**Systems of artificial wetlands**

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# **Abstract**

**Key words:**

# **Introduction**

# **Materials and methods**

## ***Study area***

The research was conducted between August and October 2019 in an Alternative Wastewater Treatment System (AWTS) in Veterinary School located on the Benjamin Nuñez campus, Universidad Nacional, Costa Rica (9°58′37″N - 84°07′37″W). According to [IMN (2019)](#_ENREF_14), average monthly temperature, precipitation, and relative humidity of air during the study period were between 18.5-28°C, 0-186.5 mm, and 79-97% (max.-min.), respectively. AWTS was built in an area of 2050 m2 and receives water from the toilets, the student restaurant and the research and teaching laboratories of the Veterinary School, therefore, there is an important contribution of chemical substances (organic and inorganic). The system consists of a set of grilles, an *Inmhoff* tank, four cells (system) of artificial wetlands with horizontal subsurface flow, a sludge drying bed, and a disinfection unit. All systems are operating in parallel, *i.e.* each of the wetlands receives the same amount of wastewater with hydraulic capacity of 40 m3 d-1 flow. The subsurface flow wetlands are formed by emerging macrophyte plants (*Pennisetum alopecuroides*, hereafter referred to as Pennisetum), which has a filter filled with stone, where the plants are sown, which cross it horizontally, through which, the wastewater flows from an entry through the wetlands to the effluent collection (see details in Fig. 1). All wetlands have a total of 12 piezometers per system, evenly distributed in four rows and three columns, each piezometer have a depth of 60 cm and with lateral perforations at different depths. To finish, the Control system simply consisted of a system without plants and with the same structural characteristics mentioned above.

## ***Sampling of physicochemical parameters***

Oxidation-reduction potential (ORP), dissolved oxygen (OD, accuracy ± 0.2 mg L-1), conductivity, pH (accuracy ± 0.2 pH) and temperature (accuracy ± 0.02 °C) of the water, were performed in each of the piezometers (Control and Pennisetum, 24 piezometros in total). Likewise, measurements at the water in-flows and out-flows of the two systems (Control and Pennisetum), were made. All previous samplings were conducted for three weeks (three days) with a frequency hourly between 9:00 h and 14:00 h (*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 three-way analysis of variance (ANDEVA) for the factors: sampling date, sampling time and system (water in- and out-flows of systems). Non-interactions had between the aforementioned factors due to no repetitions in the systems. Fisher's *Least significant difference* (LSD) tests were applied to compare the means between the systems (water in- and out-flows of systems). Kruskall-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 to along the flow of water within the systems. All statistical assumptions were checked. Likewise, quadratic interpolations between the piezometers were performed, 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. Spearman (*rρ*) correlations between all the pairs physicochemical parameters per system and together, were made. Finally, 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, 2003). PCA was performed using the R package factorextra (Kassambara & Mundt, 2017). All statistical analyses were performed using R programming language, version 3.6.1 (Crawley, 2002; RCoreTeam, 2017); R Core Team, 2019) with a significance level of α=0.05.

# **Results**

# **Discussion**

## ***Conclusion***

# **Acknowledgements**

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# **References**

# **Table**

**Table 1. Comparison of physicochemical parameters comparison in the water in- and out-flows in the subsurface artificial wetland systems using three-way ANDEVA (factors: date, time, and system)**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Parameters** | **Date** | **Time** | **Systems** | ***In-flows*** | ***Out-flows*** | | ***R2*** | ***F*** | ***P*** |
| **Control** | **Pennisetum** |
| ***ORP*** | *n.s.* | **\*\*\*** | **\*\*** | -12.8 ± 31 **a** | 57.1 ± 21.25 **ab** | 75.6 ± 17 **b** | 0.64 | 6.7 | \*\*\* |
| ***Conductividad*** | **\*\*\*** | *n.s.* | **\*\*\*** | 353.4 ± 11.93 **b** | 274.3 ± 8.35 **a** | 300.3 ± 10.43 **a** | 0.61 | 6.3 | \*\*\* |
| ***OD*** | **\*\*** | *n.s.* | **\*\*\*** | 4.3 ± 0.23 **a** | 5.4 ± 0.18 **b** | 4.7 ± 0.17 **a** | 0.43 | 3.6 | \*\* |
| ***pH*** | **\*\*** | **\*** | *n.s.* | 7.1 ± 0.1 | 7.3 ± 0.09 | 7.4 ± 0.08 | 0.42 | 3.5 | \*\* |
| ***Temperatura*** | *n.s.* | **\*\*\*** | *n.s.* | 24.5 ± 0.24 | 24.8 ± 0.47 | 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 the systems (*LSD*, p> 0.05); *n.s.*: non-significative; **\***: p<0.05; **\*\***: p<0.01; **\*\*\***: p<0.001.

**Table 2.** Spearman's (*rρ*) correlation coefficient matrix between pairs of physicochemical parameters at the system level (C: control; or P: Pennisetum; *bottom diagonal*) and both systems together (*top diagonal*)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameters** | ***pH*** | ***OD*** | ***Temperature*** | ***ORP*** | ***Conductivity*** |
| ***pH*** |  | 0.07 *n.s*. | -0.33 \*\*\* | -0.10 *n.s*. | -0.31 \*\*\* |
| ***OD*** | 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.

# **Figure**