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Pharmaceuticals Removal from Wastewater with Microalgae: A Pilot Study

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Abstract: Urban wastewaters contain pharmaceuticals that are not appropriately removed in conventional wastewater treatments, limiting treated water reuse. Microalgae have been shown to remove pharmaceuticals from urban wastewater in laboratory trials, but few studies have been conducted under natural conditions. In this work, pharmaceutical removal was assessed in a pilot-scale microalgal tertiary wastewater treatment in real conditions. Even after secondary treatment, the water contained measurable amounts of pharmaceuticals (an average of 218.4 ng/L) that significantly decreased to 39.83 ng/L at the exit of the microalgal system. Pharmaceuticals' average removal rates were slightly higher in the summer (79.1%) than in autumn (71.1%). Antibiotics and antipsychotics were better removed (88.8 and 86.4%, respectively) than antihypertensives (75.3%) and others (Bezafibrate and Diclofenac; 64.0%). Physicochemical characteristics of the wastewater influenced pharmaceutical removal; significant positive correlations were observed between anti-hypertensive drug removal and ammonium concentration ($r = 0.53$; $p < 0.05$), total nitrogen and total pharmaceutical removal ($r = 0.46$; $p < 0.05$), and total nitrogen and antipsychotic drug removal ($r = 0.47$; $p < 0.05$). The results demonstrate the effectiveness of microalgal tertiary treatment in the removal of pharmaceuticals.

Keywords: microalgae; wastewater treatment plant; pharmaceuticals; environment contaminants

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1. Introduction

Wastewater contains a complex mixture of contaminants such as organic matter, nitrogen and phosphorus nutrients, pathogens, and emerging contaminants. Emerging contaminants are a wide range of unregulated chemicals of synthetic origin or derived from natural sources that pose a great threat to humans and the ecosystem [1]. Pharmaceutical active compounds are one of the major groups of emerging contaminants that do not degrade easily and persist for long periods in the environment due to their stable structure. The occurrence of pharmaceuticals in wastewater results from their intake by humans or animals and excretion through urine and feces, since some of them are only partially metabolized and absorbed [2]. Several mechanisms and techniques have been studied

for pharmaceutical removal from wastewater, being the biological treatment the most studied and used [3–5]. However, as wastewater treatment plants (WWTPs) were not designed to eliminate persistent pharmaceutical compounds, they are not able to completely remove the pharmaceuticals and their metabolites [2,6]. Therefore, these compounds are disseminated into aquatic compartments via treated effluent discharges and land application of biosolids [7]. Their potential for negative ecotoxicological effects, even at sublethal concentrations, in the aquatic environment has been of concern [8,9]. Risk has been observed in all aquatic compartments, evidencing the importance of mitigation measurements [7]. Moreover, its presence in treated water can also hinder its reuse [10].

Considering that around 50% of the population in the Mediterranean experiences water stress in the summer, wastewater reuse would help the EU to achieve Sustainable Development Goal 6, related to the availability and sustainable management of water [11]. In this line of thought, wastewater treatment using microalgae can strongly contribute to the sustainable reuse of wastewater. Microalgae are unicellular or colonial photoautotrophic microorganisms that grow using solar energy, CO_2, and nitrogen and phosphorus, which are nutrients abundantly found in wastewater. They are also able to remove most of the organic (e.g., pharmaceuticals and personal care products, pesticides, and aromatic hydrocarbons) and inorganic pollutants (e.g., nitrogen, phosphorus, and metals) from these waters. Often growing in mixotrophic systems, together with bacteria, microalgae have been successfully used for the bioremediation of nutrients, such as nitrogen, phosphorus, and carbon from municipal wastewater [12]. In microalgae-based treatment systems, coupling nutrient removal with emerging contaminant bioremediation further improves the cost-efficiency of wastewater treatment [12,13]. This produces cleaner treated water than most conventional wastewater treatment systems, warranting water reuse [14]. Being photosynthetic, microalgal systems rely on solar energy and consume much less energy than bacterial-based systems, which, together with the fact that they consume rather than emit CO_2, makes their life-cycle assessment more favorable than the bacterial-based ones [15]. Additionally, microalgal biomass is produced, which can be valorized to produce biofuels reducing the costs of the wastewater treatment process and helping to mitigate fuel shortage in Europe [14,16].

Maryjoseph and Ketheesan reviewed the removal efficiencies of pharmaceuticals at the laboratory scale by microalgae [17]. They concluded that the operation of batch reactors at long hydraulic retention time (HRT) is still considered uncertain, and a challenge exists for validating the removal efficiencies of emerging contaminants obtained at laboratory conditions into larger systems using real wastewater. Therefore, this work aimed to assess, for the first time, the removal of pharmaceuticals during the tertiary treatment of urban wastewater using microalgae. The experiments were conducted in a pilot installation at a WWTP under realistic environmental conditions. Different conditions known to affect microalgal productivity and that could thus affect pharmaceutical removal by these microorganisms were tested. These included nutrient concentrations (e.g., nitrogen and phosphorus) and environment temperature and light intensity by performing the experiments in summer and autumn conditions.

2. Materials and Methods

2.1. Experimental Conditions, Sampling Site, and Collection

The experiments were conducted outdoors in the WWTP of Quinta do Lago, Algarve, in the South of Portugal, between late July and October. Microalgae cultivation was conducted in experimental pilot GreenDune photobioreactors (GD PBRs) developed by Bluemater, S.A., a Portuguese company, which were designed for wastewater treatment. The experimental setup of the photobioreactors and their efficiency in the tertiary treatment of wastewater has been described elsewhere [15]. Briefly, the GD PBRs are open modular systems, and, in this case, they were connected in lines of 3 modules, giving a total treatment volume of 1440 L. The system was fed with partially treated water (after biological secondary treatment of activated sludge with phosphorus precipitation) coming from the

secondary settlers of the WWTP. No inoculation was performed; instead, a natural forming consortium of microalgae was allowed to develop in the PBRs for one week prior to the beginning of the experiments. Two experiments of five weeks were conducted, the first in the summer (2 August 2021–31 August 2021) and the second in the autumn (27 September 2021–26 October 2021). The PBRs were operated in continuous mode with an HRT of 24 h. During that time, the concentration of nitrogen and phosphorus nutrients and organic matter was determined in the inflowing water in composed samples of 24 h, collected every week. Biomass concentration was also measured. Pharmaceuticals' concentration was measured both in the influent and effluent of the microalgae system to assess removal.

2.2. Analysis of Nutrients, Chemical Oxygen Demand, and Biomass Concentration

The influent was analyzed for ammonium, nitrates, total nitrogen, total phosphorus, and chemical oxygen demand (COD), in duplicate, using commercial NANOCOLOR kits (e.g., Ammonium 3, Nitrate 250, Total Nitrogen TNb 22, Ortho and Total Phosphate 15, and COD LR 150 from Macherey Nagel, Düren, Nordrhein-Westfalen, Germany), following the manufacturer's instructions. The absorbance of the colored solutions was measured with a PF-12 Plus photometer (Macherey Nagel, Düren, Germany). Total nitrogen, total phosphorus, and chemical oxygen demand (COD) were measured after the digestion of the samples. Biomass concentration inside the GD PBRs was determined by converting the oxygen demand (OD) measured at 750 nm (Biotek Synergy 4, Agilent, Santa Clara, CA, USA) to biomass concentration using a calibration curve.

2.3. Analysis of Pharmaceuticals

2.3.1. Standards and Chemicals

To obtain the final extracts for analysis, all reagents and solvents used were of analytical grade, but LC-MS purity grade solvents were purchased for mobile phase preparation. Ultrapure water was supplied daily by the Millipore water purification system (Milli-Q, Merck KGaA, Darmstadt, Germany) to obtain the quality required to be used in the mobile phase preparation. Formic acid, methanol, and acetonitrile were purchased from Merck (Darmstadt, Germany), while the pharmaceutical standards listed in Table S1 (Supplementary Materials) were purchased from Sigma-Aldrich (Madrid, Spain), including the internal standard sulfameter. Pharmaceutical stock solutions were prepared by diluting the exact amount of standard to obtain a concentration of 10 mg/mL in methanol or water for beta-lactams in order to improve the stability of these compounds. The necessary dilutions and working solutions were then prepared in methanol. All standard solutions were stored at −20 °C.

2.3.2. Experimental Procedure

The analytical procedure for the identification and quantification of 63 pharmaceuticals of 8 different therapeutic classes already validated (analgesic, antibiotics, anticonvulsants, antidepressants, antihypertensives, beta-blockers, lipid regulators, and anti-inflamatories) was based on previous studies [7,18].

Samples were frozen at −20 °C after being collected and stored this way until the day of analysis. Then, water samples were defrosted and acidified with formic acid until pH 2 and two consecutive filtration steps were performed with 0.45 and 0.22 µm pore size filters. For the extraction procedure, 500 mL of water was measured and added to 500 µL of internal standard solution comprising 10 mg/mL of sulfameter. The next step was a purification with solid phase extraction (SPE) cartridges, achieved with Oasis HLB (200 mg, 6 mL). After the water sample was loaded in the cartridges, a washing step followed with 5 mL of methanol:water (10:90), and then drying at low vacuum pressure. The elution was performed with methanol, 6 mL, and this extract was evaporated under N_2 flow at 40 °C, and then redissolved with 500 µL of 0.1% of formic acid (mobile phase A) and filtered with a PVDF Mini-uniprep TM filter (0.45 µm). The detection and quantification were performed by injecting 10 µL of this final extract into the UHPLC-ToF-MS system. This

equipment comprises UHPLC Nexera X2 Shimadzu coupled with a high-Resolution mass spectrometer, a Time-of-Flight Triple TOFTM 5600+ from Sciex. The UHPLC equipment is composed of a vacuum degasser, an autosampler with a programmed temperature (10 °C), a binary pump, and a compartment for the chromatographic column at a controlled temperature (40 °C). The column used in this method was a reverse-phase one, Acquity UPLC HSS T3 1.8 µm, 2.1 × 100 mm (Waters). The mobile phases used were A—formic acid 0.1% (*v*/*v*) in water and B—acetonitrile, at a flow rate of 500 µL/min and following the gradient as described: the first 5 min from 97% to 40% [A], from 5 to 9 min from 40% to 0% [A], until 10 min from 0% back to 97% [A], and maintained this way till the end of the chromatographic run of 12 min total.

For the detection, the mass spectrometer ToF-MS operated with ionization in positive mode and an electrospray ion source (ESI+) working in full-scan data acquisition mode from 100 to 920 Da. For acquisition, the software used was the Analyst® TF software and for data analysis, three software were used: PeakViewTM, LibraryViewTM, and MultiQuantTM all from SCIEX (Framingham, MA, USA). In terms of criteria of identification, the parameters evaluated were the exact mass, with a maximum acceptable error of 5 ppm, relative retention time deviation below 1% (Commission Regulation (EU) 2021/808), and isotope pattern similarity with a maximum of 10% difference (between the obtained and the theoretical).

The developed method was previously validated, and the obtained limits of detection ranged between 0.01 and 3.47 ng/L (Table S1, Supplementary Materials).

2.4. Statistical Analysis

Complete statistical analysis was performed using GraphPad Prism (8.4.3 GraphPad Software, Inc., San Diego, CA, USA). To test whether the dataset was of Gaussian distribution, D'Agostino–Pearson normality test was used. Since most of the data set was not normally distributed and had non-homogeneous variances, nonparametric tests were applied. Kruskal–Wallis tests with Dunns post-tests were used to assess statistical differences between three or more groups of data. For the comparison between two groups of data, the Mann–Whitney test was used. Pharmaceuticals with 0% of detection frequency were excluded from this evaluation. The correlation matrix was performed using Spearman r. The statistical significance level was set to $p < 0.05$.

Removal rates were calculated in two different ways:

- Average removal was determined by the average of the removal rates for each occurrence of each pharmaceutical;
- The total average was the percentage of the load at the exit compared with the load at the entrance, and the load was the sum of all occurrences of each pharmaceutical at the entrance or the exit of the algae system.

Analysis of nutrients in the inflow water was done in duplicate, yielding very similar results; therefore, only the average of the obtained results are presented.

3. Results and Discussion

3.1. Occurrence and Removal of Pharmaceuticals

Of the 63 pharmaceuticals tested, 19 were detected. All the samples presented at least 7 and up to 19 pharmaceuticals, with an average of 14 at the entrance and 10 at the exit of the microalgae system. As expected, average concentrations at the entrance (218.4 ng/L) were significantly ($p < 0.0001$) higher than the values at the exit (39.83 ng/L) (Figure 1 and Table S2, Supplementary Materials). Moreover, the maximum values were also much different at the entrance and exit, with values of up to 2950 and 1369 ng/L (venlafaxine), respectively.

As for the therapeutic groups at the entrance, the antipsychotics presented a higher average of 381.0 ng/L and maximum concentration. They were followed by antihypertensives (average of 225.8 ng/L), others (average of 215.5 ng/L), and antibiotics (average of 47.9 ng/L) (Figure 1a). At the exit of the WWTP, the order shifted, and the therapeutic

group with higher concentrations comprised antihypertensives (average of 60.7 ng/L), followed by antipsychotics (average of 54.4 ng/L), others (average of 46.8 ng/L), and antibiotics (average of 5.5 ng/L) (Figure 1b).

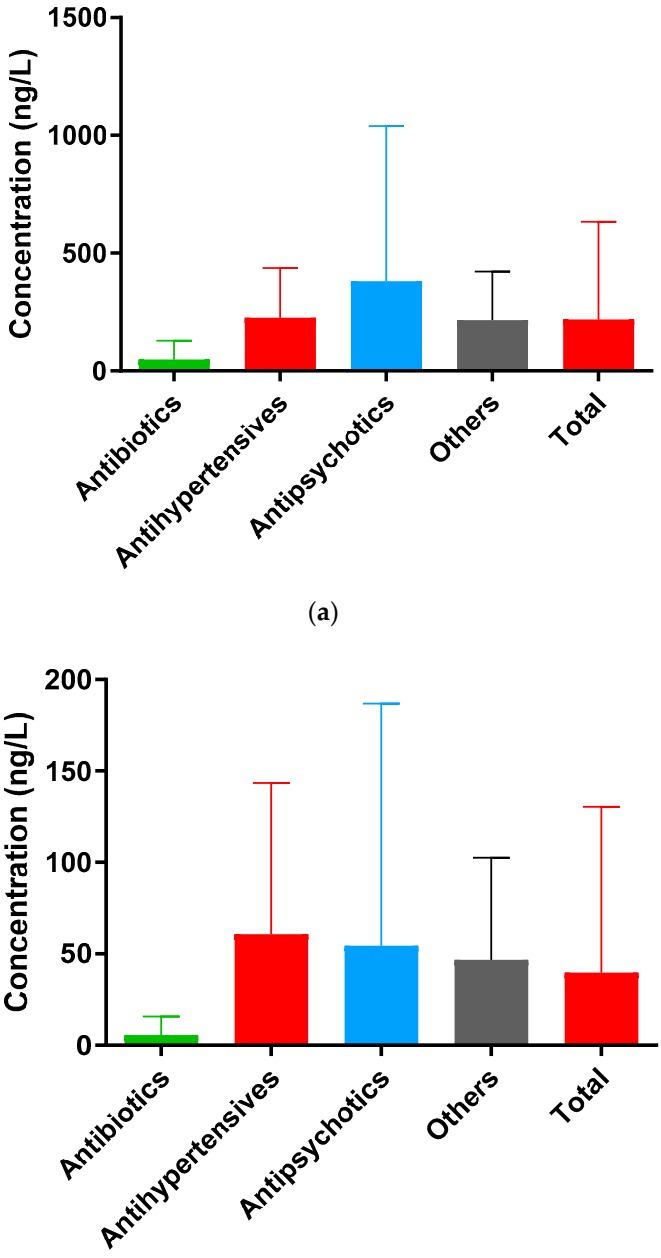

Figure 1. Therapeutic groups' concentrations (mean and standard deviation) at the entry (**a**) and exit (**b**) of the microalgae system.

Although this was the therapeutic group's pattern, within each group there are major variations since there are several factors that impact the concentrations of each pharmaceutical. These disparities are due to different therapeutic dosages, consumptions, metabolization, degradation, and removal rates. For example, in the antipsychotics therapeutic group at the entrance, venlafaxine, gabapentin, carbamazepine, and citalopram stand out from the others, with higher average concentrations (867.7, 525.6, 466.8, and 394.2 ng/L). At the exit, venlafaxine, gabapentin, and carbamazepine still stand out from the others, with averages above 68 ng/L (Table S2, Supplementary Materials). As for antihypertensives at the en-

trance, irbesartan and losartan presented higher values (averages of 430.8 and 368.5 ng/L) than the rest, which presented averages below 176 ng/L. On the other hand, at the exit, losartan and atenolol presented higher average concentrations (145.5 and 75.5 ng/L). In the antibiotics therapeutic group, trimethoprim and sulfapyridine presented the highest averages at the entrance (109.9 and 90.4 ng/L), compared with the other pharmaceuticals with values under 28 ng/L. However, at the exit, all of them presented averages below 12 ng/L. These values were lower than those of similar studies, namely due to COVID, which imposed traveling restrictions, thereby reducing the population in Algarve [9]. Moreover, since this is a WWTP receiving wastewater (WW) from a resort, the data obtained is more prone to this effect than other WWTPs. Regarding experiences 1 (Exp1) and 2 (Exp2), one performed in summer and the other in autumn, there were also higher average values at the entrance of the WWTP for Exp1 and Exp2 (304.0 and 116.5 ng/L, respectively) than at the exit of the WWTP in both experiences (39.75 and 39.92 ng/L, respectively) (Figure 2). There were also significant differences ($p < 0.0001$) between the entrance and the final effluent; however, no differences were found between entrances 1 and 2 and between exits 1 and 2.

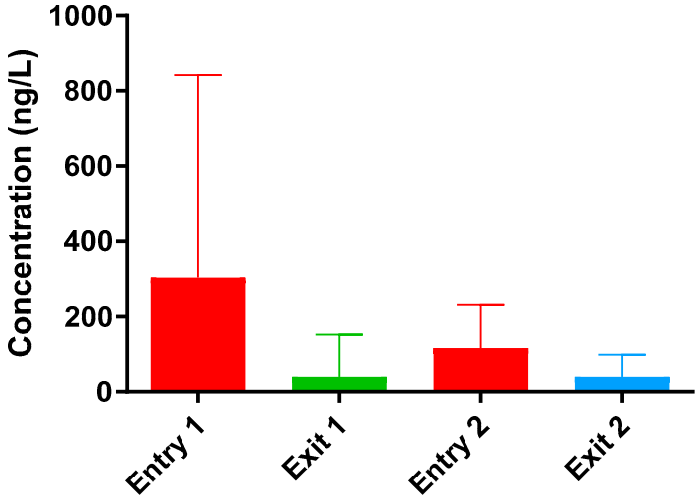

Figure 2. Mean and standard deviation of pharmaceutical concentrations in both experiments (1 and 2) at the entry and exit of the microalgae system.

As for the discrepancy at the entrance between both experiences, they are related to the sampling dates, since Exp1 was performed in July and August and Exp2 in September and October. Since August is the month with most people in Algarve, entry 1 values were impacted by this fact [14].

Since the data on pharmaceutical concentrations could bias the interpretation, removal was also calculated to compare the different experiments and therapeutic groups (Figures 3 and 4). The two experiments presented similar average removal values of 79.1% (Exp1) and 71.1% (Exp2), with no significant differences (Figure 3). When calculating the total removal, a global value of 82.5% was achieved; additionally, Exp1 presented 87.9% and Exp2 65.7% for the total removal rates. This difference occurs due to the higher initial load in Exp1 and the higher removal in the pharmaceuticals with higher concentrations. Curiously, the exit load is similar in both Exp1 and Exp2 (4154 and 3792 ng/L, respectively). Therefore, we can observe that the microalgae system has good general removal rates but appears to have a limit in the residual pharmaceuticals left in the water. Additionally, Exp1 was performed in summer with higher temperatures and sunlight, which can increase not only algal growth and therefore removal by sorption but also algae metabolic activity, which could affect removal by absorption.

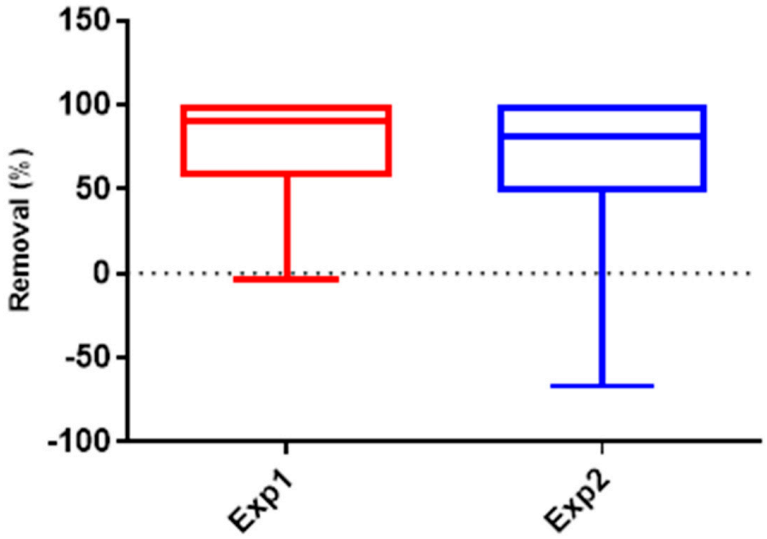

Figure 3. Box plot (minimum, Q1, median, Q3, and maximum) of removal in both experiments (1 and 2).

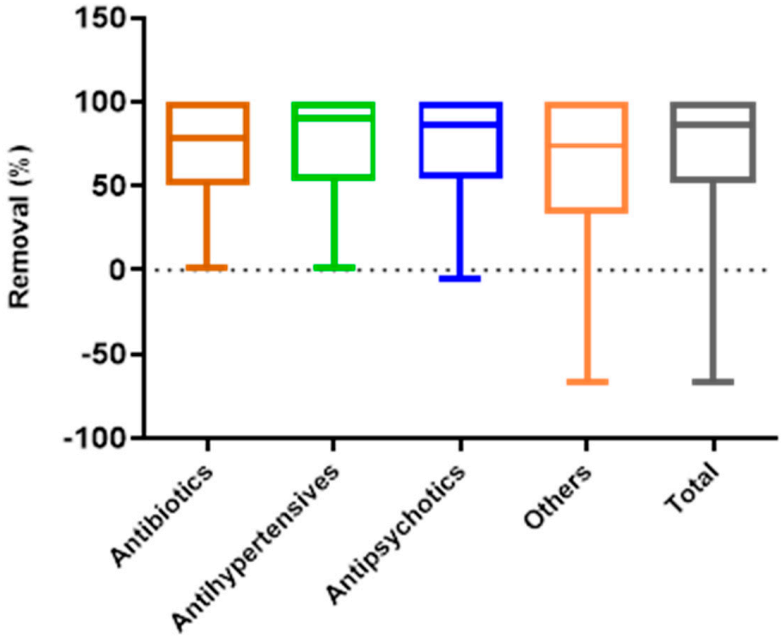

Figure 4. Removal (minimum, Q1, median, Q3, and maximum) by therapeutic group.

As for the different therapeutic groups, the average removal ranged from 64.0% to 75.3%, for the others and antihypertensive groups, respectively, with the remaining groups (antibiotics and antipsychotics) with values near 74% (Figure 4). When analyzing the total average, antibiotics and antipsychotics presented higher values (88.8 and 86.4%, respectively), followed by others and antihypertensives (79.3 and 74.3%, respectively).

Nonetheless, within each group, there was also great variability. In the antihypertensive group average removal values ranged from 54.6% (atenolol) to 98.7% (propranolol). In the antipsychotics, the same pattern was observed with values ranging from 59.8% (venlafaxine) to 100% (sertraline). As for the antibiotics' removal averages, the lower value was 44.7% for ofloxacin, and the higher was 100% (azithromycin and sulfamethoxazole). Due to practical reasons, pharmaceuticals are grouped by therapeutic properties. However, within each group, they can have very different physicochemical properties. These variations

imply that they will be impacted differently by the microalgae and other WWTP treatments, which explains the large variations observed.

The pharmaceuticals with the lowest removal rates (lower than 60%) were the antibiotics ofloxacin (44.7%) and ciprofloxacin (53.2%), the antihypertensives atenolol (54.6%) and losartan (56.2%), the antidepressant venlafaxine (59.8%), and diclofenac (52.7%). Some of them have also low removal rates in other types of wastewater treatment [7]. However, to increase their removal, we could conjugate this methodology with others, thereby complementing its efficacy.

Other authors with similar works [19] reported removals ranging from not detected to higher than 90% in pilot high-rate algal ponds (HRAP) using HRTs of 4 to 8 days in different seasons. Hom-Diaz et al. (2017) also performed a pilot outdoor experiment using a closed photobioreactor that was able to remove 30–80% of pharmaceuticals from toilet water [20]. This system was operated using HRTs of 8 to 12 days. García Galán et al. (2020) also used HRAP operating at an HRT of 4.6 days, achieving moderate removal rates (40–60%) or higher, depending on the class of pharmaceuticals [21]. Remarkably, the pilot installed in this study was able to achieve high removal rates for several pharmaceuticals using an HRT of only 24 h. This lower HRT translates into smaller and cheaper equipment for algae treatment and lower occupation of WWTP space. An increase in the HRT can lead to higher biomass concentrations inside the PBRs, which theoretically would improve removal [22]. It is possible that the application of higher HRTs in our system could result in even better pharmaceutical removal.

As most studies indicate that the removal of pharmaceuticals by microalgae proceeds via sorption to microalgal biomass [21] and is therefore dependent on microalgal productivity [22], the concentration of nitrogen (N) and phosphorous (P) nutrients was monitored during the experiments (Figure 5 and Table S3, Supplementary Materials). N and P nutrients' concentrations in the inflow of the algal system varied considerably between both experiments. While no significant differences were observed between total nitrogen (TN), ammonium, and total phosphorous (TP) concentrations between both experiments, during Exp2 (autumn), the concentration of nitrates was significantly higher (average 19.8 mg NO_3/mL) than in Exp1, conducted during the summer (average 5.07 mg NO_3/mL; $p < 0.05$). The concentration of biomass was also higher during the autumn experiment (average 83.2 mg/L) than during the summer experiment (average 31.7 mg/L; $p < 0.001$). This could be linked to the higher concentration of nitrates detected in Exp2 (autumn) or the high temperatures registered in the summer (23.0–32.5 °C; Exp1) compared with those registered in autumn (between 18.0 and 24.5 °C; Exp2). Although light intensity was not measured, it is expected to be also higher in the summer. High light intensities and extreme temperatures, such as those registered in the summer in the South of Portugal, may decrease microalgal growth productivity, as they may hamper photosynthesis.

Different microalgal species can also display different performances on the removal of pharmaceuticals. Kiki et al. tested 4 different microalgae against 10 antibiotics in synthetic wastewater under controlled laboratory conditions and found that *Haemaococcus pluvialis* was the most efficient strain among those tested for antibiotic degradation. However, the affinity of microalgae to the antibiotics was dependent on the compounds' structure and different species exhibited distinct affinities to the different compounds [23]. Hence, the microalgal strain used can influence pharmaceuticals' removal. The current study did not use a monoalgal culture but rather relied on the development of naturally developed microalgal consortia. While this strategy may not be the most efficient for pharmaceutical removal in case the consortium lacks the most appropriate strain, microalgal natural consortia are usually more advantageous for wastewater treatment than monoalgal cultures. Being composed of different strains with distinct growth performances and demands in nutritional and environmental requirements, natural consortia can recover faster from culture collapse events, due to grazers, for example, and adjust easier to climate or wastewater composition changes than monoalgal cultures [15].

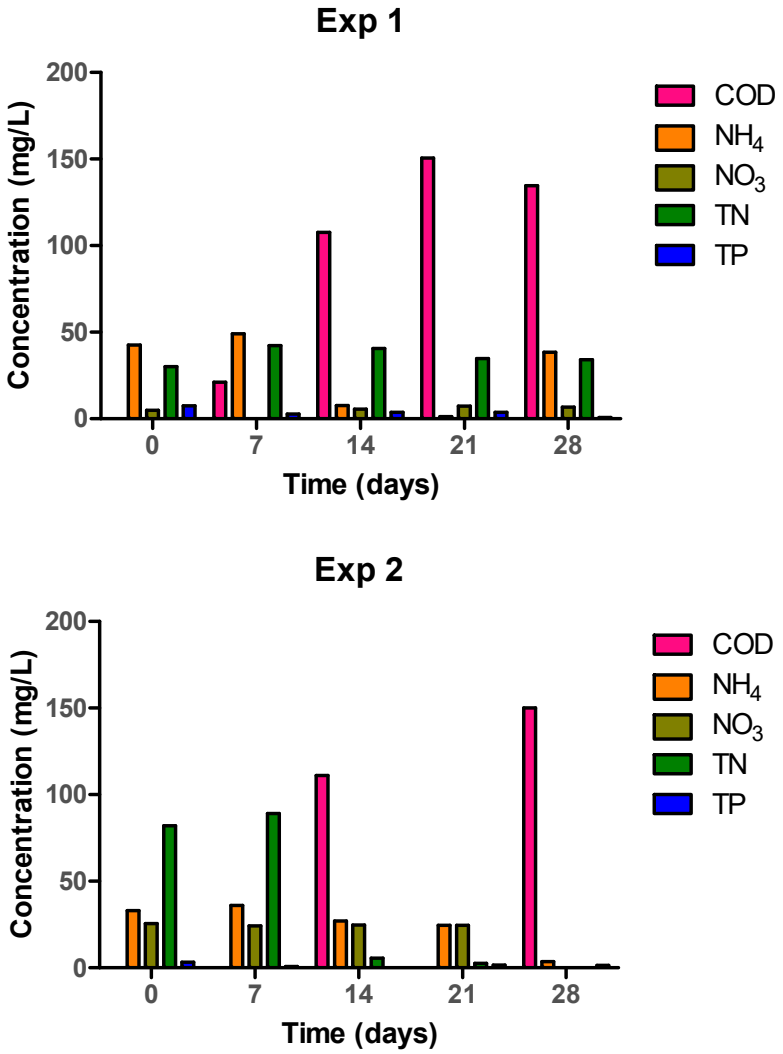

Figure 5. Concentration of nitrogen (total nitrogen, ammonium, and nitrates) and phosphorus (total P) nutrients and chemical oxygen demand (COD) in the inflow water of the GreenDune photobioreactors during the 5 weeks of both experiments.

3.2. Correlation between Different Factors

To better assess the impact of nutrient concentration and biomass productivity on pharmaceutical removal, a correlation analysis between all parameters was performed (Figure 6). Some positive correlations, already expected, are evident as those between the "Average removal" and the removal of individual pharmaceutical groups. Interestingly, a negative significant correlation was observed between the sum of all pharmaceuticals' concentrations and the average pharmaceutical removal or the removal of anti-hypertensives, suggesting that the removal of pharmaceuticals by microalgae may be compromised at high concentrations.

As already expected, a significant positive correlation exists between nitrates and biomass concentration. However, unexpectedly, no correlation was observed between biomass concentration and the removal of any of the pharmaceutical groups. Nonetheless, some significant positive correlations were observed between anti-hypertensive drugs' removal and ammonium concentration ($r = 0.53$; $p < 0.05$) and between TN and total pharmaceutical removal ($r = 0.46$; $p < 0.05$) and antipsychotic drugs' removal ($r = 0.47$; $p < 0.05$). Usually, higher concentrations of nutrients in the inflow of the photobioreactors would be linked with higher microalgal biomass productivities, which would explain the positive correlations obtained. However, no correlation between TN and NH_4 concentrations and

biomass concentration was observed. Nonetheless, the link between nitrogenated nutrient concentration and the removal rates of some pharmaceutical groups may open new avenues for research with the supplementation of wastewater with other nitrogen-rich wastes as agricultural wastes [24].

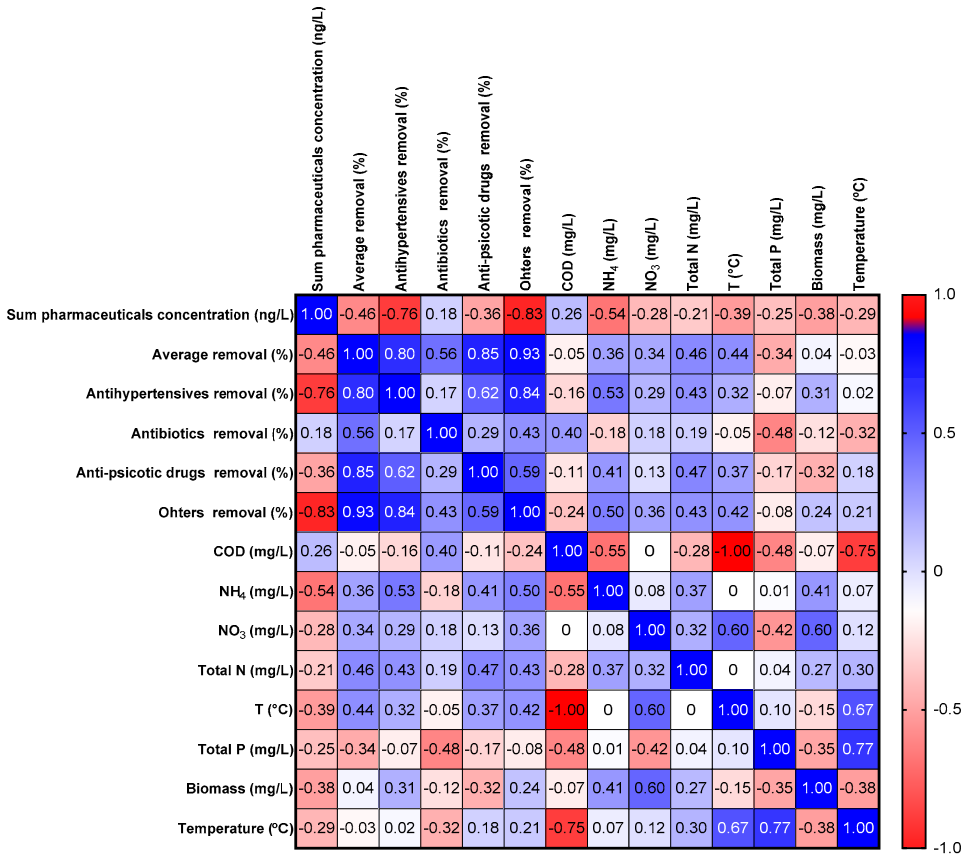

Figure 6. Correlation between pharmaceutical removal and other parameters.

Most studies involving the removal of pharmaceuticals from wastewater using microalgae are performed under carefully controlled conditions in the laboratory and not many outdoor pilot studies exist in the literature. Hence, conditions affecting pharmaceutical removal by these systems are still poorly understood. In this case, it would appear that nutrient concentrations, particularly TN and NH_4 concentrations, can promote better removal of pharmaceuticals.

4. Conclusions

In this study, we observed the presence of pharmaceuticals (between 7 and 19) in all the samples with an average of 218.4 ng/L. This confirms that WWTPs are not able to completely remove these compounds and are a hotspot of pharmaceutical contamination in the aquatic environment.

At the entrance of the microalgae system, the therapeutic groups, by decreasing average concentrations, were antipsychotics (381.0 ng/L), antihypertensives (225.8 ng/L), others (215.5 ng/L), and antibiotics (47.9 ng/L). However, at the exit, the order changed to antihypertensives (60.7 ng/L), antipsychotics (54.4 ng/L), others (46.8 ng/L), and antibiotics (5.5 ng/L). Moreover, there were significant variations within each group. There were no significant differences between Exp1 and Exp2, except for the initial load, which was related to a higher number of people in the area served by the WWTP at this time of the year.

Removal rates ranged from 64.0% (others) to 75.3% (antihypertensive) for the therapeutic groups. Nonetheless, between the different pharmaceuticals, the removal was between 44.7% (ofloxacin) and 100% (sertraline, azithromycin, and sulfamethoxazole). These results

highlight that pharmaceuticals, even those belonging to the same therapeutic group, are removed at very different rates. These promising results were obtained with an HRT of 24 h, lower than that of other similar studies. This allows it to treat the same amount of water with at least half the space and equipment as the published works.

As for the correlation between pharmaceutical removal and other factors, we could observe a negative correlation between the sum of all pharmaceuticals' concentrations and the average pharmaceutical removal or the removal of anti-hypertensives. This implies that the removal process by microalgae might be impaired at high concentrations. Positive correlations were observed between nitrates and biomass concentration, anti-hypertensive drugs removal and ammonium concentration, and TN and total pharmaceuticals removal, highlighting that particularly TN and NH_4 concentrations can promote better removal of pharmaceuticals.

This work supports that the use of a microalgae system can remove pharmaceuticals from an effluent of a WWTP, which can have implications in the design and upgrade of WWTPs, increasing the water quality and, therefore, water reuse. Additionally, since it is a methodology with low installation and operation costs it can also be used in low- and middle-income countries and small communities.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app13116414/s1. Table S1: pharmaceutical compounds included in the scope of the method, and mass detection parameters and limits of detection (LOD) for each compound in water; Table S2: pharmaceuticals' occurrence and removal in experiences 1 (a) and 2 (b) in the microalgae system; Table S3: chemical oxygen demand (COD) and nutrients concentration (mg/L) in the inflow water of the microalgal photobioreactors.

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