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Research article

Removal of Emergent Pollutants by a Vertical Flow Constructed Wetland with Vetiveria Zizanioides: A Case Study for Caffeine

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Abstract.
This work evaluated caffeine removal in a vertical flow constructed wetland (VFCW), planted with *Vetiveria zizanioides*. The feeding was continuous (synthetic influent: mineral medium and caffeine) to reduce the concentration variations in the bed. Two influent concentrations (0.75 ± 1.0 mg·L^{-1}and 1.5 ± 1.0 mg·L^{-1}) were used with a constant hydraulic load (100 ± 10 L·m^{-2}·d^{-1}). Plant growth was monitored weekly, and characterization was carried out to determine the levels of chlorophyll *a* and *b*, pigments and carotenoids, and nutrients (nitrogen, phosphorus, magnesium, calcium, sodium and potassium). HPLC-MS was used to determine the caffeine concentration. The caffeine removal efficiency reached averages of 93 ± 1% and 87 ± 1% (retention time: 6.23 ± 0.23 hours). The caffeine contents in the influent did not affect chlorophyll *a*, total chlorophyll or carotenoids, and an increasing trend throughout the tests was observed. Sodium and potassium contents also showed an increase with higher caffeine concentration. This preliminary study showed that removing caffeine from wastewater using a VFCW is promising.

Keywords: wastewater, emergent pollutants, caffeine, vertical flow constructed wetlands, *Vetiveria zizanioides*

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1. Introduction

Most pharmaceutical products are not completely metabolized after human ingestion resulting in the metabolites excretion and other similar compounds that reach wastewater treatment plants (WWTPs) [1]. Since these plants are not designed with the specific purpose of removing pharmaceutical compounds, their detection is often found in effluents and surface water [2]. Among the pharmaceuticals, caffeine has been widely studied in wastewater treatment systems. As an alkaloid, a psychoactive substance, legal is one of the most consumed in the world, being present in different drinks, such as coke soft drinks, coffee, tea, and energy drinks. In humans, caffeine acts on the

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central nervous system, stimulating the body's alertness and consequently preventing sleepiness. In developed countries, for example, 90% of the adults consume caffeine in a daily basis. Thus, caffeine is present both in domestic wastewater and in some industrial effluents. Due to their high solubility and low volatility, caffeine is quite persistent in water bodies [3]. These characteristics allow caffeine detection in the environment which are essential to correlate the risk it may offer to humans and ecosystems [4-7], and its presence in environmental matrices were confirm in recent studies [8]. Per example, wastewater from two WWTPs in Barbados, a city with about 300,000 inhabitants, were analyzed and it was obtained a range of caffeine concentrations from 0.10 to 6.90 μg L^{-1} [9]; in Italy, 13 WWTPs located in northern, central, and southern (responsible for the collection and treatment of effluents from 50 to 500,000 inhabitants) were evaluated and the results showed caffeine concentrations between 17.6 and 67.6 μg L^{-1} [10].

Constructed wetlands are considered economical compared to other types of wastewater treatment systems like ozonation, chlorination, membrane filtration or the use of activated carbon (high cost, energy demand, and maintenance and therefore only economical for large plants). The complex processes that occur in these systems are conducted through the filter material, plants, and mainly by microorganisms [4]. Treatment wetlands are nature-based technologies with a porous substrate and typically planted with macrophytes. The water passes through the system horizontally or vertically in a certain time, also known as nominal hydraulic retention time [11]. The substrate, as well as the roots of the plants, serve here as a filter and a natural growth body for the microbial community. These engineered wetlands thus mimic natural processes to ultimately improve water quality [12]. In recent years, several studies have deal with the removal of micropollutants through constructed wetlands. A wide range of wetland projects, operational conditions, and influent compositions to efficiently remove micropollutants were examined and intensified wetlands showed a great potential [2; 13-14]. Currently, the use of vertical flow has been increasing to remove both organic matter and nitrogen forms due to the aerobic conditions in this system. [15].

Among a wide range of macrophytes used in these types of projects stands out *Vetiveria Zizanioides*, with specific characteristics such as its high resistance to variations of pH (from 3.0 to 10.5) and temperature of the soil (-10 to 60°C), and to high concentrations of heavy metals (As, Cd, Pb, Hg, Ni, Se and Zn) [16]. Other features that may stand out are its asexual mode of reproduction and its long root system [17]. Studies carried out to assess the removal mechanisms of pharmaceutical compounds in VFCWs have been shown that this kind of compound, as caffeine, is partially accumulated by plants [18]. However, it is necessary to understand the main mechanisms involved in caffeine removal and its effect of increasing concentrations on plant biomass and system performance in general. This work aimed to evaluate the caffeine removal capacity (under two different concentrations) in vertical flow constructed wetlands planted with *Vetiveria zizanioides* in expanded clay aggregates as filter material to understand the removal of this type of pollutant.

2. Material and methods

The experimental work was carried out in a well-established 8-year-old pilot-scale vertical flow constructed wetland (VFCW) (0.24 m^2 × 0.70 m), planted with *Vetiveria zizanioides* (plant density higher than 120 plants m^{-2}), filled with light expanded clay aggregates (Filtralite® NR 10/20), with a bottom slope of 2%, applied to enable the hydraulic collection of the effluent. A layer of gravel (diameter 10–50 mm) was placed around the outlet valve to prevent clogging by fine particles. The flooding levels were maintained at 14% through a siphon in the outlet. The pilot bed was fed in continuous mode, through network sprinklers, equidistantly located over the whole VFCW using a submersible pump (Eheim- 1250, Deizisan, Germany) in feeding tank (Fig. 1). A synthetic wastewater was prepared with mineral medium composed by 28 $mg{\cdot}dm^{-3}$ $CaCl_2$, 52 $mg{\cdot}dm^{-3}$ $MgSO_4{\cdot}7H_2O$, 17.40 $mg{\cdot}dm^{-3}$ KH_2PO_4, 11 $mg{\cdot}dm^{-3}$ K_2SO_4, 0.03 $mg{\cdot}dm^{-3}$ $CuCl_2{\cdot}2H_2O$, 0.18 $mg{\cdot}dm^{-3}$ $MnCl_2.4H_2O$, 0.08 $mg{\cdot}dm^{-3}$ $ZnCl_2$, 1.7 $mg{\cdot}dm^{-3}$ $FeSO_4{\cdot}7H_2O$ diluted in tap water [19], and caffeine to minimize variations in concentration of influent in the batch's reservoirs [Caffeine (>99%) was purchased from Sigma–Aldrich (Germany)].

The VFCW was acclimatized during one month before the beginning of trials by the application of 0.75 $mg{\cdot}L^{-1}$ of caffeine and hydraulic load (C_h) was kept constant at 100±10 $L{\cdot}m^{-2}{\cdot}d^{-1}$. The experiment was designed to rise caffeine concentration at two different concentrations (0.75 $mg{\cdot}L^{-1}$ and 1.5 $mg{\cdot}L^{-1}$), ignoring any ordinary low levels occurring in wastewater [3]. Each trial occurs approximately during one month for each caffeine concentration (from April till July). Each new trial was initiated by increasing the caffeine concentration. Wastewater samples were collected at the inlet and outlet of the VFCW from Monday to Friday, at 10:00 a.m, wherefore a minimum of ten samples were taken from influent and effluent wastewater in each trial. The electrical conductivity (EC), pH, redox potential (Eh) and dissolved oxygen (DO) were immediately measured *in situ*, using a portable probe multiparameter (HI9829 HANNA). Aliquots were frozen (-20^{o}C) to determine the caffeine. The studied caffeine concentration at the inlet (0.75±1 $mg{\cdot}L^{-1}$; 1.5±1 $mg{\cdot}L^{-1}$) and outlet, as well as in the plant biomass after leaves extraction, was

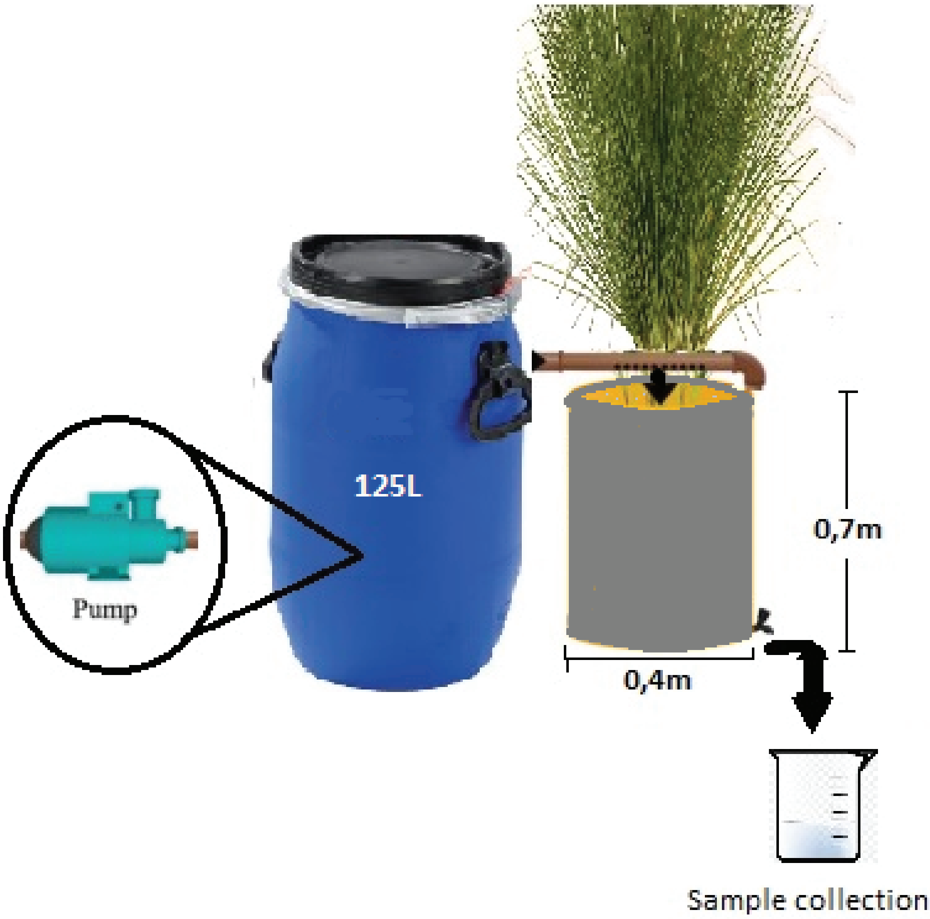

Figure 1: Schematic representation (not at scale) of the VFCW (0.24 $m^2 \times$ 0.70 m).

determined using a HPLC-MS *UltiMate 3000 HPLC series* da *Thermo Scientific* (Thermo Scientific, EUA) with ionization source (ESI), [3; 20]. The reverse phase analytical column used was an Accucore aQ C18 de 100 x 2,1 mm, at 25 ^oC. Separation was performed in isocratic mode, and the mobile phase used was using a linear composed by 30:70 acetonitrile:water, acidified with 0.1% formic acid (v:v), with a flow of 0.25 mL·min^{-1} and the infection volume was 25 µL. Three replicate of injections were made for each sample. HPLC-grade acetonitrile, water and formic acid a were obtained from Fisher Scientific (USA). All chemicals used were analytical grade.

Vetiveria zizanioides plants were visually inspected on a weekly basis for toxicity signals. The plant biomass growth (leaves) was monitored at beginning and at the end of each trial, with different caffein concentration, being determined the levels of chlorophyll *a* and *b* (Chl *a* and Chl *b*), carotenoids, the concentrations of nutrients (nitrogen, phosphorus, magnesium, calcium, sodium, and potassium) [21-22].

Results were statistically verified using software "Statistica 12.0" (StatSoft, Inc., USA). Differences in wastewater quality between influent and effluent of the VFCW were determined using ANOVA at the significance level of $p<0.05$. Tukey's test was used to determine differences between means of specific variables. The means and the standard deviation (S.D.) were calculated with n≥10.

3. Results and discussion

The biomass growth was not negatively influenced by the presence of caffeine in the influent. There was an increment in the growth of biomass during the second trial (caffeine concentration: 1.5±1 mg·L^{-1}).

The level of Chl *a*, and carotenoids showed an increased tendency due to the feeding of plants with caffeine (Fig. 2a). However, the Chl *b* level showed a very strong tendency to decrease during the trials.

Sodium (Na) and potassium (K) levels showed an increasing trend due to increased caffeine level in the influent of the VFCWs (Fig. 2b).

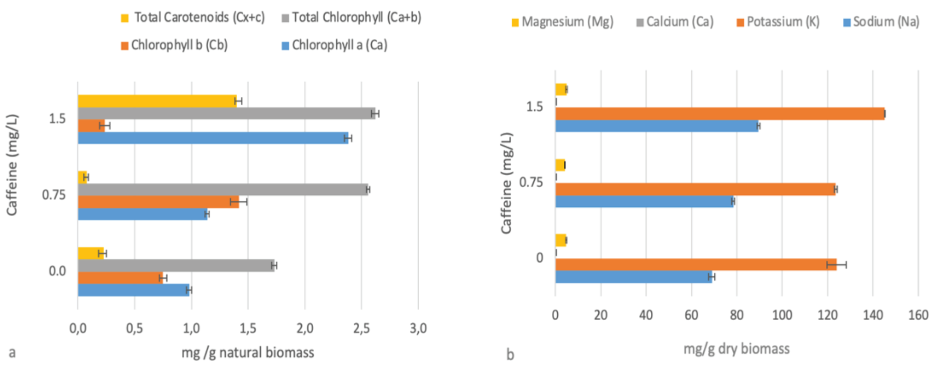

Figure 2: (a) Chlorophyll level [a, b and total], and (b) biomass composition [Mg, Ca, K, and Na] determined at beginning and at the end of each trial.

The results obtained for potential redox, dissolved oxygen and pH are presented at Figure 3.

The potential redox measured in the inlet and outlet of the VFCWs was always positive. There was not significant difference when compared the average values obtained for the influent and effluent ($p>0.05$) (Fig.3a). The dissolved oxygen of the effluent increased in both trials (Fig.3b), possibly due to the capacity of the plants or the type of flow that allowed the replacement of oxygen consumed by the microbial presented in the beds. The outlet pH decreased, without statistical significance at both trials ($p>0.05$), when compared with inlet synthetic wastewater (Fig.3c). Additionally, it was not observed significative difference at electrical conductivity between the influent and effluent at the beds monitored ($p>0.05$). It this sense, it was deduced that TSS

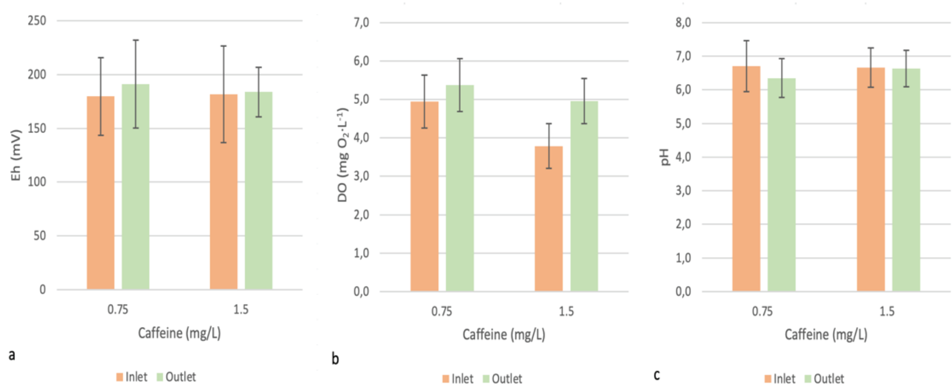

Figure 3: Evolution of potential redox - Eh (a), Dissolved oxygen (b), and pH (c) during experiment.

concentration remained constant over the trials and did not occur precipitation of salts during the experiment.

Regarding efficiency and effective monitoring of beds (HRT=6.23±0.23 hours), it was obtained, considering the different and increasing concentrations of caffeine (0.75±1 mg·L^{-1}; 1.5±1 mg·L-1), an average removal of 93±1% and 87±1%, respectively (Fig. 4).

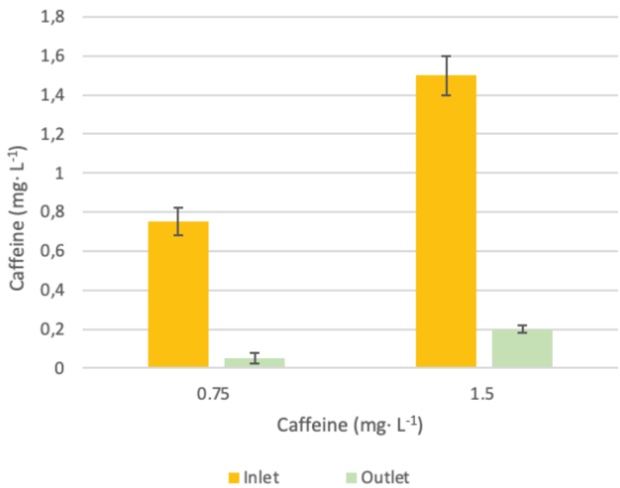

Figure 4: Caffeine concentration at VFCW (inlet and outlet), in each trial.

As mentioned before, although our HRT was 6.23±0.23 hours, the results obtained in our study were like others presented at literature [24]. Performing lab trials with a VFWC with an HRT of 7 days, it was achieved removal efficiencies of 97%. Other authors [3] using a retention time of 21 days obtained a removal efficiency in hydroponic conditions until 99 % [3].

The caffeine content in the leaves of *Vetiveria zizanioides* suggested that the plant assimilated during the experiment about 10 μg·g^{-1} of natural biomass. Therefore, it was also observed that with the increase in the caffeine concentration, the pollutant mass load input increased, leading to a reduction in the plant's capacity to removal the emergent pollutant in the system evaluated.

4. Conclusions

The results obtained showed a removal efficiency up to 93% of caffeine using vertical flow constructed wetlands with *Vetiveria zizanioides*, suggesting an assimilation of 10 µg·g-1 by plants. However, given the high caffeine removed content, this was not the main removal mechanism, pointing to the possible formation of metabolites and their assimilation by plants. In this sense, it will be important consider for future research, the internal sampling and analysis of the microbiota involved in the removal mechanism. This preliminary study showed that removing caffeine from wastewater using VFCW is promising.

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