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Effectiveness and Temporal Variation of a Full-Scale Horizontal Constructed Wetland in Reducing Nitrogen and Phosphorus from Domestic Wastewater

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Abstract: Cultural eutrophication stimulated by anthropogenic-derived nutrients represents one of most widespread water quality problems worldwide. Constructed wetlands (CWs) have emerged as an aesthetic, sustainable form of wastewater treatment, but, although they have shown adequate levels of organic matter removal in wastewaters, the effectiveness of nutrient removal has been less successful. An eleven-month monitoring program was undertaken in a horizontal subsurface flow CW (HSSF-CW) treating domestic wastewater from a village in Centre Region of Portugal, to evaluate the influence of climatic conditions (Continental-Mediterranean Climate region) and seasonal variations on removal. This CW uses gravel and sand as substrate and *Phragmites australis* as wetland plants. Samples were collected at the inlet and outlet from wetland bed and analyzed for pH, TN, Org-N, NH_4^+-N, NO_x-N, TP and DP. The removal efficiencies (RE) of nitrogen and phosphorus compounds were relatively poor, but the results allow us to conclude that season had a significant ($p < 0.05$) effect on the RE of TN, NH_4^+-N, NO_x-N, TP and DP, with higher values in warmest period (10.4%, 10.4%, 3.4%, 27.5% and 26.1%, respectively) than in coldest period (0%, −7.7%, −9.8%, 12.9% and 0%, respectively). Although lower hydraulic loading rate (HLR) generally resulted in better RE of all N and P compounds analyzed, no significant linear relationship was observed between these two variables. TN and NH_4^+-N concentrations in the effluent tend to significantly ($p < 0.05$) decrease with increasing respective incoming mass load rates for whole monitoring period and during spring–summer period, while the correlation between outlet TP concentrations and the inlet loading rate are not significant. The results indicate that the system is not effective for removal of nutrients, probably because it operated on overload and with a low hydraulic retention time (HRT) (average = 2.4 days). The results also showed that the RE of N and P followed seasonal trends, with higher values during spring–summer period.

Keywords: constructed wetland; horizontal subsurface; nitrogen; phosphorous; removal efficiency; seasonal variations

1. Introduction

Horizontal subsurface flow Constructed Wetlands (HSSF-CWs) are biological treatment systems that seek to mimic the biogeochemical processes occurring in the natural wetlands to remove the contaminants present in wastewater. When compared to conventional wastewater treatment systems, HSSF-CWs have shown to be a sustainable technology, which has been widely used to treat different types of wastewater and is simple to build and easy to operate and maintain [1,2]. They are also characterized by being low energy and chemical reagents demanding systems; consequently, they have become an alternative to conventional systems for the sanitation of small scale treatment plants. They seem to be appropriate to remove many types of pollutants [3].

In Portugal, similar to in many other parts of the world, nutrient enrichment of water bodies is a major environmental problem, thus nutrient removal during wastewater treatment in sewage-treatment plants is vital to minimize the impact of nitrogen and phosphorus pollution on water bodies and to achieve the environmental objectives of the Water Framework Directive (WFD; Directive 2000/60/EU) in Portugal [4]. Due to usually long retention times, HSSF-CWs can provide a reliable secondary level of treatment, and it has been shown that they are effective in removing total suspended solids (TSS) and organic matter (OM), and also allow the removal of nutrients, although with variable and lower efficiencies [5–7].

The idea behind nutrient removal is that plants absorb these elements and incorporate them into their tissues during the growth period [3,8]. However, this mechanism is only one very small part of nutrient removal in these systems. In fact, in CWs, nitrogen removal is subject to a wide range of transformations that include physical, chemical and biological processes involving various mechanisms such as ammonification, nitrification with further denitrification, microbial immobilization and matrix adsorption [2,9,10]. Unlike nitrogen, which could be eliminated of the system by nitrification/denitrification, phosphorous in wastewater is usually removed by retaining in the CW system [11]. Phosphorous removal efficiency varies considerably due to the complex combination of physical, chemical and biological processes involving mainly adsorption/desorption, precipitation/dissolution, sedimentation in pores of substrate media, peat accretion and burial, and to a lesser extent biomass uptake [10–12]. HSSF-CWs often exhibit inconsistent and highly variable nitrogen removal efficiency and has been reported in long term studies ranging from 20% to 70% [9,13]. Regarding P removal, values ranging from 30% to 60% have been reported, depending on factors such as the wastewater P loading rate, the type of media used and the hydraulic loading rate [11,14]. In fact, HSSF-CWs phosphorus removal must be limited because the materials used in substrate (usually gravel) are poor in iron and aluminum hydrous oxides minerals as well as in calcium and magnesium concentrations, elements essentials for adsorption and precipitation of insoluble forms in wetlands, the most important mechanisms for removal P in those systems [10]. Among all nitrogen removal mechanisms, ammonification followed by nitrification–denitrification is usually accepted to be the most important in constructed wetlands [6,15]. Since the heterotrophic organic matter removal bacteria compete with the autotrophic nitrification bacteria for oxygen and natural aeration in HSSF-CW does not often meet the oxygen demand required for both types of microorganisms, variable and undesirable levels of nitrogen in HSSF-CW effluents can be produced, influencing negatively the performance of this type of systems [3,13].

Thus, in CWs, the efficiency of N and P removal is generally limited and dependent on aspects such as the type of plants, seasons and climatic conditions. Thus, even though there have been several studies on the processes involved in the removal of nitrogen and phosphorus, the results obtained in terms of removal efficiency are highly variable, which makes it difficult to judge the extent to which HSSF-CWs are an efficient technology to reduce the nutrient concentration in wastewater and, consequently, to contribute to reducing water bodies eutrophication.

In fact, although many studies have contributed to understanding the mechanisms associated with removal process in CWs, inconsistencies in the results suggest the importance of further studies for the optimization of this technology, thus ensuring that they comply with the increasingly strict discharge standards for treated effluents and for their reuse. In Portugal, the use of HSSF-CWs is recent in comparison with other European countries and, although, in recent years, some studies on the treatment performance of HSSF-CWs systems have been conducted [16–19], it appears that there is still a lack of data in sufficient detail, both temporally and spatially, on full-scale CWs for wastewater treatment. Furthermore, although the effects of climate and seasonality are considered relevant for the performance of the treatment of CWs, their roles remain unclear [20,21]. Therefore, further investigations are needed to clarify the influence of climatic and season on the removal of pollutants in Mediterranean climatic conditions.

The aim of the present study was to examine the seasonal variations in the removal efficiencies of nitrogen (TN, Org-N, NH_4^+-N, and NO_x-N), and phosphorus (TP and DP) in a seven-year-old full-scale HSSF-CW system receiving sewage wastewater from a small village located in Interior Centre Region of Portugal, operated under temperate Mediterranean climate with strong continental influence.

2. Materials and Methods

2.1. Study Site

The study was conducted at the Sarnadas Rodão sewage treatment plant, a full-scale horizontal subsurface constructed wetland located in the Centre Interior region of Portugal (39°45′32″ N 7°38′35″ W). The region is in the warm temperate zone and, according to the Classification System of Köppen-Geiger, the climate is Csa, corresponding to a Mediterranean climate, clearly influenced continentally [22]. The climate is characterized by cold winters alternating with hot and dry summers. The two warmest months, July and August, have temperatures above 24 °C on average, while winters are harsh, with the months of December, January and February, showing average temperatures below 10 °C. The high temperature range is a clear mark of strong continental influence. The difference between the warmest month (July) and coldest month (January) is 16.2 °C. Average annual temperature is 15.6 °C, with average minimum and maximum temperatures of 11 and 20 °C, respectively. The number of days of maximum temperature >25 °C was 118, and the number of days of minimum temperature <0 °C was four [22].

Another important regional characteristic is the occurrence of episodic heavy rains, contrasting with a rather moderate total rainfall. According to IPMA [22], approximately 50% of the average annual rainfall occurs between November and February, while July, August and September are usually the driest months. The average annual precipitation for the period under review (1961–1986) is 780.7 mm, ranging between 500 and 900 mm.

The system has been operating since 2006, with design capacity of treating 73 $m^3 \cdot day^{-1}$ of sewage collected from a community of 550 equivalent inhabitants at horizon-year (2020). The system consists of pre-treatment, which involves physical separation to remove large objects and sands that includes a single manually cleaned screen bars and a sand channel followed by a primary treatment in a three compartmentalized septic tank. The septic tank has a total capacity of about 171 m^3 and provides two days of hydraulic retention time (HRT) to ensure sedimentation of part of the suspended solids; to separate oils and grease present in the raw influent; perform an equalization of the wastewater; ensure a constant flow entering in the wetland system; and prevent clogging.

As secondary level of treatment, the wastewater treatment plant (WWTP) studied has a HSSF-CW with a single wetland cell that is 40 m × 28 m (length and width), with a total surface area of 1120 m^2, which corresponds to an area per equivalent inhabitant of about 2 m^2 (assuming 60 g BOD_5 per inhabitant and per day), which is lower than the range of values using a simple "rule of thumb" of 3–6 m^2/equivalent-inhabitant recommended for HSSF-CWs systems [23–25]. However, this is not an isolated case in Portugal, Pascoal and Sousa [26] also found that for ten HSSF-CWs systems studied, 80% had an area of the bed per inhabitant ≤ 2 m^2. In addition, Duarte et al. [27], in a study of twenty HSSF-CWs systems across the country, reported that the average area of the bed per person served is 1.9 ± 0.9 m^2.

The drainage bottom layer of wetland was filled with gravel (20 cm of 15/30 mm in diameter), then with sand (30 cm of 3/10 mm of diameter) and finally 10 cm of topsoil is used on the top layer. The medium porosity of filter media is 0.38. This wetland filter bed has a mean depth of 60 cm, which is in agreement with the values recommended for Portugal by Relvão [28] and is design to be operated at a hydraulic loading rate (HLR) of 14 cm·day^{-1} in 2006 and 17 cm·day^{-1} in 2020, which falls within the hydraulic load values (2–20 cm/day) referred to HSSF-CWs systems [24,29,30].

Wetland was design to have a maximum organic loading rate (OLR) of approximately 10 g BOD_5 m^{-2}·day^{-1} both for the project-year and for the horizon-year, a TSS loading rate of 22 g TSS m^{-2}·day^{-1} and a nitrogen loading rate (NLR) of 2.2 g TN m^{-2}·day^{-1}. A total phosphorous loading rate of (PLR) of 0.44 g TP m^{-2}·day^{-1} is expected. A theoretical HRT of 4.3 days in 2006 and 3.5 days in 2020 are expected, which are out the recommended values (5–15 days) according to Vymazal [24] and IWA [29]. The bed was built with a slope of 1% at the bottom to allow easier water flow and the entire system was operated under gravity.

The average wastewater inflow rate during the study period was 83 m^3·day^{-1} in spring–summer period and 144 m^3·day^{-1} in autumn–winter period, while the average HRT was approximately 2.4 days, with average values of 1.2 and 3.2 days for cold and warm periods, respectively, which means that the system studied is not achieving its design HRTs.

This system is planted with common reed plants (*Phragmites australis*), which were well-established and covered the entire surface of the wetland bed. This wetland macrophyte plant seems to be the most widely used in Portugal. In fact, Duarte et al. [27] studied twenty Portuguese HSSF-CWs and, based on data provided by different systems, they found that, in 70% of those systems, the plant used was *Phragmites australis* and they also found that these systems were mostly (90%) in monoculture. Similar results were found by Pascoal and Sousa [26].

2.2. Sampling and Physiochemical Analysis

The sampling took place monthly from August 2012 to June 2013 at inlet of the HSSF (after septic tank) and at the outlet of the wetland bed. All samples were collected on the same date at the same time and analyzed at each sampling point. The wastewater samplings were undertaken always in the morning (8:00–10:00) and were collected manually for a polyethylene bottles and kept refrigerated (4 °C) during transportation to the Water and Wastewater Analysis Laboratory at High School of Agriculture of Polytechnic Institute of Castelo Branco, where they were analyzed within 24 hours and always maintained at temperatures of 4 °C. Samples for phosphorus analysis were kept in plastic bottles that were pre-cleaned with phosphorus-free detergent. The samples for water temperature, electrical conductivity (EC) and pH were analyzed in the field with portable sensors.

All collected water samples were analyzed for total nitrogen (TN), ammonium nitrogen (NH_4^+-N), Organic nitrogen (Org-N), oxidized nitrogen (NO_2-N + NO_3^--N: NO_x-N), total phosphorous (TP) and total dissolved phosphorus (DP) according to the Standard Methods for the Examination of Water and Wastewater [31]. Total nitrogen was calculated as the sum of the three nitrogen components. Total P was determined as soluble molybdate-reactive P after acid oxidation with potassium persulfate ($K_2S_2O_8$). Dissolved phosphorus was measured after filtration of the sample on filter membrane with mesh size of 0.45 µm. The difference between TP in nonfiltered and filtered samples was considered to be particulate phosphorous (PP). All samples were analyzed in duplicate.

For flow measurements, the WWTP installed an ultrasonic meter level, associated with V-notch weirs, just off the septic tank (which corresponds to the entrance of the macrophytes bed). The data relating to average daily flow rate of affluent to the wetland bed over the different months during the sampling period were provided by the company Águas do Centro, responsible for management and operation of the WWTP. The HLR were calculated based on acquired inflow rate at wetland bed and according the expression $q = Q/A$, where q is the HLR (m·day^{-1}), Q is the average wastewater inflow rate (m^3·day^{-1}), and A (m^2) is the total surface wetland a rea of the bed calculated using the porosity of the wetland media. The HRT was calculated by equation $HRT = V/Q$, where HRT is the detention time (days), V is pore water volume (m^3), and Q is the average wastewater inflow rate (m^3·day^{-1}).

The ESACB meteorological station, located 15 km from the study site, was consulted for meteorological data on rainfall and temperature that were used to evaluate the influence of weather conditions on the treatment performance of the Sarnadas Rodão WWTP. To acquire information about the possible influence of seasonality on the performance of the system, the dataset was divided into two subsets: spring–summer (August 2012 and April–June 2013) and autumn–winter (September–December 2012 and January–March 2013), which correspond to warm and cold periods, respectively.

2.3. Statistical Analysis

All statistical analyses were performed using SPSS version 21 for windows. Prior to the analysis, the data were subjected to normality distribution and homogeneity of variance with a Kolmogorov–Smirnov with Lilliefors Significance Correction and with Shapiro-Wilk W tests ($n \leq 50$). The combined non-normal distribution and a constrained sample size for the data led us to use non-parametric Mann-Whitney U tests to determine differences between inflow and outflow values for the different parameters analyzed in each step of treatment and for each monitoring period. Since the raw data were not normally distributed, non-parametric Kruskal-Wallis ANOVA tests were carried out to test the differences between both concentrations in the influent and effluent for both seasonal periods and also for analysis of the significance of differences between average efficiencies recorded for the periods of spring–summer and autumn–winter. The statistical analysis was conducted at a 95% confidence level.

3. Results and Discussion

3.1. Nitrogen Removal

Ammonium nitrogen (NH_4^+-N) is the N form that dominated in the influent to the bed and accounted for average 79% of TN in whole monitoring period (12 August–13 June), 88% and 61% in warm and cold periods, respectively. The same trend was observed in the outflow of the CW bed with average NH_4^+-N concentrations accounting for 98.7% (12 August–13 June), 87.7% (spring–summer period) and 65.7% (autumn–winter period) of TN. For the influent to the wetland bed, high fluctuation through time was observed, especially for entire monitoring period (12 August–13 June) and for spring–summer season period, as can be seen by the Figure 1a,b.

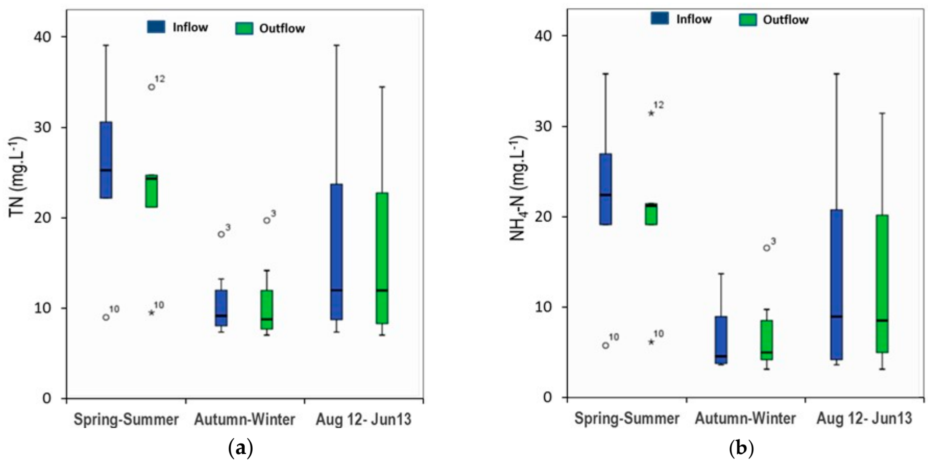

Figure 1. Variability of inflow and outflow concentrations at Sarnadas Rodão system: (**a**) TN; and (**b**) NH_4^+-N.

The quality of discharged wastewater was also unstable in time for all N compounds, which was confirmed by very high coefficients of variation (CV) with values above 43.8% (warm period), 55.7% (cold period), and 64.7% (whole period) for TN, and 49.4% (warm period), 84.8% (cold period), and 84.7% for NH_4^+-N (whole period). The same trend was observed for NO_x-N and Org-N, although the CV was lower and ranged from 31% to 37%. These results seem indicated that variability observed in the inflow concentrations was not significantly reduced in outflow, even though, in spring–summer period, lower variability in effluent quality was observed when compared with autumn–winter period (Figure 1).

Compared with cold periods, average influent concentrations during warm period was higher ($p < 0.05$) for NH_4^+-N and TN, while average influent of Org-N concentration was similar for both periods, and NO_x-N concentration was slightly lower in warm period. Effluent concentrations were also higher ($p < 0.05$) in warm period compared to colder seasons for NH_4^+-N and TN, but NO_x-N in contrast showed higher concentrations during cold period. These results could be derived by dilution of the wetland inlet and outlet of the bed by rainfall during colder and rainy period and by the highest evapotranspiration rates in warm period that may also contribute to increasing the concentration of pollutants in the treated wastewater.

Over the whole period (12 August–13 June), the CW bed showed very low average removal efficiency for all N compounds. RE for TN, Org-N, NH_4^+-N and NO_x-N was 6.9%, 29.6%, 5.8% and −4.3%, respectively (Figure 2). These removals efficiencies were lower than those normally reported by the international and national literature [16,19,23,25,32].

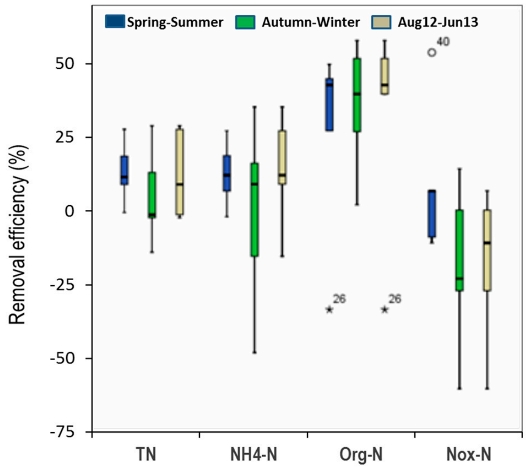

Figure 2. Removal efficiency for the different nitrogen compounds during the monitoring period.

In a study on the performance of 11 HSSF-CWs systems in Catalonia (Spain), Vera et al. [33] reported mean removal percentages for TN that are in the range of 48% and 66%, values that are consistent with the values found by Puigagut et al. [34] who indicated a mean RE of 51% for TN in HSSF-CWs also in Spain. In addition, Mietto and Borin [35], when evaluating the performance of HSSF-CW treating domestic wastewater in northern Italy in the first two years of operation, obtained a TN removal efficiency of 59% for an HRT of eight days.

Regarding systems operating in Portugal, Duarte et al. [27] found RE of TN ranging from 38.5% to 74.2%, when evaluating eight full-scale CWs systems throughout of Portugal, while Simões [36] reported a removal efficiency of 74.6% and 76.7% for TN and NH_4^+-N, respectively, in a HSSF wetland system located in the same region of the studied system in this work. However, Oliveira [37], in four HSSF systems operating in the northern region of Portugal, found an average removal efficiency of NH_4^+-N ranging between 4% and 51%, while Marecos do Monte and Albuquerque [18], based on a nine-month campaign in an HSSF bed located at Interior Centre of Portugal, reported an average removal efficiency of 76.3% and 78.8% for TN and NH_4^+-N, respectively.

Figure 2 also allows observing that the RE was more unstable in autumn–winter when compared to spring–summer period and, although the values obtained are very low for both seasonal periods, we found that generally the average removal efficiency was higher for all parameters in the warmest period, except for organic nitrogen. A statistical analysis of the seasonality variable has shown there was significant effect of season in TN, NH_4^+-N and NO_x-N removal efficiencies. In fact, we observed that autumn–winter average removal efficiencies were significantly ($p < 0.05$) lower for TN (0%),NH_4^+-N

(−7.7%) and NO_x-N (−9.8%) than for spring–summer seasons, for which the percentage of removal were, respectively, 10.4%, 10.4% and 3.4%.

These results match with some other studies that found nitrogen removal efficiencies in warm seasons tend to be higher than those obtained for colder periods [19,38]. These removal efficiencies were lower than that observed in similar system and climate conditions by Albuquerque et al. [16] and by Marecos do Monte and Albuquerque [18] also in Interior Central Region of Portugal: Albuquerque et al. [16] reported a removal efficiency for TN, NH_4^+-N and NO_3^--N of 86%, 89% and 80%, respectively, during a spring–summer period (May to August), while the Marecos do Monte and Albuquerque [18] found an average removal efficiencies of 76% and 78.8% for TN and NH_4^+-N, respectively, over a period from March to December.

Highest removal percentage ($p < 0.05$) in removing TN, NH_4^+-N and NO_x-N during the spring–summer period might be associated with greater absorption of these compounds by plants during this period, coinciding with the growing season and greater density of plants on the system under study, which in turn stimulates ammonia removal by the development of biofilms in plant roots. The high removal of TN and NH_4^+-N during the warmest period also highlights the influence of temperature on the nitrification and denitrification processes that has been accepted as the major removal mechanisms for nitrogen compounds in HSSF-CWs [39,40].

In fact, Akratos and Tsihrintzis [39] and Silveira et al. [40] have reported that, for wastewater temperature below 15 °C, the mean TN removal decreased, while Burchell et al. [41] highlight the fact that with average temperatures of 7.5 °C denitrification activity did not respond to added organic matter. A temperature between 20 and 25 °C has been referenced in several studies as being the optimum range for the denitrification process [42]. The mean air temperature in this study was of 12.1 °C for cold period, which may indicate that the conditions of nitrification/denitrification were worse than those that occurred in the warmer months, for which was obtained a monthly mean air temperature of 19.8 °C that could have benefited both nitrifying and denitrifying bacteria.

As we have mentioned, higher measured HLR were observed along the whole time coverage in the present study and we observed that lowest and negative removal efficiency for TN and NH_4^+-N was obtained for the months in which higher HLR was observed (October to March), probably because the contact time between wastewater and biofilm responsible for the nitrogen removal is inadequate. Pan et al. [13] also observed that, when HLR is increased, the removal efficiencies of NH_4^+-N and TN decreased, which is similar to what was found by others authors [43,44]. Although lower HLR generally resulted in better removal efficiencies of N compounds, RE did not significantly ($p > 0.05$) correlate with HLR and the linear relationships between HLR as independent variable and removal percentage were rather weak for all nitrogen compounds and for all monitoring periods, with coefficient of determination (R^2) always less than 0.4356.

Generally, HRTs of 2–10 days has been reported to improve N removal in HSSF systems [45]. However, Akratos and Tsihrintzis [39] stated that, at low temperature, a HRT of eight days is not appropriate for nitrogen removal. In our case, during the coldest period, the average HRT was 2.4 days, which seemed insufficient considering the overall unsatisfactory removal efficiency for all nitrogen compounds analyzed. The negative average removal in NH_4^+-N in autumn–winter may also be related to high flushing due to the high flow rate of water through the wetland bed during the rainy months.

Several authors reported that increase in N loads within certain limits tends to contribute to grater removal rates in HSSF-CWs [16,18,43,46]. The results obtained at the present study have shown that the concentrations of TN and NH_4^+-N in the effluent tend to significantly ($p < 0.05$) decrease with increasing respective incoming mass load rates, although the linear relationship between the two variables for the whole period (12 August–13 June) is moderate, as shown by the values found for the coefficient of determination (TN: $R^2 = 0.3738$; NH_4^+-N: $R^2 = 0.3521$).

The same trend was observed for spring–summer period, despite not having found a significant linear relationship between the two variables neither for TN nor to the NH_4^+-N. For autumn–winter period, this tendency was less clear, since the measured data were spread more widely in this seasonal period. Similar tendencies were also observed for NO_x-N, even though the linear function used to describe the relationship between NO_x-N loading rate and respective effluent concentration was not statistically significant ($p > 0.05$). For organic nitrogen, any trend curve could be drawn out of the results and thereby its concentration into the effluent does not seem to be affected by the inlet Org-N loading rate.

The lower average removal rate obtained in this bed for N compounds for both seasons periods can be associated to the average COD organic load that was relatively high for all sampling periods (12 August–13 June: 22.4 g CODt $m^{-2}\cdot day^{-1}$; warm period: 14.9 g CODt $m^{-2}\cdot day^{-1}$; cold period: 26.8 g CODt $m^{-2}\cdot day^{-1}$) and generally outside the interval normally recommended both by International design criteria to size HSSF-CWs (5–20 g CODt $m^{-2}\cdot day^{-1}$) [32] and by German guidelines ATV-A 262 (16 g CODt $m^{-2}\cdot day^{-1}$) [16]. The oxygen available for microorganisms is used first by heterotrophic organic removal bacteria and later by nitrifying bacteria and, thus, the observed high organic loading rate may have contributed to the oxygen availability being insufficient for the two mechanisms [2,3].

The lower removal of TN and NH_4^+-N might also be explained by the relatively small area of the bed considered in design project (2 m^2/p.e.) of the system studied, which also contributed to lower residence time. According to Wallace and Knight [47], to obtain significant reductions in N compounds, the unit area values must be above 12 m^2/p.e., while Vymazal [24] established that the value of 5 m^2/p.e. is recommended for eliminating organic matter and suspended solids, but is insufficient to achieve complete nitrification. However, Vera et al. [33], in a study on 11 HSSF-CWs in Spain, did not find a clear and direct relationship between TN removal and the surface area.

The quality of treated effluent was unstable in time and, considering the discharge requirements imposed by Portuguese Decrees Nos. 152/97 and 236/98, during the spring–summer period, the average emission of N compounds (TN and NH_4^+-N) did not meet the legal limits. TN concentrations on the discharge site were 1.4 times higher than TN limit (15 $mg\cdot L^{-1}$) and NH_4^+-N concentrations were 1.8 times higher than NH_4^+-N limit (10 $mg\cdot L^{-1}$).

3.2. Phosphorous Removal

TP inlet concentration did not exceed 4.55 $mg\cdot L^{-1}$ along the entire monitoring period, although the concentrations values have changed throughout the study period (Figure 3) from 0.13 $mg\cdot L^{-1}$ (February and March) to 4.55 $mg\cdot L^{-1}$ (June). On average, during spring–summer and autumn–winter periods, the TP concentrations in the influent to the wetland bed were of 2.87 ± 1.23 $mg\cdot L^{-1}$ and 1.01 ± 1.00 $mg\cdot L^{-1}$, respectively. TP effluent concentrations from wetland bed were generally slightly lower than at the inlet, except in October, February and March. On average, HSSF bed does not provide statistically significant abatement ($p > 0.05$) in TP concentration for any sampling periods considered, and effluent also maintaining a wide variability (Figure 3).

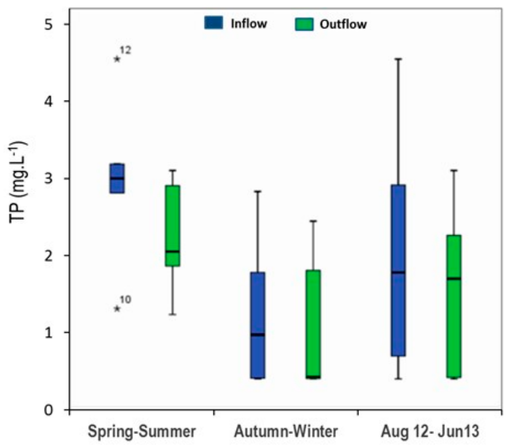

Figure 3. Seasonal variations of total phosphorus (TP) concentrations in inflow and outflow of the Sarnadas Rodão HSSF system during the study period and for the three monitoring periods.

HSSF bed system influent and effluent concentrations of dissolved phosphorus (DP) showed a pattern in accordance with TP concentration; however, lower concentrations were measured at different sampling points (Figure 4). No statistically significant difference ($p > 0.05$) was found between average influent and effluent concentration at the bed for the three monitoring periods, even though the mean outflow DP concentrations were lower than the inflow concentrations (Figure 4).

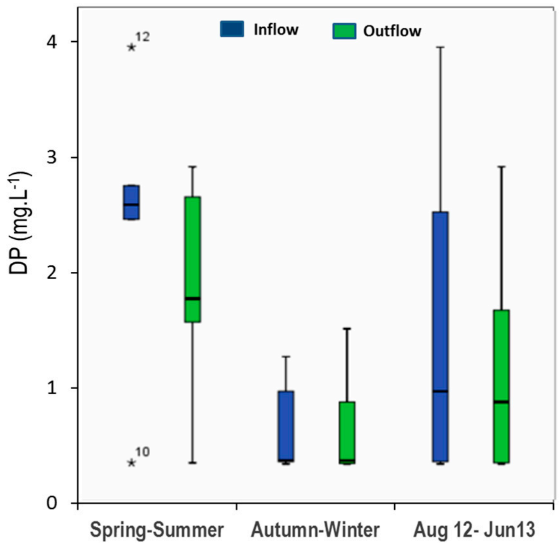

Figure 4. Seasonal variations of dissolved phosphorus (DP) concentrations in inflow and outflow of the Sarnadas Rodão HSSF system, during the study period and for the three monitoring periods.

Significantly lower mean values for the concentration of TP and DP were observed in autumn–winter period, at both the entrance and the output of the bed, which may be explained by the dilution effect caused by rainfall during that period, while evapotranspiration rate may account for the higher values found during the spring–summer period. In fact, during the spring–summer period, average concentrations of TP and DP in influent were, respectively, two and five times higher compared to autumn–winter period. Average effluent concentrations showed similar trend to influent, with highest values during spring–summer (2.5 and 3.5 times higher respectively to TP and DP). The average concentrations of TP in the effluent were below the discharge limit (10 $mg \cdot L^{-1}$) according to the Portuguese Decree No. 236/98.

The RE of phosphorus compounds was relatively poor when compared with TSS and organic compounds, but better than N compounds removal. Average removal rates of TP were 23%, 27.5% and 12.9% during the whole monitoring period, the spring–summer and autumn–winter periods, respectively. Regarding DP, the system removed 20% along the entire sampling period, 26.1% in spring–summer period, and 0% in autumn–winter period.

Thus, the results allow us to conclude that there were significant differences ($p < 0.05$) in the rate of removal of TP and DP between two seasonal periods considered, with highest percentage removal occurring in warmest period. The results showed that there was a sharp decrease of 14.6% in the removal efficiency of TP from spring–summer to autumn–winter, whereas, regarding DP, this reduction was 26.1% (Figure 5). This figure also shows that there was a trend toward greater instability in the efficiency of removal of both the TP and DP during the autumn–winter.

The variety of P RE for domestic wastewater treatment observed in HSSF-CWs in Portugal is high and generally ranged from 26% to 94% [18,19,27,36,37]. These differences may be linked to differences in phosphorus concentration in the influent to the beds or on the difference in the HLR, HRT, material used for bed construction and how long the system has operated. However, the low TP removal efficiency observed in this study are similar to what is reported in the literature [48,49]. In a study based on data collected worldwide regarding the removal of P in HSSF-CWs, Vymazal [24] reported an average mass-based efficiency of 32%, while IWA [29] reported a better performance (50%) for systems operating in Europe. For 11 HSSF-CWS in Catalonia, Spain, Vera et al. [33] a maximum mean of 58% for TP removal efficiency was achieved. In addition, Gagnon [49] reported a poor P removal in three wetlands where DP was the dominant P form in the inflow and found in fact the wetlands acted as net sources of P on an annual basis during establishment. Generally, phosphorous RE reported in the literature is variable and usually range from 26% to 70% [12,23,45,50].

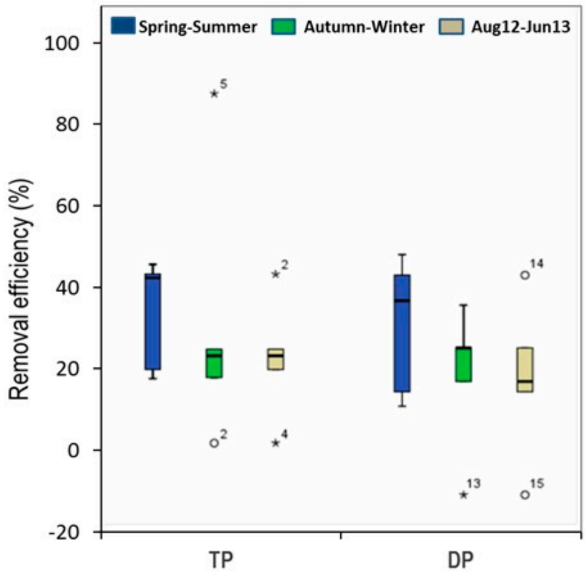

Figure 5. Removal efficiency for TP and DP along the different sampling periods in HSSF-CW at Sarnadas Rodão, Portugal.

A possible explanation for higher removal efficiency in spring–summer period regarding autumn–winter can be linked to the fact that the average concentration of P (total and soluble phosphorus) have decreased sharply in the winter time at the entrance of the bed, which could have contributed to an apparent release of accumulated P from the adsorption sites due to exchange ions reactions which might resulted into a sharp decrease in phosphorus removal efficiency. Similar results are also reported by other authors [45,50–52]. On the other hand, higher evapotranspiration rates in spring–summer period which will result in a lengthening of the retention times (on average 3.2 days), which ensures more time for phosphorus removal by both physical and chemical processes as biological processes [39,42]. In addition, plant uptake (despite being a minor route for P removal) also reaches a maximum in spring–summer months. Furthermore, the combination of the role of plants in oxygen transfer to the rhizosphere and water level fluctuations caused by higher evapotranspiration due to higher temperatures during spring–summer period could have contributed to enhance the potential redox, which in turn seems to favor the increase of phosphorus removal by sorption and co-precipitation on elements minerals in the rhizosphere [30].

The lower removal efficiency that have been found at autumn–winter months may also be attributed to the annual die-off of the plants that could potentially be an additional source of phosphorus release due to the biodegradation from that organic material [10,12]. Similar to concentrations, high fluctuation on average inlet loads were observed for TP and for DP throughout the all study period. Average loads in influent to the CW bed was of 0.19 g·m^{-2}·day^{-1} for TP and 0.13 g·m^{-2}·day^{-1} for DP for the whole period, whereas, in the spring–summer period, mean values of TP and DP loads applied to the system of 0.21 g·m^{-2}·day^{-1} and 0.17 g·m^{-2}·day^{-1}, respectively, were recorded, which were higher than those observed for the autumn–winter period (0.13 g·m^{-2}·day^{-1} for TP and 0.06 g·m^{-2}·day^{-1} for DP). TP loading rate in this wetland bed was more than six times larger than the loading rate of

0.03 g·m^{-2}·day^{-1} suggested by [53] for HSSF-CWs, but smaller than that considered at the project (0.44 g TP m^{-2}·day^{-1}).

The correlation between the TP concentration in effluent and inlet TP loading rates for entire sampling period has shown that there was a negative linear ($p > 0.05$) relationship ($R^2 = 0.315$, p-value = 0.07), but for the two seasonal periods no trend curve could be drawn based on the results, probably due to the relatively few samples per period. Regarding to the DP, the same statistical analysis also showed a negative ($p > 0.05$) linear relationship between the loads of DP applied and the concentration of dissolved phosphorus in the treated effluent with $R^2 = 0.186$ and p-value = 0.185 for entire coverage time, $R^2 = 0.381$ and p-value = 0.268 for spring–summer period and $R^2 = 0.1134$ and p-value = 0.514 for autumn–winter months. In addition, Maltais-Landry et al. [54], in a mesocosm HSSF-CWs treating trout farm wastewater, observed that TP removal efficiency decreased with increasing loading rates.

4. Conclusions

The present study investigated the performance of a full-scale HSSF-CW system, after seven years of operation. The results indicate that the system is not effective for removal the nutrients (N and P) when used as secondary treatment. However, this study highlighted that removal efficiencies exhibited seasonal trends.

Regarding nitrogen compounds, we found that average removal efficiencies for all nitrogen species were generally higher ($p < 0.05$) during the warmest period when compared to those during autumn–winter period, except for organic nitrogen. Factors that probably include lower flowrates and HLRs and therefore increased HRTs as well as more favorable temperatures for nitrification and denitrification processes, along the intense growth of plants might have contributed to enhance the nitrogen removal during warmer period. In fact, unfavorable values of HRT (on average 2.1 days) during autumn–winter could have limited the nitrification/denitrification processes in conjunction with the lower temperatures observed in this seasonal period.

In addition, results from this study indicate that removal efficiencies were also marked by seasonality for phosphorus compounds (TP and DP), with highest ($p < 0.05$) percentage removal occurring during spring–summer period. A higher removal rate is expected when the plants are in the exponential growth phase (spring–summer period) due to its role in direct uptake of phosphorus. The high HLRs observed in this study, especially during autumn–winter period, also means shorter retention time, thus lower adsorption efficiency of phosphorous onto the substrate media.

The results also indicate that the removal percentage of phosphorus compounds were relatively low, indicating that phosphorus release from the wetland bed may occur due to the soluble reactive phosphorus (orthophosphate) adsorbed and/or precipitated with iron and manganese hydroxides, which may be released due to high reactivity and solubility of iron and manganese hydroxides in environments with low values of potential redox that are usually lower in autumn–winter due to lower oxygen availability.

Thus, we conclude that, in addition to the influence of differences in vegetative cycle of the macrophytes species and seasonal temperature on the seasonally cyclic performance of HSSF-CW systems, we should also pay attention to the influence of seasonal changes in the HLR, and subsequently HRTs on the treatment efficiency by these systems.

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