v. 5, p. 131-142, 2004.

CHEN, Z. J.; TIAN, Y. H.; ZHANG, Y.; SONG, B. R.; LI, H. C.; CHEN, Z. H. Effects of root organic exudates on rhizosphere microbes and nutrient removal in the constructed wetlands. **Ecological Engineering,** v. 92, p. 243-250, 2016. https://doi.org/10.1016/j.ecoleng.2016.04.001

COSTA, J. F.; PAOLI, A. C.; SEIDL, M.; VON SPERLING, M. Performance and behaviour of planted and unplanted units of a horizontal subsurface flow constructed wetland system treating municipal effluent from a UASB reactor. **Water Science and Technology**, v. 68, n. 7, p. 1495-1502, 2013. https://doi.org/10.2166/wst.2013.391

DALLAS, S.; HO, G. Subsurface flow reedbeds using alternative media for the treatment of domestic greywater in Monteverde, Costa Rica, Central America. **Water Science & Technology,** v. 51, n. 10, p. 119-128, 2005. https://doi.org/10.2166/wst.2005.0358

FIA, F. R. L.; MATOS, A. T.; FIA, R.; BORGES, A. C.; CECON, P. R. Efeito da vegetação em sistemas alagados construídos para tratar águas residuárias da suinocultura. **Engenharia Sanitária e Ambiental**, v. 22, n. 2, p. 303-311, 2017. https://doi.org/10.1590/s1413-41522016123972

KADLEC, R. H.; WALLACE, R. D. **Treatment Wetlands**. 2. ed. Florida: CRC Press, 2009. 1016 p.

KARATHANASIS, A. D.; POTTER, C. L.; COYNE, M. S. Vegetation effects on fecal bacteria, BOD, and suspended solid removal in constructed wetlands treating domestic wastewater. **Ecological Engineering,** v. 20, n. 157-169, 2003. https://doi.org/10.1016/S0925-8574(03)00011-9

LEE, C. Y.; LEE, C. C.; LEE, F. Y.; TSENG, S. K.; LIAO, C. J. Performance of subsurface flow constructed wetland taking pretreated swine effluent under heavy loads. **Bioresource Technology**, v. 92, n. 173-179, 2004. https://doi.org/10.1016/j.biortech.2003.08.012

LIU, X.; HUANG, S.; TANG, T.; LIU, X.; SCHOLZ, M. Growth characteristics and nutrient removal capability of plants in subsurface vertical flow constructed wetlands. **Ecological Engineering**, v. 44, p. 189-198, 2012. https://doi.org/10.1016/j.ecoleng.2012.03.011

IPABH

MATOS, A. T.; FREITAS, W. S.; LO MONACO, P. A. V. Eficiência de sistemas alagados construídos na remoção de poluentes de águas residuárias da suinocultura. **Revista Ambiente &Água**, v. 5, n. 2, p. 119-132, 2010. https://dx.doi.org/10.4136/ambi-agua.142

MATOS, A. T.; ABRAHÃO, S. S.; LO MONACO, P. A. V. Eficiência de sistemas alagados construídos na remoção de poluentes de águas residuárias de indústria de laticínios. **Engenharia Agrícola**, v. 32, n. 6, p. 1144-1155, 2012. https://doi.org/10.1590/S0100-69162012000600016

MATOS, A. T. **Manual de análise de resíduos sólidos e águas residuárias**. 1. ed. Viçosa: Editora UFV, 2015. 149 p.

MENDONÇA, H. V.; RIBEIRO, C. B. M.; BORGES, A. C.; BASTOS, R. R. Remoção de nitrogênio e fósforo de águas residuárias de laticínios por sistemas alagados construídos operando em bateladas. **Revista Ambiente & Água**, v. 7, n. 2, p. 75-87, 2012. https://doi.org/10.4136/ambi-agua.805

MENDONÇA, H. V.; RIBEIRO, C. B. M.; BORGES, A. C.; BASTOS, R. R. Sistemas alagados construídos em batelada: remoção de demanda bioquímica de oxigênio e regulação de pH no tratamento de efluentes de laticínios. **Revista Ambiente &Água**, v. 10, n. 2, p. 442-453, 2015. https://doi.org/10.4136/ambi-agua.1511

MIRANDA, S. T.; MATOS, A. T.; MATOS, M. P.; SARAIVA, C. B.; TEIXEIRA, D. L. Influence of the substrate type and position of plant species on clogging and the hydrodynamics of constructed wetland systems. **Journal of Water Process Engineering,** v. 31, p. 1-8. 2019. https://doi.org/10.1016/j.jwpe.2019.100871

PEDESCOLL, A.; UGGETTI, E.; LLORENS, E.; GRANÉS, F.; GARCIA, D.; GARCIA, J. Practical method based on saturated hydraulic conductivity used to assess clogging in subsurface flow constructed wetlands. **Ecological Engineering**, v. 35, n. 8, p. 1216–1224, 2009. https://doi.org/10.1016/j.ecoleng.2009.03.016

SARAIVA, C. B.; MATOS, A. T.; MATOS, M. P.; MIRANDA, S. T. Influence of substrate and species arrangement of cultivated grasses on the efficiency of horizontal subsurface flow constructed wetlands. **Engenharia Agrícola,** v. 38, n. 3, p. 417-425, 2018. https://doi.org/10.1590/1809-4430-eng.agric.v38n3p417-425/2018

SARAIVA, C. B.; MATOS, A. T.; MATOS, M. P. D. Extraction capacity of grasses grown in constructed wetland systems using different arrangements and substrates. **Engenharia Agrícola**, v. 39, n. 5, p. 668-675, 2019. https://doi.org/10.1590/1809-4430-eng.agric.v39n5p668-675/2019

SKLARZ, M. Y.; GROSS, A.; YAKIREVICH, A.; SOARES, M. I. M. A recirculating vertical flow constructed wetland for the treatment of domestic wastewater. **Desalination**, v. 246, n. 1-3, p. 617-624, 2009. https://doi.org/10.1016/j.desal.2008.09.002

STOTTMEISTER, U.; WIEBNER, A.; KUSCHK, P.; KAPPERLMEYER, U.; KASTNER, M.; BEDERSKI, O.; MÜLLER, R. A.; MOORMANN, H. Effects of plants and microorganisms in constructed wetlands treatments. **Biotechnology Advances,** v. 22, n. 1-2, p. 93-117, 2003. https://doi.org/10.1016/j.biotechadv.2003.08.010

TANNER, C. T.; SUKIAS, J. P. Accumulation of organic solids in gravel-bed constructed wetlands. **Water Science and Technology**, v. 32, n. 3, p. 229-239, 1995. https://doi.org/10.1016/0273-1223(95)00624-9

THOMAS, P. R.; GLOVER, P.; KALAROOPAN, T. An evaluation of pollutant removal from secondary treated sewage effluent using a constructed "wetland" system. **Water Science Technology**, v. 32, n. 3, p.87-93, 1995. https://doi.org/10.1016/0273-1223(95)00608-7

WANG, M.; ZHANG, D. Q.; DONG, J. W.; TAN, S. K. Constructed wetlands for wastewater treatment in cold climate - A review. **Journal of Environmental Science**, v. 57, p. 293-311, 2017. https://doi.org/10.1016/j.jes.2016.12.019

WU, J. Q.; RUAN, X. H.; WANG, X. Selection and function of aquatic plants in constructed wetlands. **Water Resources Protection,** v. 21, n. 1, p. 1-6, 2005.

WU, S.; KUSCHK, P.; BRIX, H.; VYMAZAL, J.; DONG, R. Development of constructed wetlands in performance intensifications for wastewater treatment: a nitrogen and organic matter targeted review. **Water Research**, v. 57, p. 40-55, 2014. https://doi.org/10.1016/j.watres.2014.03.020

ZHANG, D. Q.; JINADASA, K. B. S. N.; GERSBERG, R. M.; LIU, Y.; NG, W. J.; TAN, S. K. Application of constructed wetlands for wastewater treatment in developing countries– a review of recent developments (2000-2013). **Journal of Environmental Management,** v. 141, p. 116-131, 2014. https://doi.org/10.1016/j.jenvman.2014.03.015

ZHANG, S.; XIAO, R.; LIU, F.; ZHOU, J.; LI, H.; WU, J. Effect of vegetation on nitrogen removal and ammonia volatilization from wetland microcosms. **Ecological Engineering**, 97, 363-369, 2016. https://doi.org/10.1016/j.ecoleng.2016.10.021