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Behavior of Selected Pharmaceuticals in Subsurface Flow Constructed Wetlands: A Pilot-Scale Study

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Subsurface flow constructed wetlands (SSFs) constitute a wastewater treatment alternative to small communities due to the low operational cost, reduced energy consumption, and no sewage sludge production. Although much information is available about conventional water quality parameters in SSF constructed wetlands, few data are available regarding specific contaminants. In this paper, we focus on the behavior of three widely used pharmaceuticals (clofibric acid, ibuprofen, and carbamazepine) in two pilot SSF constructed wetlands planted with *Phragmites australis* and characterized by different water depths (i.e., 0.3 and 0.5 m). These SSFs partially treat the urban wastewater from a housing development (ca. 200 inhabitants). The three pharmaceuticals and bromide were continuously injected into the two SSFs during a period of 150−200 h, and the effluent concentration was simultaneously measured as 6 h composite samples. Their removal efficiency was calculated from the injected concentration, and the hydraulic parameters were evaluated and compared to bromide as tracer. In this regard, the behavior of clofibric acid was similar to that of bromide, and no sorption into the gravel bed occurred. On the other hand, carbamazepine showed a higher sorption than bromide and clofibric acid, which is attributable to its interaction on the gravel bed. Accordingly, the use of clofibric acid as a hydraulic tracer is proposed, taking into account its low residence time. Ibuprofen removal was 81% in the shallow SSF and 48% in the deep one. Differences in removal efficiency could be explained by the less anaerobic environment of the shallow wetland.

Introduction

Constructed wetlands are land-based wastewater treatment systems that consist of shallow ponds, beds, or trenches that contain floating or emergent-rooted wetland vegetation (*1*). In subsurface flow constructed wetlands (SSFs), wastewater is infiltrated through vegetated gravel beds confined by a liner. The wastewater treatment relies on biological, chemical, and physical processes in a natural environment. The potential of SSFs for the removal of contaminants occurring in urban wastewater has attracted increasing interest over the past decade with a view of treating wastewaters from small populations to comply with environmental regulations such as the European Union Directive 91/271 and the U.S. EPA Clean Water Act and to attenuate diffuse agricultural contamination runoff to surface waters (*2*, *3*). Nevertheless, the available information on the performance of these systems is limited to common contamination parameters, such as suspended solids, COD, BOD_5, nutrients, and bacteria (*4*). At present, very little information is available on specific organic contaminants, and it mainly deals with herbicides, pesticides (*5*−*7*), and nonionic (*8*) and anionic surfactants (*9*).

Pharmaceutical products are becoming a new environmental problem due to the widespread use of antiinflammatory, lipid regulator, and analgesic drugs. In recent years, several authors have reported the occurrence of pharmaceuticals in the aquatic environment (*10*). Pharmaceutical substances have been detected in wastewater treatment plant (WWTP) effluents, even at low parts-per-billion concentrations (*11*−*19*). Moreover, it is known that if pharmaceutical compounds are not totally removed, then variable concentrations of pharmaceutical drugs can reach surface, ground, and coastal waters (*20*−*22*).

In this paper, we focus on the fate of clofibric acid (the active metabolite of the blood lipid regulators clofibrate, ethofyllin clofibrate, and ethofibrate), ibuprofen (an analgesic), and carbamazepine (an antiepileptic, antineuralgic, and antipsychotic drug) in SSFs. These drugs were chosen according to their high production volume and widespread use (ca. 100 t $year^{-1}$ for ibuprofen and carbamazepine and 16 t $year^{-1}$ for clofibric acid in Germany (*23*)) and their widespread occurrence in WWTP effluents.

Furthermore, their different physicochemical structure and characteristics shown in Table 1 can presumably lead to different behavior in SSFs. In fact, whereas clofibric acid and carbamazepine show a remarkably high persistence in the aquatic environment, ibuprofen is readily removed in conventional WWTPs (*20*, *24*).

The objectives of this work were to evaluate the response curves and removal behavior of clofibric acid, ibuprofen, and carbamazepine in two pilot SSFs with different water depths serving a housing development. In a former paper, we showed that water depth is a key design parameter affecting the redox status of the SSFs and therefore the relative importance of the biochemical reactions responsible for the degradation of organic matter (*25*). Moreover, because of the sorption of contaminants in the organic matter retained in the gravel bed and in the biofilm, a continuous injection experiment of the pharmaceuticals was designed to equilibrate the sorption sites of the bed and thus obtain a more reliable estimate of their biodegradation potential. Furthermore, the use of some of these pharmaceuticals as a hydraulic tracer is evaluated and compared to widely used bromide.

Experimental Procedures

Chemicals. HPLC grade methanol, acetonitrile, and water were obtained from Merck (Darmstadt, Germany). Analytical grade formic acid and trifluoracetic acid were obtained from Panreac (Barcelona, Spain). Suprasolv grade isooctane, hexane, and ethyl acetate were obtained from Merck. Pyromellitic acid, triethanolamine, hexamethonium hydroxide, clofibric acid, ibuprofen, carbamazepine, and dihydrocarbamazepine were purchased from Sigma-Aldrich (Steinheim, Germany). Mecoprop and 2,4,5-trichlorophenoxy-

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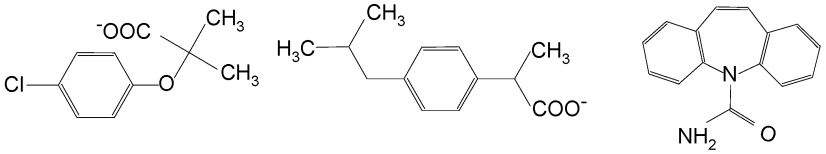
TABLE 1. Chemical Structures and Physicochemical Properties of the Pharmaceutical Products

Compound	Clofibric acid	Ibuprofen	Carbamazepine
function	lipic regulator	anti-inflammatory	antiepileptic
pka	2.84	4.31	7.00
log Kow[a]	2.84	3.97	2.45
log Kow (pH 8)[b]	-0.97	-0.11	2.25
Structure[c]			
CAS RN.	882-09-7	15687-27-1	298-46-4

[a] Experimental values of neutral compounds (*40*). [b] Estimated value calculated for the compounds in the injection experiment conditions (*40*). [c] Obtained from ChemSketch 5.0.

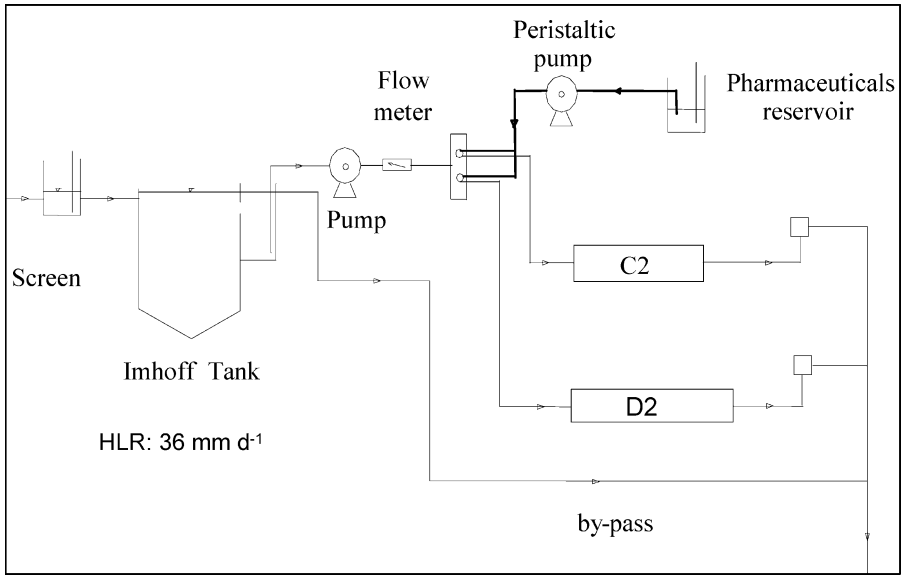


FIGURE 1. Schematic diagram of the pharmaceutical injection experiment in two wetlands of the SFCW pilot plant at Les Franqueses del Vallès, Barcelona. The hydraulic loading rate was 36 mm day^{-1}.

propionic acid (2,4,5-TPA) were obtained from Reidel-de-Haen (Seelze, Germany) and sodium bromide from Fluka (Buchs, Switzerland). Bis(trimethylsilyl)-trifluoro-acetamide (BSTFA) was purchased from Merck.

SSF Pilot Plant. The pilot plant consists of eight parallel horizontal SSFs and is located in the municipality of Les Franqueses del Vallès (Barcelona, Spain). The plant partially treats the urban wastewater generated by a housing development (ca. 200 inhabitants). The water is screened and then flows to an Imhoff tank (Figure 1). This tank is connected to another one from which the water is pumped to a distribution chamber where the flow is split by means of a weir into eight lines flowing to each wetland. The flow rate was adjusted through the operation time of the pump and a valve that allowed the flow rate of the influent to be controlled. An electromagnetic flow meter was installed to measure the instantaneous flow rate. These mechanisms allow the flow rate in the SSF plant to be controlled. Each bed has a surface area of 54−56 m^2 and was planted with *Phragmites australis*. The plant began operation in March 2001. In this study, only two SSFs were used, one with an aspect ratio (length × width) of 2:1 (named C2) and one with an aspect ration of 2.5:1 (named D2). The size of the granular granitic medium measured as D_{60} (D_x is the size of the material that corresponds to %x of the total material weight) was 3.5 mm, and the uniformity coefficient was 1.7. The average water depth is approximately 0.3 m in bed D2 and 0.5 m in bed C2. The plant was operated at a hydraulic loading rate of 36 mm day^{-1} during the injection experiment.

Experimental Design. About 20 L of distilled water contained in a glass bottle was spiked with 250 mg of each pharmaceutical compound and 200 g of sodium bromide. This mixture was homogenized and injected into the weir lines corresponding to both SSFs using a Minipuls 2 peristaltic pump (Gilson, Villiers le Bel, France) synchronized to the wastewater pumping system. A previous conditioning of the silicon tubing and connectors for 72 h with the experimental pharmaceutical concentration was carried out to minimize the analyte sorption losses during the experiment. Flow rates were adjusted to obtain a final influent concentration of 25 μg L^{-1} for each pharmaceutical and 15 mg L^{-1} for bromide. The injection experiment was run for 9 days in the D2 wetland and 7 days in the C2 wetland from April to May 2004.

Sampling. Effluent composite samples were collected every 6 h with an American Sigma 900 autosampler (Sigma, Loveland, CO) for a time period of 21 days. To ensure the absence of the injected pharmaceuticals in the influent, samples of the inflow were also collected and analyzed. All the samples were collected in 500 mL amber glass bottles and kept refrigerated during transportation to the laboratory, where they remained refrigerated at 4 °C until analysis. The sample holding time was less than 2 days. Furthermore, a gravel sample with biofilm was sampled at 2 m from each wetland inlet and kept under −20 °C prior to analysis.

Wastewater Analysis. All sewage samples, influents, and effluents were filtered through 0.45 μm glass filter of 47 mm from Millipore (Bedford, MA) and then acidified to pH 2 with concentrated hydrochloric acid. A sample volume of 200 mL was mixed with a solution of surrogate standards, 2,4,5-TPA (25 mg L^{-1}) and dihydrocarbamazepine (25 mg L^{-1}), giving a final concentration of 25 μg L^{-1}. The spiked sample was percolated through a polymeric SPE cartridge packed with 100 mg of Strata X from Phenomenex (Torrance, CA) conditioned previously with 5 mL of *n*-hexane, 5 mL of ethyl acetate, 10 mL of MeOH, and 10 mL of MilliQ water (pH = 2). The flow rate was approximately adjusted to 10 mL min^{-1}. Thereafter, the cartridges were allowed to dry for 30 min; finally, the analytes were eluted with 5 mL of ethyl acetate. The extract was evaporated to dryness under a gentle nitrogen stream, reconstituted in 175 μL of methanol, and mixed with 5 μg of mecoprop as an internal standard since it was not detected in the influent wastewater.

The resulting extracts were analyzed using a dual pump HPLC equipped with a UV diode array detector from Shimadzu (Kyoto, Japan) at 230 nm. Injection was carried out using a Rheodyne valve (Rohnert Park, CA) with a sample loop volume of 20 μL fitted to a Shimadzu autosampler. Chromatographic separation was performed on a Lichro-CART column 125 × 4 mm packed with Lichrospher 100 RP-18 (5 μm) from Merck. The mobile phase used in the chromatographic separation consisted of a binary mixture of solvents A (acetonitrile) and B (water adjusted to pH 3 with formic acid) at a flow rate of 0.8 mL min^{-1}. Initial conditions were 40% A for 20 min, then linearly programmed up to 80% A for 10 min followed by a linear program to 100% A for 5 min, and then held for 5 min. The LODs, LOQs (μg L^{-1}), and RSDs ($n = 3$) were analyte-dependent, ranging from 0.15 to 0.24, 0.48 to 1.05, and 1.4 to 4.2%, respectively, and recoveries were from 80 to 98%. LOD and LOQ of the analytical procedure were carried out by using a water effluent from a microcosm pilot plant effluent without pharmaceuticals. LOD was calculated 3 times for the area of the procedural blank and 10 times for the LOQ. Linear regression coefficients from a 10-point linear calibration curve (concentration rage 0.6−50 μg mL^{-1}) were always higher than 0.998 for all compounds.

The bromide determination was carried out with a Hewlett-Packard (Palo Alto, CA) 3DCE capillary electrophoresis (CE) system equipped with a UV diode array detector. A 60 cm capillary length of 50 μm i.d. from Agilent Technologies was used. The electrolyte solution was pyromellitic acid (2.25 mM), triethanolamine (1.6 mM), and hexamethonium hydroxide (0.75 mM) adjusted to pH 8.5. The injection was performed at 50 mbar for 4 s, at a constant voltage of −30 kV, and a column temperature of 30 °C was used with a detection signal of 350 nm and a reference of 245 nm. Quantitation was performed as described elsewhere (*26*).

Gravel Analysis. Following a freeze-drying step for 24 h, 40 g of dry wt gravel samples were extracted by a pressurized solvent extraction apparatus (PSE, Applied Separations, Allentown, PA) using a 33 mL extraction cell. The extraction conditions were 80 °C, 100 bar, and 3 cycles of 5 min with ethyl acetate containing 0.2% TFA acid (v/v) added into the extraction cell. The obtained extract was concentrated to 0.2 mL with a rotary evaporator (Heidolph Instruments, Schwabach, Germany) and reconstituted with 200 mL of distilled water (*27*), adjusted to pH 2 with concentrated hydrochloric acid, and cleaned up by percolation through a 100 mg Strata X cartridge (Phenomenex, Torrance, CA) eluting with 5 mL of ethyl acetate.

The cleaned extracts were evaporated to dryness and derivatized with 200 μL of BSTFA for 1 h at 70 °C. Then, the BSTFA excess was evaporated to dryness and reconstituted with isooctane. The sample was injected onto a TRACE-GC-MS (Thermo-Finnigan, Dreieich, Germany) fitted with a 30 m × 0.25 mm i.d. × 0.25 μm DB-5 (J&W Scientific, Folsom, CA). The carrier gas was helium (purity: 99.9995%) at a constant linear average velocity of 40 cm s^{-1}. The oven temperature was held at 90 °C for 1 min, then temperature programmed at 20 °C min^{-1} to 120 °C, then to 3 °C min^{-1} to 160 °C followed by 12 °C min^{-1} to 260 °C, and finally held for 12 min. A volume of 2 μL of sample was injected in the splitless mode at an injector temperature of 260 °C with an injector purge activation time of 1 min. The transfer line was set at 280 °C and the ion source at 200 °C. The electron impact energy was 70 eV. Acquisition was performed in the scan mode ranging from m/z 50 to 350 at two scans s^{-1}. The following diagnostic ions were monitored for target analyte quantification (in bold) and identification: ibuprofen **161**/2347263; clofibric acid, **128**/169/143; carbamazepine, **193**/236/192; mecoprop, **169**/286/142; 2,4,5-TPA, **196**/282/198; and dihydrocarbamazepine,**195**/238/160. The LODs and LOQs (ng g^{-1}) were compound-dependent, ranging from 1.1 to 1.3 and 4.8 to 8.4, respectively. The recoveries for the acid pharmaceuticals were ca. 75%, and the correlation coefficients (r^2) of the calibration curve were always higher than 0.998.

TABLE 2. Average Concentration and SD of Several Contaminants in Influent and Effluents ± SD of Pilot SSFs

parameter	*N*	influent	effluent C2	effluent D2
TOC, mg/L[a]	20	50 ± 20	21 ± 7.1	17 ± 7.4
BOD_5, mg/L[a]	103	136 ± 54	55 ± 28	20 ± 19
NH_4^+, mg N/L[a]	103	45 ± 16	31 ± 12	18 ± 12
LAS, mg/L[b]	20	3.65 ± 0.97	2.6 ± 0.89	1.07 ± 0.84

[a] Garcia et al. 2005. [b] Huang et al. 2004.

Results

Pharmaceutical Behavior in Injection Experiment. The injection experiment was carried out in shallow SSF (bed D2) because after 3 years of operation, it was found to have a better performance in terms of the removal efficiency of a broad spectrum of contaminants (i.e., BOD_5, TOC, NH_4^+, and linear alkylbenzenes sulfonates) (Table 2). A deeper SSF (bed C2) was also considered in the injection experiment to compare its performance with bed D2. The aspect ratio of the two wetlands is also different, but it has been demonstrated that in the range used in the present study, it is not a relevant design parameter in terms of contaminant removal efficiency (*9*, *25*).

Effluent concentrations were corrected by the actual influent flow and the background target pharmaceutical concentration, ibuprofen being the only target pharmaceutical that occurred in the SSF influent at low concentrations (i.e., 4.7−5.8 μg L^{-1}). Effluent concentrations of target compounds were normalized by their influent concentration, and the results are shown in Figure 2. There is a different behavior of the pharmaceutical compounds response curves, which is attributable to their different physicochemical characteristics (Table 1). The anionic form of clofibric acid showed a similar curve to bromide because it has the lowest log K_{ow} (−0.97), and no degradation was observed in two wetlands. On the other hand, the behavior of carbamazepine is different to that of bromide and attributable to its sorption behavior, which is higher than that of clofibric acid with a log K_{ow} of 2.25. The behavior of ibuprofen was affected by its degradation, and its trend is not similar to that of bromide.

Hydraulic Behavior. The effluent concentrations were used to obtain the hydraulic parameters, namely, the mean hydraulic retention time (τ), the normalized variance (σ^2/τ^2), and the normalized delay time (ϕ_θ) (*28*). Moreover, these parameters were correlated with the different behavior of

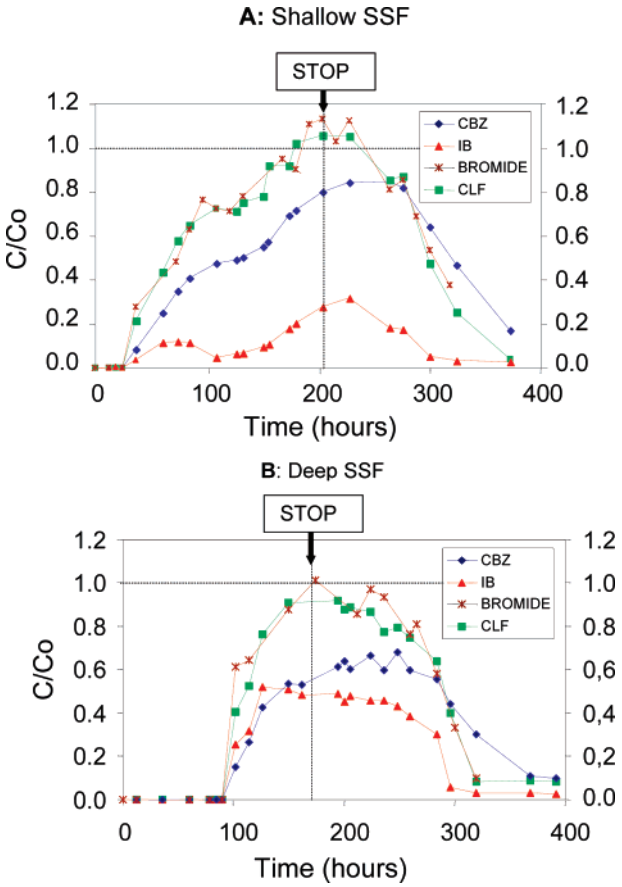


FIGURE 2. Behavior of the pharmaceuticals (carbamazepine: CBZ; ibuprofen: IB; clofibric acid: CLF) and bromide in wetlands type D2 and C2 (STOP: end of pharmaceutical injection). Horizontal line at $C/C_o = 1$ denotes the equilibration for conservative tracers.

TABLE 3. Values of the Hydraulic Parameters Estimated from Different Substances Used as Tracers

	deep bed (C2)			shallow bed (D2)		
	τ (h)	σ^2/τ^2	ϕ_θ	τ (h)	σ^2/τ^2	ϕ_θ
bromide	154.9	0.80	0.58	114.4	1.10	0.21
clofibric acid	151.8	0.80	0.59	111.5	1.16	0.22
carbamazepine[a]	226.7	0.92	0.40	167.7	1.10	0.14

[a] Sorption observed.

these pharmaceuticals as compared to bromide. The first step was to transform the response curves to residence time distribution curves (RTD or E curves), from which the hydraulic parameters were obtained (Table 3). The similarity in the shape of the response curve, τ, and σ^2/τ^2 for both clofibric acid and bromide in the two SSFs indicates that clofibric acid can be used as a nonreactive tracer. The normalized variance can be used to measure the internal dispersive process in SSFs (*29*). In SSFs with a similar depth, the normalized variance will decrease with the increase in aspect ratio, while the normalized delay time will increase (*28*). In the present experiment, the opposite trend was observed because the shallow SSF had a higher aspect ratio than the deep SSF, and the shallow one had a higher normalized variance and a lower normalized delay time. Moreover, carbamazepine and ibuprofen acid will be described in this work as reactive compounds by sorption and degradation, respectively, and cannot be used as conservative tracers.

Gravel Bed Accumulation. Since suspended particles from the wastewater are retained in a wetland bed (*30*), sorption of dissolved organic contaminants in that organic matter and on the biofilm coating the gravel bed can be a significant mechanism for their removal (*4*). Therefore, the concentration of pharmaceutical products was measured near the inlet because, in accordance with previous results, this is the place where most of the organic matter is retained (*32*). The highest retention in the gravel bed was obtained for carbamazepine (97 ng g^{-1}; 77%) in relation to clofibric acid (14 ng g^{-1}; 11%) and ibuprofen (15 ng g^{-1}; 12%). These results are consistent with its response curves and could be explained by its higher hydrophobicity (Table 1) and the electrostatic interactions between the acidic compounds charged negatively and the negative charged biofilm covering the gravel bed (*31*).

Degradation. The removal rate (Table 4) was evaluated using the cumulative mass recovery curves in the effluents (Figure 3). Recoveries of clofibric acid and bromide in the deep bed were nearly 100%, but in the shallow bed it was 108%. The approximately 8% in excess may be attributable to evapotranspiration in this bed. The different behavior of the two SSF wetlands could be attributable to a higher rhizome contact with the water in the shallower bed. Accordingly, the removal rate was corrected. After more than 400 h from the pharmaceutical injection, a different removal efficiency for ibuprofen was observed in two wetlands: 81 and 48% for the shallow and deep beds, respectively. Carbamazepine was not completely recovered, in agreement with its higher sorption in the bed (*33*). Clofibric acid has been identified as a refractory contaminant in several WWTPs (*14*) and in river biofilm systems (*34*). No removal was observed in the two wetlands studied.

Discussion

The removal of contaminants in SSFs involves a variety of processes including biodegradation, sorption onto the bed, sedimentation, and plant uptake (*4*). The complexity of such processes makes it difficult to understand the primary removal mechanism for each class of contaminants, and design parameters are usually based on empirical approaches. Further understanding of the removal and degradation pathways and mechanisms of organic matter is needed to improve the efficiency of such systems.

In this regard, we have demonstrated that the redox potential (E_H) was higher in the water inside the shallow beds (ranging from −144 to −131 mV) than in the deep ones (from −183 to −151 mV) (*31*). More oxidized conditions in the shallow SSFs can promote more energetically favorable biochemical reactions, leading to a higher removal efficiency of the organic matter. Therefore, in the present study, we considered two pilot SSFs with a different depth, and the shallow bed showed a better ibuprofen removal than the deep bed. Similar results were reported in an experimental biofilm microcosm reactor (*35*). The lower concentration of the pharmaceutical in the bed under the equilibrium conditions indicates that ibuprofen is biodegraded in the wetland environment. Although much information is available on the removal of this pharmaceutical in conventional WWTPs, to the best of our knowledge, this is the first time that its removal has been reported in a SSF system. The removal efficiency of the SSF system used in this study is not directly comparable with that of a conventional WWTP because the inlet concentration is usually lower than in our case; however, the results can be compared in Table 4, and it can be seen that the efficiencies are quite similar in the case of the shallow bed.

A completely different behavior was obtained in the case of carbamazepine. First, it exhibited a different response curve to the other pharmaceutical compounds (Figure 2), consistent with its higher hydrophobicity (Table 1). Second, its removal is lower than that of ibuprofen, being more efficient in the deep bed wetland than in the shallow one (Table 4). The

TABLE 4. Removal Efficiency (%) of Pharmaceuticals during Injection Experiment Obtained from Total Mass Recovery and Comparison with Conventional WWTPs

	SSFs		WWTPs				
	deep	shallow	ref *18*	ref *14*	ref *20*	ref *17*	
country	this paper	this paper	Spain	Germany	Germany	Brazil[a]	Brazil[b]
ibuprofen	48	81	60−70	n.d.[c]	90	75	22
clofibric acid	n.r.	n.r.	n.d.	n.r	51	34	15
carbamazepine	26	16	n.d.	8	7	n.d.	n.d.
samples (*n*)	*d*	*d*	3[e]	20−27[f]	6[e]	6[e]	6[e]

[a] Actived sludge. [b] Biological filter (trickling filter). [c] n.r.: no removal observed; n.d.: no data available. [d] Continuous injection. [e] Integrated samples of 24 h. [f] Integrated samples of 24 h from three different WWTPs in Berlin.

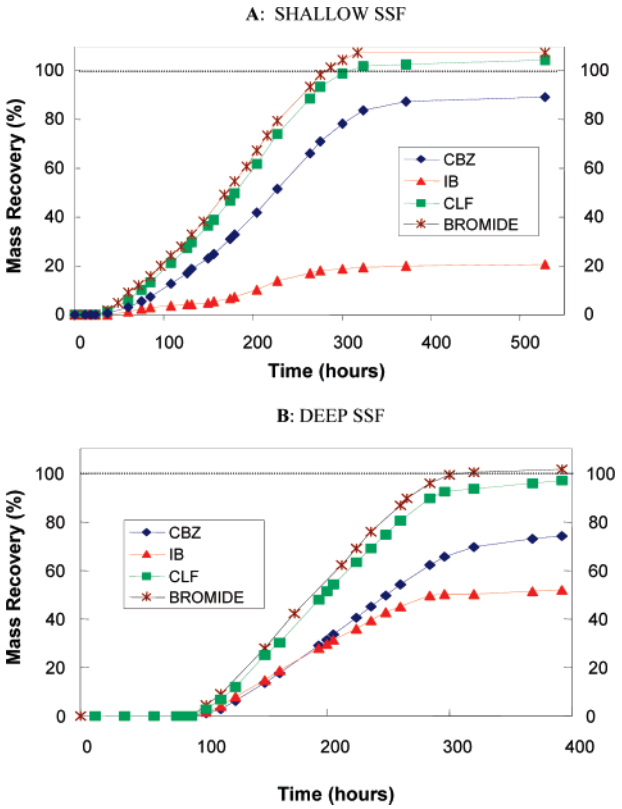


FIGURE 3. Cumulative percent mass recovery for pharmaceuticals and bromide for two wetlands. See Figure 2 caption for abbreviations.

different behavior of this pharmaceutical is attributable to its higher hydrophobicity and its refractory behavior to biodegradation. In fact, carbamazepine is one of the most recalcitrant pharmaceutical compounds occurring in the aquatic environment (*36*). It has even been suggested as a conservative tracer of urban pollution in ground and surface waters (*37*). According to its refractory character to biodegradation and its high concentration in the gravel, the removal of this compound in the wetlands could be explained by the sorption onto the available organic surfaces. The difference between the two wetlands could be explained because of the greater volume of the deep wetland than the shallow one, which implies a higher specific surface for sorption processes than in the shallow bed. The comparison of its removal in the SSF and in conventional WWTPs is favorable to the former, probably due to the presence of media for sorption.

Finally, clofibric acid behaves in a different way than the other two pharmaceuticals. Its low hydrophobicity leads to a low sorption onto the wetland bed, and its refractory behavior leads to negligible removal. Clofibric acid was described as a refractory compound in WWTPs (*13*). Nevertheless, in WWTPs in Berlin and Brazil (*20*, *17*), moderate removal is reported (Table 4). However, no removal was also reported in an exhaustive study in Berlin WWTP (*14*). Additional microcosm studies support the recalcitrant behavior of clofibric acid (*34*, *35*), as found in our study. A possible explanation of the different results could be explained by the required microbiological adaptation to achieve a significant degradation rate or/and the high fluctuation of the pharmaceutical concentrations in the WWTP influents; this fluctuation is avoided when the pharmaceuticals are injected as in our study. Moreover, the presence of clofibric acid in the North Sea (*22*) supports the recalcitrant behavior of this compound.

In summary, SSF depth is a key design factor for the removal of pharmaceuticals. Less refractory compounds such as ibuprofen are removed more efficiently in the shallow SSF, presumably linked to more oxidized conditions. On the other hand, the more refractory pharmaceuticals such as clofibric acid show no removal, in good agreement to the limited removal observed in WWTPs.

The retention of pharmaceuticals in wetlands is dependent on their physicochemical properties and is close to the behavior of bromide for acidic pharmaceuticals. In this regard, clofibric acid has a similar retention behavior to bromide in the two wetlands studied. Therefore, it can be used as a tracer to describe the hydraulic parameters. In addition, using clofibric acid in a lower concentration (ng mL^{-1}) than bromide (mg mL^{-1}) avoids the effect of density stratification in the characterization of the hydraulic parameters (*38*) and bromide uptake by plants (*39*), which has not been demonstrated in the case of pharmaceuticals.

Acknowledgments

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