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Removal of Pharmaceuticals in a Macrophyte Pond-Constructed Wetland System and the Effect of a Low Effluent Recirculation

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Abstract: Waste stabilization ponds and constructed wetlands (CWs) are effective at eliminating pharmaceutical residues, but removals are not usually complete. Their combination is regarded as an efficient, robust wastewater treatment method, but their efficiency in the removal of pharmaceuticals and the effect of a mild effluent recirculation has not been sufficiently studied in full-scale systems. Effluent recirculation can help to improve performance by increasing hydraulic residence time and, eventually, dissolved oxygen concentration. In this work, the presence of pharmaceuticals in wastewater from a university campus, their removal in a macrophyte pond–CW system, and the effect of effluent recirculation on removal and ecological risk were evaluated. Stimulants (caffeine and nicotine) and non-steroidal anti-inflammatories (naproxen and ibuprofen) were the most detected compounds in the influent and showed the highest concentrations, ranging from 0.5 to 300 $\mu g \cdot L^{-1}$. The pond–CW combination showed notable elimination for these compounds, achieving 87% on average. The ecological risk was also reduced by between 5.5 and 12.4 times, but it was still over values that indicates high ecological risk, mainly because of the concentrations of nicotine and ibuprofen. The effect of effluent recirculation was not as high as expected since the removals of caffeine, paraxanthine and naproxen were significantly improved, but those of atenolol and ibuprofen were lower. These results suggest that a higher recirculation ratio should be tested.

Keywords: wastewater treatment; pharmaceuticals; pond; constructed wetland; effluent recirculation; risk assessment

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1. Introduction

Waste stabilization ponds and constructed wetlands (CWs) are particularly suitable alternatives for wastewater treatment in small communities because of their minimum or zero energy consumption, low maintenance requirements, limited or no use of chemical products, and high visual and ecological integrability [1]. Additionally, their combination has been proposed as a robust wastewater treatment [2].

On the other hand, the consumption of pharmaceutical products has increased worldwide in recent decades, and residues of pharmaceutical compounds (PhCs) are now common pollutants in urban and domestic wastewaters [3–5]. The widespread release, persistence, and ecotoxicological effects of PhCs in aquatic environments have become a major concern in the scientific community. Consequently, legislators such as the European Commission have included PhCs in different surveillance programs [6,7]. In this regard, numerous works have been published on the efficiency of wastewater treatments in the elimination of PhCs, especially in conventional wastewater treatment plants (WWTPs), and in recent years an increasing number of studies have been performed on the efficiency of

CWs in removing PhCs [8–11]. As also observed in WWTPs, the main elimination mechanisms of PhCs in CWs are microbial activity and sorption [12–14]. For this reason, it has been reported that CWs are efficient in the removal of PhCs [15], and in many cases removal efficiencies are similar to those of WWTPs [16]. Nevertheless, the degradation of most PhCs is not complete, and the trends in this area are focused on augmenting removal. In this regard, the biodegradation of PhCs is improved when dissolved oxygen (DO) availability is increased [15]. Other positive effects, such as the enhancement of N and P removal, have been observed when dissolved oxygen concentration is increased. This effect could lead to the possible reduction in land area requirements of CWs [17,18]. Artificial aeration has been used as a strategy to increase dissolved oxygen in CWs with good results in terms of PhC removal [19,20]. A dissolved oxygen increase can also be achieved by generating turbulence, for example by the recirculation of effluent. Some authors have studied the effect of effluent recirculation on the removal of total nitrogen, biological oxygen demand, and suspended solids [21–23], but little information is available on the removal of PhCs. For example, Suárez et al. performed a mass balance of PhCs in a system when an internal recirculation was performed from an anaerobic fluidized bed reactor [24], while in a work by Dutta et al. the removal of PhCs was evaluated when the recirculation was performed between two of the treatment stages of a purification plant, specifically between the anoxic and anaerobic tanks [25]. Nevertheless, to the best of our knowledge, the effect of recirculation on the removal of PhCs in a macrophyte pond–CW system has not been studied. We hypothesized that effluent recirculation can be an easy-to-apply and economic method to improve PhC removal, particularly in full-scale systems in operation. Additionally, high effluent recirculation ratios can achieve better removals but at a higher economic cost. Thus, they should be determined experimentally.

Therefore, the objectives of this research were to evaluate the presence of PhCs in wastewater from a university campus, the effectiveness of a macrophyte pond–CW system in the removal of PhCs, and whether a low effluent recirculation was able to improve PhC removal and ecological risk. Monitoring was performed in two periods: (i) the year 2018 without effluent recirculation and (ii) the year 2019 with a 50% effluent recirculation ratio (recirculated flow vs. influent flow). A statistical analysis of the concentrations and removal rates was performed with the 11 PhCs found at measurable concentrations.

2. Materials and Methods

2.1. Materials and Reagents

The studied pharmaceuticals (Table S1) showed purities over 97% and were purchased from Sigma-Aldrich (Madrid, Spain). To overcome the signal changes of the matrix effect, three internal standards were used: atenolol–d7 (Toronto Research Chemical Inc., Toronto, ON, Canada), ibuprofen–d3 (Sigma–Aldrich, Madrid, Spain) and sulfamethoxazole–d4 (Dr. Ehrenstorfer GmbH, Ausgburg, Germany). Stock solutions were prepared in methanol at a concentration of 1000 $mg{\cdot}L^{-1}$ and stored at -20 °C prior to use. From them, a mixture solution was prepared at a concentration of 10 $mg{\cdot}L^{-1}$, and this mixture was used to prepare daily working solutions.

The solvents used in the chromatographic separation were water and methanol, LC–MS grade, both from Panreac (Barcelona, Spain). Acetic acid used as mobile-phase modifier was also from Panreac (Barcelona, Spain). Ultrapure water (type I) used in solid phase extraction was obtained using a water purification system from Millipore (Bedford, MA, USA).

2.2. Wastewater Treatment System Description and Sample Collection

The studied system (Figure 1) is situated at the campus of Tafira of the University of Las Palmas de Gran Canaria (Canary Islands, Spain). It receives the sewage from a part of the campus which includes cafeterias, laboratories, sport facilities and toilets from different buildings. Hazardous laboratory residues are selectively collected and are not discharged into the system. Although the analyzed wastewater is characterized by high inflow variability due to the low affluence of students and university staff during weekends, exam periods, and holidays, and the influent can be considered a particular type of urban wastewater. The campus is located 270 m above sea level. The average annual temperature and rainfall are 19.5 °C and 194 mm, respectively. The treatment system was designed to treat the effluent of 150 population equivalent (p.e.), i.e., 7.5 m^3 d^{-1}, considering 50 L $p.e.^{-1}$ d^{-1} for a campus p.e. Its approximate surface area and volume are 292 m^2 and 321 m^3, respectively. However, in this study the inflow was calculated to be 5–5.5 m^3 d^{-1} because of the lower activity in the campus. Thus, the overall theoretical hydraulic retention time would be 58 days.

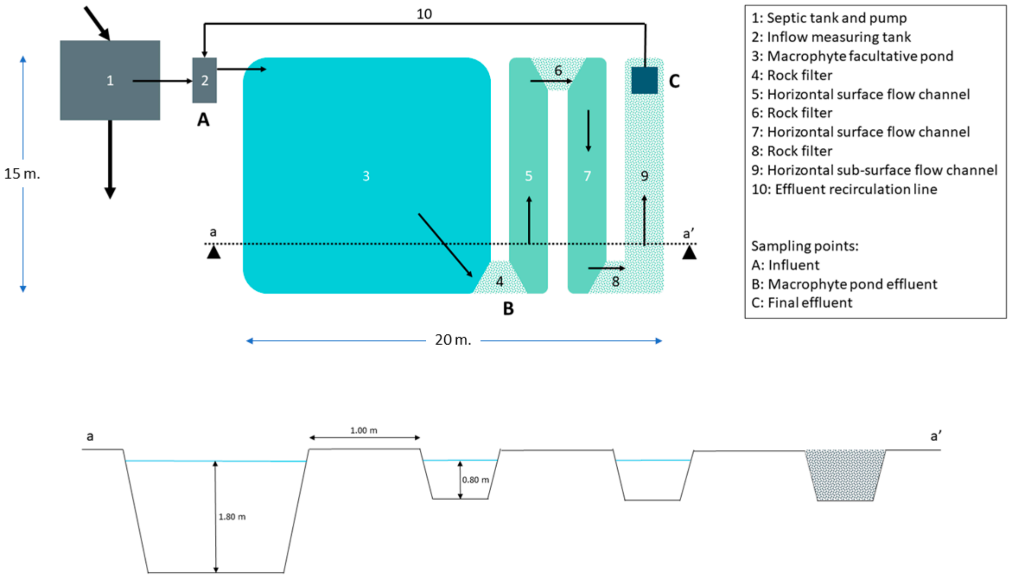

Figure 1. Scheme and vertical section (not to scale) of the studied system.

Raw wastewater from a part of the campus was conducted to a 17 m^3 septic tank. A timer-controlled pump, placed at the bottom of the septic tank, was programmed to work for 10 min every 2 h. Thus, 12 times a day from Monday to Sunday, the influent was pumped into a 0.395 m^3 tank located above the system and equipped with a draining tube placed at the bottom. The influent was thus allowed to flow down to the treatment system with reduced pressure. This inlet tank was also used as the sampling point for the influent and to assess the daily inflow. The treatment system is composed of a macrophyte facultative pond and a horizontal flow CW in series. The pond is 1.6 m deep and has a surface area of 157 m^2 and a volume of approximately 235 m^3. Water flows horizontally from the pond to the CW and, following the water flow, it comprises a stone filter, a free water channel, a second stone filter, a second free water channel, and a final subsurface flow channel. Stone filters can be regarded as short horizontal subsurface flow CWs. The mean depth of the CW is 0.8 m. Basaltic stones (Ø~10 cm) were used to construct the filters and the final subsurface flow channel. Specimens of *Phragmites*, *Cyperus*, *Pontederia*, *Canna*, and *Typha* were planted around the edges of the system. The plants, mainly common reed and *Cyperus*, have completely invaded the entire system including the pond surface, except for *Typha*, which has almost disappeared. Grab samples were taken at 8 am from the influent, pond effluent, and CW effluent during the second academic semester, a period with a high presence of people in the campus. Sampling was performed every 3 weeks from the first week of February to late June in 2018 (no effluent recirculation) and 2019 (50% effluent recirculation). Thus, the total number of samples in each year was 6. PhC samples were collected in 1-L amber glass bottles. Before storing at 5 °C, the samples were acidified at pH below 3 using hydrochloric acid to inhibit bacterial activity. Samples were not taken from the system after rainy days to avoid error by dilution.

Effluent recirculation was performed with a small, submergible, timer-controlled pump located at the outlet of the system. The pump was operated for 15 min at 1, 5, 9, 11, 13, 15, 17, and 21 h. The resulting daily recirculation flow was 2.4–2.6 $m^3 \cdot d^{-1}$, i.e., about 50% of the influent.

2.3. Analysis of Conventional Water Quality Parameters

All the parameters were analyzed according to standard methods in unfiltered samples. Chemical oxygen demand (COD) was determined using open reflux digestion with dichromate and titration and a ferrous standard. Five-day biochemical oxygen demand (BOD) was measured with the manometric method with nitrification inhibition (Velp, Italy). TSS was determined gravimetrically. Turbidity was determined with a portable nephelometer (Velp, Usmate, Italy). Ammonium-N (NH4-N) was determined with a selective electrode (Metrohm, Herisau, Switzerland). Total N (TN) was determined photometrically as nitrites after (i) alkaline peroxydisulfate digestion at 120 °C for 90 min, (ii) reduction with hydrazine-Cu-Zn solution, and (iii) the formation of a pink diazo dye with sulfanilamide and N N-(1-naphthyl)-ethylene-diamine. A Zuzi UV–Vis spectrophotometer 4201/50 (Auxilab, Navarre, Spain) was used.

2.4. Pharmaceutical Analysis Procedure

A previously optimized solid phase extraction (SPE) methodology was used to extract and preconcentrate the target pharmaceuticals [26]. Briefly, 250 mL of filtered wastewater at pH 7 was extracted using OASIS HLB cartridges (500 mg, 6 mL, Waters, Barcelona, Spain). After loading the samples, the cartridges were washed with 5 mL of Milli-Q water to eliminate the interferences retained in the cartridge, and then the pharmaceuticals were eluted with 5 mL of methanol. To enhance the preconcentration factor, the extracts were evaporated under a gentle stream of nitrogen and reconstituted with 1 mL of Milli-Q water with 100 $\mu g \cdot L^{-1}$ of internal standards. Before analysis, the extracts were filtered using Chromafil Xtra PET-20/25 syringe filters with a pore size of 0.20 µm from Machery–Nagel (Düren, Germany). After the extraction, 100 µL of the extracts was analyzed using high performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS). The system consisted of two solvent pumps which impulse the mobile phase, an autosampler capable of injecting up to 84 samples, and a Varian 320-MS triple quadrupole detector (TQD) with electrospray interface (ESI) working in both positive and negative mode, all from Varian (Madrid, Spain). All modules were controlled using System Manager software, also from Varian. To carry out the chromatographic separation, the analytical column used was a SunFire C_{18} column (100 × 3.0 mm, 3.5 µm) from Waters Chromatography (Barcelona, Spain). The mobile phase used consisted of LC–MS grade water and methanol, both with 0.5% of acetic acid. Separation of the target compounds was carried out in gradient mode at a flow rate of 0.2 $mL \cdot min^{-1}$. The detection of the target compounds was conducted using electrospray ionization (ESI) in both positive and negative mode, with source and desolvation temperatures of 60 and 250 °C, respectively. Nitrogen was used as a drying gas and nebulizer gas at 30 and 65 psi, respectively, and the capillary voltage was set at 5.0 kV in positive mode (ESI+) and −4.5 kV in negative mode (ESI-). Collision-induced dissociation (CID) was performed with argon as collision gas at a pressure of 2.00 psi. This analytical methodology reports high recovery rates, mostly over 70%, with appropriate quantification limits of between 0.033 and 628 $ng \cdot L^{-1}$.

2.5. Environmental Risk Assessment

An environmental risk assessment was performed for the three sampling points by following the recommendations of the Technical Guidance Document (TGD) on Risk Assessment of the European Commission in support of Commission Directive 93/67/EEC on risk assessment for new notified substances. In this regard, the risk quotients (RQs) were calculated by dividing the maximum environmental concentration (MEC) for each sampling point and period by the predicted non-effect concentration (PNEC). The PNEC values were obtained from the literature and modeling programs for different organisms such as *Daphnia* and algae and are specified in Table S3. The overall RQ was calculated as the sum of the individual RQs obtained for each pharmaceutical. The aforementioned TGD specifies the following RQ-based classification of environmental risk: low ecological risk: $0.01 < RQ < 0.1$; medium ecological risk: $0.1 < RQ < 1$; high ecological risk: $RQ > 1$.

2.6. Statistical Analysis

The statistical analysis of the results was performed with the open-source R-Commander program. The first step in the analysis was to identify and remove the outliers (see the box-and-whiskers representation of the data in Figure S1).

A 1-tailed ANOVA test was then performed to compare the average pharmaceutical removal efficiency of the periods with and without effluent recirculation. Since this is a parametric test, the conditions of normal distribution (Shapiro–Wilk test) and homoscedasticity, i.e., equality of variances (Bartlett test), must be met. If either of these conditions was not met, the non-parametric Kruskal–Wallis test was applied. In all cases a 95% confidence level was adopted, i.e., the means of the different data groups were considered to be different when the obtained *p*-values were lower than 0.05.

3. Results and Discussion

3.1. Influent Concentrations and Removals of Conventional Parameters

Some studies have demonstrated that in CWs, the recirculation of a fraction of the effluent improves the removal of nitrogen and reduces investment costs and surface area demand as well [21]. In addition, the degradation of emerging pollutants such as PhCs is faster in aerobic conditions [27]. Thus, the concentrations in the influent of oxygen demanding compounds (organic matter and NH_4-N) should be similar so that the surface loadings of both periods are comparable. Figure 2 illustrates the average concentrations of COD, BOD, TSS, turbidity, NH_4-N, and TN for the periods without effluent recirculation (ER) and 50% effluent recirculation.

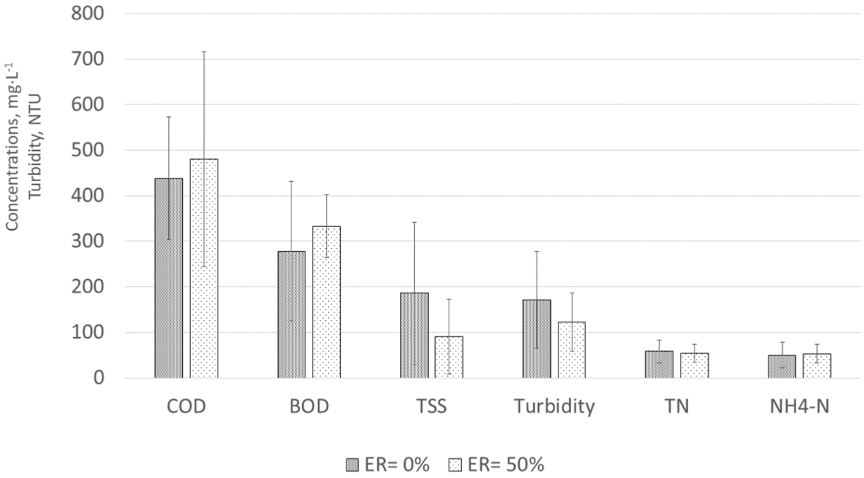

Figure 2. Average concentrations of conventional parameters for the periods of 0% effluent recirculation and 50% effluent recirculation All units in $mg\cdot L^{-1}$, except turbidity (NTU). The error bars are standard deviation values.

As can be observed, the average concentrations are quite similar within both periods for COD (438–480 $mg\cdot L^{-1}$), BOD (278–333 $mg\cdot L^{-1}$), turbidity (171–123 NTU), TN (58–54 $mg\cdot L^{-1}$), and NH_4-N (50–53 $mg\cdot L^{-1}$), except for TSS (186–91 $mg\cdot L^{-1}$) with a much lower concentration in the period with effluent recirculation. This result can be explained by the fact that the influent pump was placed a few cm above the cesspit bottom with the goal of reducing its clogging produced by wet towels. Nevertheless, there was no significant difference regarding average concentrations between both sampling periods, most probably because of the wide dispersion of the data. Figure 3 shows the average removals achieved for the conventional parameters for both periods.

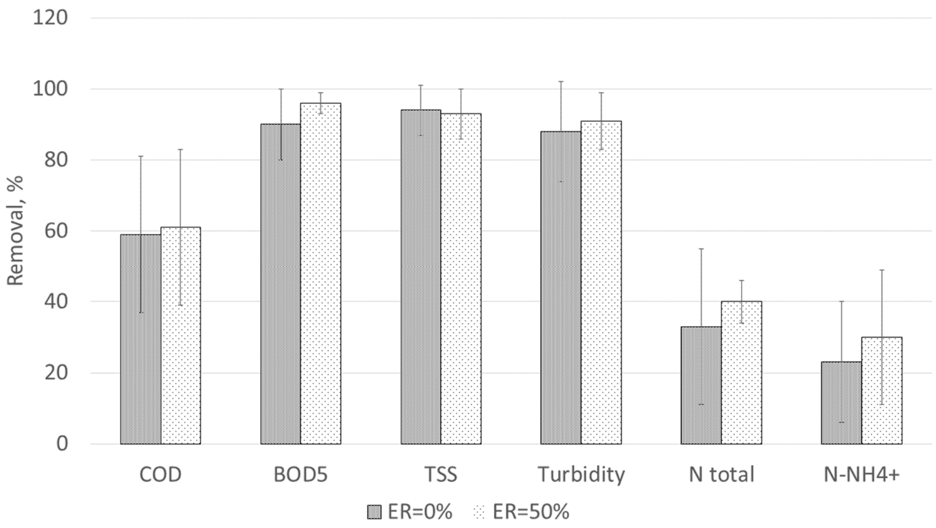

Figure 3. Average removal of conventional parameters without and with 50% effluent recirculation.

The removals of organic matter, in particular BOD, TSS, and turbidity achieved high values, above 80% in both periods. However, those of TN and NH_4-N, were modest, below 40%. Dissolved oxygen concentration was not measured in this study, but the removal of NH_4-N was taken as an indirect measurement of nitrification and dissolved oxygen availability. A general improvement of average removals was observed with recirculation, with COD passing from 59% without recirculation to 61% with recirculation, BOD from 90% to 96%, turbidity from 88% to 91%, TN from 33% to 44%, and NH4-N from 23% to 30%. In the case of TSS, the average removals were 94% (no effluent recirculation) and 93% (50% effluent recirculation). Nevertheless, there is no significant difference between average removals for any variable between both experimental periods.

The obtained results are in agreement with those obtained by other authors with hybrid, HF, and VF CWs. For example, Ilyas and Masih (2017) reviewed the performances of HF, VF, and hybrid CWs with ER and observed TSS removals of 76–95% for VF and 92–99% for hybrid CWs. COD removals were 85–88%, 67–90%, and 58–97% for HF, VF, and hybrids, respectively [18]. In a review by Wu et al. (2014), VFs and HFs that treated different types of wastewater and ER ratios between 25% and 250% obtained COD removals between 43% and 92% [28].

Thus, it can be said that in the present study ER enhanced interactions between pollutants and microorganisms, resulting in improved treatment performance [28]. Nevertheless, the fact that NH_4-N was not significantly improved suggests that the applied ER ratio in this study was not high enough to increase dissolved oxygen concentration for nitrification.

3.2. Evaluation of Pharmaceutical Occurrence and Removal without Effluent Recirculation

The 11 PhCs subject to analysis were chosen on the basis of previous studies which revealed their presence in wastewaters in Gran Canaria [26,29]. These were nicotine, caffeine, and paraxanthine (stimulants present in pharmaceuticals and other products such as coffee, energy drinks, and tobacco), naproxen, ibuprofen, and diclofenac (non-steroidal anti-inflammatory drugs (NSAIDs), trimethoprim and erythromycin (antibiotics), atenolol, gemfibrozil, and carbamazepine. As can be seen in Figure 4 and Table S2, 9 of the 11 compounds under study were present at measurable concentrations, while carbamazepine was detected under the quantification limits in a significant number of samples. The PhCs found were categorized in three groups depending on their concentration. In the first group (Figure 4), the concentrations were under 0.3 $\mu g \cdot L^{-1}$ in all cases. The pharmaceuticals that constitute this group are prescribed for specific illnesses: cardiovascular diseases in the cases of atenolol (ATE) and gemfibrozil (GEM) and urinary infections in the case of trimethoprim (TRIM). Regarding diclofenac (DIC), the low concentrations detected could be attributed to the restrictions of this PhC in Spain in recent years because of associated cardiovascular problems [30]. In the second group, the concentrations ranged from 0.5 to 30 $\mu g \cdot L^{-1}$. In this group, two stimulants, nicotine (NICO) and paraxanthine (PRX), the main metabolite of caffeine, were found as well as two widespread NSAIDs, naproxen (NPX) and ibuprofen (IBU). Caffeine (CAFF) and nicotine can be considered as lifestyle compounds. Since they are consumed worldwide in large quantities and due to their frequent presence in many pharmaceutical preparations, they have been included in the group of PhCs. The sources of nicotine in WWTP influents may include direct inputs of nicotine-containing products, such as runoff and indiscriminate disposal of items containing this compound (e.g., cigarette stubs, beverages, dietary supplements, and tobacco cultivation waste) [31].

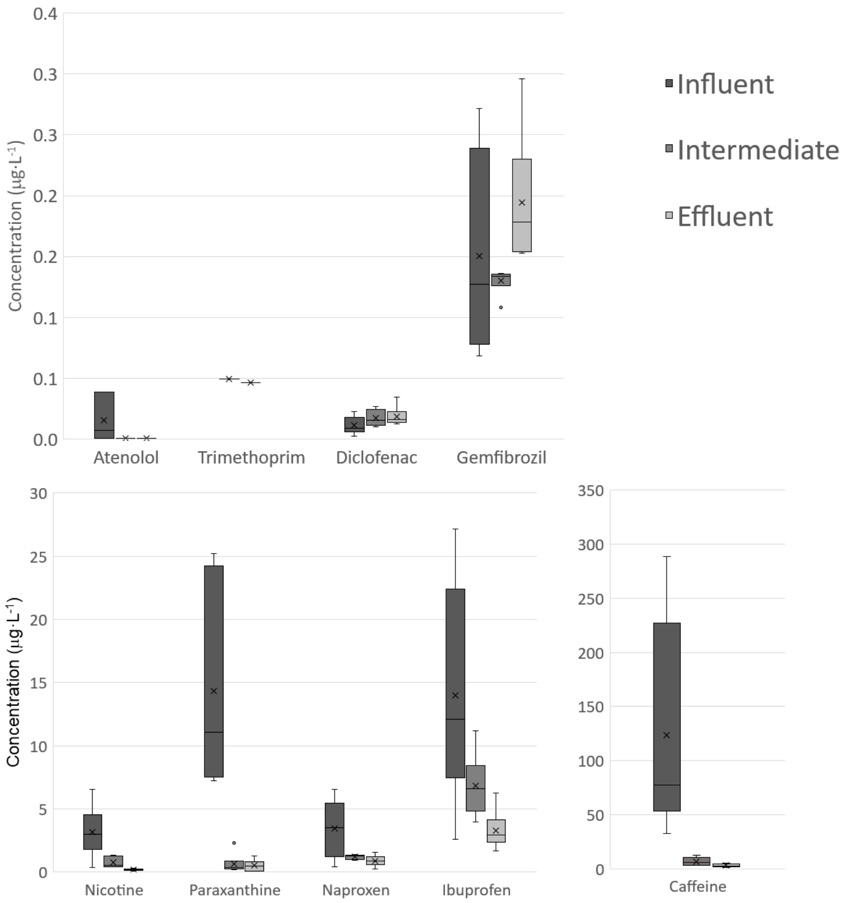

Figure 4. Boxplot of concentrations of analyzed pharmaceuticals at the three sampling points.

As can be seen in Figure 4, the concentrations in the influent samples were higher than those of the other sampling points. Also notable is the wide range in the concentrations of paraxanthine and ibuprofen, which decreases as the treatment process advances. Finally, the concentrations of caffeine were found to be higher than those of the other compounds, especially in the influent samples where concentrations ranged from 30 to 300 $\mu g \cdot L^{-1}$. This result can be explained by considering the high consumption of this compound, which in adults from Europe ranges between 36.5 and 319.4 mg per day [32]. For this reason, caffeine has been confirmed as a good biomarker of anthropogenic pollution [33].

Although the removal rates of the two parts of the treatment system cannot be compared, since the macrophyte pond and the CW are connected in series and do not treat the same influent, it is interesting to determine the contribution of each part of the system. Figure 5 shows the obtained results for six of the detected pharmaceuticals. As can be observed, the pond achieved significant removal of the three stimulants under study, with average values over 60%. For these compounds, the relative elimination rates were different in the CW. In this regard, the average elimination of nicotine in the CW was 72%, but the average eliminations of caffeine and its metabolite, paraxanthine, were lower. The average elimination of caffeine was 39%, while for paraxanthine the value was −80%, which means that in this stage of the treatment process, the concentrations of this compound increased. This could be attributable to the low concentrations of caffeine and paraxanthine found after the pond treatment, resulting in a major dispersion of the calculated relative recoveries. Considering that stimulants are highly hydrophilic (log K_{OW} between −0.22 and 1.17), biodegradation is proposed as the major removal mechanism, as reported for other PhCs [34]. High removals of stimulants such as caffeine and nicotine have also been observed in other CWs located in Gran Canaria and abroad [11,26,29]. Regarding the compounds with the lowest concentrations, notable removals were achieved for atenolol, which was detected only in the influent samples but at very low concentrations (below 0.039 $\mu g \cdot L^{-1}$). Xu et al. found a correlation between atenolol biodegradation and ammonium nitrification [35]. However, in the present study the removal of ammonium has been low (average 23% without ER).

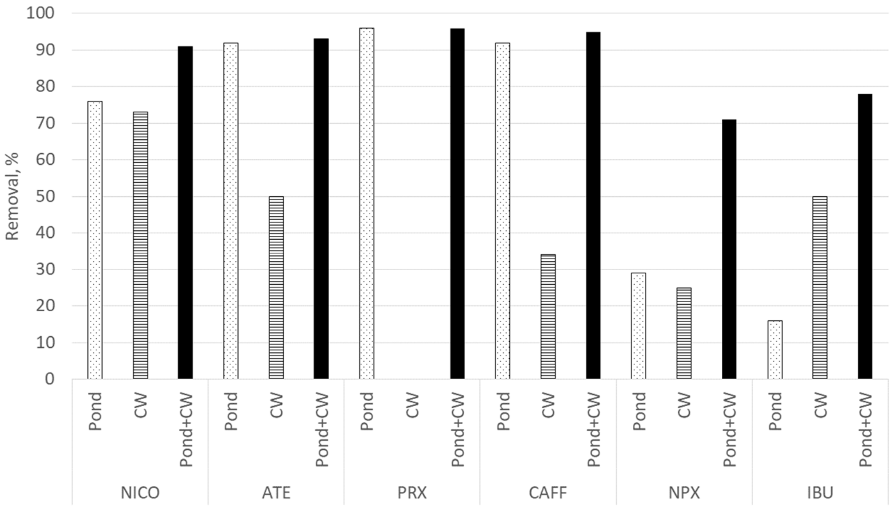

Figure 5. Removal (%) of PhCs in the pond, CW, and the complete system (Pond + CW) without recirculation.

For the rest of the compounds (gemfibrozil, diclofenac, trimethoprim, and carbamazepine), removals could not be calculated due to the low number of positive results. Nevertheless, carbamazepine, which was detected in most of the influent and effluent samples but at concentrations under its quantification limit (0.7 $ng \cdot L^{-1}$), has been reported in various studies to show recalcitrant behavior [36,37], which may be due to the poor biodegradability and hydrolysis of carbamazepine from a glucuronide conjugate [38].

The complete system provided removals of between 48% and 95% for the six most detected compounds (nicotine, caffeine, paraxanthine, atenolol, naproxen, and ibuprofen). The highest average removals were for the three stimulants and were over 91%, while the elimination efficiencies for the NSAIDs were between 48% and 64%. For erythromycin, diclofenac, and gemfibrozil, the overall removals were not calculated because these compounds were not detected at the influent point.

3.3. *Effect of the Effluent Recirculation on Pharmaceutical Removal*

As indicated above, one of the objectives of this study was to evaluate the effect of effluent recirculation on PhC removal. Thus, in 2019 a 50% effluent recirculation was implemented aimed at increasing the hydraulic retention time and the concentration of dissolved oxygen in the system to favor the aerobic biodegradation of contaminants. Several authors have reported that effluent recirculation improved the removal of TN [21,39] and metals such as Na, Ca, and Fe [40] in CWs. Nevertheless, studies on recirculation strategies to increase the removal efficiency of emerging pollutants are scarce. For this reason, in the second sampling period, an effluent recirculation of about 2.4 $m^3 \cdot d^{-1}$ (~50% of the influent) was implemented.

As can be seen in Figure 6, the concentrations of PhCs in the influent were similar in both sampling periods, except for a significant increase (p-value < 0.05) in the concentrations of trimethoprim, diclofenac, and paraxanthine. Thus, considering that the influent concentrations of conventional parameters and those of most PhCs remained similar, PhC removals for both periods can, in general, be compared with confidence. This result can be explained by the similar PhC production patterns of both periods and the fact that recirculation did not affect the influent composition, i.e., it was not diluted with the recirculated effluent.

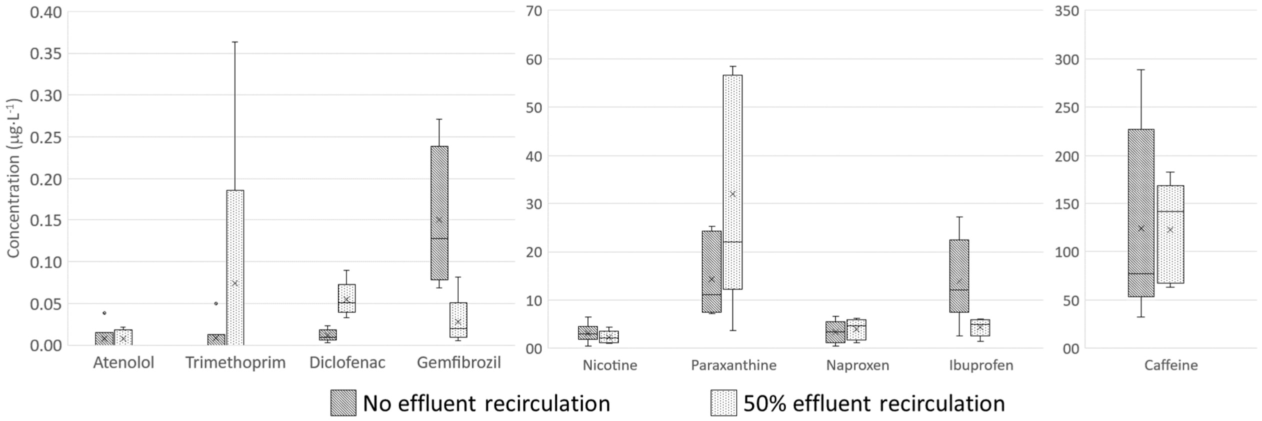

Figure 6. Concentrations of detected compounds in raw influent samples for the periods of 0% recirculation (2018) and 50% recirculation (2019).

To evaluate the effect of effluent recirculation on the removal efficiencies of target pharmaceuticals in the pond–CW system, the effluent of the system was recirculated to the pond. Figure 6 illustrates the average removals achieved in the macrophyte pond, the CW, and the complete system when this strategy was applied. As can be observed, effluent recirculation had similar effects on the performance of the pond and the CW. In the case of the pond, the effect was slight on the removal of stimulants (changes between −5% and 15%) and produced up to a 4-fold decrease in the average removal of atenolol and ibuprofen. Only naproxen removal was moderately improved, with a removal enhancement of 145%. A similar trend can be observed for the CW with recirculation. All the compounds presented removal enhancements of between 35% and 216%, except for nicotine and ibuprofen which both had decreases in the average removal rate of around 20%. Improved removal rates were especially evident for paraxanthine and caffeine, which presented moderate or even negative removal rates in the period without effluent recirculation (2018) due to the lower concentrations detected in this part of the system. When effluent recirculation was applied, the removal of these two stimulants in the CW increased to over 90%, a slightly higher value than that reported by Avila et al. for caffeine in a CW without recirculation [21]. After evaluating the whole system, it can be concluded that the implementation of effluent recirculation benefited the removal of most PhCs, especially the stimulants. Nicotine, caffeine, and paraxanthine were more degraded when effluent recirculation was applied, and in the case of caffeine complete degradation was achieved in most samples. These results are similar to those of other studies that have reported better biodegradation of stimulants such as caffeine in aerobic conditions [15], though the macrophyte pond–CW system of the present study was predominantly anaerobic as suggested by the low ammonium removal obtained (Figure 3). For the NSAIDs, different trends were observed. As can be seen in Figure 7, effluent recirculation enhanced the removal of naproxen, achieving average removal rates of over 80%, but the overall removal of ibuprofen decreased significantly.

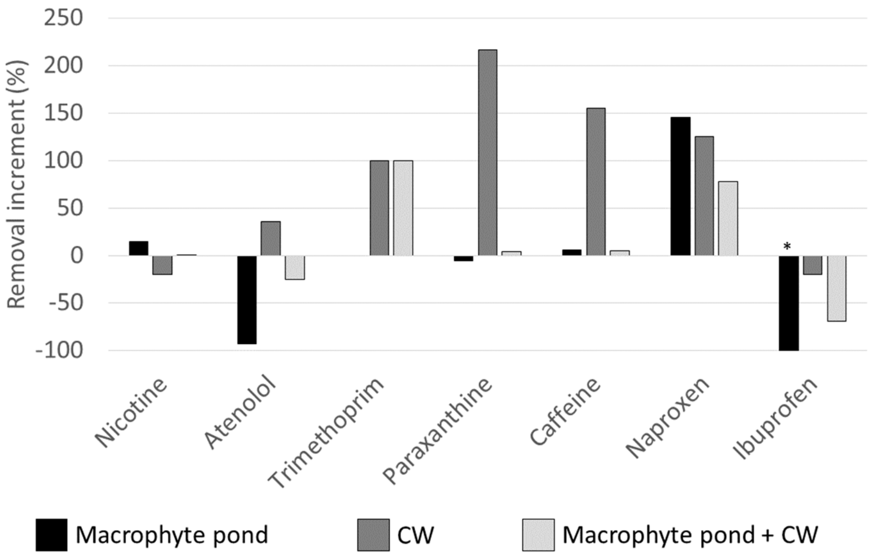

Figure 7. Increment (%) in average removals with effluent recirculation. * Removal increment: −420%.

In contrast, for gemfibrozil and diclofenac, the detection rate increased after effluent recirculation as they were detected in more samples. For these two compounds, effluent recirculation had a slight effect on CW performance. Nevertheless, in the overall treatment, these two compounds showed higher concentrations at the final stages of the treatment process, demonstrating their recalcitrant behavior in CWs [41,42].

Finally, to have an overall view of the effect of recirculation on PhC removal, all the removal values obtained for both periods (Table S2) were tested for significant differences. The boxplots of removals for both periods (Figure S1) indicated that those below 70% were outliers, and they were removed in this analysis. Consequently, removal efficiency was evaluated only for those PhCs with higher eliminations (caffeine, paraxanthine, nicotine, and naproxen). A comparative analysis of the data revealed that the average removal rate for these compounds was significantly higher when using effluent recirculation (p-value = 0.032, Kruskal–Wallis). Nevertheless, the difference between these average removal rates was not high: 90.9 ± 9.1% without recirculation and 94.4 ± 8.95% with recirculation, indicating a moderate removal improvement with recirculation.

The application of effluent recirculation improved removals of organic matter and N in VF, HF, or hybrid CWs [28,39,43]. It is believed that the hydraulic residence time and/or dissolved oxygen concentration are increased, favoring removal [18]. Moreover, recirculation enhances interactions between pollutants and microorganisms, resulting in improved treatment performance, particularly for the effective removal of TN [28]. Additionally, in the case of toxic influent such as landfill leachate, ER has a dilution effect that reduces the toxicity to microbial activity and plants, and regulates the pollutant composition in the system, optimizing pollutant removal [44]. However, the recirculation ratio (effluent recirculated flow rate/influent flow rate) is a key parameter. Recirculation ratio (Rr) usually falls in the range 50–250%. Nevertheless, Lin et al. (2020) tested the effect of Rr as high as 14.3 and 3.0 in HSSF CWs [45]. In this study, a low Rr was tested because the treatment system is full scale, and the real application of a high Rr would not be practical considering the high flow to be pumped and the energy expended. Although the results obtained with recirculation are interesting, it is clear that the Rr applied should be increased to achieve a significantly improved effect on the removal of conventional and pharmaceutical pollutants.

3.4. *Environmental Risk Associated with Pharmaceutical Concentrations*

An environmental risk assessment was evaluated of all sampling points for the two sampling campaigns. It can be seen in Table 1 that the environmental risks in influent samples were up to 75 times greater than the high-risk threshold for daphnids and over

20 times greater for algae. Higher ecological risks for daphnids than for algae were also observed at the other sampling points. This is mainly due to the presence of nicotine in all the samples, which is acutely toxic for daphnids at concentrations of μg·L^{-1} [46]. Nevertheless, the system provided a great reduction in risk quotient (RQ), even without effluent recirculation. For daphnids, the ecological risk was 12.4 times lower in effluent samples in comparison with influent samples, while the reduction in risk considering algae was 5.5 times. It was also observed that both with and without effluent recirculation, the system achieved a reduction in ecological risk, especially in the pond. In the pond effluent, the ecological risk was 4.5 to 8 times lower than at the influent for both daphnia and algae. However, the effect of effluent recirculation was less appreciable in the CW. In this case, the treatment effluent presented ecological risks up to 4 times lower than at the inlet.

Table 1. Risk quotients (RQs) of the target pharmaceuticals for daphnids and algae in the different studied points with and without effluent recirculation. Red cells: high ecological risk, yellow cells: medium ecological risk, green cells: low ecological risk.

DAPHNIDS						
	No Effluent Recirculation			**50% Effluent Recirculation**		
	Influent	**Pond Effluent**	**CW Effluent**	**Influent**	**Pond Effluent**	**CW Effluent**
Nicotine	65.43	13.46	3.13	43.51	4.96	3.17
Atenolol	0.00	0.00	0.00	0.00	0.00	0.00
Trimethoprim	0.00	0.00	0.00	0.00	0.00	0.00
Paraxanthine	0.14	0.01	0.01	0.33	0.02	0.00
Caffeine	6.28	0.27	0.11	3.98	0.05	0.00
Erythromycin	0.00	0.01	0.00	0.00	0.00	0.00
Carbamazepine	0.00	0.00	0.00	0.00	0.00	0.00
Naproxen	0.44	0.09	0.10	0.41	0.10	0.06
Ibuprofen	3.01	1.24	0.69	0.68	0.55	0.69
Diclofenac	0.00	0.00	0.00	0.00	0.00	0.01
Gemfibrozil	0.03	0.01	0.03	0.01	0.02	0.01
TOTAL	75.32	15.09	4.08	48.92	5.70	3.94

ALGAE						
	No Effluent Recirculation			**50% Effluent Recirculation**		
	Influent	**Pond Effluent**	**CW Effluent**	**Influent**	**Pond Effluent**	**CW Effluent**
Nicotine	6.54	1.35	0.31	4.35	0.50	0.32
Atenolol	0.00	0.00	0.00	0.00	0.00	0.00
Trimethoprim	0.00	0.00	0.00	0.02	0.00	0.00
Paraxanthine	0.25	0.02	0.01	0.58	0.04	0.00
Caffeine	6.28	0.27	0.11	3.98	0.05	0.00
Erythromycin	0.00	0.00	0.00	0.00	0.00	0.00
Carbamazepine	0.00	0.00	0.00	0.00	0.00	0.00
Naproxen	0.30	0.06	0.07	0.28	0.07	0.04
Ibuprofen	6.79	2.80	1.56	1.53	1.23	1.57
Diclofenac	0.00	0.00	0.00	0.01	0.00	0.01
Gemfibrozil	0.07	0.03	0.07	0.02	0.04	0.02
TOTAL	20.23	4.54	2.14	10.77	1.93	1.95

Considering the contribution of each compound to the overall RQ, Table 1 reveals that nicotine provided the highest RQ, followed by ibuprofen. Furthermore, the high RQ values for daphnids are caused mainly by nicotine, while for algae, in addition to nicotine, other compounds such as caffeine or ibuprofen also provided a significant contribution. Regarding the final effluent of the system, the overall RQ is close to the threshold values of high ecological risk, especially for algae and mainly produced by the presence of nicotine. Moreover, effluent recirculation had a limited effect in terms of decreasing the ecological risk for daphnids, although a slight reduction was observed for algae due to the total elimination of caffeine achieved in the recirculation period. As also observed recently by other authors [47], the ecological risk associated with ibuprofen was also significant in the present study.

4. Conclusions

This research evaluated the presence of PhCs in wastewater from a university campus, their removal in a macrophyte pond–CW system, and the effect of low effluent recirculation on removal and ecological risk. The highest concentrations of PhCs belong to stimulants such as caffeine and nicotine and other pharmaceuticals which are not restricted to medical prescription, such as ibuprofen and naproxen. The pond–CW system achieved remarkable removals, especially for stimulants and non-steroidal anti-inflammatories. The sum of the concentrations of these compounds showed a total value in the influent of up to 300 $\mu g \cdot L^{-1}$ and overall removal rate efficiencies of over 70%. The effect of 50% effluent recirculation was satisfactory for stimulants but nil for recalcitrant compounds such as carbamazepine or diclofenac. A remarkable reduction in the influent risk was observed (up to 12.4 times), but the effluent still presented a non-negligible value ($RQ > 1$), mainly caused by nicotine and ibuprofen. This study proves that effluent recirculation can improve the performance of a full-scale pond–CW system without major economic and constructive issues, but higher than 50% recirculation ratios should be applied.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w14152340/s1, Table S1: Therapeutic classification and molecular structure of target pharmaceuticals; Table S2: Average concentrations ($\mu g\ L^{-1}$) ± std. dev. and (number of positive readings) in 2018 (no recirculation) and 2019 (50% recirculation); Figure S1: Box plots of obtained removals during the whole study; Table S3: Values of PNEC of the target pharmaceuticals for *Daphnids* and algae (References [48–51] are cited in the supplementary materials.

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