Behavior of UV Filters, UV Blockers and Pharmaceuticals in High Rate Algal Ponds Treating Urban Wastewater

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Abstract: The present study evaluated the efficiency of a high rate algal pond (HRAP) at pilot scale to remove pharmaceuticals and personal care products (PPCPs) from urban wastewater, including UV-filters and parabens (10), benzotriazoles (4), antibiotics (15), anti-inflammatory drugs (3) and other pharmaceuticals (3). A total of 35 compounds were targeted, of which 21 were detected in the influent wastewater to the HRAP. Removals (RE%) for pharmaceuticals were variable, with efficient eliminations for atenolol (84%) and sulfathiazole (100%), whereas the anti-inflammatory drugs naproxen and ketoprofen were only partially removed <50%. Benzotriazoles showed elimination rates similar to those of conventional WWTPs, with RE% ranging from no elimination to 51% for the UV filter benzophenone-3 (BP3) and 100% for 4-methylbenzilidenecamphor (4MBC). Hazard quotients (HQs) were estimated for those compounds not fully eliminated in the HRAP, as well as the cumulative ecotoxicity in the resulting effluent. The majority of the compounds yielded HQs < 0.1, meaning that no environmental risk would be derived from their discharge. Overall, these results clearly indicate that HRAPs are a reliable, green and cost-effective alternative to intensive wastewater treatment, yielding promising results removing these contaminants.

Keywords: emerging contaminants; sunscreens; metabolites; microalgae; wastewater treatment; green treatments

1. Introduction

Nature-based, low-cost treatment systems are gradually becoming feasible alternatives to conventional secondary/biological treatment (activated sludge) in wastewater treatment plants (WWTP), especially to cover the needs of small populations (up to 10,000 inhabitants) with no access to sanitary facilities. Microalgae-based systems, and specifically high rate algal ponds (HRAP), have already been used for decades for wastewater treatment [1,2]. They were introduced to improve the efficiency of stabilization ponds, being generally shallower to ensure light penetration, having a higher rate of oxygen production and operating under shorter retention times. As a result, the area requirements were also reduced.

Compared to conventional WWTPs, HRAPs do not require external aeration due to microalgae photosynthesis nor any chemical inputs during the treatment process [3,4]. Microalgae biomass
grows fixing CO\textsubscript{2} and assimilating the nutrients (mostly nitrogen (N) and phosphorus (P)) present in the influent wastewater. Oxygen is generated through photosynthesis and used by heterotrophic aerobic bacteria to degrade the organic matter present in the water. This way, these systems have the capacity of treating wastewater efficiently whereas producing microalgal biomass which, after an appropriate harvesting/separation technique from the aqueous phase, can be further profited to produce bioenergy (biogas) or other added-value bioproducts such as pigments, biofertilizers or bioplastics [5–9]. In consequence, if this biomass is managed properly, the waste generated during microalgae treatment (biomass) is considerably reduced, as well as the operation and maintenance costs when compared to conventional WWTPs.

Several recent studies have demonstrated the efficiency of HRAPs in terms of nutrients, organic matter and pathogens elimination [10–12]. The mechanisms to eliminate these pollutants in HRAPs are similar to those occurring in stabilization ponds. Specifically for particulated organic matter, sorption to the algae/bacteria flocs is the main elimination pathway [13]. HRAPs become more efficient when they operate at pH below 9. Organic matter removal (in terms of BOD\textsubscript{5}) of up to 94% has been reported in HRAP systems with pH control (by means of CO\textsubscript{2} injection) below 9 [14]. Regarding nutrients (N and P), volatilization and photosynthetic assimilation are the main removal pathways for N, and precipitation in the case of P, with a strong correlation between pH values and residual levels of N-NH\textsubscript{4} and phosphates [15].

Currently, these systems are being intensively investigated regarding their capacity to eliminate organic micropollutants such as pharmaceuticals and personal care products (PPCPs), obtaining promising results [11,16,17]. PPCPs comprise a broad number and variety of compounds, which are neither routinely monitored in wastewaters, nor regulated [18,19]. These compounds are used on a daily basis and, after usage, they enter the environment regularly mostly via WWTP effluents. Despite their low concentrations, their bioactivity and recalcitrance can cause natural imbalances in the receiving water bodies, including aquatic toxicity, genotoxicity, endocrine disruption and selection of resistant pathogenic bacteria amongst others [18]. The reduction of microinvertebrates diversity in rivers and behavioral changes in the ichthyofauna have also been reported [20]. Ponsatí et al. [21] evaluated the effect on the biodiversity of the presence of inorganic and organic compounds, including pesticides and pharmaceuticals, and observed a considerable decrease in biodiversity and simplification of the biological structure of both biofilms (algae, bacteria and fungi) and invertebrates. However, the lack of long-term studies and chronic effects is evident in the literature and further research should be carried out [22].

On the other hand, personal care products are less frequently studied than pharmaceuticals, and the research on this topic is generally focused on some types of disinfectants (triclosan), insect repellents (DEET) and fragrances, whereas there is little data on the occurrence and fate of other compounds such as UV filters and/or UV blockers [23,24]. Based on these gaps in the literature and knowing that the main entrance pathway of PPCPs in the aquatic environment are WWTP effluents, the need to find alternative and more efficient treatments is evident. Within this context, HRAPs are gaining a renewed popularity due to their demonstrated high efficiency treating wastewater [10–12] within a more sustainable frame than activated sludge treatments. Efficient removal of different PPCPs such as hormones, anti-inflammatories and other pharmaceuticals in HRAPs has recently been demonstrated [11,16], yet the number of studies on the removal capacity of microalgae-based systems against these organic contaminants is scarce, especially in real scale systems.

Thus, the present study aims to evaluate the removal capacity of a HRAP at pilot scale, as secondary treatment, for 35 different PPCPs, including UV-filters and parabens (10), benzotriazoles (4), antibiotics (15) and other pharmaceuticals (6). The different removal pathways have been discussed for each pollutant, and the potential ecotoxicity of the HRAP effluent has been evaluated, estimating the risk quotients (HQs) associated to the PPCPs to ascertain a safe discharge in the receiving surface waters. To the best of the author’s knowledge, it is the first time that an open algae-based system (HRAP) is investigated regarding the specific removal of UV filters and UV Blockers in wastewater.
2. Materials and Methods

2.1. Microalgae-Based Treatment System Description and Operation

Samples were taken from a pilot HRAP installed outdoors the UPC premises (Barcelona, Spain). The pilot plant has been previously described in detail elsewhere [10]. Briefly, the wastewater treatment line was composed by a screening pre-treatment, a primary settler in a cylindrical PVC settling tank (effective volume: 3 L, HRT: 41 min), a HRAP and a secondary settler (effective volume: 3.3 L, HRT: 46 min) to separate the biomass produced from the treated effluent (Figure 1). The system was continuously fed with wastewater directly pumped from the public sewer to a homogenization tank (1.2 m³), which was constantly stirred to avoid solids sedimentation. The HRAP had a volume of 470 L (surface area of 1.54 m², 0.3 m depth) and was equipped with a paddle wheel (working at 5 rpm approx.) for mixed liquor mixing. The system was operating continuously for almost one year with an HRT of 4.5 days.

![Diagram of the high rate algal pond (HRAP) pilot plant used in the study. Samples were taken after the primary settler and after the secondary settler (indicated with a star).](image)

2.2. Sampling Campaign

The sampling campaign was carried out when the HRAP had already been in operation for almost one year and reached steady state (which requires from 3 to 5 HRT in completely mixed systems). Sampling was carried out during two consecutive weeks in July, three days per week. Grab samples of the HRAP influent were taken after primary treatment, right before entering the pond, and from the effluent after the secondary settler (Figure 1). As the HRAP works as a completely mixed reactor, the HRT was not considered when taking influent and effluent samples. For physicochemical characterization of the water, samples were taken in PVC bottles and directly analyzed in the laboratory on the same day. For the analysis of environmental levels of PPCPs, samples were collected, immediately filtered through 0.45 μm PVDF membrane filters (Millipore, Burlington, MA, USA) and frozen (amber glass bottles) until analysis.

2.3. Chemicals and Reagents

High purity standards (>99%) for 4 benzotriazoles (1H-benzotriazole (BZT), 5-methyl-1-H-benzotriazole (MeBZT), 5,6-dimethyl-1H-benzotriazole (DMBZT) and 2-(2′-hydroxy-5′-methylphenyl)benzotriazol (UVP), 6 benzophenones (benzophenone-1 (BP1), benzophenone-2 (BP2), benzophenone-3 (BP3), 4-hydroxybenzophenone (4HB), 4,4-dihydroxybenzophenone (4DHB) and 2,2′-dihydroxy-4-methoxybenzophenone (DHMB)), 1 camphor derivative (4-methylbenzilidenecamphor (4MBC)), 1 cinnamate derivative (ethylhexyl methoxyccinnamate (EHMC)), 2 p-aminobenzoic acid derivatives (benzocaine (EtPABA), and ethylhexyl-4-(dimethylamino)benzoate (ODPABA)) and their corresponding isotopically labelled compounds were purchased from Sigma Aldrich (Augsburg, Germany) and Merck (Darmstadt, Germany). Regarding pharmaceuticals, 2 macrolides (clarithromycin and tylosin), 2 fluoroquinolones (flumequine and ofloxacin), 1 quinolone (oxolinic acid), 9 sulfonamides (sulfabenzamide, sulfadimethoxine, sulfamerazine, sulfamethizole, sulfamethoxazole, sulfanitran,
sulfapyridine, sulfathiazole and sulfisomidin), trimethoprim, 3 antiinflammatories (ketoprofen, naproxen and mfenamic acid), the lipid regulator gemfibrozil, the β-blocking agent atenolol and the stimulant caffeine and their corresponding isotopically labelled compounds were obtained from Sigma-Aldrich (St. Louis, MO, USA) and TRC (Toronto Research Chemicals Inc., Ontario, Canada). Detailed information for all the studied compounds is given in Table S1 of the Supplementary Materials. Standard solutions of the mixtures of all compounds were made at appropriate concentrations and used to prepare the aqueous calibration curve and to perform the recovery studies. Similarly, stock standard solutions for the internal standards were prepared. Aqueous standard solutions always contained <0.1% of methanol (MeOH).

2.4. Analytical Methodologies

2.4.1. Physico-Chemical Characterization

Both HRAP influent and effluent samples were analyzed for the following wastewater quality parameters: DO and temperature (EcoScan DO 6, ThermoFisher Scientific, Waltham, MA, USA); pH (portable pH-meter 506, Crison Instruments, Barcelona, Spain); turbidity (Hanna HI 93703, Woonsocket, RI, USA); total suspended solids (TSS), volatile suspended solids (VSS), alkalinity and chemical oxygen demand (COD) following Standard Methods [25]; NH$_4^+$-N according to the Solórzano method [26]. The ions N-NO$_2^-$, N-NO$_3^-$ and P-PO$_4^{3-}$ were measured by ion chromatography (ICS-1000, Dionex Corporation, Sunnyvale, CA, USA). Total organic carbon (TOC), total phosphorus (TP) and total nitrogen (TN) were measured by a TOC analyzer (multi N/C 2100S, Analytik Jena, Germany). All the analyses were done in triplicate and results are given as average values. Mixed liquor samples were examined under an optic microscope (Motic, China) for qualitative evaluation of microalgae populations, employing taxonomic books and databases for their identification [27,28].

Average biomass productivity (g VSS m$^{-2}$·day$^{-1}$) in the HRAP was calculated based on the VSS concentration in the mixed liquor, using Equation (1):

$$\text{Biomass productivity} = \frac{\text{VSS} (Q - Q_E + Q_P)}{A}$$  (1)

where VSS is the volatile suspended solids concentration of the HRAP mixed liquor (g VSS L$^{-1}$); Q is the wastewater flow rate (L d$^{-1}$); $Q_E$ is the evaporation rate (L d$^{-1}$); $Q_P$ is the precipitation rate (L d$^{-1}$), and A is the surface area of the system. The evaporation rate was calculated using Equation (2):

$$Q_E = E_P A$$  (2)

where $E_P$ is the potential evaporation (mm d$^{-1}$), calculated using Equation (3) [29].

$$E_P = a \frac{T_a}{(T_a + 15)} (R + 50)$$  (3)

where a is a dimensionless coefficient which varies depending on the sampling frequency (0.0133 for daily samples); R is the average solar radiation in a day (MJ m$^{-2}$), and T$_a$ is the average air temperature (°C). Meteorological data (solar radiation, temperature and precipitation) were obtained from the network of local weather stations in Barcelona Metropolitan Area (www.meteo.cat) and are given in Table S2 of the Supplementary Materials.

2.4.2. Online-SPE-HPLC-MS/MS Analysis of the Target Compounds

The target analytes were analyzed using an adapted methodology based on previous analytical strategies, by means of high resolution liquid chromatography coupled to mass spectrometry in tandem (HPLC-MS/MS) [30,31]. Briefly, pre-concentration and chromatographic separation were done using on-line solid phase extraction (SPE) coupled to liquid chromatography (on-lineSPE–LC)
(Symbiosis™ Pico, Spark Holland (Emmen, The Netherlands)). On-line SPE pre-concentration of all samples, including the calibration curve (5 mL volume), was carried out using PLRP-s cartridges (Agilent, St. Clara, CA, USA). HPLC-MS/MS analyses were performed using a 4000 Q TRAP™ MS/MS system (Applied Biosystems-Sciex (Foster City, CA, USA). MS/MS detection was performed in both positive and negative ionization modes, under the selected reaction monitoring (SRM) mode. Table S3 summarizes the HPLC-MS/MS conditions for the targeted compounds. Linearity and limits of quantification (LOQ) of the methodology are given in Table S4.

2.5. Environmental Risk Assessment

In order to evaluate the potential ecotoxicological risk of those PPCPs still present in the HRAP effluent, hazard quotients (HQs) were estimated as indicated in Equation (4), following the guidelines of the European Medicines Agency (EMA),

$$HQ = \frac{MEC}{PNEC}$$  \hspace{1cm} (4)

where MEC is the measured environmental concentration, and PNEC is the predicted-no effect concentration. When PNEC data are not available, alternative PNECs can be derived by dividing the toxicity endpoint values found in the literature (EC50 or LC50) by an uncertainty factor of up to 1000 [32]. HQ values < 0.1 mean that no adverse effects are expected. When 0.1 < HQ < 1, the risk is low, but it should not be neglected; when 1 < HQ < 10, a moderate risk is implied, and HQ > 10 mean a relevant ecological hazard.

Eventually, for the purpose of evaluating the overall ecotoxicity risk of the HRAP effluent, cumulative HQs were calculated for each trophic level considered, adding all HQs calculated for each individual PPCP detected in the effluent.

3. Results

3.1. Physico-Chemical Water Quality Parameters

The physico-chemical properties of the influent and effluent from the HRAP are summarized in Table 1. The VSS/TSS ratio in the mixed liquor was 85% in average, which is in accordance with previous studies [10,33]. The TSS removal efficiency of the HRAP was 77.8%, which is higher than other results obtained previously (52.6%) in bigger HRAPs [34]. The average influent COD was 199 mg L⁻¹, with an average removal of 60% in the HRAP. The COD removal observed in the HRAP (60%) was in accordance with the results obtained in the same system operated and monitored for one year [10] and also with those observed in different HRAPs [35,36]. The removal for N-NH₄⁺ was 97%. Microalgae biomass assimilation is the main removal pathway of N-NH₄⁺ in algae systems but also nitrification and volatilization as secondary routes [3].

3.2. Biomass Productivity

The average biomass productivity in the HRAP was 20.71 ± 6 g VSS m⁻² day⁻¹ (equivalent to 67.9 ± 17 mg VSS L⁻¹ day⁻¹), which is in accordance with results obtained in previous studies (ranging from 5 to 33 g VSS m⁻² day⁻¹ [6,13]. Regarding the different microalgae species identified in the HRAP, Chlorella sp. was predominant in the HRAP (Figure 2).

3.3. Occurrence of PPCPs in the HRAP Influent

3.3.1. Benzotriazoles and UV-Filters

Eight UV filters and benzotriazoles (UV blockers) were detected in the urban wastewater feeding the HRAP (Figure 3A). The metabolite of BP3, 4DHB, was detected only one day of sampling (frequency of detection ≤ 17%) and has been included in Figure S2 of the SM. For UV
filters, none of the p-aminobenzoic acid and cinnamate derivatives were detected. The highest concentrations corresponded to BZT (935 ng L⁻¹–2292 ng L⁻¹) followed by its derivative, MeBZT (220 ng L⁻¹–1982 ng L⁻¹) (see Figure 3A). Both were detected in 5 of the 6 samples, and these concentrations were in agreement with levels reported in previous studies of urban wastewaters [30,37–40]. Another derivative of BZT, DMBZT was also detected in 50% of the samples, but at much lower concentrations (45 ng L⁻¹–107 ng L⁻¹). Benzotriazoles are generally used as UV-blockers in industrial products such as plastics and paints to protect polymers and pigments against photodegradation [41], but also in combination with UV-filters such as BP3 to extend the UV protection range of sunscreens and other cosmetics. Regarding UV filters, BP3 was present in all samples analyzed, in the range of 26–106 ng L⁻¹, which also agrees with previous studies in the area [30,40,43]. Concerning the other BP3 metabolites, 4HB was present only in two samples of the HRAP influent, at concentrations <48 ng L⁻¹, and 4DHB and DHMB were detected only in one of the 6 samples. These three metabolites of BP3 are not usually included in monitoring studies, and their frequency of detection and concentration levels are generally very low [30,40]. On the contrary, BP4 was not detected in any of the influent samples, despite being frequently found in urban wastewater at concentrations >1000 ng L⁻¹ [30]. Regarding the camphor derivatives, 4MBC was detected in 3 of the 6 samples analyzed, in the range of 26–106 ng L⁻¹, which also agrees with previous studies in the area [30,40,43].

### Table 1. Physicochemical characterization of the influent and effluent of HRAP.

| Parameters (n = 6) | HRAP⁺influent Mean ± SD | HRAP⁺effluent Mean ± SD |
|-------------------|-------------------------|------------------------|
| TSS (mg L⁻¹)      | 192.44 ± 114.65         | 323.15 ± 88.4 */42.59 ± 12.8 |
| VSS (mg L⁻¹)      | 154.67 ± 102.9          | 274.30 ± 77.5 */42.89 ± 13.9 |
| COD (mg O₂ L⁻¹)   | 199.92 ± 75.11          | 78.85 ± 33.13           |
| pH                | 8.2 ± 0.2               | 8.4 ± 0.4               |
| DO (mg L⁻¹)       | -                       | 8.80 ± 1.7             |
| Temperature (°C)  | 19.5 ± 1.3              | 18.9 ± 1.6             |
| N-NH₄⁺ (mg L⁻¹)   | 24.1 ± 3.0              | 6.0 ± 0.4              |
| TN (mg L⁻¹)       | 56.9 ± 17.8             | 38.7 ± 9.4             |
| TIN (mg L⁻¹)      | 29.3 ± 9.94             | 17.4 ± 6.5             |
| TC (mg L⁻¹)       | 253.5 ± 95.6            | 160.6 ± 21.6           |
| N-NO₂⁻ (mg L⁻¹)   | 5.2 ± 4.5               | 1.8 ± 2.1              |
| N-NO₃⁻ (mg L⁻¹)   | 0.15 ± 0.36             | 15.2 ± 8.6             |
| P-PO₄³⁻ (mg L⁻¹)  | 3.9 ± 2.1               | 3.2 ± 2.1              |
| S-SO₄²⁻ (mg L⁻¹)  | 76.2 ± 84.9             | 39.9 ± 20.1            |

TSS—Total Suspended Solids; VSS—Volatile Suspended Solids; COD—Chemical oxygen demand (soluble); DO—Dissolved Oxygen; TN—Total Nitrogen; N-NH₄⁺—Ammonium; TN—Total Nitrogen; TC—Total Carbon. (*) values measured in the mixed liquor of the reactors.

**Figure 2.** Microscope images of mixed liquor of the HRAP observed in bright light microscopy (x1000).
3.3.2. Pharmaceuticals and Stimulants

Eleven out of the 21 pharmaceuticals targeted were detected in HRAP effluent. For antibiotics, only sulfonamides and trimethoprim were detected. The highest concentrations corresponded to the stimulant caffeine (8050 ng L\(^{-1}\)–23,126 ng L\(^{-1}\)), one of the most consumed worldwide and frequently used as tracking marker for wastewater pollution [44,45] due to its low metabolization in the human body [46]. These results are in accordance with previous monitoring studies in HRAPs [17,47]. High concentrations were detected also for the non-steroidal anti-inflammatory drugs (NSAID) naproxen and ketoprofen, with concentrations in the range of 1529–7900 ng L\(^{-1}\) and 165–1406 ng L\(^{-1}\), respectively, in agreement with the levels reported in previous monitoring studies on wastewater [48–50]. Antibiotics were present at much lower concentrations, generally <50 ng L\(^{-1}\). Six out of the nine sulfonamides targeted were detected, but only one or two days of sampling. Only sulfapyridine was detected in the raw wastewater 5 of the 6 days of sampling, with average concentrations of 25 ng L\(^{-1}\). Sulfapyridine is usually found at levels ranging from 10 ng L\(^{-1}\) to 100 ng L\(^{-1}\) in wastewater [51,52] but also at concentrations higher than 1000 ng L\(^{-1}\) [53]. Sulfathiazole was present (two days of sampling) at average concentrations of 15 ng L\(^{-1}\). Frequencies
of detection for sulfathiazole are generally low in urban wastewater, as its main use is veterinary [51,54]. Nevertheless, it was detected in 50% of the influent samples analyzed by García-Galán et al. [55] in different WWTPs in Catalonia, at levels in the range of 7.3–142 ng L$^{-1}$, and also at concentrations up to 300 ng L$^{-1}$ in an Australian WWTP [56]. On the contrary, sulfamethoxazole is clearly the sulfonamide most frequently detected in urban wastewater, considered even as a water quality indicator by different authors due to its environmental ubiquity and recalcitrance. However, in the present study, it was determined only in one sample and at a very low concentration (27 ng L$^{-1}$), similarly to sulfaquinoxaline and sulfabenzamide (20 ng L$^{-1}$ and 23 ng L$^{-1}$, respectively). Trimethoprim was frequently present (5 of the 6 days of sampling) at an average concentration of 76 ng L$^{-1}$, which is in accordance with the levels reported in previous studies for raw wastewater [48,51]. The β-blocker atenolol was detected in 3 out of the 6 samples analyzed, at an average concentration of 107 ng L$^{-1}$. Results in previous studies were more than one order of magnitude higher (µg L$^{-1}$), which may be related to local consumption trends [46,48,57].

3.4. Removal Efficiency in the HRAP

The removal efficiency in the HRAP was calculated according to Equation (5):

$$\text{RE(\%)} = 100 \times \left(1 - \frac{C_{\text{eff}}}{C_{\text{inf}}}\right)$$

where RE(%) is the removal efficiency, and $C_{\text{inf}}$ and $C_{\text{eff}}$ are the concentrations (ng L$^{-1}$) in the influent and effluent water, respectively.

Figure 3A,B shows the removal efficiencies (RE%) obtained for different families of contaminants evaluated.

3.4.1. Benzotriazoles

BZT was not efficiently removed in the HRAP, with RE not higher than 22% and obtaining also negative eliminations (see Figure 3A). To the author’s knowledge, only Matamoros et al. [17] had previously studied the removal of BZT in HRAPs, obtaining eliminations in the range of 33–74% under the same HRT (4 d). Vassalle et al. [58] investigated the removal of this compound in closed photobioreactors, obtaining similar efficiencies (15% removal in average). The results obtained in different conventional WWTPs are highly variable and can range from negative RE up to 70% [40]. For instance, Liu et al. obtained a RE of 7% [38], Asimakopoulos et al. obtained RE values in the range of 25–37% [37], Reemtsma et al. achieved eliminations of 20–59% [59] and Liu et al. obtained removals of 47% [60]. The low removal of this compound in WWTP explains their ubiquity and impact in the receiving surface waters [61,62].

Removal of MeBZT was similar (0–19%), and higher concentrations in the effluent than in the influent were frequently observed. MeBZT is also frequently found in surface waters and groundwaters in urban areas, at concentrations up to 7181 ng L$^{-1}$ and 1980 ng L$^{-1}$, respectively [40,43].

Another benzotriazole derivative, DMBZT, was also only barely removed, with RE% between 0% and 8%. RE% in conventional WWTP are again highly variable for these two compounds, with removals in the range of 0–72% for MeBZT and 0–16% for DMBZT [37,59,63].

3.4.2. UV Filters

The elimination of BP3 was variable, with removals ranging from −28% to 51%. This results agree with those obtained by Díaz-Garduño et al. [47], using HRAP as tertiary treatment for WWTPs effluents, with RE% ranging from −50% to 70%, and also with those obtained in a closed photobioreactor operating as tertiary treatment (40% removal on average) [58]. BP3 has been even found in tap water at concentrations up to 290 ng L$^{-1}$ [64] and also in sewage sludge (0.79 µg g$^{-1}$) [65]. Its metabolite BP1 was not removed in the HRAP, with only one positive value (44%) out of 3. Eliminations reported
in the literature after activated sludge treatments are also variable and in the range of 48%–97% for BP1 and 58%–91% for BP3 [40]. Regarding the other BP3 metabolites, 4HB was completely eliminated, and 4DHB was removed by 45% (Figure S2). The environmental occurrence of these metabolites is not as relevant as that of BP1 or BP3, as their occurrence in wastewater is less frequent and at much lower levels. Their removal in WWTPs is also efficient, and both metabolites have been detected in sewage sludge at concentrations of 0.15 µg g\(^{-1}\) for 4HB and up to 0.62 µg g\(^{-1}\) for 4DHB, despite their low K\(_{ow}\) [65,66].

The camphor derivative 4MBC was fully removed in the HRAP. High removal efficiencies are usually registered in conventional WWTPs for this compound (given its high K\(_{ow}\)), where it is frequently detected in sewage sludge [65]. Nevertheless, it is still also in surface water and groundwater at low concentrations [40,43] and even in tap water (10 ng L\(^{-1}\)–35 ng L\(^{-1}\)) [64]. Table S5A of the Supplementary Materials gives more detailed information on the levels detected on each sampling day.

### 3.4.3. Pharmaceuticals

Negative RE% were obtained for trimethoprim in the HRAP (~32% in average), meaning that higher concentrations were found in the effluent than in the influent. Previous studies also obtained negative and low RE% for trimethoprim (3.7%) in HRAPs [47], but elimination efficiencies up to 78% have been obtained in a closed photobioreactor working as tertiary treatment [58,67,68]; in that same study, however, the elimination of sulfapyridine was negative, whereas a 4% removal in average has been obtained in his work. On the other hand, sulfathiazole was fully removed within the HRAP.

A removal efficiency of 84% for atenolol was observed, which is in agreement with previous studies in HRAPs [47,68]. Regarding NSAIDs, RE values were highly variable. Ketoprofen was eliminated by 33% in average, with RE ranging from negative to 100%. Previous studies also obtained elimination efficiencies, ranging from 0% to 50% in HRAPs [47], also in closed photobioreactors [58]. Similarly, naproxen was only moderately removed (45% on average), with RE% ranging from ~3% to 100%. Díaz-Garduño et al. [47] reported RE% in the range of 0–35% in laboratory-scale reactors; Vassalle et al. [11] obtained a RE% of 54% in a pilot HRAP (205 L) operating with a HRT of 8 days, and Matamoros et al. [17] obtained removals in the range 60–90%, with better efficiencies during the warm season and with longer HRTs (8 vs. 4 days).

Last of all, caffeine was efficiently removed (63–80%), except for one day (14%). Matamoros et al. [17] obtained RE% > 90% for this compound, but lower and even negative RE% have been also reported [47,58]. Table S5B gives more detailed information on the pharmaceutical levels detected on each sampling day.

### 3.5. Ecotoxicity in the HRAP Effluent

The environmental risk estimated for those PPCPs still present in the HRAP effluent (FD ≥ 33%) was evaluated by means of HQs, as indicated in Section 2.5. This quotient estimates the potential adverse effects of the drug concentration detected in the effluent on non-target organisms present in the receiving water body. Environmental assessments based on the calculation of HQs are frequently carried out [47,55]. Microalgae, invertebrates (Daphnia magna) and fish were considered to calculate HQs as representative species of three of the main trophic levels in aquatic ecosystems. Table 2 summarizes the results obtained, as well as the endpoints used. Given that the different considered species are exposed regularly to low concentrations of the PPCPs studied, PNECs were calculated using EC\(_{50}\)-LC\(_{50}\) as indicators of acute toxicity, divided by an uncertainty factor of 1000 to transform these endpoints into values more representative of the real situation under environmental conditions (longer periods of exposure) [32,55].
Table 2. Average HRAP effluent concentrations (mg L\(^{-1}\)) (used as measured environmental concentrations, MEC), ecotoxicity endpoints used for the different trophic levels considered (mg L\(^{-1}\)) and hazard quotients (HQ) estimated. HQs have been estimated for those PPCP present in the effluent with a frequency of detection \(\geq 33.3\% \ (n \geq 2 \ over \ 6)\).

| Effluent Concentration (mg L\(^{-1}\)) | Toxicity Endpoints (mg L\(^{-1}\)) | Hazard Quotients (HQs) |
|----------------------------------------|-----------------------------------|------------------------|
|                                        | Green Algae | Crustaceans | Fish | Ref. | Green Algae | Crustaceans | Fish |
| Ben佐triazoles                         |              |              |      |      |              |              |      |
| 1H-benzotriazole (BZT)                 | 0.0001769    | 2.51        | 107  | 654  | 1.65 \times 10^{-2} |
| 5-methyl-1-H-benzotriazole (MeBZT)    | 0.0000998    | 2.861       | 51.6 | 224  | 1.93 \times 10^{-2} |
| Benzophenones                          |              |              |      |      |              |              |      |
| BP1                                    | 0.000346     | 10.52       | 3.9  | 49.194 | 4.54 \times 10^{-2} |
| BP3                                    | 0.000239     | 0.961       | 1.6  | 7495  | 1.81 \times 10^{-2} |
| Camphor derivatives                    |              |              |      |      |              |              |      |
| 4MBC*                                  | 0.000049     | –           | 2.3  | 4155  | –           |
| Antibiotics                            |              |              |      |      |              |              |      |
| Sulfapyridine (SPY)                    | 0.000026     | 1912        | –    | <39.836 | 6.21 \times 10^{-4} |
| Trimethoprim (TMP)                     | 0.000073     | 80.32       | 92   | >1007  | 7.34 \times 10^{-4} |
| Analgesics/Anti-inflammatories         |              |              |      |      |              |              |      |
| Ketoprofen (KPF)                       | 0.000954     | 1642        | 248  | 32    | 2.40 \times 10^{-2} |
| Naproxen (NPX)                         | 0.005373     | 223         | 15   | 34    | 2.40 \times 10^{-2} |
| Stimulants                             |              |              |      |      |              |              |      |
| Caffeine (CAF)                         | 0.004924     | >1502       | 160  | 1514  | 3.26 \times 10^{-2} |
| Cumulative HQs                         | 1.62         | 0.36        | 0.27 |      |              |              |      |

(1) Desmodesmus subspicatus; (2) Pseudokirchneriella subcapitata; (3) Selenastrum capricornutum; (4) Pimephales promelas; (5) Oncorhynchus mykiss; (6) Danio rerio. (7) Orizyas latipes. [1] ECOTOX database of the Environmental Protection Agency (EPA); [2] Molins-Delgado et al. (2017); [3] Sanderson et al. (2003).
4. Discussion

The role of WWTP effluent discharges as main contributors to the presence of benzotriazoles in aquatic ecosystems is generally highlighted in the bibliography [37,38,59], and the low biodegradability of these substances makes these concentrations cumulative in sediments and also in living organisms at different trophic levels [62]. Indeed, concentrations up to 8529 ng L\(^{-1}\) for BZT have been found in highly-impacted rivers in the Barcelona Metropolitan area [40], but it is generally detected at lower concentrations in surface waters. Generally, as demonstrated by Loos et al. [61], BZT is frequently detected (>94%) in surface waters at average concentrations of 493 ng L\(^{-1}\). On the other hand, Liu et al. demonstrated that BZT was photodegradable, and obtained removals in stabilization ponds (with HRT of 27 days) of 47% [38,60]. Nevertheless, photodegradation, which is usually enhanced in microalgae-based treatment systems [16,17], was not significant for BZT under the HRAP configuration in the present study. Given its high solubility and low \(K_{\text{ow}}\), its retention/sorption tendency on the microalgae biomass was also low. Regarding its two main metabolites, MeBZT and DMBZT, better RE% were obtained in a closed photobioreactor for both metabolites [58], reaching average eliminations up to 62% and 40%, respectively, compared to the results <19% in the studied HRAP. The presence of other benzotriazole derivatives in the influent wastewater (not considered as target compounds of the present study) that could biotransform into BZT or MeBZT could also explain the overall low removals obtained for these compounds in the pond. For instance, xylyltriazole demethylates are known to release both MeBZT and BZT as transformation products, and 5-chloro-benzotriazole can lose the chlorine moiety to transform back into BZT [39].

Regarding UV-filters and the variable and low RE obtained for BP3, Gago-Ferrero et al. demonstrated that photodegradation was not a relevant degradation pathway for this compound [66]. If photodegradation of BP3 is discarded in the HRAP, the higher concentrations of BP1 in the effluent of the pond could be explained in terms of biodegradation of BP3 and new synthesis of BP1 as a by-product. Indeed, in the same study by Gago et al., the authors identified BP1 as a biodegradation by-product of BP3 by the white-rot fungi *Trametes versicolor*. The camphor derivative 4MBC was fully removed in the HRAP. It is a highly lipophilic compound, and due to its low solubility and high \(K_{\text{ow}}\), biosorption to microalgae biomass seems to be the main removal pathway in the pond.

The low biodegradability of the antibiotics trimethoprim and sulfapyridine, combined with their high ionization potential and hydrophilicity, could explain the poor RE% obtained. Indeed, the high \(pK_a\) values of these substances and their low \(K_{\text{ow}}\) (Table S1) explain their low tendency to adsorb to the biomass and their presence in the aqueous phase. A recent study evaluated the bioconcentration of trimethoprim and different sulfonamides in microalgae biomass, not finding any of the compounds in the cell wall [67]. These authors in that study also stated that sulfonamides and trimethoprim were resistant to microalgae-based treatments. However, sulfathiazole was fully eliminated. Generally, sulfonamides are not prone to photodegradation [69], so biodegradation seems the most feasible removal pathway for these antibiotics.

Similarly, atenolol is highly hydrophilic and shows a very low tendency to sorption, but different studies have demonstrated that it is susceptible to photodegradation [70], which could be the main removal pathway for this compound (84% removal). Indeed, it shows low and variable RE% in WWTPs, which leads to its frequent presence in river and even coastal waters.

Naproxen is generally considered an easily biodegradable drug, with RE% >80% in conventional WWTPs [71,72]. Its photodegradability has also been demonstrated [73] and should be considered as one of the main removal pathways within the pond. Sorption processes are not considered due to its low \(K_{\text{ow}}\) value [74–76].

Last of all, and based on the established methodologies [77,78], the HQs calculated for the compounds detected in the effluent showed that no environmental risk would be derived from the discharge of the HRAP effluent. Amongst the different PPCP still present in the HRAP effluent, none of them yielded HQ > 1. HQ values between 0.1 and 1 (low risk) were obtained for BZT and MeBZT against green microalgae, for BP3 against green microalgae and *Daphnia magna* and for naproxen in the
three trophic levels. As it can be observed, the majority of the compounds yielded HQs < 0.1, meaning that no environmental risk would be derived from their discharge after HRAP treatment.

Final Remarks

Considering the results obtained, it should be noted that the comparison of the removal efficiency among microalgae-based wastewater treatment systems is complex. First of all, different mixed cultures can lead to different elimination routes and pathways in the systems. Indeed, different publications on the elimination of a single compound using different microalgae cultures have yielded very different results [20,79,80]. Biomass productivity in the photobioreactors can also be determinant in terms of bioadsorption, and so does the HRT, and higher temperatures may lead to faster biodegradation processes and removal routes [17]. This should be considered when comparing, for instance, closed and open systems, as the former will usually keep higher temperatures. The control of pH could also aid to the removal via bioadsorption of the target analytes by changing their protonation state.

5. Conclusions

The efficiency of an innovative natural and low-cost treatment system, based on microalgae biomass productivity and operating as secondary treatment of urban wastewater, was evaluated focusing on their capacity to remove different PPCPs, including benzotriazoles, benzothiazoles and pharmaceuticals amongst others. BP3 was detected in all the HRAP influent wastewater samples investigated and also the benzotriazoles BZT and MeBZT in all except one and at concentrations usually > 1000 ng L⁻¹. The HRAP was not efficient in removing these two compounds, with average elimination not better than those obtained in conventional WWTPs. Their limited photodegradability and low sorption to biomass tendency could account for this lack of improvement, with elimination rates only attributable to biodegradation. The removal of BP3 was better than that of the benzotriazoles but still not higher than 51%. Nevertheless, further studies should be developed to confirm these results, including biomass analysis in order to establish complete mass balances. The ecotoxicity of the resulting effluent was neglectable; on the other hand, the role of the HRAP in reducing the environmental risk of the treated wastewater was not relevant either, considering either the low concentrations in the influent or the low removal efficiencies for some of the contaminants studied. Overall, data on the removal capacity of microalgae-based systems under real conditions is still scarce, with most of the studies developed under laboratory-controlled conditions and not considering the concentration in the biomass, which would help to understand the main removal mechanisms and predominant routes within these systems.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4441/12/10/2658/s1.

Table S1: Target PPCPs, classified by their chromatographic retention time, and their optimized UPLC–QqLIT-MS/MS parameters by positive ionization mode. Table S2: Solar irradiation and air temperatures registered during the days of the experiment. Table S3: Chromatographic retention time and optimized MS/MS transitions for the target analytes. Table S4: Solar irradiation and air temperatures registered during the days of the experiment. Table S5: Lineararity and method limits of quantification (LOQ), given in ng L⁻¹, for the targeted analytes. Table S5: Concentrations detected in the HRAP influent and effluent on every day of sampling. -_ not detected. Figure S1: Concentrations of the targeted compounds detected with frequencies of detection (F%) ≤ 17% (1 sample out of 6) in the HRAP.

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