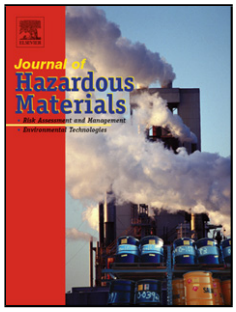

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Capability of microalgae-based wastewater treatment systems to remove emerging organic contaminants: a pilot-scale study

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Highlights (max. 85 characters per highlight including spaces):

- The effect of hydraulic retention time and seasonality has been evaluated.
- Removal efficiency ranged from undetectable removal to more than 90%.
- Biodegradation and photodegradation were the most important removal pathways.
- We suggested that microalgae enhance the biodegradation of emerging contaminants.
- Up to 90% of the contaminant toxicity risk was removed by microalgae treatment.

0. Abstract

The effect of hydraulic retention time (HRT) and seasonality on the removal efficiency of 26 organic microcontaminants from urban wastewater was studied in two pilot high-rate algal ponds (HRAPs). The targeted compounds included pharmaceuticals and personal care products, fire retardants, surfactants, anticorrosive agents, pesticides and plasticizers, among others. The pilot plant, which was fed at a surface loading rate of 7-29 g of COD m^{-2} d^{-1}, consisted of a homogenisation tank and two parallel lines, each one with a primary settler and an HRAP with a surface area of 1.5 m^2 and a volume of 0.5 m^3. The two HRAPs were operated with different HRTs (4 and 8 d). The removal efficiency ranged from negligible removal to more than 90% depending on the compound. Microcontaminant removal efficiencies were enhanced during the warm season, while the HRT effect on microcontaminant removal was only noticeable in the cold season. Our results suggest that biodegradation and photodegradation are the most important removal pathways, whereas volatilization and sorption were solely achieved for hydrophobic compounds (log Kow>4) with a moderately high Henry's law constant values (11-12 Pa m^{-3} mol^{-1}) such as musk fragrances. Whereas acetaminophen, ibuprofen and oxybenzone presented ecotoxicological hazard quotients (HQs) higher than 1 in the influent wastewater samples, the HQs for the effluent water samples were always below 1.

Keywords: *emerging organic contaminants; microalgae; high-rate algal pond; photodegradation; biodegradation; volatilization.*

1. Introduction

Emerging organic contaminants (EOCs) include a wide range of compounds belonging to different chemical classes, such as pharmaceuticals, personal care products, plasticizers, flame retardants, surfactants, and certain pesticides, among others, the ecotoxicological effects of which are relatively unknown [1]. Since conventional wastewater treatment plants (WWTPs) are not designed to remove emerging and related contaminants, many of these compounds occur at different concentrations in natural water bodies [2], where they may exert ecotoxicological effects at relatively low concentrations [3, 4]. Although some of the compounds have been proposed for inclusion on regulatory lists of contaminants (European Commission, 2006), there is relatively little information on the ecotoxicological effects of complex mixtures at environmental levels, and, to date, they have not been regulated [1]. Known environmental effects of some EOCs include the reduction of macroinvertebrate diversity in rivers [3], behavioural changes in mosquito fish [4] and reproductive disruption in fish [5], among others. Due to the difficulty of assessing the effects of EOCs on ecosystems, the use of hazard quotients (HQs) based on the chemical composition of water samples and tabulated predicted non-effect concentrations (PNECs) for different aquatic organisms has been postulated as a good screening strategy [6].

Microalgae-based wastewater treatment technologies such as high-rate algal ponds (HRAPs) have received considerable attention in recent years due to the resource recovery of algal biomass, for use as fertilizer, protein-rich feed or biofuel, and a high-quality effluent (treated wastewater)[7]. HRAPs are shallow raceway reactors in which microalgae and bacteria grow in symbiosis. In such systems,

organic matter is degraded by heterotrophic bacteria, which consume oxygen provided by microalgal photosynthesis; therefore, no aeration is needed [8]. Although the capability of microalgae wastewater treatment systems to remove nutrients, heavy metals, bacteria, and helminthic eggs has been studied since the 1950s, few studies have focused on the removal of organic contaminants, namely, phenolic compounds, surfactants, biocides and polycyclic aromatic hydrocarbons [9-12]. Indeed, no attention has been paid to the effectiveness of HRAPs for removing EOCs of environmental concern.

The removal of EOCs by conventional activated sludge WWTPs has been widely studied, but the effectiveness of HRAPs for removing EOCs from wastewater has not yet been addressed. There is only one study dealing with HRAPs' capacity to remove tetracyclines, and it was performed at laboratory-scale with synthetic wastewater [13]. Other studies dealing with microalgae's capacity to remove organic contaminants, such as polycyclic aromatic hydrocarbons (PAHs), biocides (e.g. organotin compounds), surfactants and phenolic compounds, suggest that microalgae–based wastewater technologies may remove microcontaminants by both abiotic (sorption, volatilization or photodegradation) and biotic (biodegradation, microalgae uptake or metabolization) processes [14-16].

The aim of this study was to evaluate for the first time, the effect of hydraulic retention time (HRT) and ambient temperature / sunlight irradiation (seasonality) on the removal efficiency of 26 EOCs in two HRAP pilot plants fed with real urban wastewater. The selected compounds were high production volume chemicals (e.g. fire retardants, surfactants, anticorrosive agents, pesticides, plasticizers, pharmaceuticals and personal care products, among others). Finally, aquatic risk assessment was performed based on the concentrations of the detected EOCs in the influent and effluent water samples, and the listed EC50 values for *Daphnia magna*.

2. Material and Methods

2.1. Chemicals and reagents

Gas chromatography (GC) grade (Suprasolv) hexane, methanol, and ethyl acetate were obtained from Merck (Darmstadt, Germany). Analytical-grade hydrogen chloride was obtained from Panreac (Barcelona, Spain). Caffeine, acetaminophen, ibuprofen, methyl dihydrojasmonate, oxybenzone, ketoprofen, hydrocinnamic acid, 5-methylbenzotriazole, naproxen, carbamazepine, galaxolide, benzothiazole, diclofenac, methylparaben, benzotriazole, tonalide, OH-benzothiazole, tributyl phosphate, tris(2-chloroethyl) phosphate, triphenyl phosphate, triclosan, cashmeran, octylphenol, diazinon, celestolide, atrazine, bisphenol A, 2,4-D, atrazine D5, mecoprop D3, tonalide D3 and dihydrocarbamazepine were purchased from Sigma-Aldrich (Steinheim, Germany). Trimethylsulfonium hydroxide (TMSH) was obtained from Fluka (Buchs, Switzerland). Strata-X polymeric SPE cartridges (200 mg) were purchased from Phenomenex (Torrance, CA, USA) and the 0.7 µm glass fibre filters (ø 47 mm) were obtained from Whatman (Maidstone, UK).

2.2. Description of the HRAP pilot plant

The experimental set-up was located outdoors at the laboratory of the GEMMA research group (Universitat Politècnica de Catalunya-BarcelonaTech, Spain). The system has been operated since March 2010. The microalgae production system was composed of a screening pre-treatment and two identical parallel lines, each one equipped with a primary settler, a pilot high-rate algal pond and a final settler for biomass separation (Fig. 1). Paddle wheel was set at 5 rpm giving mixed liquor with a linear velocity of recirculation of 11 cm s^{-1}, enough to ensure complete mixing. Urban wastewater was pumped from a municipal sewer to a homogenisation tank (1.2 m^3), which was continuously stirred to avoid solids sedimentation. From there, the wastewater was pre-treated and conveyed to each line. The primary treatment included a settler with an internal diameter of 0.3 m, a total height of 0.4 m and an effective volume of 7 L that was operated at an HRT of 0.9 h. Primary effluent from the settlers was pumped to the HRAPs by means of peristaltic pumps. The

123 experimental HRAPs were PVC raceway ponds equipped with a paddle wheel for stirring the mixed
124 liquor (Fig.1). The two HRAPs had a nominal volume of 0.47 m^3, a surface area of 1.54 m^2 and a
125 water depth of 0.3 m, and they were operated simultaneously with different HRTs (4 and 8 days
126 corresponding to 117.5 and 58.8 L d^{-1} respectively). The final settlers for biomass separation had an
127 internal diameter of 0.15 m, a total height of 0.3 m and an effective volume of 3.5 L that were
128 operated at an HRT of 0.7 and 1.4 h for the HRAP set at 4 days HRT and 8 days, respectively. Note
129 that these settlers were only used for biomass separation, which was not recycled back to the
130 HRAPs.

131

132 *2.3. Sampling strategy*

133 Two sampling campaigns were carried out, one in July 2013 (warm season) and the other in
134 December 2013 (cold season). In each campaign, influent and effluent grab samples were collected
135 from both HRAPs at the same time each day (9:00 am) for a period of 10 days (n=8), from Monday
136 to the Wednesday of the next week, Saturday and Sunday were not sampled. The samples were
137 collected in the primary effluent from the settler and at the effluent from both HRAPs (Fig. 1). No
138 rainfall events were recorded at any time during the sampling period. All water samples were
139 collected in 1000 mL amber glass bottles, which were transported under refrigeration to the
140 laboratory, where they were stored at 4 ºC until analysis. The sample holding time was less than 12
141 hours.

142

143 *2.4. Analytical procedures*

144 Conventional wastewater quality parameters, including ammonium nitrogen (NH_4-N), total
145 suspended solids (TSS) and chemical oxygen demand (COD), were determined using the Standard
146 Methods (APHA, 2001). Onsite measurements of water temperature, dissolved oxygen (DO) and

147 pH were taken using a Checktemp-1 Hanna thermometer, an Eutech Ecoscan DO6 oxygen meter
148 and a Crison pH-meter, respectively.

149 For each campaign, 2 well-mixed 25 mL samples from each HRAP were examined by light
150 microscopy and the predominant microalgae were identified and quantified. Microalgae genus were
151 identified from classical specific literature [17, 18].

152 All water samples were filtered and processed as previously reported [19]. A 100 mL sample was
153 spiked with 50 ng of a surrogate standard (atrazine D5, mecoprop D3, tonalide D3, and
154 dihydrocarbamazepine). The spiked sample was percolated through a previously activated
155 polymeric solid-phase extraction cartridge (200 mg Strata X). Elution was performed with 10 mL of
156 hexane/ethyl acetate (1:1). The eluted extract was evaporated under a gentle nitrogen stream until
157 ca. 100 µL remained, at which point 20 ng of triphenylamine was added as an internal standard.
158 Finally, the vial was reconstituted to 300 µL with ethyl acetate.

159 The TSS collected in the glass fibre filters (0.7 µm) were processed according to a previously
160 reported analytical method [19]. Briefly, the filters were freeze-dried and extracted in an ultrasonic
161 bath with hexane/acetone (3:1) for 15 minutes. The extracts were then further processed as water
162 samples.

163 Methylation of the acidic carboxyl group was performed in a hot GC PTV injector (270 ºC) by
164 adding 10 µL of TMSH solution (0.25 mol L^{-1} in methanol) to a 50 µL sample before injection.
165 Derivatized samples were analysed into a Bruker 450-GC gas chromatograph coupled to a Bruker
166 320-MS triple quadrupole mass spectrometer (Bruker Daltonics Inc., Billerica, MA, USA) fitted
167 with a 20 m × 0.18 mm, 0.18 µm film thickness Sapiens X5-MS capillary column coated with 5%
168 diphenyl 95% dimethyl polysiloxane from Teknokroma (Sant Cugat del Vallès, Spain) operated in
169 the multiple reaction mode (MRM). Validation of the analytical methodology has been described
170 elsewhere [20]. The limit of detection (LOD) and limit of quantification (LOQ) of the analytical
171 methodology were determined (using ultra-pure water) based on the mean background noise plus 3

or 10 times the standard deviation of the background noise, respectively. The LOD and LOQ ranged from 1 to 40 ng L^{-1} and from 3 to 80 ng L^{-1}, respectively. Recoveries and repeatability were always higher than 80% and lower than 20%, respectively.

2.5. Data analysis

The removal efficiencies of conventional water quality parameters and EOCs were calculated as follows (equation 1):

$$Removal = \frac{1}{n}\sum_{i=1}^{n}\frac{C'a - \left(Ci - Ci \times \frac{EVR}{HLR}\right)}{C'a} \times 100 \qquad \textit{(Equation 1)}$$

where *C'a* is the average concentration of a selected compound in the HRAP influents in each sampling campaign, *Ci* is the concentration in the HRAP effluents on each sampling day, and *n* is the number of samples collected per sampling campaign (n=8). HLR are 83 or 43 L m^{-2} d^{-1} at a HRT of 8 and 4 days, respectively. Evaporation rates (EVRs) are 21 and 9 L m^{-2} d^{-1} in warm and cold season, respectively calculated from Turc's equation.

The experimental results were statistically evaluated using the SPSS v.13 package (Chicago, IL, USA). According with the data set size, non-parametric statistics were applied. The comparison of means was conducted by means of the Kruskal–Wallis test. Spearman's coefficients were used for correlations between variables (physicochemical parameters, removal efficiencies and influent concentration). Significance was defined as $p < 0.05$.

3. Results and Discussion

3.1 Assessment of conventional water quality parameters

The HRAPs' performance was monitored in warm and cold seasons (Table 1). COD, TSS and NH_4-N concentrations in the primary settlers' effluents were within the typical range of a conventional primary effluent [21]. Note that DO concentration was quite high for a primary effluent due to wastewater stirring in the homogenisation tank. The actual surface organic loading rates (OLRs) applied to the HRAPs were, on average, 13 and 26 g total COD m^{-2} d^{-1} (HRT=8 and 4 d respectively) in the warm season and 29 and 58 g total COD m^{-2} d^{-1} (HRT=8 and 4 d respectively) in the cold season.

The microalgae present in the HRAPs were mostly species of the Phylum *Chlorophyta*, and varied depending on seasonal conditions. In the warm season, predominant algae species were *Stigeoclonium* sp. (filamentous algae); diatoms, *Chlorella* sp. and *Monoraphidium* sp. In the cold season, predominant algae species were *Chlorella* sp.; diatoms, *Stigeoclonium* sp. In both campaigns, the number of species was higher in the HRAP set at HRT of 8 days.

In the present study, mixed liquor TSS was analysed as an indicator of biomass concentration in the HRAP (Table 1). Note that biomass in such type of systems corresponds to microalgae as well as bacteria, and the relative proportion of them can be approached by means of mass balances on an estimated net yield of heterotrophic bacteria [22]. Thus, if the yield for domestic wastewater aerobic treatment is around 0.25 g VSS/g bCOD (biodegradable COD), assuming bCOD = 0.8 COD and TSS = 1.35 VSS; the heterotrophic bacteria concentration expected is approximately 30 mg TSS L^{-1} in summer and 75 mg TSS L^{-1} in winter (for both HRT). This then implies an algae biomass concentration near 290 (HRT 4 days) -320 (HRT 8 days) mg TSS L^{-1} in summer against 35 (HRT 4 days) -75 (HRT 8 days) mg TSS L^{-1} in winter. These numbers agree with experimental observations

and meteorological data: average microalgal biomass concentration and production were clearly higher in the warm season than in the cold season in relation with the higher solar radiation (Table 1). In addition, put into evidence that algal activity was clearly lower in winter than in summer, although enough to produce changes in the DO concentration and the pH of the mixed liquor (Table 1). Biomass concentration was slightly higher in the HRAP with a HRT 8 days where lower flow-rate gave place to less biomass wash-up, as already observed in other studies [23-25]. Despite this, biomass production was higher in the HRAP operated at a HRT of 4 days, in these systems biomass production usually increases inversely with the HRT [26].

Biomass production values are in accordance with those previously reported [27]. De Godos et al. [28] observed a biomass production of 21.3-27.7 g TSS m^{-2} d^{-1} in summer (average daily solar radiation of 282 W m^{-2}) for HRAPs operating at 10 HRT fed with diluted swine manure. In winter period (average daily solar radiation of 74 W m^{-2}) biomass production decreased to 5.7-6.1 g TSS m^{-2} d^{-1}. García et al. [29] using the same HRAPs as in the present study reported a production between 12.7 and 14.8 m^{-2} d^{-1}. The HRAPs' performance (Table 1) was consistent throughout the experimental period, with removal efficiencies similar to those reported in previous studies for this pilot plant [8] and others previously reported [27]. COD removal was moderate (66-85%) and its removal in these systems depends on influent concentration because background concentration remains around 50-70 mg L^{-1} [29]. Up to 99% of NH_4-N was removed in the HRAPs in the warm season at both HRTs, whereas the removal rate was lower and different at both HRTs (90 *vs* 98%) in the cold season. Hence, environmental conditions (i.e. temperature and solar radiation) played an important role in NH_4-N removal, whereas HRT was only relevant in the cold season. Mechanisms for nitrogen removal have been studied in detail in the past and the most predominant include volatilisation, biological uptake and nitrification [27, 30]. NH_4-N removal values were similar to those found in the literature. De Godos et al. [28] found in summer a COD and NH_4-N removal of 76 and 96% respectively, whereas in winter those removal decreased to 57 and 92% for HRAPs

operating at a HRT of 10 days. Sutherland et al. [31] found a decrease on NH_4-N removal between summer (77%) and winter (53%) in a HRAP operating at a HRT of 4 and 9 days respectively.

3.2. Occurrence and removal efficiency of EOCs

For this study, we selected the emerging contaminants with the highest concentrations that are most often detected in raw wastewaters [32]. The concentration of EOCs in HRAP influent (primary effluent) ranged from undetected to 24 μg L^{-1} (Fig. 2). Caffeine, acetaminophen and ibuprofen were usually detected at concentrations higher than 9 μg L^{-1}, in keeping with the reported literature [33]. Although the influent COD showed a high seasonal variability (Table 1), the concentration of the studied EOCs showed no statistically significant seasonal difference ($p=0.84$). This may be explained by the presence of a primary treatment that favours a more constant influent wastewater in terms of quality, as can be seen in the low variability of the EOC concentrations (Fig. 2).

Table 2 shows the removal efficiencies of the target EOCs. They can be classified into four groups in accordance with the corresponding overall average removal efficiency in the HRAPs: high removal (>90%: caffeine, acetaminophen, ibuprofen, methyl dihydrojasmonate and hydrocinnamic acid), moderate-to-high removal (from 60% to 90%: oxybenzone, ketoprofen, 5-methyl/benzotriazole, naproxen, galaxolide, tonalide, tributyl phosphate, triclosan, bisphenol A and octylphenol), moderate-to-low removal (from 40 to 60%: diclofenac, benzotriazole, OH-benzothiazole, triphenyl phosphate, cashmeran, diazinon, benzothiazole, celestolide, 2,4-D and atrazine) and poor or no removal (<40%, carbamazepine, methyl paraben, tris(2-chloroethyl) phosphate).

Taking into account the configuration of the HRAPs, the most relevant removal processes that may occur in these systems can be biodegradation, photodegradation, volatilization and sorption to microalgae biomass. Uptake by microalgae is an important removal process, and it was assessed by analysing the occurrence of EOCs in the TSS (solids retained in the filters). Table 1 in the Supplementary Material (SM) shows that the most abundant compounds in the biomass (mostly

271 microalgae) were the most hydrophobic ones, such as galaxolide and tonalide (log Kow>5). Hence,
272 HRAPs may remove hydrophobic compounds by sorption, similarly to other wastewater treatment
273 technologies such as constructed wetlands (CWs) and activated sludge systems [34, 35]. Despite the
274 higher concentration of TSS in the HRAPs during the warm season, due to the greater biomass
275 production and evaporation losses (Table 1), the concentration of musk fragrances in the TSS was
276 higher in winter. Therefore, it may be postulated that the increase in biomass (microalgae,
277 heterotrophs and non-photosynthetic autotrophs organisms) improved the biodegradability of these
278 compounds or that the higher sunlight irradiation and temperatures in warm season improved the
279 volatilization rates. This is in keeping with the moderate biodegradability found for musk fragrances
280 (>75%) in lab-scale activated sludge reactors [36] and the tabulated high Henry's law constants (a
281 measure of air-water partitioning) for musk fragrances (11-12 Pa m^{-3} mol^{-1}). The occurrence of most
282 of the studied EOCs in the filters was below their LOD. This may be due to the fact that these EOCs
283 were not uptaken by microalgae or because they were removed by microalgal metabolism. A
284 microalgae removal effect due to the release of exudates likewise cannot be disregarded [37]. In
285 fact, it has been proved that the consortia of cyanobacteria/microalgae and bacteria can be efficient
286 in detoxification of organic and inorganic pollutants, and removal of nutrients from wastewaters,
287 compared to the individual microorganisms. Cyanobacterial/algal photosynthesis provides oxygen
288 and organic exudates that serves to the pollutant-degrading heterotrophic bacteria [38].

289 The overall average removal efficiencies of the studied EOCs were plotted against their
290 physicochemical properties (log Kow, molecular weight (MW) and Henry's law constant) as is
291 shown in Fig. 1 SM. Although the plots seem to show a relationship between the EOC removal
292 efficiencies and MW (Spearman's correlation coefficient = -0.197), log Kow (Spearman's
293 correlation coefficient = -0.080) and Henry's law constant (Spearman's correlation coefficient = -
294 0.075), no significant correlations were found (significance level >0.05). This may be explained by
295 the complexity of the chemical compounds studied as well as the fact that different removal

296 processes occurred simultaneously. Conversely, a statistically significant relationship between
297 influent concentration and removal efficiency was obtained (Spearman's correlation coefficient =
298 0.627, significance level =0.002). This may be explained by the fact that biodegradation needs a
299 certain compound concentration before microbial degradation is stimulated. Nevertheless, this
300 general rule must be applied with care and further work is necessary in this field.

301 The removal efficiency of HRAPs is comparable to that of conventional activated sludge WWTPs,
302 as can be seen in Table 2 (overall removal efficiencies of 84% and 59% in warm and cold season
303 respectively). Hijosa-Valsero et al. [39] found that waste stabilization ponds (WSPs) were capable
304 of moderately removing pharmaceuticals such as naproxen (33%) and ibuprofen (56%). Matamoros
305 et al. [40] found that unsaturated CWs were capable of removing up to 90% of pharmaceuticals and
306 personal care products such as ibuprofen, naproxen and galaxolide, but did not remove
307 carbamazepine. Hence, HRAPs seem to be as or more efficient than other biological wastewater
308 treatment technologies, such as CWs or WSPs, with regard to EOC removal. Therefore, HRAPs can
309 be considered a suitable technology for the treatment of wastewaters containing EOCs, with the
310 added advantage that they produce microalgal biomass, do not require aeration and have smaller
311 land area requirements than other engineered natural wastewater treatment technologies (i.e. CWs
312 and WSPs). Nevertheless, since the effectiveness of engineered natural wastewater treatment
313 technologies for removing EOCs has been shown to rely on different key design and environmental
314 factors, such as HRT and seasonality [41], the next two sections will explore the influence of these
315 factors on HRAPs.

316

317 *3.2.1. Effect of hydraulic retention time (HRT)*

318 HRT is a key design parameter for achieving proper removal efficiency of biodegradable organic
319 contaminants from wastewaters engineered natural treatment systems such as constructed wetlands
320 and waste stabilization ponds [33]. In fact, it has already proved that EOC removal in engineered

natural treatment systems and activated sludge WWTPs increases as HRT increases due to the increase of biodegradation and sorption processes [34, 42]. No significant differences in their performance were observed between HRTs in the warm season ($p>0.05$), but significant differences were found in the cold season for those compounds that has already been described in the literature [41] as been removed by biodegradation (i.e. caffeine, 4%; ibuprofen, 7%; methyl dihydrojasmonate, 5%; oxybenzone, 13%; naproxen, 8% and triphenyl phosphate, 44%), photodegradegradation (i.e. ketoprofen; 25% and triclosan, 20%) and sorption or volatilization (i.e. galaxolide, 24% and tonalide, 16%). As already noted, conventional water quality parameters such as COD and NH_4-N behaved similarly. From these results, it can be postulated that biodegradation, photodegradation, sorption and volatilization removal mechanism were likely affected by the increase of HRT in the cold season. García-Rodríguez et al. [41] reported that biological wastewater treatment technologies for removing EOCs are highly dependent on HRT because it enhances biodegradation, photodegradation and sorption removal processes. In general, the higher the HRT, the greater the EOC removal efficiency. However, our results suggest that an HRT of 4 days is enough to remove most of the compounds in both seasons. Therefore, while this technology is competitive in terms of HRT compared to CWs and WSPs, activated sludge WWTPs are generally set at an HRT of 12-24 hours or lower. Notwithstanding the foregoing, activated sludge WWTPs also have higher energy requirements (0.6 kWh m^{-3} for activated sludge WWTPs *vs.* 0.02 kWh m^{-3} for HRAPs). Finally, the lower microcontaminant sorption onto the biomass (table 1-SM) than in conventional activated sludge WWTPs biosolids [43] is relevant for risk management and sludge valorisation. This low bioaccumulation of microcontaminants into the biomass have already been reported for vegetables [44], but this is the first time that it has been assessed for microalgae.

3.2.2 Seasonality (environmental conditions)

Seasonality is relevant to achieving adequate EOC removal efficiency in mild climates such as that

of the NW Mediterranean because it affects temperature, daylight duration and intensity, and biomass production, four important factors influencing biodegradation, photodegradation, volatilization and sorption EOC removal processes [41]. Significant differences ($p<0.05$) between the warm and cold seasons were observed for the removal of above described biodegradable compounds (i.e. caffeine, ibuprofen, methyl dihydrojasmonate, oxybenzone, naproxen, benzothiazole, methylparaben, benzotriazole, 5-methyl-benzotriazole, OH-benzothiazole, and triphenyl phosphate), photodegradable compounds (i.e. ketoprofen, diclofenac, and triclosan) and highly hydrophobic / moderately volatile compounds (i.e. galaxolide and tonalide). The higher temperature (11 *vs.* 26 ºC, on a daily average) and greater average daily solar radiation (74 *vs.* 282 W m^{-2}) in the warm season may explain these differences. It should be noted that the effect of seasonality on the pollutant removal performance of this technology for the most abundant compounds was low (around 10-20%) or null (i.e. for caffeine, acetaminophen, ibuprofen and methyl dihydrojasmonate). In contrast, various authors [45-47] have reported higher seasonal variability for EOC removal by other engineered natural wastewater treatment technologies (CWs or WSPs). Hence, although the HRAP technology seems to be a robust and reliable wastewater treatment technology in terms of EOC removal efficiency, further studies are required to provide more insight.

3.3. Aquatic risk assessment

Aquatic risk assessment throughout the HRAP treatment was performed based on the concentrations of the detected EOCs in the influent and effluent water samples, and the listed EC50 values for *Daphnia magna.* Hazard quotient indexes (HQs) were calculated according to the following equation (2):

$$HQ = \frac{MEC}{PNEC} \quad \textit{(Equation 2)}$$

where PNEC is the predicted non-effect concentration and MEC is the measured environmental concentration at the influent or effluent of each HRAP reactor. PNEC values were estimated for *Daphnia magna*, dividing the EC50 values (48 hours) by a recommended arbitrary safety factor of 1000 [48]. The EC50 values used in this study were collected from the literature and are summarized in Table 2SM. When more than one EC50 value was reported for a single compound, the lowest value was used. When no experimental values were available, the EC50 values were estimated with ECOSAR v1.10 (EPI Suite software, US EPA).

Fig. 3 shows the individual HQ for each of the studied EOCs in the HRAP influent and effluent water samples. As the difference between the EOC removal efficiencies at both of the studied HRTs was minimal, the risk assessment was only performed at an HRT of 4 days (under the most critical operating conditions). Acetaminophen, ibuprofen and oxybenzone exhibited higher HQs in influent wastewater samples (HQ>1), mainly due to their high concentration. Following the treatment in the HRAP system, all of the studied EOCs had an HQ<1. The most relevant compounds in the treated wastewater effluents, apart from those observed at the influent, were triclosan and galaxolide. These HQ values for the EOCs are in keeping with those reported in the literature for treated wastewater effluents [42]. In fact, triclosan has already been postulated as a critical compound in terms of contribution and environmental risk in wastewater effluents [49]. Hence, von der Ohe et al. [50] argued that triclosan should be seriously considered as a candidate for regulatory monitoring and prioritization on a European scale on the basis of realistic PNECs.

As previously described by various authors, HQs should follow an additive model [6]. Hence, the final HQ for each water sample can be calculated as the sum of each individual HQ. The cumulative HQs for the influent wastewater samples were 8.45 and 6.20 for the summer and winter campaigns, respectively. However, they fell 93% (warm season) and 72% (cold season) following the treatment with the HRAPs. Consequently, in summer the cumulative HQ was less than 1 (0.62), whereas in

winter it was significantly higher (HQ=1.73). These results are in keeping with the high reduction in acute toxicity achieved by other biological wastewater treatment technologies, such as CWs [51]. Nevertheless, this ecological risk assessment was only performed for the target EOCs; therefore, further studies may be needed to include other EOCs and related transformation products.

4. Conclusions

This study has shown that microalgae-based wastewater treatment systems (such as HRAPs) enable the removal of a wide range of EOCs from urban wastewater. Removal efficiency ranged from none to up to 99%. The EOCs were classified into four groups in accordance with their average removal efficiency in HRAPs: high removal (>90%: caffeine, acetaminophen, ibuprofen, methyl dihydrojasmonate and hydrocinnamic acid), moderate-high removal (from 60% to 90%: oxybenzone, ketoprofen, 5-methyl/benzotriazole, naproxen, galaxolide, tonalide, tributyl phosphate, triclosan, bisphenol A and octylphenol), moderate-low removal (from 40 to 60%: diclofenac, benzotriazole, OH-benzothiazole, triphenyl phosphate, cashmeran, diazinon, celestolide and atrazine) and poor or no removal (<30%, carbamazepine, benzothiazole, methyl paraben, tris(2-chloroethyl) phosphate, and 2,4-D). The removal of emerging contaminants in HRAPs was only affected by the HRT during the cold season, whereas no differences where observed in the warm season. The most frequently occurring compounds (caffeine, acetaminophen and ibuprofen) had removal efficiencies of up to 90% that were minimally affected by seasonality and HRT. The ecotoxicological risk assessment study revealed that the HQ for the influent wastewater was removed by up to 90%, indicating no acute toxicity risk associated with the studied EOCs at the water effluents.

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Capability of microalgae-based wastewater treatment systems to remove emerging organic contaminants: a pilot-scale study

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0. Abstract

The effect of hydraulic retention time (HRT) and seasonality on the removal efficiency of 26 organic microcontaminants from urban wastewater was studied in two pilot high-rate algal ponds (HRAPs). The targeted compounds included pharmaceuticals and personal care products, fire retardants, surfactants, anticorrosive agents, pesticides and plasticizers, among others. The pilot plant, which was fed at a surface loading rate of 7-29 g of COD m^{-2} d^{-1}, consisted of a homogenisation tank and two parallel lines, each one with a primary settler and an HRAP with a surface area of 1.5 m^2 and a volume of 0.5 m^3. The two HRAPs were operated with different HRTs (4 and 8 d). The removal efficiency ranged from negligible removal to more than 90% depending on the compound. Microcontaminant removal efficiencies were enhanced during the warm season, while the HRT effect on microcontaminant removal was only noticeable in the cold season. Our results suggest that biodegradation and photodegradation are the most important removal pathways, whereas volatilization and sorption were solely achieved for hydrophobic compounds (log Kow>4) with a moderately high Henry's law constant values (11-12 Pa m^{-3} mol^{-1}) such as musk fragrances. Whereas acetaminophen, ibuprofen and oxybenzone presented ecotoxicological hazard quotients (HQs) higher than 1 in the influent wastewater samples, the HQs for the effluent water samples were always below 1.

Keywords: *emerging organic contaminants; microalgae; high-rate algal pond; photodegradation; biodegradation; volatilization.*

1. Introduction

Emerging organic contaminants (EOCs) include a wide range of compounds belonging to different chemical classes, such as pharmaceuticals, personal care products, plasticizers, flame retardants, surfactants, and certain pesticides, among others, the ecotoxicological effects of which are relatively unknown [1]. Since conventional wastewater treatment plants (WWTPs) are not designed to remove emerging and related contaminants, many of these compounds occur at different concentrations in natural water bodies [2], where they may exert ecotoxicological effects at relatively low concentrations [3, 4]. Although some of the compounds have been proposed for inclusion on regulatory lists of contaminants (European Commission, 2006), there is relatively little information on the ecotoxicological effects of complex mixtures at environmental levels, and, to date, they have not been regulated [1]. Known environmental effects of some EOCs include the reduction of macroinvertebrate diversity in rivers [3], behavioural changes in mosquito fish [4] and reproductive disruption in fish [5], among others. Due to the difficulty of assessing the effects of EOCs on ecosystems, the use of hazard quotients (HQs) based on the chemical composition of water samples and tabulated predicted non-effect concentrations (PNECs) for different aquatic organisms has been postulated as a good screening strategy [6].

Microalgae-based wastewater treatment technologies such as high-rate algal ponds (HRAPs) have received considerable attention in recent years due to the resource recovery of algal biomass, for use as fertilizer, protein-rich feed or biofuel, and a high-quality effluent (treated wastewater)[7]. HRAPs are shallow raceway reactors in which microalgae and bacteria grow in symbiosis. In such systems, organic matter is degraded by heterotrophic bacteria, which consume oxygen provided by microalgal photosynthesis; therefore, no aeration is needed [8]. Although the capability of microalgae wastewater treatment systems to remove nutrients, heavy metals, bacteria, and helminthic eggs has been studied since the 1950s, few studies have focused on the removal of organic contaminants, namely, phenolic compounds, surfactants, biocides and polycyclic aromatic

633 hydrocarbons [9-12]. Indeed, no attention has been paid to the effectiveness of HRAPs for
634 removing EOCs of environmental concern.

635 The removal of EOCs by conventional activated sludge WWTPs has been widely studied, but the
636 effectiveness of HRAPs for removing EOCs from wastewater has not yet been addressed. There is
637 only one study dealing with HRAPs' capacity to remove tetracyclines, and it was performed at
638 laboratory-scale with synthetic wastewater [13]. Other studies dealing with microalgae's capacity to
639 remove organic contaminants, such as polycyclic aromatic hydrocarbons (PAHs), biocides (e.g.
640 organotin compounds), surfactants and phenolic compounds, suggest that microalgae–based
641 wastewater technologies may remove microcontaminants by both abiotic (sorption, volatilization or
642 photodegradation) and biotic (biodegradation, microalgae uptake or metabolization) processes [14-
643 16].

644 The aim of this study was to evaluate for the first time, the effect of hydraulic retention time (HRT)
645 and ambient temperature / sunlight irradiation (seasonality) on the removal efficiency of 26 EOCs
646 in two HRAP pilot plants fed with real urban wastewater. The selected compounds were high
647 production volume chemicals (e.g. fire retardants, surfactants, anticorrosive agents, pesticides,
648 plasticizers, pharmaceuticals and personal care products, among others). Finally, aquatic risk
649 assessment was performed based on the concentrations of the detected EOCs in the influent and
650 effluent water samples, and the listed EC50 values for *Daphnia magna*.

651

652 **2. Material and Methods**

653 *2.1. Chemicals and reagents*

654 Gas chromatography (GC) grade (Suprasolv) hexane, methanol, and ethyl acetate were obtained
655 from Merck (Darmstadt, Germany). Analytical-grade hydrogen chloride was obtained from Panreac
656 (Barcelona, Spain). Caffeine, acetaminophen, ibuprofen, methyl dihydrojasmonate, oxybenzone,

ketoprofen, hydrocinnamic acid, 5-methylbenzotriazole, naproxen, carbamazepine, galaxolide, benzothiazole, diclofenac, methylparaben, benzotriazole, tonalide, OH-benzothiazole, tributyl phosphate, tris(2-chloroethyl) phosphate, triphenyl phosphate, triclosan, cashmeran, octylphenol, diazinon, celestolide, atrazine, bisphenol A, 2,4-D, atrazine D5, mecoprop D3, tonalide D3 and dihydrocarbamazepine were purchased from Sigma-Aldrich (Steinheim, Germany). Trimethylsulfonium hydroxide (TMSH) was obtained from Fluka (Buchs, Switzerland). Strata-X polymeric SPE cartridges (200 mg) were purchased from Phenomenex (Torrance, CA, USA) and the 0.7 µm glass fibre filters (ø 47 mm) were obtained from Whatman (Maidstone, UK).

2.2. Description of the HRAP pilot plant

The experimental set-up was located outdoors at the laboratory of the GEMMA research group (Universitat Politècnica de Catalunya-BarcelonaTech, Spain). The system has been operated since March 2010. The microalgae production system was composed of a screening pre-treatment and two identical parallel lines, each one equipped with a primary settler, a pilot high-rate algal pond and a final settler for biomass separation (Fig. 1). Paddle wheel was set at 5 rpm giving mixed liquor with a linear velocity of recirculation of 11 cm s^{-1}, enough to ensure complete mixing. Urban wastewater was pumped from a municipal sewer to a homogenisation tank (1.2 m^3), which was continuously stirred to avoid solids sedimentation. From there, the wastewater was pre-treated and conveyed to each line. The primary treatment included a settler with an internal diameter of 0.3 m, a total height of 0.4 m and an effective volume of 7 L that was operated at an HRT of 0.9 h. Primary effluent from the settlers was pumped to the HRAPs by means of peristaltic pumps. The experimental HRAPs were PVC raceway ponds equipped with a paddle wheel for stirring the mixed liquor (Fig.1). The two HRAPs had a nominal volume of 0.47 m^3, a surface area of 1.54 m^2 and a water depth of 0.3 m, and they were operated simultaneously with different HRTs (4 and 8 days corresponding to 117.5 and 58.8 L d^{-1} respectively). The final settlers for biomass separation had an

682 internal diameter of 0.15 m, a total height of 0.3 m and an effective volume of 3.5 L that were
683 operated at an HRT of 0.7 and 1.4 h for the HRAP set at 4 days HRT and 8 days, respectively. Note
684 that these settlers were only used for biomass separation, which was not recycled back to the
685 HRAPs.

686

687 *2.3. Sampling strategy*

688 Two sampling campaigns were carried out, one in July 2013 (warm season) and the other in
689 December 2013 (cold season). In each campaign, influent and effluent grab samples were collected
690 from both HRAPs at the same time each day (9:00 am) for a period of 10 days (n=8), from Monday
691 to the Wednesday of the next week, Saturday and Sunday were not sampled. The samples were
692 collected in the primary effluent from the settler and at the effluent from both HRAPs (Fig. 1). No
693 rainfall events were recorded at any time during the sampling period. All water samples were
694 collected in 1000 mL amber glass bottles, which were transported under refrigeration to the
695 laboratory, where they were stored at 4 ºC until analysis. The sample holding time was less than 12
696 hours.

697

698 *2.4. Analytical procedures*

699 Conventional wastewater quality parameters, including ammonium nitrogen (NH_4-N), total
700 suspended solids (TSS) and chemical oxygen demand (COD), were determined using the Standard
701 Methods (APHA, 2001). Onsite measurements of water temperature, dissolved oxygen (DO) and
702 pH were taken using a Checktemp-1 Hanna thermometer, an Eutech Ecoscan DO6 oxygen meter
703 and a Crison pH-meter, respectively.

For each campaign, 2 well-mixed 25 mL samples from each HRAP were examined by light microscopy and the predominant microalgae were identified and quantified. Microalgae genus were identified from classical specific literature [17, 18].

All water samples were filtered and processed as previously reported [19]. A 100 mL sample was spiked with 50 ng of a surrogate standard (atrazine D5, mecoprop D3, tonalide D3, and dihydrocarbamazepine). The spiked sample was percolated through a previously activated polymeric solid-phase extraction cartridge (200 mg Strata X). Elution was performed with 10 mL of hexane/ethyl acetate (1:1). The eluted extract was evaporated under a gentle nitrogen stream until ca. 100 µL remained, at which point 20 ng of triphenylamine was added as an internal standard. Finally, the vial was reconstituted to 300 µL with ethyl acetate.

The TSS collected in the glass fibre filters (0.7 µm) were processed according to a previously reported analytical method [19]. Briefly, the filters were freeze-dried and extracted in an ultrasonic bath with hexane/acetone (3:1) for 15 minutes. The extracts were then further processed as water samples.

Methylation of the acidic carboxyl group was performed in a hot GC PTV injector (270 ºC) by adding 10 µL of TMSH solution (0.25 mol L^{-1} in methanol) to a 50 µL sample before injection. Derivatized samples were analysed into a Bruker 450-GC gas chromatograph coupled to a Bruker 320-MS triple quadrupole mass spectrometer (Bruker Daltonics Inc., Billerica, MA, USA) fitted with a 20 m × 0.18 mm, 0.18 µm film thickness Sapiens X5-MS capillary column coated with 5% diphenyl 95% dimethyl polysiloxane from Teknokroma (Sant Cugat del Vallès, Spain) operated in the multiple reaction mode (MRM). Validation of the analytical methodology has been described elsewhere [20]. The limit of detection (LOD) and limit of quantification (LOQ) of the analytical methodology were determined (using ultra-pure water) based on the mean background noise plus 3 or 10 times the standard deviation of the background noise, respectively. The LOD and LOQ ranged from 1 to 40 ng L^{-1} and from 3 to 80 ng L^{-1}, respectively. Recoveries and repeatability were always

729 higher than 80% and lower than 20%, respectively.

730

731 2.5. Data analysis

732 The removal efficiencies of conventional water quality parameters and EOCs were calculated as
733 follows (equation 1):

734

735

736 $$Removal = \frac{1}{n}\sum_{i=1}^{n}\frac{C'a - \left(Ci - Ci \times \frac{EVR}{HLR}\right)}{C'a} \times 100 \qquad \textit{(Equation 1)}$$

737

738 where *C'a* is the average concentration of a selected compound in the HRAP influents in each
739 sampling campaign, *Ci* is the concentration in the HRAP effluents on each sampling day, and *n* is
740 the number of samples collected per sampling campaign (n=8). HLR are 83 or 43 L m^{-2} d^{-1} at a HRT
741 of 8 and 4 days, respectively. Evaporation rates (EVRs) are 21 and 9 L m^{-2} d^{-1} in warm and cold
742 season, respectively calculated from Turc's equation.

743

744 The experimental results were statistically evaluated using the SPSS v.13 package (Chicago, IL,
745 USA). According with the data set size, non-parametric statistics were applied. The comparison of
746 means was conducted by means of the Kruskal–Wallis test. Spearman's coefficients were used for
747 correlations between variables (physicochemical parameters, removal efficiencies and influent
748 concentration). Significance was defined as $p < 0.05$.

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752

3. Results and Discussion

3.1 Assessment of conventional water quality parameters

The HRAPs' performance was monitored in warm and cold seasons (Table 1). COD, TSS and NH_4-N concentrations in the primary settlers' effluents were within the typical range of a conventional primary effluent [21]. Note that DO concentration was quite high for a primary effluent due to wastewater stirring in the homogenisation tank. The actual surface organic loading rates (OLRs) applied to the HRAPs were, on average, 13 and 26 g total COD m^{-2} d^{-1} (HRT=8 and 4 d respectively) in the warm season and 29 and 58 g total COD m^{-2} d^{-1} (HRT=8 and 4 d respectively) in the cold season.

The microalgae present in the HRAPs were mostly species of the Phylum *Chlorophyta*, and varied depending on seasonal conditions. In the warm season, predominant algae species were *Stigeoclonium* sp. (filamentous algae); diatoms, *Chlorella* sp. and *Monoraphidium* sp. In the cold season, predominant algae species were *Chlorella* sp.; diatoms, *Stigeoclonium* sp. In both campaigns, the number of species was higher in the HRAP set at HRT of 8 days.

In the present study, mixed liquor TSS was analysed as an indicator of biomass concentration in the HRAP (Table 1). Note that biomass in such type of systems corresponds to microalgae as well as bacteria, and the relative proportion of them can be approached by means of mass balances on an estimated net yield of heterotrophic bacteria [22]. Thus, if the yield for domestic wastewater aerobic treatment is around 0.25 g VSS/g bCOD (biodegradable COD), assuming bCOD = 0.8 COD and TSS = 1.35 VSS; the heterotrophic bacteria concentration expected is approximately 30 mg TSS L^{-1} in summer and 75 mg TSS L^{-1} in winter (for both HRT). This then implies an algae biomass concentration near 290 (HRT 4 days) -320 (HRT 8 days) mg TSS L^{-1} in summer against 35 (HRT 4 days) -75 (HRT 8 days) mg TSS L^{-1} in winter. These numbers agree with experimental observations and meteorological data: average microalgal biomass concentration and production were clearly higher in the warm season than in the cold season in relation with the higher solar radiation (Table

1). In addition, put into evidence that algal activity was clearly lower in winter than in summer, although enough to produce changes in the DO concentration and the pH of the mixed liquor (Table 1). Biomass concentration was slightly higher in the HRAP with a HRT 8 days where lower flow-rate gave place to less biomass wash-up, as already observed in other studies [23-25]. Despite this, biomass production was higher in the HRAP operated at a HRT of 4 days, in these systems biomass production usually increases inversely with the HRT [26].

Biomass production values are in accordance with those previously reported [27]. De Godos et al. [28] observed a biomass production of 21.3-27.7 g TSS m^{-2} d^{-1} in summer (average daily solar radiation of 282 W m^{-2}) for HRAPs operating at 10 HRT fed with diluted swine manure. In winter period (average daily solar radiation of 74 W m^{-2}) biomass production decreased to 5.7-6.1 g TSS m^{-2} d^{-1}. García et al. [29] using the same HRAPs as in the present study reported a production between 12.7 and 14.8 m^{-2} d^{-1}. The HRAPs' performance (Table 1) was consistent throughout the experimental period, with removal efficiencies similar to those reported in previous studies for this pilot plant [8] and others previously reported [27]. COD removal was moderate (66-85%) and its removal in these systems depends on influent concentration because background concentration remains around 50-70 mg L^{-1} [29]. Up to 99% of NH_4-N was removed in the HRAPs in the warm season at both HRTs, whereas the removal rate was lower and different at both HRTs (90 *vs* 98%) in the cold season. Hence, environmental conditions (i.e. temperature and solar radiation) played an important role in NH_4-N removal, whereas HRT was only relevant in the cold season. Mechanisms for nitrogen removal have been studied in detail in the past and the most predominant include volatilisation, biological uptake and nitrification [27, 30]. NH_4-N removal values were similar to those found in the literature. De Godos et al. [28] found in summer a COD and NH_4-N removal of 76 and 96% respectively, whereas in winter those removal decreased to 57 and 92% for HRAPs operating at a HRT of 10 days. Sutherland et al. [31] found a decrease on NH_4-N removal between summer (77%) and winter (53%) in a HRAP operating at a HRT of 4 and 9 days respectively.

3.2. Occurrence and removal efficiency of EOCs

For this study, we selected the emerging contaminants with the highest concentrations that are most often detected in raw wastewaters [32]. The concentration of EOCs in HRAP influent (primary effluent) ranged from undetected to 24 μg L^{-1} (Fig. 2). Caffeine, acetaminophen and ibuprofen were usually detected at concentrations higher than 9 μg L^{-1}, in keeping with the reported literature [33]. Although the influent COD showed a high seasonal variability (Table 1), the concentration of the studied EOCs showed no statistically significant seasonal difference ($p=0.84$). This may be explained by the presence of a primary treatment that favours a more constant influent wastewater in terms of quality, as can be seen in the low variability of the EOC concentrations (Fig. 2).

Table 2 shows the removal efficiencies of the target EOCs. They can be classified into four groups in accordance with the corresponding overall average removal efficiency in the HRAPs: high removal (>90%: caffeine, acetaminophen, ibuprofen, methyl dihydrojasmonate and hydrocinnamic acid), moderate-to-high removal (from 60% to 90%: oxybenzone, ketoprofen, 5-methyl/benzotriazole, naproxen, galaxolide, tonalide, tributyl phosphate, triclosan, bisphenol A and octylphenol), moderate-to-low removal (from 40 to 60%: diclofenac, benzotriazole, OH-benzothiazole, triphenyl phosphate, cashmeran, diazinon, benzothiazole, celestolide, 2,4-D and atrazine) and poor or no removal (<40%, carbamazepine, methyl paraben, tris(2-chloroethyl) phosphate).

Taking into account the configuration of the HRAPs, the most relevant removal processes that may occur in these systems can be biodegradation, photodegradation, volatilization and sorption to microalgae biomass. Uptake by microalgae is an important removal process, and it was assessed by analysing the occurrence of EOCs in the TSS (solids retained in the filters). Table 1 in the Supplementary Material (SM) shows that the most abundant compounds in the biomass (mostly microalgae) were the most hydrophobic ones, such as galaxolide and tonalide (log Kow>5). Hence, HRAPs may remove hydrophobic compounds by sorption, similarly to other wastewater treatment

828 technologies such as constructed wetlands (CWs) and activated sludge systems [34, 35]. Despite the
829 higher concentration of TSS in the HRAPs during the warm season, due to the greater biomass
830 production and evaporation losses (Table 1), the concentration of musk fragrances in the TSS was
831 higher in winter. Therefore, it may be postulated that the increase in biomass (microalgae,
832 heterotrophs and non-photosynthetic autotrophs organisms) improved the biodegradability of these
833 compounds or that the higher sunlight irradiation and temperatures in warm season improved the
834 volatilization rates. This is in keeping with the moderate biodegradability found for musk fragrances
835 (>75%) in lab-scale activated sludge reactors [36] and the tabulated high Henry's law constants (a
836 measure of air-water partitioning) for musk fragrances (11-12 Pa m^{-3} mol^{-1}). The occurrence of most
837 of the studied EOCs in the filters was below their LOD. This may be due to the fact that these EOCs
838 were not uptaken by microalgae or because they were removed by microalgal metabolism. A
839 microalgae removal effect due to the release of exudates likewise cannot be disregarded [37]. In
840 fact, it has been proved that the consortia of cyanobacteria/microalgae and bacteria can be efficient
841 in detoxification of organic and inorganic pollutants, and removal of nutrients from wastewaters,
842 compared to the individual microorganisms. Cyanobacterial/algal photosynthesis provides oxygen
843 and organic exudates that serves to the pollutant-degrading heterotrophic bacteria [38].

844 The overall average removal efficiencies of the studied EOCs were plotted against their
845 physicochemical properties (log Kow, molecular weight (MW) and Henry's law constant) as is
846 shown in Fig. 1 SM. Although the plots seem to show a relationship between the EOC removal
847 efficiencies and MW (Spearman's correlation coefficient = -0.197), log Kow (Spearman's
848 correlation coefficient = -0.080) and Henry's law constant (Spearman's correlation coefficient = -
849 0.075), no significant correlations were found (significance level >0.05). This may be explained by
850 the complexity of the chemical compounds studied as well as the fact that different removal
851 processes occurred simultaneously. Conversely, a statistically significant relationship between
852 influent concentration and removal efficiency was obtained (Spearman's correlation coefficient =

0.627, significance level =0.002). This may be explained by the fact that biodegradation needs a certain compound concentration before microbial degradation is stimulated. Nevertheless, this general rule must be applied with care and further work is necessary in this field.

The removal efficiency of HRAPs is comparable to that of conventional activated sludge WWTPs, as can be seen in Table 2 (overall removal efficiencies of 84% and 59% in warm and cold season respectively). Hijosa-Valsero et al. [39] found that waste stabilization ponds (WSPs) were capable of moderately removing pharmaceuticals such as naproxen (33%) and ibuprofen (56%). Matamoros et al. [40] found that unsaturated CWs were capable of removing up to 90% of pharmaceuticals and personal care products such as ibuprofen, naproxen and galaxolide, but did not remove carbamazepine. Hence, HRAPs seem to be as or more efficient than other biological wastewater treatment technologies, such as CWs or WSPs, with regard to EOC removal. Therefore, HRAPs can be considered a suitable technology for the treatment of wastewaters containing EOCs, with the added advantage that they produce microalgal biomass, do not require aeration and have smaller land area requirements than other engineered natural wastewater treatment technologies (i.e. CWs and WSPs). Nevertheless, since the effectiveness of engineered natural wastewater treatment technologies for removing EOCs has been shown to rely on different key design and environmental factors, such as HRT and seasonality [41], the next two sections will explore the influence of these factors on HRAPs.

3.2.1. Effect of hydraulic retention time (HRT)

HRT is a key design parameter for achieving proper removal efficiency of biodegradable organic contaminants from wastewaters engineered natural treatment systems such as constructed wetlands and waste stabilization ponds [33]. In fact, it has already proved that EOC removal in engineered natural treatment systems and activated sludge WWTPs increases as HRT increases due to the increase of biodegradation and sorption processes [34, 42]. No significant differences in their

878 performance were observed between HRTs in the warm season ($p>0.05$), but significant differences
879 were found in the cold season for those compounds that has already been described in the literature
880 [41] as been removed by biodegradation (i.e. caffeine, 4%; ibuprofen, 7%; methyl
881 dihydrojasmonate, 5%; oxybenzone, 13%; naproxen, 8% and triphenyl phosphate, 44%),
882 photodegradegradation (i.e. ketoprofen; 25% and triclosan, 20%) and sorption or volatilization (i.e.
883 galaxolide, 24% and tonalide, 16%). As already noted, conventional water quality parameters such
884 as COD and NH_4-N behaved similarly. From these results, it can be postulated that biodegradation,
885 photodegradation, sorption and volatilization removal mechanism were likely affected by the
886 increase of HRT in the cold season. García-Rodríguez et al. [41] reported that biological wastewater
887 treatment technologies for removing EOCs are highly dependent on HRT because it enhances
888 biodegradation, photodegradation and sorption removal processes. In general, the higher the HRT,
889 the greater the EOC removal efficiency. However, our results suggest that an HRT of 4 days is
890 enough to remove most of the compounds in both seasons. Therefore, while this technology is
891 competitive in terms of HRT compared to CWs and WSPs, activated sludge WWTPs are generally
892 set at an HRT of 12-24 hours or lower. Notwithstanding the foregoing, activated sludge WWTPs
893 also have higher energy requirements (0.6 kWh m^{-3} for activated sludge WWTPs *vs.* 0.02 kWh m^{-3}
894 for HRAPs). Finally, the lower microcontaminant sorption onto the biomass (table 1-SM) than in
895 conventional activated sludge WWTPs biosolids [43] is relevant for risk management and sludge
896 valorisation. This low bioaccumulation of microcontaminants into the biomass have already been
897 reported for vegetables [44], but this is the first time that it has been assessed for microalgae.

898

899 *3.2.2 Seasonality (environmental conditions)*

900 Seasonality is relevant to achieving adequate EOC removal efficiency in mild climates such as that
901 of the NW Mediterranean because it affects temperature, daylight duration and intensity, and
902 biomass production, four important factors influencing biodegradation, photodegradation,

volatilization and sorption EOC removal processes [41]. Significant differences ($p<0.05$) between the warm and cold seasons were observed for the removal of above described biodegradable compounds (i.e. caffeine, ibuprofen, methyl dihydrojasmonate, oxybenzone, naproxen, benzothiazole, methylparaben, benzotriazole, 5-methyl-benzotriazole, OH-benzothiazole, and triphenyl phosphate), photodegradable compounds (i.e. ketoprofen, diclofenac, and triclosan) and highly hydrophobic / moderately volatile compounds (i.e. galaxolide and tonalide). The higher temperature (11 *vs.* 26 ºC, on a daily average) and greater average daily solar radiation (74 *vs.* 282 W m^{-2}) in the warm season may explain these differences. It should be noted that the effect of seasonality on the pollutant removal performance of this technology for the most abundant compounds was low (around 10-20%) or null (i.e. for caffeine, acetaminophen, ibuprofen and methyl dihydrojasmonate). In contrast, various authors [45-47] have reported higher seasonal variability for EOC removal by other engineered natural wastewater treatment technologies (CWs or WSPs). Hence, although the HRAP technology seems to be a robust and reliable wastewater treatment technology in terms of EOC removal efficiency, further studies are required to provide more insight.

3.3. Aquatic risk assessment

Aquatic risk assessment throughout the HRAP treatment was performed based on the concentrations of the detected EOCs in the influent and effluent water samples, and the listed EC50 values for *Daphnia magna.* Hazard quotient indexes (HQs) were calculated according to the following equation (2):

$$HQ = \frac{MEC}{PNEC} \quad \textit{(Equation 2)}$$

where PNEC is the predicted non-effect concentration and MEC is the measured environmental

928 concentration at the influent or effluent of each HRAP reactor. PNEC values were estimated for
929 *Daphnia magna*, dividing the EC50 values (48 hours) by a recommended arbitrary safety factor of
930 1000 [48]. The EC50 values used in this study were collected from the literature and are
931 summarized in Table 2SM. When more than one EC50 value was reported for a single compound,
932 the lowest value was used. When no experimental values were available, the EC50 values were
933 estimated with ECOSAR v1.10 (EPI Suite software, US EPA).
934 Fig. 3 shows the individual HQ for each of the studied EOCs in the HRAP influent and effluent
935 water samples. As the difference between the EOC removal efficiencies at both of the studied HRTs
936 was minimal, the risk assessment was only performed at an HRT of 4 days (under the most critical
937 operating conditions). Acetaminophen, ibuprofen and oxybenzone exhibited higher HQs in influent
938 wastewater samples (HQ>1), mainly due to their high concentration. Following the treatment in the
939 HRAP system, all of the studied EOCs had an HQ<1. The most relevant compounds in the treated
940 wastewater effluents, apart from those observed at the influent, were triclosan and galaxolide. These
941 HQ values for the EOCs are in keeping with those reported in the literature for treated wastewater
942 effluents [42]. In fact, triclosan has already been postulated as a critical compound in terms of
943 contribution and environmental risk in wastewater effluents [49]. Hence, von der Ohe et al. [50]
944 argued that triclosan should be seriously considered as a candidate for regulatory monitoring and
945 prioritization on a European scale on the basis of realistic PNECs.

946 As previously described by various authors, HQs should follow an additive model [6]. Hence, the
947 final HQ for each water sample can be calculated as the sum of each individual HQ. The cumulative
948 HQs for the influent wastewater samples were 8.45 and 6.20 for the summer and winter campaigns,
949 respectively. However, they fell 93% (warm season) and 72% (cold season) following the treatment
950 with the HRAPs. Consequently, in summer the cumulative HQ was less than 1 (0.62), whereas in
951 winter it was significantly higher (HQ=1.73). These results are in keeping with the high reduction in
952 acute toxicity achieved by other biological wastewater treatment technologies, such as CWs [51].

Nevertheless, this ecological risk assessment was only performed for the target EOCs; therefore, further studies may be needed to include other EOCs and related transformation products.

4. Conclusions

This study has shown that microalgae-based wastewater treatment systems (such as HRAPs) enable the removal of a wide range of EOCs from urban wastewater. Removal efficiency ranged from none to up to 99%. The EOCs were classified into four groups in accordance with their average removal efficiency in HRAPs: high removal (>90%: caffeine, acetaminophen, ibuprofen, methyl dihydrojasmonate and hydrocinnamic acid), moderate-high removal (from 60% to 90%: oxybenzone, ketoprofen, 5-methyl/benzotriazole, naproxen, galaxolide, tonalide, tributyl phosphate, triclosan, bisphenol A and octylphenol), moderate-low removal (from 40 to 60%: diclofenac, benzotriazole, OH-benzothiazole, triphenyl phosphate, cashmeran, diazinon, celestolide and atrazine) and poor or no removal (<30%, carbamazepine, benzothiazole, methyl paraben, tris(2-chloroethyl) phosphate, and 2,4-D). The removal of emerging contaminants in HRAPs was only affected by the HRT during the cold season, whereas no differences where observed in the warm season. The most frequently occurring compounds (caffeine, acetaminophen and ibuprofen) had removal efficiencies of up to 90% that were minimally affected by seasonality and HRT. The ecotoxicological risk assessment study revealed that the HQ for the influent wastewater was removed by up to 90%, indicating no acute toxicity risk associated with the studied EOCs at the water effluents.

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Table 1. Average concentration and standard deviation of conventional water quality parameters (n=8 per campaign). Removal efficiencies for COD and NH_4-N are shown in brackets for the two HRAP (4 and 8 d HRT). **COD (chemical oxygen demand); DO (dissolved oxygen); TSS (total suspended solids).**

	Warm season			**Cold season**		
	Primary effluent	HRT = 4 d	HRT = 8d	Primary effluent	HRT = 4 d	HRT = 8d
Average daily solar radiation ($W\ m^{-2}$)		282			74	
T (°C)	28±1	25±1	25±1	16±1	13±1	13±1
DO ($mg\ L^{-1}$)	2±1	6±1	8±1	7±2	10±1	12±2
pH	8±1	8±1	9±1	8±1	8±1	9±1
TSS ($mg\ L^{-1}$)	118±112	316±50	346±38	-	110±23	149±15
Biomass production ($gTSS\ m^{-2}d^{-1}$)	-	24±8	13±2	-	8±2	6±0.3
COD ($mg\ L^{-1}$)	156±79	52±9* (75**)	52±12* (84**)	342±107	67±7* (83**)	52±7* (88**)
NH_4-N ($mg\ L^{-1}$)	81±9	0.6±0.3(99**)	0.7±0.5(99**)	19±4	2±1 (90**)	0.4±0.1 (98**)

*** soluble COD; ** calculation corrected for evaporation water losses.**

Table 2. Seasonal removal efficiency (%) of target EOCs in the HRAPs operated at HRT of 4 and 8 days. EOCs have been sorted by their abundance in Fig. 2.

Name	**Warm season**		**Cold season**		**Activated sludge WWTPs**
	HRT 4 d	HRT 8d	HRT 4d	HRT 8 d	
Caffeine	97±1	98±1	85±2[ab]	91±2[ab]	50-99[c]
Acetaminophen	99±1	99±1	99±1	99±1	99-100[c]
Ibuprofen	99±1	99±1	86±4 [ab]	93±3 [ab]	72-100[c]
Methyl dihydrojasmonate	99±1	99±1	92±2 [ab]	97±1 [ab]	98[d]
Oxybenzone	97±1	99±1	75±10 [ab]	88±4 [ab]	63-98[c]
Ketoprofen	87±6 [b]	95±4	50±17 [ab]	75±9 [ab]	11-100[c]
Hydrocinnamic acid	99±1	99±1	99±1	99±1	-
5-methyl benzotriazole	83±16	95±8	74±5	77±2[a]	60[e]
Naproxen	83±4	89±4	48±5[a]	60±3[a]	43-99[c]
Carbamazepine	46±9	62±15	15±19[a]	34±15[a]	<nr-62[c]
Galaxolide	94±1b	97±1	47±1[ab]	71±2[ab]	88[c]
Benzothiazole	70±6	78±7	13±8[a]	30±14[a]	40-60[d]
Diclofenac	82±6	92±3	21±29[a]	29±14[a]	<0-81[c]
Methylparaben	59±12	75±8	12±9 [a]	25±11 [a]	82-91[h]
Benzotriazole	74±7	84±4	33±10 [a]	41±5 [a]	60[e]
Tonalide	84±1b	90±1	51±5[a]	67±7a	85[c]
OH-Benzothiazole	80±3	82±5	20±17[a]	37±11a	50-70[d]
Tributyl phosphate	82±5	86±8	69±8	78±2	55-86[g]
Tris(2-chloroethyl)phosphate	39±28	63±12	15±23	21±19	nr[c]
Triphenyl phosphate	82±2b	89±1	24±6[ab]	68±10[ab]	40[g]
Triclosan	93±1	95±1	49±5[ab]	69±2[ab]	71-99[c]
Cashmeran	70±5	79±5	61±3	64±8	50[f]
Octylphenol	90±6	93±4	58±12[a]	74±5[a]	<nr-97[c]
Diazinon	61±4	63±1	-	-	nr[c]
Celestolide	52±1	53±1	-	-	59[f]
Atrazine	76±6	85±3	41±7[ab]	69±6[ab]	nr-25[c]
Bisphenol A	72±14	85±8	66±16	78±6	63-99[c]
2,4-D	22±10	32±26	-	-	-

[a] seasonal statistical difference at p=0.05; [b] HRT statistical difference at p=0.05, c [32];d [51]; d [52]; e [53] ; f[54]; g[55]; h [56]

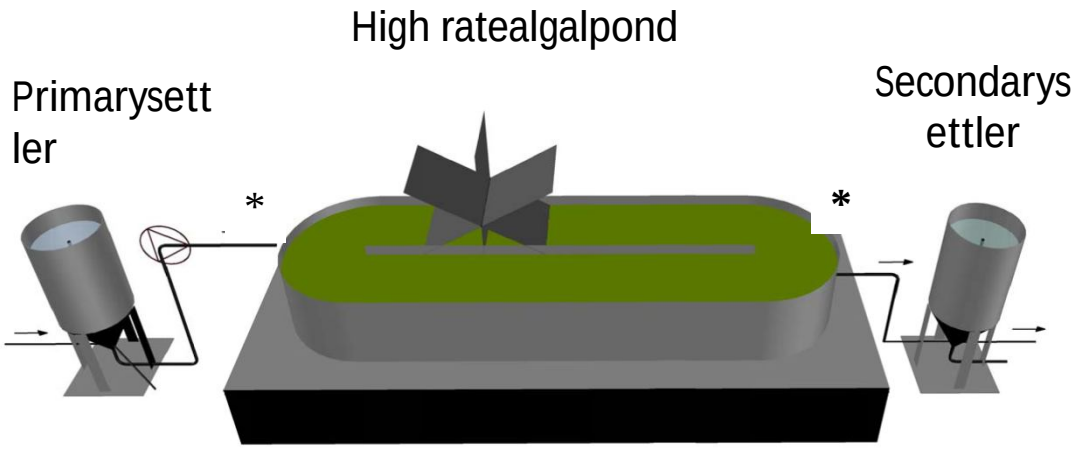

Figure 1.3D view of treatment units of one line. Primary settler is fed with screened wastewater. Secondary settler allows separation of the biomass produced in the HRAP. Sampling points are indicated (*).

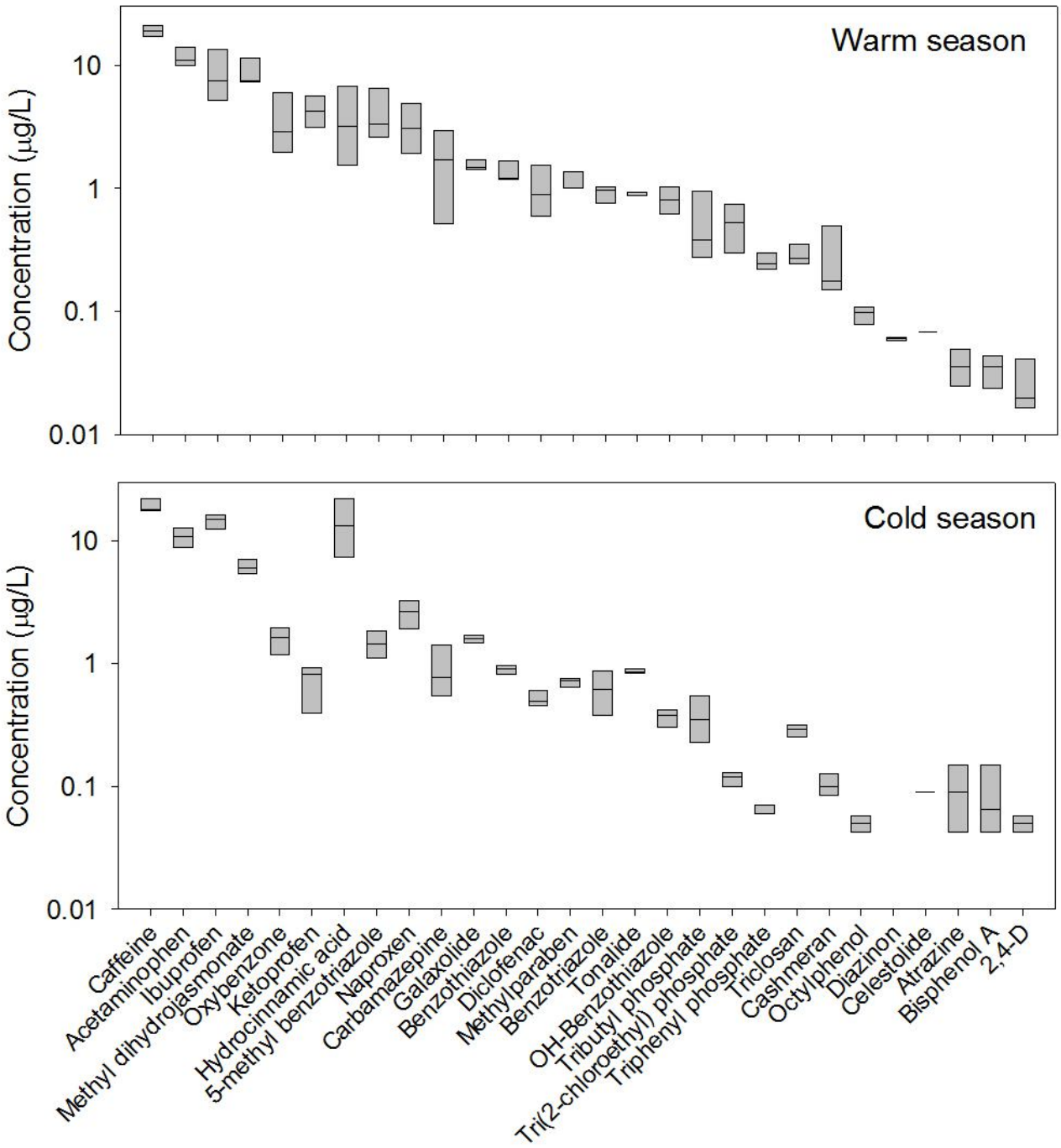

Figure 2. Logarithmic box-plot of the seasonal occurrence of emerging contaminants in the primary effluent wastewater (n=8). The box plots indicate the median, and the 25th and 75th percentiles for each compound. Note that particulate and dissolved phase were both included. Similar profiles were obtained when molar concentrations were compared (not shown).

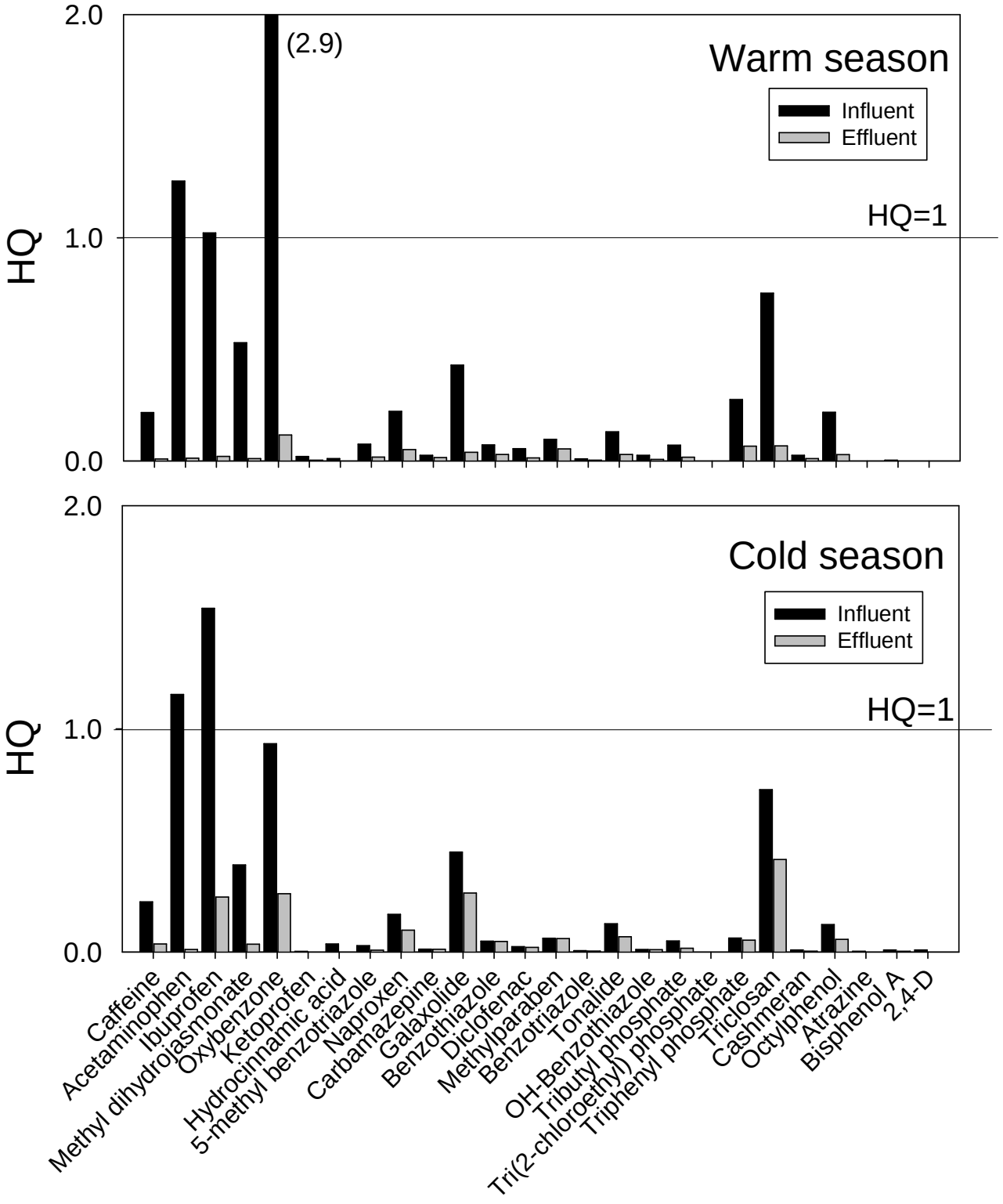

Figure 3. Seasonal hazard quotients (HQs) for the influent and effluent water samples collected from the HRAP set at a HRT of 4days.