Spatial variation of physicochemical parameters in a constructed wetland for wastewater treatment: An example of the use of the *R* programming language

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ABSTRACT. Introduction: The implementation of wastewater treatment systems such as constructed wetlands has a growing interest in the last decade due to its low cost and high effectiveness in treating industrial and residential wastewater. **Objective:** To evaluate the spatial variation of physicochemical parameters in a constructed wetland system of sub-superficial flow of *Pennisetum alopecuroides* (*Pennisetum*) and a Control (unplanted). The purpose is to provide an analysis of spatial dynamic of physicochemical parameters using *R* programming language. **Methods:** Each of the cells (*Pennisetum* and Control) had 12 piezometers, organized in three columns and four rows with a separation distance of 3,25m and 4,35m, respectively. The turbidity, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), ammoniacal nitrogen (N-NH$_4$), organic nitrogen (N-org.) and phosphorous (P-PO$_4^{-3}$) were measured in water under in-flow and out-flow of both conditions Control and *Pennisetum* (n= 8). Additionally, the oxidation-reduction potential (ORP), dissolved oxygen (DO), conductivity, pH and water temperature, were measured (n= 167) in the piezometers. **Results:** No statistically significant differences between cells for TKN, N-NH$_4$, conductivity, turbidity, BOD, and COD were found; but both Control and *Pennisetum* cells showed a significant reduction in these parameters (*P*<0,05). Overall, TKN and N-NH$_4$ removal were from 65,8 to 84,1% and 67,5 to 90,8%, respectively; and decrease in turbidity, conductivity, BOD, and COD, were between 95,1-95,4%; 15-22,4%; 65,2-77,9% and 57,4-60,3% respectively. Both cells showed ORP increasing gradient along the water-flow direction, contrary to conductivity (p<0,05). However, OD, pH and

RESUMEN. "Variación espacial de los parámetros fisicoquímicos en un humedal artificial para el tratamiento de aguas residuales: un ejemplo del uso del lenguaje de programación *R*". **Introducción:** La implementación de sistemas de tratamiento de aguas residuales como los humedales artificiales, ha tenido gran interés en la última década por su bajo costo y efectividad en el tratamiento de aguas residuales. **Objetivo:** Evaluar la variación espacial de algunos parámetros fisicoquímicos en un sistema de humedal construido de flujo sub-superficial de *Pennisetum alopecuroides* (*Pennisetum*) y un Control (no plantado). Se proporciona un ejemplo de análisis de dinámica espacial de parámetros fisicoquímicos mediante el uso del lenguaje de programación *R*. **Metodología:** Cada una de las celdas (Pennisetum y Control) tenía 12 piezómetros, organizados en tres columnas y cuatro filas con una separación de 3,25 m y 4,35 m, respectivamente. Se midieron en agua del flujo de entrada y salida de ambas condiciones Control y Pennisetum (*n* = 8): la turbidez, la demanda bioquímica de oxígeno (*DBO*), la demanda química de oxígeno (DQO), el nitrógeno Kjeldahl total (*TKN*), el nitrógeno amoniacal (*N-NH$_4$*), el nitrógeno orgánico (*N-org.*) y el fósforo (*P-PO$_4^{-3}$*). Además, se midieron el potencial de oxidación-reducción (*ORP*), oxígeno disuelto (*DO*), conductividad, *pH* y temperatura del agua (*n* = 167) en los piezómetros. **Resultados:** No se hallaron diferencias estadísticamente significativas entre celdas para TKN, N-NH$_4$, conductividad, turbidez, BOD y COD (p>0,05); pero ambas celdas presentaron una reducción significativa en estos parámetros (*p*<0,05). La eliminación de TKN y N-NH$_4$ fue de 65,8 a 84,1% y de 67,5 a 90,8%, respectivamente. La disminución de turbidez, conductividad, BOD y COD fue entre 95,1-95,4%; 15-

temperature were inconsistent in the direction of the water flow in both cells. **Conclusions:** *Pennisetum* demonstrated pollutant removal efficiency, but presented results similar to the control cells, therefore, remains unclear if it is a superior option or not. Spatial variation analysis did not reflect any obstruction of flow along the CWs; but some preferential flow paths can be distinguished. An open-source repository of *R* was provided.

Keywords: *Pennisetum alopecuroides*, Artificial Wetland System of subsuperficial Flow, oxidation-reduction potential, dissolved oxygen, conductivity.

22,4%; 65,2-77,9% y 57,4-60,3% respectivamente. Ambas celdas mostraron un gradiente creciente de ORP a lo largo de la dirección del flujo de agua, contrario a la conductividad (p<0,05). Sin embargo, OD, pH y temperatura fueron inconsistentes en la dirección del flujo de agua en ambas. **Conclusiones:** *Pennisetum* demostró eficacia en la remoción de contaminantes, pero presentó resultados similares a Control. Hay duda si a largo plazo puede demostrarse como una opción superior. El análisis de variación espacial no reflejó ninguna obstrucción del flujo a lo largo de los CWs; pero se pueden distinguir rutas de flujo preferenciales. Se proporcionó un repositorio de código abierto de *R*.

Palabras clave: *Pennisetum alopecuroides*, sistema de humedales artificiales de flujo sub-superficial, potencial de oxidación-reducción, oxígeno disuelto, conductividad.

The lack of sanitation has become a worldwide problem. This has exacerbated with the generation of increasing amounts of wastewater from industries and the residential areas (van Puijenbroek, Beusen, & Bouwman, 2019; Ahmad, Majhi, Kothari, & Singh, 2020; Gomes da Silva & Gouveia, 2020). In Costa Rica, the average of wastewater is estimated at 966 455 m^3 d^{-1} (the average per person is about 0.2 m^3 d^{-1} (Instituto Nacional de Estadística y Censos [INEC] 2015)). Only 15% of the country's wastewater has some type of treatment (Acueductos y Alcantarillados [AyA], Ministerio de Ambiente y Energía [MINAE] y Ministerio de Salud [MS], 2016). This information is calculated from industrial companies that register the produced wastewater and companies reporting to have some treatment system; which could indicate that wastewater problem sanitation within the country is more serious than is estimated.

At present, there is a progressive explosion in the development of wastewater treatment systems (Xu & Cui, 2019). Among the wastewater treatment technologies used in Costa Rica are aerobic treatment systems such as activated extended aeration sludge and conventional activated sludge. The anaerobic systems used are septic tanks, drainage, up-flow anaerobic sludge blanket digestion, pre-conditioning systems with submarine emissary, and systems by facultative ponds (Ruiz, 2012). Although these treatment technologies are very efficient, their application within the country will largely depend on the balance between their minimum acceptable result and the minimum cost of construction and maintenance.

Constructed wetlands (CW) for wastewater treatment is an emerging ecological technology of low investment, low energy consumption, and convenient management (Tang, Huang, Scholz, & Li, 2011; Cui, Ouyang, Gu, Yang, & Xu, 2013; López-Ocaña et al., 2018). During the 1980s and 1990s, CW with horizontal subsurface flow (CW-HSF) was the most common type of CW used for wastewater treatment, due to the elimination of organic compounds and suspended solids, using filtration beds with anoxic and anaerobic conditions (Vymazal, 2019). In recent years, researchers have recognized the economic and environmental benefits for treating both industrial and residential wastewater effectively (He, Peng, Hua, Zhao, & Xiao, 2018).

CW-HSF with emerging macrophyte plants performs effectively in removing conventional pollutants (*e.g.*: nitrogen, phosphorus, and organic compounds) due to their excellent sediment filtration (López-Ocaña et al., 2018). Similar systems that have operated for more than 20 years, show removal levels higher than 91,7%; 82,9% and 88,3% for biochemical and chemical oxygen demand, and total suspended solids, respectively during the last five years (Vymazal, 2019).

However, despite the diversity of researches conducted in the last decade, the strategies for the spatial monitoring of these wastewater treatment systems (WTS) for proper functioning are still poorly studied. Notwithstanding *CWs'* main drawbacks are substrate media clogging and high area requirements (Tatoulis, Akratos, Tekerlekopoulou, Vayenas, & Stefanakis, 2017).

Overall, an analysis of spatial variation of total suspended solids (*TSS*) within *CW-HSF* could indicate the location of obstruction on the flow and/or possible reduction of the removal. However, in an extensive *CW-HSF* the measurement of *TSS* could be laborious and the sample size could be unmanageable. *TSS* can be estimated from turbidity measurements (Grayson, Finlayson, Gippel, & Hart, 1996; Daphne, Utomo, & Kenneth, 2011); nevertheless, this requires a linear regression model fit, which must be recalibrated at each sample location and time point (Rügner, Schwientek, Beckingham, Kuch, & Grathwohl, 2013). Therefore, we presume that the measurement of physicochemical parameters such as water electrical conductivity could be a promising indicator. Because this is strictly linked to turbidity and total dissolved solids. According to Villa, Fölster, & Kyllmar (2019), the site turbidity and conductivity-total phosphorus relationship was significant at 78% of sites (*n*= 84; mean r^2 = 0,62). Other physicochemical parameters such as dissolved oxygen and oxidation-reduction potential could also be tested. It is important to note that all above physicochemical parameters are easy and quick to measure with low-cost sensors (e.g., Sensor-Vernier-LabQuest).

This research evaluated the spatial variation of some conventional physicochemical parameters on *CW-HSF* (1,5 years) of cultivated with *Pennisetum alopecuroides* and control (not plants). To the best of our knowledge, this is the first study conducted on *CW* using spatial variability analysis with *R* programming language. The main *R* package used was "*fields*"; this allows to adjust of curves, surfaces, and functions with an emphasis on splines, to later be applied to the interpolation of spatial data and spatial statistics (Nychka, Furrer, Paige, & Sain, 2017). The scope of this research was to provide an example of applications and recommendations for spatial variation analysis using *R* open-source tools.

MATERIALS AND METHODS

Study area: The research was conducted between May 2018 and October 2019 in a *CW-HSF* at the Escuela de Medicina Veterinaria located on the Benjamín Núñez Campus, Universidad Nacional [UNA] Costa Rica (9°58'37"N - 84°07'37"W). *CW-HSF* started to operate on April 2018. According to Instituto Metereológico Nacional [IMN] (2020), average monthly temperature, precipitation, and relative humidity of air during the study period were between 18,5-28°C, 0-186,5mm, and 79-97% (max.-min.), respectively. *CW-HSF* was built in an area of ~ 800m² (each cell of ~ 200m²) and receives water from toilets, student dining, and research and teaching laboratories of the Escuela de Medicina Veterinaria – UNA. Therefore, there is an important presence of chemical substances (organic and inorganic) from the mentioned facilities. The system in this research consists of a set of grilles, an *Inmhoff* tank, four cells of constructed wetlands with a horizontal subsurface flow, a sludge drying bed, and a disinfection unit. It is important to note that only two cells out of a total of four cells were used for this study.

All systems are operating in parallel, *i.e.,* each of the wetlands receives the same amount of wastewater with a total hydraulic capacity of 20m³d⁻¹ flow for each cell. A cell of *CW-HSF* is formed by emerging macrophyte plants (*Pennisetum alopecuroides*, hereafter referred to as *Pennisetum*). This has a filter filled with stone, where the plants are sown to, crossing it horizontally, through which the wastewater flows from its entry through the wetlands and to the effluent collection (see details in Fig. 1). *CW* has a total of 12 piezometers per cell, evenly distributed in four rows and three columns. Each piezometer has a depth of 60 cm and with lateral perforations at different depths.

The Control cells simply consisted of an unplanted system with the same structural characteristics mentioned above.
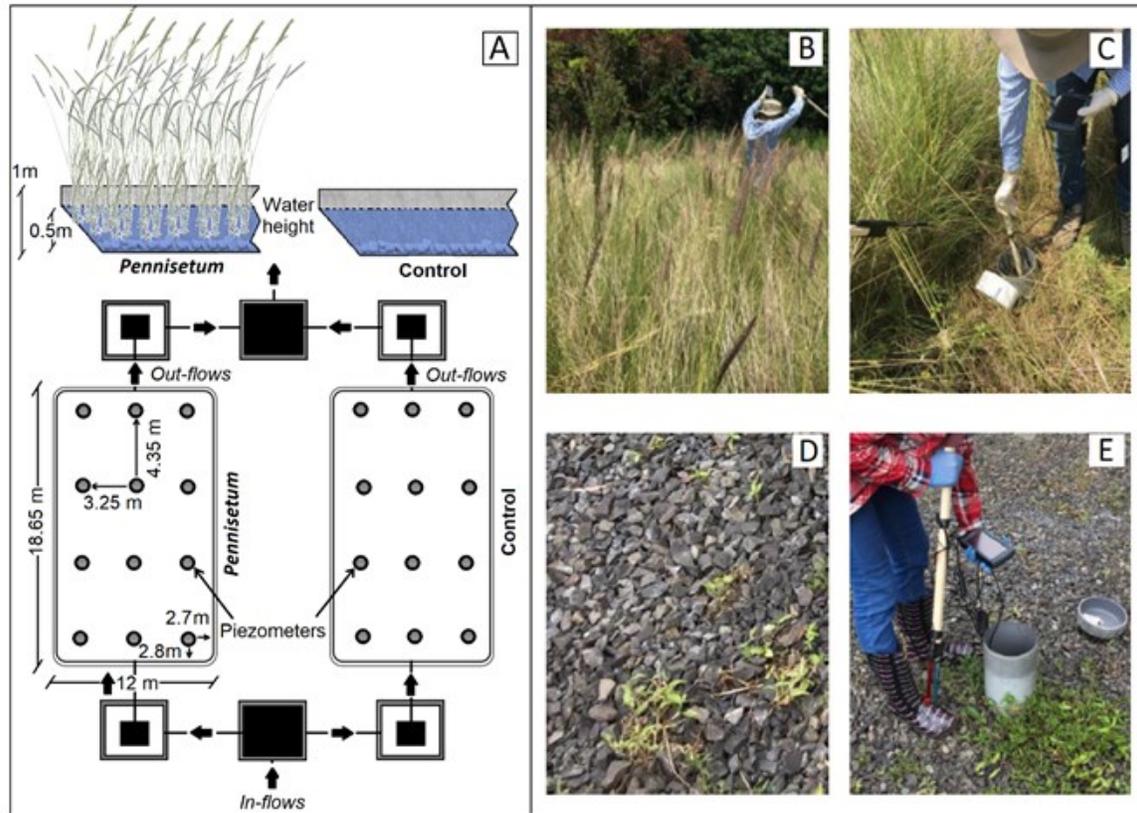

Fig. 1. Scheme of CW-HSF for wastewater treatment (A: *Pennisetum* and Control). Images of the substrate and piezometers in both systems (B-C: *Pennisetum*; and D-E: Control).

Sampling of physicochemical parameters: Three sampling sites were established (Fig. 1A): *in-flow* and two *out-flow* (Control and *Pennisetum*). The physicochemical parameters measured were (n= 8): turbidity (nephelometric turbidity units - NTU), biochemical oxygen demand (*BOD*, mg L^{-1}), chemical oxygen demand (*COD*, mg L^{-1}), total Kjeldahl nitrogen (*TKN*, mg L^{-1}), ammoniacal nitrogen ($N-NH_4$, mg L^{-1}), organic nitrogen (*N-org.*, mg L^{-1}), and phosphorous ($P-PO_4^{-3}$, mg L^{-1}). All the physicochemical parameters were measured strictly following the established by *Standard Methods for the Examination of Water and Wastewater* (Rice, Baird, & Eaton, 2017).

The electrical conductivity, oxidation-reduction potential (*ORP*), dissolved oxygen (*DO*, accuracy ± 0,2 mg L^{-1}), *pH* (accuracy ± 0,2 *pH*) and temperature (accuracy ± 0,02 °C) of water, were measured in each of the piezometers (Control and *Pennisetum*, 24 piezometers in total). Likewise, measurements at water *in-flows* and *out-flows* of the two systems (Control and *Pennisetum*), were made. All previous these samplings were conducted for three weeks (three days, between August and October 2019) with a frequency hourly between 9:00h and 14:00h (n= 167). Each physicochemical parameter was measured by means of an independent sensor connected and distributed in two portable consoles of the Vernier type (LabQuest®2, Version 2.8.5, Vernier Software & Technology, Beaverton, OR, USA). Before each sampling, all sensors were calibrated using their respective manufacturer's protocol.

Data analysis: All variables were evaluated using analysis of variance (ANOVA) or Kruskal-Wallis test. Fisher's *Least significant difference* (*LSD*) tests were applied to compare the means between the systems (water in- and out-flows of systems). Spearman (r_p) correlations were made between all the pairs physicochemical parameters per system and together. Multivariate associations among all physicochemical parameters were analyzed using a principal component analysis (*PCA*). Piezometers that produced similar responses were clustered using a multivariate technique of grouping analysis according to the method of Tocher, which is based on Euclidean average distances (Rencher, 2005). *PCA* was performed using the *R* package "*factorextra*" (Kassambara & Mundt, 2020).

Kruskal-Wallis tests were carried out with Bonferoni tests to compare between the rows of the piezometers positions for all the physicochemical parameters for each system (Control or *Pennisetum*), this to verify the change of the parameters along the flow of water within the systems. All statistical assumptions were checked. Likewise, bilinear interpolators between the piezometers were done, to obtain a distribution map of the levels of each physicochemical parameter to understand the dynamics within the systems to due to the direction of the water flow (see "*fields*" *R* package; Nychka et al. (2017)). All statistical analyses were performed using *R* programming language, version 3.6.1 (RCoreTeam, 2020) with a significance level of α=0,05. Finally, a *GitHub* repository was made to the access to *R* script, database and results of statistical analyses (see DOI: http://doi.org/10.5281/zenodo.3726063).

RESULTS

***CW-HSF* efficiency:** Water out-flow of *Pennisetum* and Control showed a significant reduction with respect to water in-flow, for turbidity, *BOD*, *COD*, *TKN*, and *N-NH$_4$* ($P<0,05$, Table 1). No significant difference was found between water in- and out-flow for *N-org.* and *P-PO$_4^{-3}$* ($P>0,05$), a high variability was registered in these parameters. In addition, statistically significant differences were found between the sampling dates for conductivity, *DO* and *pH*, and the time of measurement for *ORP*, *pH*, and temperature ($P<0,05$, Table 2). These parameters in the time-course did not followed a determined pattern concerning the time of day except for temperature (Fig. 2). Nevertheless, statistically significant differences between water in- and out-flows cells (Control and *Pennisetum*) for conductivity, *ORP* and *DO* parameters, were found ($P<0,05$; Table 2).

Both systems showed a significant reduction in electrical conductivity and water turbidity (Table 3). Also, significant removals between 65,8% and 84,1% of *TKN* were found for the Control and *Pennisetum*, respectively. Both systems reported significant reductions in both *BOD* and *COD* ($P<0,05$). Control evidenced an increase in *DO* (20%, $P<0,05$), contrary to *Pennisetum* (1,1%). In contrast, *ORP* showed an increase in *Pennisetum* (114,8%, $P<0,05$).

The interplay between physicochemical parameters: Conductivity of water was correlated negatively with all independent parameters of the system and for all the data together, except for temperature and *ORP* in *Pennisetum* and Control, respectively (Table 4). The variability explained by the first two axes of the principal component (PC) analysis for all combinations of physicochemical parameters was 67,3% (Fig. 3A), which, it was not possible to separate the systems (Control vs *Pennisetum*). A more detailed analysis by distance in the direction of the out-flow (row of piezometers position) showed a variability explained by the first two axes of PC between 65,6% and 71,7% (Fig. 3B-E), additionally, the separation between the systems from the measured parameters, was not possible.

TABLE 1

Physicochemical parameters comparison in water *in-* and *out-flows* (Control and *Pennisetum*) in the *CW-HSF*, using one-way ANOVA

Parameters (abbreviation, unit)	In-flow	Out-flow Control	Out-flow Pennisetum	R2	F or KW (†)	P
Turbidity (NTU)	32,8 ± 4,3 a	1,6 ± 0,4 b	1,5 ± 0,4 b	---	15,4 †	***
Biochemical oxygen demand (BOD, mg L-1)	68,7 ± 6,7 a	23,9 ± 5,1 b	15,2 ± 4,2 b	0,73	27,9	***
Chemical oxygen demand (COD, mg L-1)	111,2 ± 19,6 a	44,1 ± 6,9 b	47,4 ± 6,6 b	---	13,8 †	**
Total Kjeldahl nitrogen (TKN, mg L-1)	19,3 ± 2,6 a	6,6 ± 1,4 b	3,1 ± 0,7 b	0,69	23,4	***
Ammoniacal nitrogen (N-NH4, mg L-1)	16,0 ± 2,7 a	5,2 ± 1,3 b	1,5 ± 0,7 b	0,63	17,5	***
Organic nitrogen (N-org., mg L-1)	3,3 ± 0,7	1,4 ± 0,7	1,6 ± 0,4	0,21	2,9	n.s.
Phosphorous (P-PO4-3, mg L-1)	4,6 ± 2,3	1,4 ± 0,5	1,7 ± 0,8	---	1,7 †	n.s.

Mean ± Standard Error; R2: coefficient of determination; F: Fisher value; KW (†): Kruskal-Wallis test; P: probability; Equal letters indicate no statistically significant difference between water out-flows and in-flows **(Control and *Pennisetum*;** LSD, P>0,05); n.s.: non-significative; *: P<0,05; **: P<0,01; ***: P<0,001.

TABLE 2

Physicochemical parameters comparison in water *in-* and *out-flows* (Control and *Pennisetum*) in the *CW-HSF*, using three-way ANOVA (factors: date, time, and system)

Parameters (abbreviation, unit)	Date	Time	Systems	In-flows	Out-flows Control	Out-flows Pennisetum	R2	F	P
Conductivity (µS cm-1)	***	n.s.	***	353,4 ± 11,9 b	274,3 ± 8,3 a	300,3 ± 10,4 a	0,61	6,3	***
Oxidation-reduction potential (*ORP*, mV)	n.s.	***	**	-12,8 ± 31,0 b	57,1 ± 21,2 ab	75,6 ± 17,0 a	0,64	6,7	***
Dissolved oxygen (DO, mg L-1)	**	n.s.	***	4,3 ± 0,2 b	5,4 ± 0,2 a	4,7 ± 0,2 b	0,43	3,6	**
pH	**	*	n.s.	7,1 ± 0,1	7,3 ± 0,1	7,4 ± 0,1	0,42	3,5	**
Temperature (°C)	n.s.	***	n.s.	24,5 ± 0,2	24,8 ± 0,5	24,7 ± 0,4	0,61	6,3	***

Mean ± Standard Error; R2: coefficient of determination; F: Fisher value; P: probability; Equal letters indicate no statistically significant difference between water out-flows and in-flows **(Control and *Pennisetum*;** LSD, P> 0,05); n.s.: non-significative; *: P<0,05; **: P<0,01; ***: P<0,001.
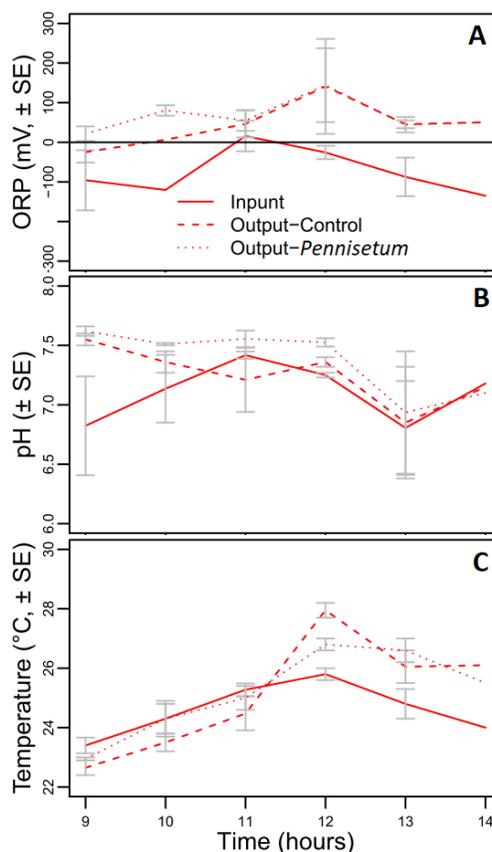
Fig. 2. Time-course of ORP (A), pH (B), and temperature (C) for water in- and out-flows of two systems (Control and P*ennisetum*). Standard Error (SE). Statistical analysis sees Table 2.

TABLE 3

Statistically significant difference and percentage`s removal (*LSD*, *P*<0,05) of physicochemical parameters between water *out-flows* and *in-flows* (Control and *Pennisetum*) in the *CW-HSF*

Parameters (abbreviation, unit)	Control		Pennisetum	
	Difference	Removal (%)	Difference	Removal (%)
Turbidity (NTU)	-31,2	95,1	-31,3	95,4
Conductivity (µS cm-1)	-79,1	22,4	-53,1	15,0
Nitrogen *To*tal Kjeldahl (NTK, mg L-1)	-12,7	65,8	-16,3	84,1
Ammoniacal nitrogen (N-NH4, mg L-1)	-10,8	67,5	-14,6	90,8
Biochemical oxygen demand (BOD, mg L-1)	-44,8	65,2	-53,5	77,9
Chemical oxygen demand (COD, mg L-1)	-67,1	60,3	-63,8	57,4

Spatial variation of physicochemical parameters: Conductivity and *ORP* showed statistically significant differences along the direction of water flow in both systems (KW> 5,07; *p*<0,05; see Fig. 4-5). The conductivity showed a gradient reduction along the flow water direction, contrary to *ORP*. Nevertheless, *DO*, *pH*, and temperature were inconsistent along the direction of the water flow, *i.e.*: *DO* in the systems were irregular along the flow, however, it was noted that the Control showed a slight increase in *DO* at the end of the system (out-flows; Fig. 6); pH and temperature showed non-significant changes throughout the systems with average difference of 0,2 pH and 1°C (see figures of temperature and pH in repository, DOI: http://doi.org/10.5281/zenodo.3726063).

TABLE 4

Spearman's (r_ρ) correlation coefficient matrix between pairs of the physicochemical parameters at the system level (C: Control; or P: *Pennisetum*; bottom diagonal) and both systems together (top diagonal)

Parameters	pH	DO	Temperature	ORP	Conductivity
pH		0,07 [n.s.]	-0,33 [***]	-0,10 [n.s.]	-0,31 [***]
DO	C= 0,10 [n.s.] P= 0,07 [n.s.]		0,18 [***]	0,17 [***]	-0,31 [***]
Temperature	C= 0,02 [n.s.] P= -0,49 [***]	C= 0,36 [***] P= -0,03 [n.s.]		0,47 [***]	-0,27 [***]
ORP	C= 0,15 [*] P= -0,19 [*]	C= 0,18 [*] P= 0,09 [n.s.]	C= 0,21 [**] P= 0,12 [n.s.]		-0,39 [***]
Conductivity	C= -0,46 [***] P= -0,38 [***]	C= -0,31 [***] P= -0,27 [***]	C= -0,14 [*] P= 0,04 [n.s.]	C= -0,15 [n.s.] P= -0,29 [***]	

n.s.: non-significative; *: $p < 0,05$; **: $p < 0,01$; ***: $p < 0,001$.
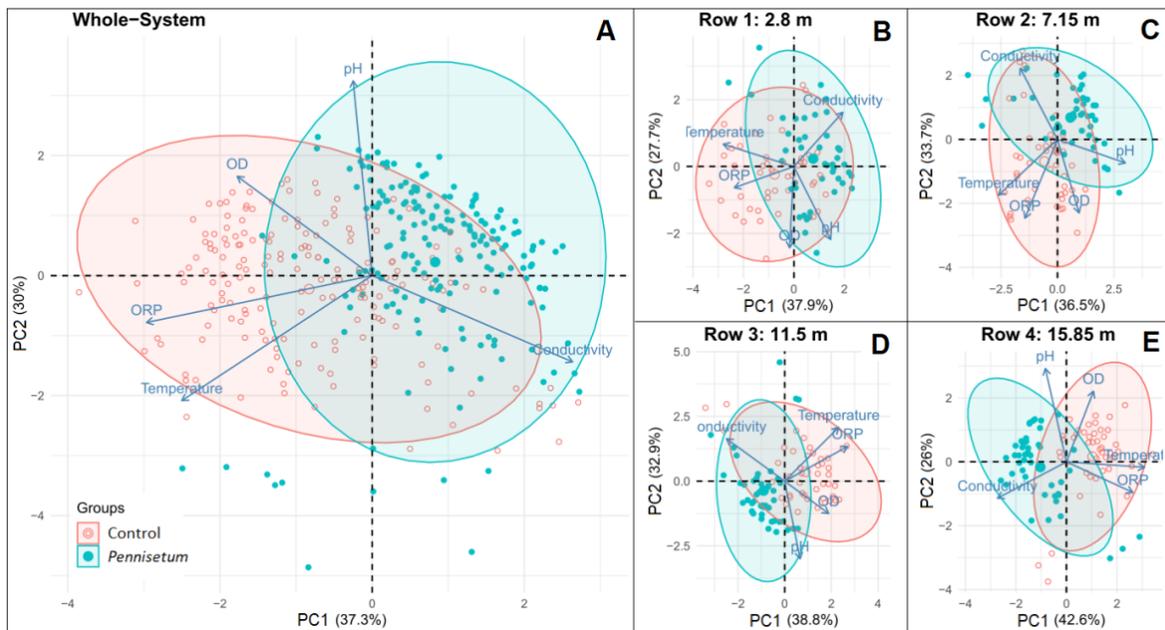

Fig. 3. The first two axes of a principal component (PC) analysis for all combinations of physicochemical parameters for all system (A) and by each row of the position of the piezometers (B, C, D, and E).
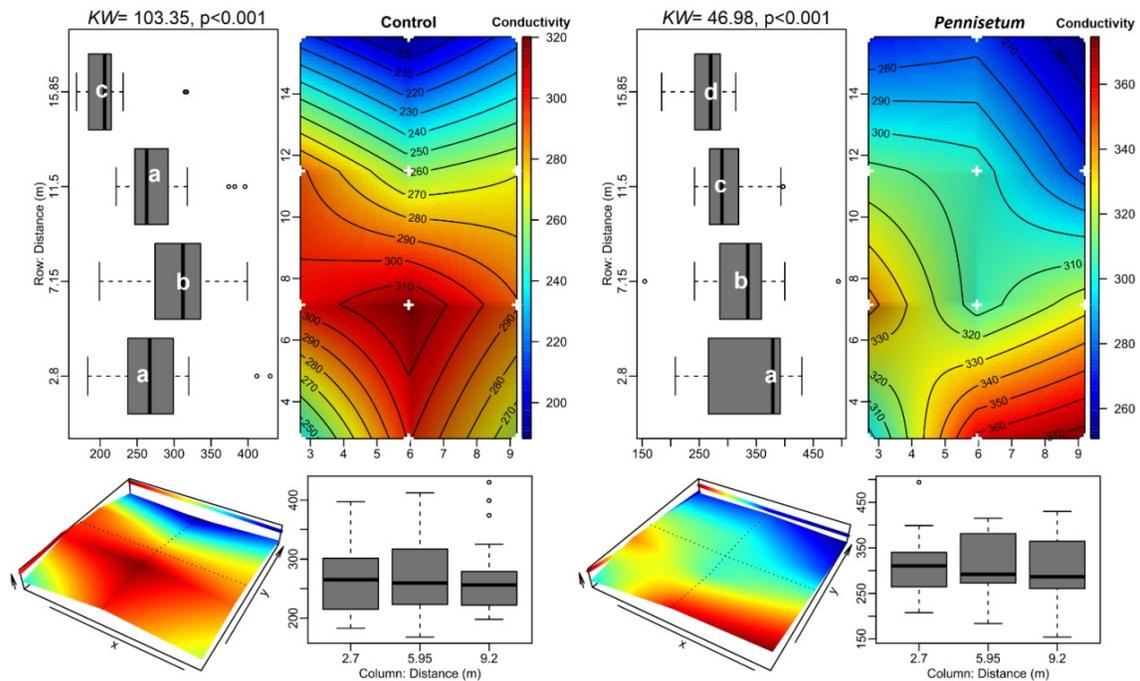
Fig. 4. Spatial variation of electrical conductivity of water within the systems (Control and *Pennisetum*) based on bilinear interpolations between piezometers. Colour gradient and contour lines indicate parameter intensity from low (blue) to high (red), and white crosses indicate the position of piezometers. Boxplots show the comparison between rows and columns of the piezometers position where box marks Q1 and Q3, the black line is median (Q2), lines shown maximum and minimum values, and circles are values outliers with three times greater than the mean. Subplot with colour gradient indicate flow direction in y axis. KW: Krukall-Wallis; p: probability; equal letters indicate no statistically significant difference between the position of the piezometers in rows (Bonferroni, p>0,05).
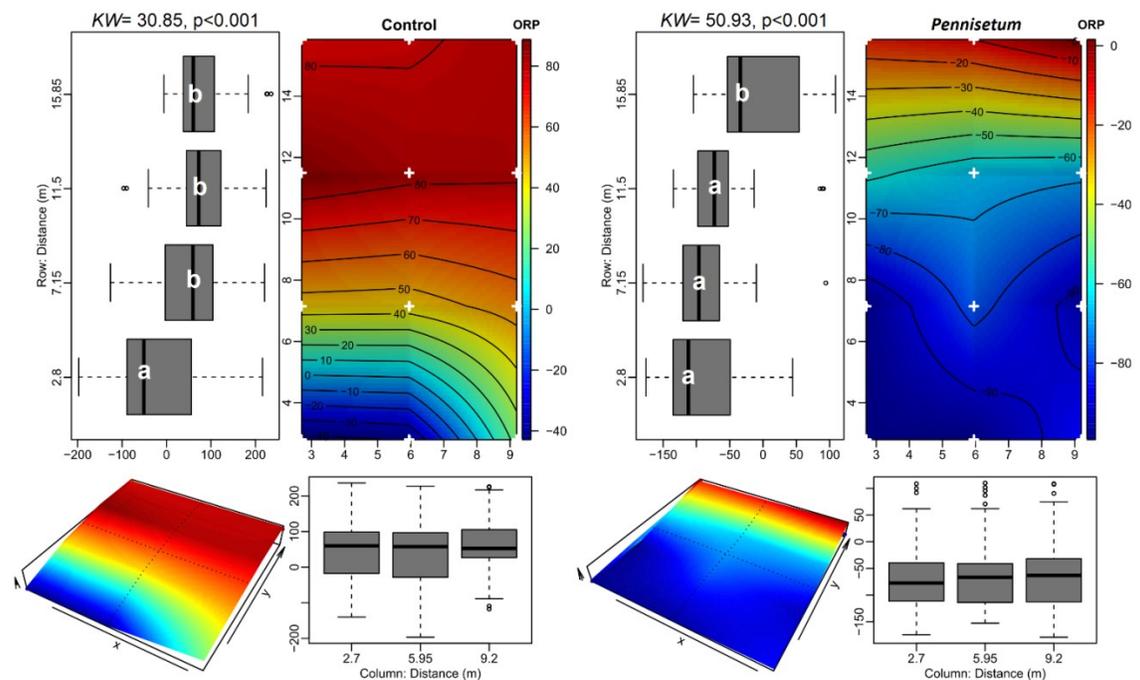

Fig. 5. Spatial variation of ORP within the systems (Control and *Pennisetum*) based on bilinear interpolations between the piezometers. See legend explanation in Fig. 4.
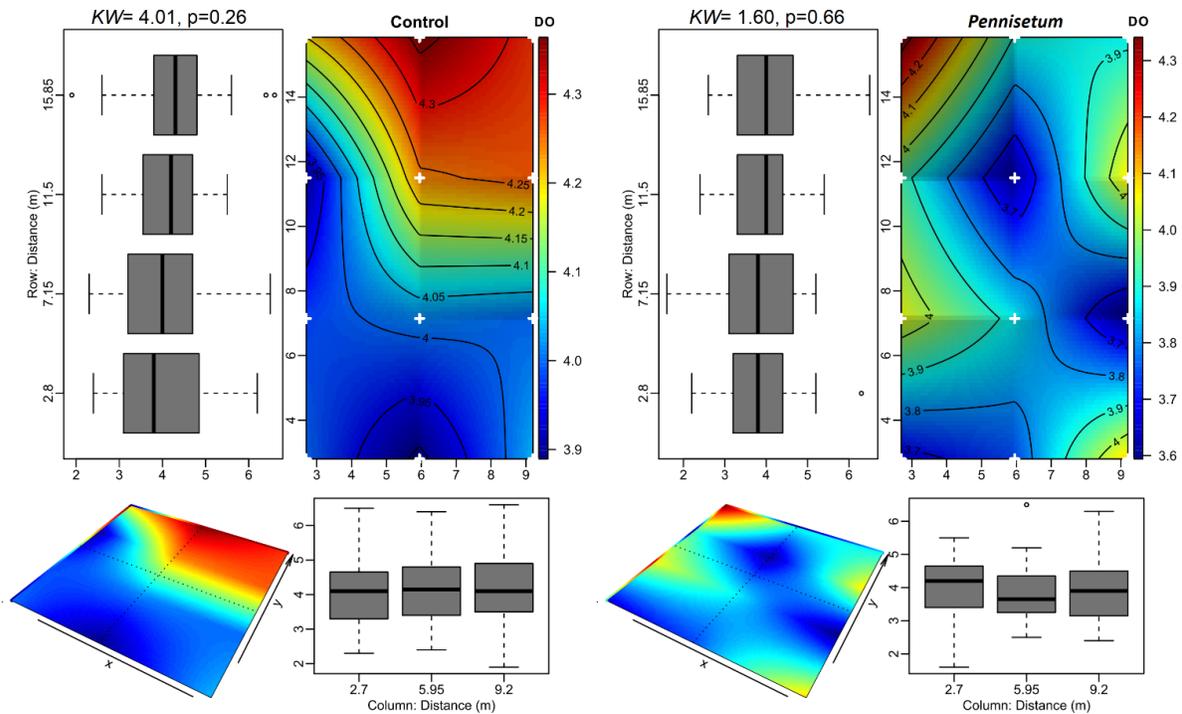
Fig. 6. Spatial variation of the DO within the systems (Control and *Pennisetum*) based on bilinear interpolations between the piezometers. See legend explanation in Fig. 4.

DISCUSSION

Flow conditions and other practical issues: A challenge in studying these systems is to keep in-flow disturbances to a minimum to reduce variation in *BOD* and hydraulic load rates (Haddis, Van der Bruggen, & Smets, 2020). Two problems in this investigation caused alterations in the in-flow in the *CWs*: (i) large waste pieces (clothing, bags, plastic material, laboratory waste, etc.) caused the obstruction of the flow in the set of grilles in the *Inmhoff* tank; and (ii) around a year of *Pennisetum* growth, the root system clogged the pipes. Both problems were solved by periodic cleaning maintenance. The control system was the one that presented the minimum problems of changes in in-flow. The only problem observed was the natural growth of herbaceous plants within the *CW*, but it was solved by their removal during the maintenance.

Despite all the problems, the preliminary advances of this research evidence for both systems (Control and *Pennisetum*) acceptable performances in the general removal rates of organic pollutants. Even both *CW-HSF* from the first month of construction complied with the provisions of Costa Rican national legislation (Perez-Salazar, Alfaro-Chinchilla, & Scholz, 2019). Furthermore, preliminary data (unpublished data) shows that both systems report a reduction of ~ 29% of faecal coliform (log) and ~ 44% of *Escherichia coli* (log), where *Pennisetum* highlighted with 34% microorganism removal compared to Control. Overall, we do not have enough information to ensure that the use of *Pennisetum* emerging plants could have superior efficiency on an unplanted system, however, the results that will be discussed below show that both *CWs* have promising performances in wastewater treatment.

CW-HSF efficiency and the interplay between physicochemical parameters: The differences found between sampling dates for conductivity, *DO* and *pH*, the time of measurement for *ORP*, *pH*, and temperature, possibly indicate an irregularity in water in-flow quality (Haddis et al., 2020). The temperature (21,1min. – 28,2max.) and *pH* (6,1min. – 7,7max.). Recorded between

8:00 and 14:00 were within optimal conditions for the development of all heterotrophic microbial activities (Viessman & Hammer, 2005). *CW* performance is temperature dependent, as it has a strong effect on nitrogen removal, but may have less effect on *BOD* and phosphorous removal (Kadlec & Reddy, 2001; Tatoulis et al., 2017).

Both systems showed a significant reduction in electrical conductivity and water turbidity, explained by the rocky bed that filters most of the sediments during the horizontal flow through the system (Vymazal, 2019). In contrast, the *ORP* shows an increase in *Pennisetum*; it is a slight improvement in the ability to reduce some of the organic compounds in the medium; similar results found in Srivastava, Abbassi, Garaniya, Lewis, & Yadav (2020). Evapotranspiration is a factor influencing the redox condition, as are the flow regimes of the system (García et al., 2010). Planted wetlands produce up to 20% higher redox gradient as compared to unplanted ones (Doherty et al., 2015); where, it is in the CW depth profile, *ORP* less negative values near the surface have lower anaerobic conditions, while *ORP* more negative values in the lower layer indicate more anaerobic conditions (Srivastava et al., 2020).

It is well documented that *CW-HSFs* have organic compound removal rates, with average rates from 80% to 90% of nitrogen, phosphorus, and suspended solids (López-Ocaña et al., 2018; Tatoulis et al., 2017; Vymazal, 2019). Significant reductions of *TKN, BOD,* and *COD* were found for the Control and *Pennisetum*. These removal efficiencies of vegetated beds in *CW-HSF* has been reported of 75% (Vymazal, 2010); it is an unanimously agreed outcome on *CW-HSFs* with emerging macrophyte plants for wastewaters treatment from municipal, agriculture, industry and landfill leachate (Vymazal, 2010; Vymazal & Kröpfelová, 2009). All these results are explained by the prevailing anoxic and anaerobic conditions in the filter bed of these systems (Vymazal, 2019).

The weak but significant correlations of water electrical conductivity with the analysed variables reflect the plasticity of the conductivity and the potential use that could be made as an indicator variable of important changes within the system. Among other uses, the total dissolved solids can be estimated from conductivity (Villa et al., 2019), since it is a measure of the volume of ionized solids, such as salts and minerals. Finally, the variability explained by the *PCs* of all the variables measured in each of the piezometers did not allow to separate the two treatments (Control *versus Pennisetum*), reflecting a similar consistency of all the variables throughout the flow of water within *CW*.

The bilinear interpolations of the physicochemical parameters between the piezometers allowed the establishment of a gradient of the measured levels of conductivity, *ORP* and *OD*. The spatial variation of conductivity in the control showed a slight increase in the position of the second row of the piezometers, possibly an irregularity mediated by the retention times of the water within the systems. Meanwhile, in *Pennisetum* a gradual reduction of the average conductivity between the rows of the piezometer positions was recorded; at the same time, a reduction in its variability was observed. A change in water electrical conductivity throughout the treatment system indicates that plants` salts extraction can overcome the increase in conductivity caused by their evapotranspiration (Teixeira, Matos, Matos, Hamakawa, & Teixeira, 2020). In conclusion, the preliminary results of this research showed no spatial obstruction of the flow and/or possible reduction of the removal. However, some preferential flow paths can be distinguished throughout the system.

Applications and recommendations for spatial variation analysis with *R* script: This research provides the *GitHub* repository where it is explained step by step how to use the *R* script (see DOI: http://doi.org/10.5281/zenodo.3726063). Likewise, the references of the main packages used are provided; all packages used are free and constantly updated. For this reason, is recommended to install the *R* packages directly from Github and verify updates before running the script. Other types of CWs, plant species, a greater number of variables, no restrictions on the

repetition number, and treatments can be applied to the R scripts and packages, with a simple modification of the original R script. There are no restrictions on the repetition number (number of piezometers).

This research is a preliminary advance of spatial variability monitoring results. Therefore, it is recommended to establish a long-term analysis of spatial-temporal variability of conventional pollutants on CWs (*e.g.*: nitrogen, phosphorous, sediments, etc.) to integrate and model the long-term removal efficiency. Also, the seasonality (dry and rainy) of tropical climate could show the performance of systems subjected to flow change effect. Finally, the main recommendation of this research for *CW-HSF* is the periodic monitoring of water in-flow rate, to detect obstructions in the pipelines and avoid changes in the hydraulic load rate.

Conclusion: *Pennisetum* demonstrated pollutant removal efficiency, but presented results like to the Control, therefore, it remains in doubt if in long-term it can be demonstrated as superior option respect to the control. Other emerging macrophyte plants should be tested. However, we can conclude that both systems had a significant effect on the removal of organic pollutants within the acceptable range (80-90%). In perspective, spatial variation analysis did not reflect any obstruction of flow along the *CWs*; but some preferential flow paths can be distinguished. Spatial variation is a tool that can be used in developing countries which wish to carry out monitoring campaigns to their treatment systems at a very low cost. Which can be used for the possibility of making decisions at the maintenance level and improvements in the systems. All *R* scripts used can be applied to other plants in *CWs* or similar treatments.

ETHICAL, CONFLICT OF INTEREST AND FINANCIAL STATEMENTS

The authors declare that they have fully complied with all pertinent ethical and legal requirements, both during the study and in the production of the manuscript; that there are no conflicts of interest of any kind; that all financial sources are fully and clearly stated in the acknowledgements section; and that they fully agree with the final edited version of the article. A signed document has been filed in the journal archives.

The declaration of the contribution of each author to the manuscript is as follows: J. P. P. M.: Experiment design, data collection, statistical data analysis, Writing - original draft, Writing - review & editing; C. S.: Experiment design, data collection, Writing - review & editing; R. P. S.: Experiment design, data collection, Writing - review & editing; C. A. C.: Experiment design, data collection, Writing - review & editing; A. A. M.: Data collection; L. A. L.: Data collection; J. C. C.: Data collection; A. E. F.: Data collection; M. E. B.: Data collection; R. A. R.: Data collection; S. F. A.: Data collection & C. S. C.: Data collection.

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