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Biotic phase micropollutant distribution in horizontal sub-surface flow constructed wetlands

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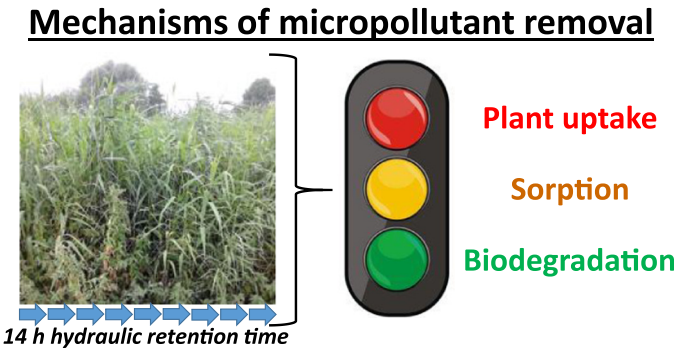
HIGHLIGHTS

- Micropollutant removal ranged from −112% (enrichment) to 98%.
- Uptake and metabolism of recalcitrant micropollutants by *P. australis* evidenced
- Direct role of plants on micropollutant removal limited at 14 h HRT
- Stereo-selective degradation of atenolol and MDMA by HSSF wetlands
- Predominant removal mechanism during treatment was biodegradation.

GRAPHICAL ABSTRACT



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ABSTRACT

The distribution of micropollutants in biotic phases of horizontal sub-surface flow (HSSF) constructed wetlands was investigated. 88 diverse micropollutants (personal care products, pharmaceuticals and illicit drugs) were monitored for in full-scale HSSF steel slag and gravel beds to assess their fate and behaviour during tertiary wastewater treatment. Of the studied micropollutants 54 were found in receiving and treated wastewaters. Treatment reduced concentrations of several micropollutants by >50% (removal range −112% to 98%) and resulted in changes to the stereo-isomeric composition of chiral species. For example, stereo-selective changes were observed for 3,4-methylenedioxymethamphetamine (MDMA) and atenolol during HSSF constructed wetland treatment for the first time. Analysis of sludge present within the HSSF beds found 37 micropollutants to be present. However, concentrations for the majority of these micropollutants were not considered high enough to suggest partitioning into sludge was a contributing mechanism of removal. Nevertheless the preservative methylparaben was found at 2772 mg bed^{-1}. Its daily removal from wastewater of 3.4 mg d^{-1} indicates partitioning and accumulation in sludge contributes to its removal. Other micropollutants found at high levels in sludge (relative to their overall removals) were the antidepressants sertraline and fluoxetine, and the metabolite desmethylcitalopram. Furthermore, process balances indicated uptake and metabolism by *Phragmites australis* (*Cav.*) *Trin. ex Steud* did not contribute significantly to micropollutant removal. However analysis of plant tissues evidenced uptake, metabolism and accumulation of recalcitrant micropollutants such as ketamine and carbamazepine. It is considered that the rate of uptake was too slow to have a notable impact on removal

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at the 14 h hydraulic retention time. Despite evidence of other removal mechanisms at play (e.g., partitioning into sludge and plant uptake), findings indicate biodegradation is the dominant mechanism of micropollutant removal in HSSF constructed wetlands.

1. Introduction

Constructed wetlands are being used as a tertiary treatment option for secondary effluent polishing to meet increasingly stringent discharge limits. Horizontal sub-surface flow (HSSF) is a popular wetland configuration in which water flows through a porous medium or substrate such as gravel (Verlicchi and Zambello 2014). These systems tend to be planted with macrophytes such as *Phragmites australis* (the common reed). They are primarily used for the removal of conventional pollutants including suspended solids, nutrients and bulk organic matter (Verlicchi and Zambello 2014). However, fortuitous removals of other pollutants such as pharmaceuticals have been observed in several studies (Verlicchi and Zambello 2014; Ávila et al. 2015; Matamoros et al. 2016).

To date, the majority of studies focused on micropollutants measure their net removal during HSSF constructed wetland treatment (i.e., determine micropollutant concentrations before and after treatment only) (Verlicchi and Zambello 2014). However, to better appreciate micropollutant fate and behaviour it is essential to determine their distribution between the various biotic phases of the process. For example the build up of sludge within the wetland bed can act as sink for the accumulation of micropollutants. Furthermore, there is a paucity of information on micropollutant uptake by *P. australis* under field conditions. The presence of plants is known to improve micropollutant removal in constructed wetlands (Hijosa-Valsero et al., 2010; Matamoros et al. 2012). For example, Matamoros et al. (2012) found removals of caffeine and ibuprofen were 40–80% in microcosm studies with plants, whereas in equivalent reactors without plants the removal was 2–30%. Plants are known to take up and assimilate nutrients, act as a surface for biofilm growth, pump and release O_2, retain suspended particles and insulate against low temperatures (Tanner 2001; Kyambadde et al. 2004). Previous studies have provided evidence of micropollutant uptake and metabolism by plants in controlled experiments (He et al. 2017; Lv et al. 2017). He et al. (2017) proposed that following uptake by *P. australis*, ibuprofen is catalyzed by cytochrome P450 mono oxygenase and glycosyltransferase. Further storage or metabolism was then mediated in vacuoles or cell walls. However, it is unknown if uptake and metabolism by plants directly plays a significant role on overall micropollutant removals in full-scale systems. Overall there has been little analysis undertaken on solid matrices of HSSF constructed wetlands (e.g., sludge and plant tissues). The lack of analysis is due to the lack of good analytical approaches available as well as the further sample preparation requirements (Petrie et al. 2015).

Despite ~50% of pharmaceuticals being chiral and likely to undergo stereo-selective changes during HSSF wetland treatment, the majority of previous studies have not considered this when assessing micropollutants removal. This is essential to monitor as stereo-specific toxicity is known to occur in the environment (Stanley et al. 2006; Stanley et al. 2007; De Andrés et al. 2009). Nevertheless, very little information is available in the literature on the stereo-selective transformation of chiral micropollutants by HSSF constructed wetlands. Only ibuprofen and naproxen have been studied in any detail previously, and have shown to undergo stereo-selective degradation (Matamoros et al. 2009; Hijosa-Valsero et al. 2010).

To further our understanding of HSSF constructed wetlands for micropollutant remediation, the aim of this study was to examine the (stereo-selective) distribution of chiral and achiral micropollutants between different biotic phases of constructed wetlands. This was achieved by studying three differently configured HSSF wetlands: steel slag bed operated for 2 months, gravel bed operated for 2 months and gravel bed operated for 12 months. These were all planted with *P. australis* and treated the same wastewater. A total of 88 chemically and biologically diverse chiral and achiral micropollutants were studied (see Table S1, Supplementary material). Receiving wastewater (trickling filter effluent), constructed wetland effluent, sludge and *P. australis* were analysed for the full suite of micropollutants.

2. Materials and methods

2.1. Materials

All materials used in the investigation are described in the Supplementary material. This includes all analytical reference standards and reagents for mobile phase preparation and sample extraction. The procedure for deactivating glassware is also described here.

2.2. Analytical methods

2.2.1. Liquid matrices

Liquid wastewater samples were filtered (GF/F filters, 0.7 μm) and 50 mL aliquots spiked with 50 ng of all internal standards. Samples were loaded onto Oasis HLB SPE cartridges pre-conditioned using 2 mL methanol (MeOH) followed by 2 mL H_2O. Samples were loaded at 5 mL min^{-1} then dried under vacuum. Analytes were then eluted using 4 mL MeOH at a flow rate of 1 mL min^{-1}. Extracts were dried under nitrogen using a TurboVap evaporator (Caliper, UK, 40 °C, N_2, <5 psi) and reconstituted in 500 μL 80:20 H_2O:MeOH (Waters, Manchester, UK) ready for UHPLC-MS/MS analysis (Petrie et al. 2016).

2.2.2. Phragmites australis

Plants were frozen and freeze dried (ScanVac, CoolSafe freeze dryer, Lynge, Denmark) prior to extraction. Samples were then homogenized using a mechanical blender (Kenwood, Havant, UK). Representative 0.5 g samples were spiked with 50 ng of all internal standards (in 50 μL MeOH) and left for a minimum of 2 h. Extraction was performed using 25 mL of 25:75 MeOH:H_2O at a temperature of 50 °C using a 800 W MARS 6 microwave (CEM, UK). Once cooled to room temperature, samples were diluted with H_2O to achieve a final MeOH concentration of <5%, and treated as a liquid sample as in Section 2.2.1. Reconstituted samples were filtered through pre-LCMS 0.2 μm PTFE filters (Whatman, Puradisc) (Petrie et al. 2017). A single plant was also divided into different sections (roots, leaves and 4 × 30 cm section of stem) to investigate within-plant micropollutant distribution. Each section of plant was treated as described previously.

2.2.3. Sludge

Sludge was frozen and freeze dried (ScanVac). 0.5 g samples were spiked with 50 ng of all internal standards and left for a minimum of 2 h. These were then extracted with 25 mL 50:50 MeOH:H_2O (pH 2) at 110 °C using a 800 W MARS 6 microwave (CEM). Sample extracts were then adjusted to <5% MeOH using H_2O (pH 2) and filtered (0.7 μm). SPE was then performed using Oasis MCX cartridges conditioned with 2 mL MeOH and 2 mL H_2O (pH 2). Samples were loaded at 5 mL min^{-1} and dried. Analytes were eluted in separate fractions to reduce matrix interferences from the high complexity of sludge extracts using 0.6% HCOOH in MeOH and 7% NH_4OH in MeOH (fraction 2).

Dried extracts were then reconstituted in 500 μL in 80:20 H_2O:MeOH and filtered through pre-LCMS 0.2 μm PTFE filters (Whatman) (Petrie et al. 2016).

2.2.4. LC-MS/MS analysis

To determine micropollutant concentrations, 2 chromatography methods were used. Acidic analytes were separated using a gradient of 1 mM NH_4F in 80:20 H_2O:MeOH (mobile phase A) and 1 mM NH_4F in 5:95 H_2O:MeOH (mobile phase B). Initial conditions of 100% A were maintained for 0.5 min before reducing to 40% over 2 min and further reduced to 0% over 5.5 min. Separation of basic analytes was performed using 5 mM NH_4OAc and 3 mM CH_3COOH in 80:20 H_2O:MeOH (mobile phase A) and MeOH (mobile phase B). Starting conditions of 100% A were reduced to 10% over 20 min and maintained for a further 6 min. Both methods utilised a reversed-phase BEH C18 column (150 × 1.0 mm, 1.7 μm particle size) (Waters, Manchester, UK) maintained at 40 °C using a Waters Acquity UPLC system (Waters). The mobile phase flow rate was 0.04 mL min^{-1} and an injection volume of 15 μL used for both methods (Petrie et al. 2016).

To determine the enantiomeric fraction (EF) of chiral micropollutants, analytes were separated using a Cellobiohydrolase column (100 × 2 mm, 5 μm particle size) (Chiral Technologies, France) and a mobile phase consisting 1 mM NH_4OAc in 85:15 H_2O:MeOH. Separation was achieved under isocratic conditions with a mobile phase flow rate of 0.1 mL min^{-1}. Here the injection volume was 20 μL (Castrignanò et al. 2016). EFs were calculated according to Eq. (1):

$$EF = \frac{(+)}{[(+) + (-)]} \quad (1)$$

where (+) is the peak area of the (+)-enantiomer or the first eluting enantiomer if the elution order is not known, corrected for the deuterated internal standard response and (−) is the peak area of the (−)-enantiomer or the second eluting enantiomer corrected for the deuterated internal standard response.

In all methods the LC system was coupled to a Xevo TQD Triple Quadrupole Mass Spectrometer (Waters), equipped with an electrospray ionisation (ESI) source. Acidic analytes were analysed in negative ionisation mode (ESI −) with a capillary voltage of 3.20 kV. Basic micropollutants were analysed in positive ionisation mode (ESI +) with a capillary voltage of 3.00 kV. The optimised MS/MS transitions can be found in Table S2 (See Supplementary material). The source temperature was 150 °C whilst the desolvation temperature was 400 °C. A cone gas flow of 100 L $h^{-1}$and a desolvation gas flow of 550 L h^{-1} was used. Finally, the nebulising and desolvation gases were nitrogen, and the collision gas was argon (Petrie et al. 2016). For validation information of all methods please see Table S3 (See Supplementary material).

2.3. Constructed wetlands

All samples were collected from a wastewater treatment plant (WTP) which serves a population equivalent of 12,500 located in the South-West of the UK (average dry weather flow = 1850 m^3 d^{-1}). It consists of primary sedimentation, secondary treatment by trickling filters and sedimentation followed by constructed wetlands as a final polishing step. The constructed wetlands consist of three different beds (in duplicate) configured with identical dimensions (55 × 10 m) treating the same wastewater. They operated as non-aerated HSSF wetlands with differing substrates planted with *P. australis* (planting density = 4–6 plants per m^2). One bed had a steel slag substrate (40.0% CaO, 28.5% Fe_2O_3, 11.2% SiO_2, 9.7% MgO, 3.4% Al_2O_3, 2.5% Mn_3O_4, 1.3% P_2O_5 and <1.0% TiO_2, Cr_2O_3, V_2O_5, Na_2O, K_2O, BaO and SO_3) whilst the others were siliceous gravel. The depth of substrate was approximately 1 m and flow was controlled to achieve an estimated hydraulic retention time (HRT) of 14 ± 2 h. One gravel bed was considered well-established as it had been operating for 12 months and *P. australis* were generally 1.5 to 2.0 m in height. In the remaining beds *P. australis* were planted 2 months prior to sampling and were ~0.5 m in height (See Fig. S1, Supplementary material). Samples were collected from the inlet of the constructed wetlands as well as final effluent from each differently configured bed (see Figs. S2 and S3, Supplementary material). 24 h time proportional composite samples (80 mL every 15 min) were collected and cooled to 4 °C. Micropollutant removals (%) were calculated by taking account of their difference in concentration between the inlet and final effluent for each differently configured bed. Sludge/sediment (~10 g) was extracted from the top 10 cm of the substrate bed, and whole plants (including roots) were collected from near the inlet and outlet of the gravel bed which had been in operation for 12 months on each sampling day (see Fig. S3, Supplementary material). Both plants and sludge samples were collected in duplicate from each sampling point. Roots were rinsed under tap water to remove excess sludge. All samples were transported to the laboratory on ice and within 30 min of collection. Sampling was performed during four consecutive days in July 2015.

2.4. Microcosm studies

Microcosm studies were prepared to examine mechanisms of removal. Microcosms were prepared in autoclaved 2 L Erlenmeyer flasks and filled with steel slag substrate collected from the constructed wetland, accounting for ~50% of the flask volume (see Fig. S3, Supplementary material). Substrate was collected at a depth of >5 cm to ensure the presence of a biofilm. Flasks were then filled with 1 L of trickling filter effluent (collected as a grab sample during July 2015). Prepared microcosms were treated to achieve the following conditions; (i) dark biotic - A, (ii) dark abiotic - B, (iii) light biotic - C, (iv) light abiotic - D, (v) light biotic (no substrate) - E, and (vi) light abiotic (no substrate) - F. "Light" microcosms were left exposed to ambient light in the laboratory (windows and overhead lighting) whereas the "dark" microcosms were wrapped in aluminium foil. The effluent in abiotic microcosms was treated with 1 g L^{-1} NaN_3 to inhibit biological activity. Sterile cotton wool plugs were used to seal each microcosm. Samples were collected at times 0, 4, 22, 26, 46 and 50 h.

3. Results and discussion

3.1. Micropollutants in liquid matrices of HSSF constructed wetlands and their speciation

A total of 88 micropollutants were monitored including a range of pharmaceutical drug sub-classes (e.g., antibiotics, anti-hypertensives, lipid regulators, anti-histamines, diabetes, beta-blockers, H_2 receptor agonists, anaesthetics, anti-depressants, analgaesics and anti-epileptics), personal care products (UV filters, parabens), steroid estrogens, the plasticizer bisphenol-A, licit stimulants and illicit drugs (stimulants and opioids), as well as metabolites. In receiving wastewater for treatment (trickling filter effluent) 54 micropollutants were present, with all major micropollutant classes and sub-classes represented (Table 1). Concentrations ranged from ng L^{-1} to the low μg L^{-1} range and were representative of previously reported levels in municipal effluents (Petrie et al. 2015). The micropollutant found at the highest average concentration was the UV filter benzophenone-4 at 4164 ± 119 ng L^{-1} (Table 1).

Novel bed substrates such as steel slag have been proposed as an alternative bed substrate in HSSF constructed wetlands to enhance phosphorus removals (Ge et al. 2016; Park et al. 2016). However, there is a paucity of information on steel slag for micropollutant remediation. Under field conditions (operational for 2 months), the steel slag HSSF constructed wetland was successful at removing some micropollutants by >50% (Table 2). This included licit stimulants (caffeine, nicotine and cotinine), non-steroidal anti-inflammatory drugs (NSAIDs, ibuprofen,

Table 1
Micropollutant concentrations determined in inlet trickling filter effluent, final effluent from the 3 differently configured beds, sludge and *P. australis*.

Micropollutant class	Micropollutant	Inlet – trickling filter effluent ($ng\ L^{-1}$)	Final effluent			Sludge ($\mu g\ kg^{-1}$ dry weight)	*P. australis* ($\mu g\ kg^{-1}$ dry weight)
			Steel slag substrate – 2 months ($ng\ L^{-1}$)	Gravel substrate – 2 months ($ng\ L^{-1}$)	Gravel substrate – 12 months ($ng\ L^{-1}$)		
UV filters	Benzophenone-1	<MQL	<MQL	<MQL	<MQL	6.4 ± 2.1	<MQL
	Benzophenone-2	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
	Benzophenone-3	46.0 ± 7.3	64.6 ± 19.3	31.1 ± 2.8	69.6 ± 17.9	–	<MQL
	Benzophenone-4	4164 ± 118.6	3803 ± 394.2	3858 ± 89.8	3719 ± 222.6	<MQL	<MQL
Parabens	Methylparaben	23.8 ± 1.8	22.6 ± 4.1	19.9 ± 1.6	19.8 ± 2.7	308 ± 119	197 ± 83.8
	Ethylparaben	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
	Propylparaben	6.1 ± 0.6	7.5 ± 2.1	6.0 ± 0.7	8.6 ± 2.0	2.7 ± 0.4	<MQL
	Butylparaben	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
Plasticizer	Bisphenol-A	245 ± 150.2	242 ± 218.0	166 ± 20.3	184 ± 40.2	356 ± 187	78.4 ± 43.4
Steroid estrogens	E1	26.0 ± 10.4	16.0 ± 4.3	<MQL	<MQL	<MQL	<MQL
	E2	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
	EE2	<MQL	<MQL	<MQL	<MQL	–	<MQL
Human antibiotics/antibacterials	Sulfasalazine	386 ± 35.2	392 ± 24.5	282 ± 39.1	173 ± 6.8	–	<MQL
	Clarithromycin	572 ± 121.9	152 ± 24.8	311 ± 48.4	301 ± 27.2	–	<MQL
	Azithromycin	<MQL	<MQL	<MQL	<MQL	–	<MQL
	Trimethoprim	646 ± 105	483 ± 42.3	353 ± 33.5	213 ± 41.4	48.4 ± 28.3	<MQL
	Sulfamethoxazole	46.8 ± 7.0	37.8 ± 9.3	33.3 ± 4.3	32.8 ± 4.2	2.5 ± 0.3	<MQL
	Triclosan	129 ± 16.3	129 ± 24.8	104 ± 15.3	102 ± 8.2	–	–
Antihypertensives	Valsartan	415 ± 72.9	336 ± 35.6	345 ± 37.8	323 ± 20.2	–	<MQL
	Irbesartan	667 ± 70.7	644 ± 84.2	701 ± 77.7	695 ± 71.4	–	<MQL
	Lisinopril	149 ± 41.7	55.5 ± 10.7	<MQL	<MQL	<MQL	–
NSAIDs	Ketoprofen	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
	Ibuprofen	432 ± 25.3	314 ± 51.9	317 ± 32.3	300 ± 47.9	17.3 ± 5.0	<MQL
	Naproxen	1497 ± 165	1146 ± 217.7	1152 ± 74.4	1013 ± 139	34.0 ± 17.9	<MQL
	Diclofenac	2099 ± 1224	1770 ± 851.0	1639 ± 568	1691 ± 594	39.0 ± 11.2	<MQL
	Acetaminophen	273 ± 158.5	152 ± 144.4	70.9 ± 18.7	95.1 ± 25.4	<MQL	<MQL
Lipid regulators	Bezafibrate	969 ± 23.5	520 ± 40.2	509 ± 30.6	383 ± 51.0	<MQL	<MQL
	Atorvastatin	418 ± 142	273 ± 55.4	139 ± 27.8	132 ± 8.4	–	<MQL
Antihistamines	Fexofenadine	1922 ± 111	1668 ± 29.7	1190 ± 143.4	664.0 ± 66.5	–	<MQL
	Cetirizine	650 ± 63.0	610 ± 57.5	588 ± 72.0	572 ± 70.8	–	<MQL
Diabetes	Gliclazide	83.8 ± 10.7	63.9 ± 13.6	71.0 ± 5.8	71.8 ± 1.8	–	<MQL
Cough suppressant	Pholcodine	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
Beta-blocker	Atenolol	287 ± 27.6	89.1 ± 7.7	61.1 ± 7.4	64.5 ± 7.4	4.6 ± 0.4	<MQL
	Metoprolol	4.9 ± 3.4	4.0 ± 1.7	3.5 ± 1.4	2.9 ± 1.5	1.3 ± 1.0	<MQL
	Propranolol	132 ± 3.5	107 ± 5.7	110 ± 5.3	92.3 ± 3.3	120 ± 35.5	29.1 ± 25.1
H_2 receptor agonists	Ranitidine	1335 ± 123.1	1005 ± 75.4	889 ± 62.1	755 ± 88.0	313 ± 157	<MQL
	Cimetidine	<MQL	<MQL	<MQL	<MQL	–	<MQL
X-ray contrast media	Iopromide	<MQL	<MQL	<MQL	<MQL	–	<MQL
Drug precursor and metabolite	Ephedrine/pseudoephedrine	78.0 ± 8.5	62.2 ± 5.5	33.1 ± 7.3	24.0 ± 3.0	<MQL	<MQL
	Norephedrine	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
Anti-cancer	Azathioprine	<MQL	<MQL	<MQL	<MQL	–	<MQL
	Methotrexate	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
	Ifosfamide	<MQL	<MQL	<MQL	<MQL	–	<MQL
	Tamoxifen	<MQL	<MQL	<MQL	<MQL	<MQL	–
Anaesthetic and metabolite	Ketamine	242 ± 70.6	269 ± 73.3	266 ± 66.1	259 ± 43.5	15.3 ± 5.7	80.2 ± 55.2
	Norketamine	22.1 ± 6.6	29.4 ± 8.1	34.0 ± 7.0	37.9 ± 5.4	4.0 ± 2.4	13.1 ± 9.5
Anti-depressants and metabolites	Venlafaxine	553 ± 9.4	502 ± 28.9	533 ± 23.4	539 ± 22.2	<MQL	23.6 ± 11.2
	Desmethylvenlafaxine	1173 ± 39.2	1181 ± 96.7	1245 ± 64.9	1223 ± 86.2	76.2 ± 38.4	3.5 ± 1.5
	Fluoxetine	47.5 ± 6.6	58.0 ± 20.6	61.9 ± 8.0	96.9 ± 27.3	171 ± 28.6	–
	Norfluoxetine	<MQL	<MQL	<MQL	<MQL	30.0 ± 6.7	–
	Sertraline	<MQL	<MQL	<MQL	<MQL	688 ± 127	–
	Mirtazapine	47.0 ± 3.7	44.4 ± 2.3	40.5 ± 3.1	44.5 ± 0.9	50.9 ± 8.1	35.2 ± 22.9
	Citalopram	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
	Desmethylcitalopram	110 ± 6.1	70.3 ± 2.9	101 ± 13.6	118 ± 4.3	300 ± 94.2	<MQL
Anti-epileptic and metabolites	CBZ	1102 ± 12.8	1061 ± 90.9	1075 ± 59.6	1100 ± 52.4	105 ± 33.2	30.2 ± 4.0
	CBZ10,11-epoxide	238 ± 13.3	242 ± 16.3	253 ± 17.0	255 ± 7.9	–	10.0 ± 2.7
	10,11-Dihydro-10-hydroxyCBZ	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
Calcium channel blocker	Diltiazem	61.0 ± 3.9	22.6 ± 2.3	25.3 ± 2.8	11.8 ± 3.4	–	4.3 ± 2.7
Hypnotic	Temazepam	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
Anti-psychotic	Quetiapine	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
Veterinary antibiotic	Tylosin	<MQL	<MQL	<MQL	<MQL	–	<MQL
Licit stimulants and metabolites	Nicotine	98.0 ± 19.0	65.9 ± 16.4	22.8 ± 3.4	25.9 ± 11.2	70.4 ± 16.8	<MQL
	Caffeine	2866 ± 174.1	1622 ± 236.0	1261 ± 92.4	747.6 ± 125.6	–	11.6 ± 7.9
	Cotinine	218 ± 29.1	148 ± 4.6	59.6 ± 5.0	41.0 ± 10.4	21.7 ± 4.3	<MQL
	1,7 dimethylxantine	<MQL	<MQL	<MQL	<MQL	–	–
Analgaesics and metabolites	Morphine	221 ± 22.4	71.6 ± 14.2	23.8 ± 5.4	3.6 ± 0.7	16.4 ± 5.2	<MQL
	Dihydromorphine	<MQL	<MQL	<MQL	<MQL	17.3 ± 5.1	<MQL
	Normorphine	<MQL	<MQL	<MQL	<MQL	72.0 ± 21.8	<MQL
	Methadone	24.3 ± 2.6	18.0 ± 2.6	23.1 ± 1.6	24.8 ± 1.9	22.6 ± 3.7	8.8 ± 3.7
	EDDP	127 ± 16.8	88.0 ± 7.5	109 ± 9.4	111 ± 6.6	98.8 ± 33.4	3.9 ± 1.1
	Codeine	794 ± 110.5	516 ± 30.0	284 ± 31.9	181 ± 30.6	16.2 ± 6.6	<MQL

(continued on next page)

Table 1 (*continued*)

Micropollutant class	Micropollutant	Inlet – trickling filter effluent (ng L^{-1})	Final effluent			Sludge (μg kg^{-1} dry weight)	*P. australis* (μg kg^{-1} dry weight)
			Steel slag substrate – 2 months (ng L^{-1})	Gravel substrate – 2 months (ng L^{-1})	Gravel substrate – 12 months (ng L^{-1})		
	Norcodeine	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
	Dihydrocodeine	287 ± 15.2	246 ± 12.9	177 ± 2.3	94.0 ± 13.1	7.3 ± 4.8	<MQL
	Tramadol	1519 ± 42.6	1406 ± 106.7	1450 ± 66.2	1456 ± 71.1	80.6 ± 20.9	64.6 ± 40.4
	N-Desmethyltramadol	1092 ± 20.9	959.6 ± 61.1	976.6 ± 117.0	962.8 ± 28.3	89.1 ± 18.5	50.0 ± 42.5
	O-Desmethyltramadol	1592 ± 45.1	1355 ± 132.1	1373 ± 65.3	1535 ± 46.0	–	<MQL
Stimulants and metabolites	Amphetamine	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
	Methamphetamine	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
	MDMA	62.5 ± 42.4	58.3 ± 21.7	52.9 ± 22.6	56.1 ± 16.6	4.6 ± 1.1	6.6 ± 4.6
	MDA	28.4 ± 9.3	21.3 ± 5.1	15.0 ± 10.1	12.3 ± 8.4	–	<MQL
	Cocaine	14.0 ± 5.9	1.8 ± 0.9	<1.1	2.5 ± 1.5	1.5 ± 0.6	<MQL
	Benzoylecgonine	312 ± 71.7	193 ± 68.4	17.8 ± 5.9	23.1 ± 12.6	0.9 ± 0.7	<MQL
	Anhydroecgonine methylester	<MQL	<MQL	<MQL	<MQL	–	<MQL
	Cocaethylene	2.3 ± 0.5	1.4 ± 0.5	1.4 ± 0.3	1.6 ± 0.5	0.5 ± 0.2	<MQL
	Mephedrone	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
	MDPV	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
Opioid and metabolite	Heroin	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
	6-Acetylmorphine	<MQL	<MQL	<MQL	<MQL	–	<MQL

Key: E1, estrone; E2, 17β-estradiol; EE2, 17α-ethinylestradiol; CBZ, carbamazepine; EDDP, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; MDMA, 3,4-methylenedioxymethamphetamine; MDA, 3,4-methylenedioxyamphetamine; <MQL, below method quantitation level; —, not determined.

naproxen and acetaminophen), the natural estrogen estrone, the antibiotic clarithromycin, the calcium channel blocker diltiazem, the antihypertensive lisinopril and the illicit stimulant cocaine. On the other hand, anti-depressants and their metabolites, and other recalcitrant compounds including ketamine (anaesthetic), carbamazepine (anti-epileptic), tramadol (analgesic), diclofenac (NSAID) and MDMA (illicit stimulant) were poorly removed or increased in concentration. An increase in concentration during biological treatment is attributed to the biotransformation of metabolites (not measured by the analytical method) to the parent micropollutant. Removals of some micropollutants were similar to those observed in the gravel HSSF constructed wetlands (2 months and 12 months operation) (Table 2). However, it should be noted that to make more substantive comparisons on the performance of the differently configured wetlands for micropollutant remediation, more rigorous sampling is required (i.e., sampling over longer time periods as well as during different seasons). Nevertheless, the removals reported are in agreement with previous studies (Verlicchi and Zambello 2014).

The same 54 micropollutants which were found in receiving wastewater for treatment were found in HSSF constructed wetland effluent. As benzophenone-4 was at the highest receiving concentration and poorly removed during treatment, it was found in effluent at the highest concentrations. Average concentrations of benzophenone-4 in effluent were in the range 3719 to 3858 ng L^{-1} (Table 1). Other micropollutants found at concentrations >1000 ng L^{-1} following treatment were naproxen, diclofenac, fexofenadine, ranitidine, desmethylvenlafaxine, carbamazepine, caffeine, tramadol and *O*-desmethyltramadol.

Of the 54 micropollutants detected several are chiral and exist as enantiomers. However, there is a paucity of information on the speciation of chiral drugs during treatment by constructed wetlands. Stereo-selective degradation has been previously reported for only ibuprofen (Matamoros et al. 2009; Hijosa-Valsero et al. 2010) and naproxen (Matamoros et al. 2009). In our study, 9 different chiral micropollutants were monitored at the enantiomeric level for the first time (MDMA, MDA, methamphetamine, amphetamine, mephedrone, atenolol, tramadol, venlafane and desvenlafaxine). Receiving inlet wastewater (trickling filter effluent) contained chiral micropollutants whose EF ranged from 0.23 ± 0.03 for MDMA to 0.67 ± 0.01 for tramadol (Table 3). These are typical of what is observed in the effluent of secondary treatment processes such as trickling filters or activated sludge (Kasprzyk-Hordern and Baker 2012).

In final effluent of the steel slag constructed wetland no substantial changes to the EF (in comparison to inlet wastewater) of any studied micropollutants was observed. However, stereo-selective changes to MDMA were observed for the gravel constructed wetland (2 month operation). Here the EF of MDMA in final effluent was 0.11 ± 0.03 (Table 3). In final effluent of the 12 month operated gravel wetland stereo-selective changes were observed for both MDMA and atenolol. The EF of MDMA was 0.12 ± 0.04 and similar to that of the other gravel constructed wetland (Table 3). However, the EF of atenolol in final effluent was 0.41 ± 0.01 (inlet EF = 0.48 ± 0.02). Despite the EF of atenolol being significantly different to the 2 month bed (EF = 0.46 ± 0.01) (ANOVA, p-value < 0.05), the total atenolol concentration (sum of both enantiomers) in the 2 effluents were not (ANOVA, p-value > 0.05). Concentrations were 64.5 ± 7.4 ng L^{-1} and 61.1 ± 7.4 ng L^{-1} for the wetlands operated for 12 and 2 months, respectively (Table 3). Such an observation is important as stereo-specific toxicity of atenolol towards aquatic micro-organisms can be observed (De Andrés et al. 2009). This demonstrates that the speciation of chiral micropollutants should be measured to better understand HSSF constructed wetland performance for micropollutant removal.

3.2. Behaviour of micropollutants in the presence of the bed substrate

To assess the behaviour of micropollutants in the presence of the wetland bed substrate, controlled laboratory microcosms (without plants) were performed. These were prepared to help establish the role of substrate on micropollutant removal and to identify possible mechanism(s) of removal. Steel slag was the chosen substrate for these experiments as it has not been studied previously. 16 micropollutants were present in the grab sample of trickling filter effluent used to prepare the microcosms (Table S4. Supplementary material). Differences observed in the grab sample compared to the 24 h composites collected in the full-scale study is attributed to the sample mode and the likely short-term variability in micropollutant concentration.

For those micropollutants which exhibited removal in microcosm studies, the range of fate behaviours observed were broadly encompassed by atenolol, bezafibrate and morphine (see Fig. S4, Supplementary material). Firstly, atenolol was reduced to <1.8 ng L^{-1} in all microcosms containing substrate following 26 h of treatment (starting concentration was 425.5 ± 1.5 ng L^{-1}, see Table S4, Supplementary material). No substantial differences in removal were observed between microcosms which were biologically active and those treated with NaN_3, confirming the dominant removal pathway here was by sorption. As atenolol is present in the cationic form and the biofilm on

Table 2
Removal efficiency (%) of micropollutants by differently configured constructed wetlands receiving the same source of treated wastewater ($n = 4$).

Micropollutant class	Micropollutant	HSSF constructed wetland configuration		
		Steel slag substrate – 2 months operation	Gravel substrate – 2 months operation	Gravel substrate – 12 months operation
UV-filters	Benzophenone-1	–	–	–
	Benzophenone-2	–	–	–
	Benzophenone-3	−41 ± 38	31 ± 11	−50 ± 21
	Benzophenone-4	8 ± 11	7 ± 3	11 ± 8
Parabens	Methylparaben	5 ± 15	16 ± 3	17 ± 11
	Ethylparaben	–	–	–
	Propylparaben	−24 ± 41	1 ± 17	−43 ± 45
	Butylparaben	–	–	–
Plasticizer	Bisphenol-A	7 ± 30	5 ± 64	3 ± 51
Steroid estrogens	E1	36 ± 15	>70	>70
	E2	–	–	–
	EE2	–	–	–
Human antibiotics/antibacterials	Sulfasalazine	5 ± 5	28 ± 9	51 ± 2
	Clarithromycin	73 ± 6	45 ± 3	46 ± 11
	Azithromycin	–	–	–
	Trimethoprim	24 ± 15	45 ± 5	67 ± 5
	Sulfamethoxazole	19 ± 18	28 ± 10	30 ± 4
	Triclosan	−2 ± 31	19 ± 13	20 ± 12
Antihypertensives	Valsartan	18 ± 14	14 ± 7	19 ± 11
	Irbesartan	3 ± 7	−5 ± 6	−4 ± 6
	Lisinopril	58 ± 22	>71	>71
NSAIDs	Ketoprofen	–	–	–
	Ibuprofen	27 ± 13	26 ± 8	31 ± 10
	Naproxen	23 ± 17	23 ± 7	32 ± 9
	Diclofenac	1 ± 44	0 ± 37	−5 ± 54
	Acetaminophen	20 ± 80	69 ± 16	51 ± 37
Lipid regulators	Bezafibrate	46 ± 4	48 ± 2	61 ± 5
	Atorvastatin	32 ± 10	65 ± 7	66 ± 10
Antihistamines	Fexofenadine	16 ± 4	40 ± 4	67 ± 3
	Cetirizine	6 ± 6	9 ± 5	12 ± 5
Diabetes	Gliclazide	21 ± 28	13 ± 20	13 ± 12
Cough suppressant	Pholcodine	–	–	–
Beta-blocker	Atenolol	69 ± 6	79 ± 3	77 ± 3
	Metoprolol	−2 ± 41	4 ± 61	22 ± 42
	Propranolol	19 ± 6	17 ± 2	30 ± 4
H_2 receptor agonists	Ranitidine	24 ± 12	33 ± 7	43 ± 6
	Cimetidine	–	–	–
X-ray contrast media	Iopromide	–	–	–
Drug precursor and metabolite	Ephedrine/pseudoephedrine	19 ± 13	58 ± 6	69 ± 4
	Norephedrine	–	–	–
Anti-cancer	Azathioprine	–	–	–
	Methotrexate	–	–	–
	Ifosfamide	–	–	–
	Tamoxifen	–	–	–
Anaesthetic and metabolite	Ketamine	−11 ± 2	−11 ± 4	−9 ± 12
	Norketamine	−34 ± 10	−57 ± 18	−78 ± 35
Anti-depressants and metabolites	Venlafaxine	9 ± 4	3 ± 6	2 ± 3
	Desmethylvenlafaxine	−1 ± 6	−6 ± 7	−4 ± 5
	Fluoxetine	−24 ± 48	−32 ± 23	−112 ± 83
	Norfluoxetine	–	–	–
	Sertraline	–	–	–
	Mirtazapine	5 ± 10	14 ± 8	5 ± 8
	Citalopram	–	–	–
	Desmethylcitalopram	36 ± 1	8 ± 14	−8 ± 6
Anti-epileptic and metabolites	CBZ	4 ± 8	2 ± 5	0 ± 4
	CBZ10,11-epoxide	−2 ± 12	−6 ± 7	−7 ± 3
	10,11-Dihydro-10-hydroxyCBZ	–	–	–
Calcium channel blocker	Diltiazem	63 ± 6	59 ± 4	81 ± 6
Hypnotic	Temazepam	–	–	–
Anti-psychotic	Quetiapine	–	–	–
Veterinary antibiotic	Tylosin	–	–	–
Licit stimulants and metabolites	Nicotine	33 ± 10	76 ± 4	74 ± 9
	Caffeine	43 ± 8	56 ± 1	74 ± 5
	Cotinine	31 ± 9	72 ± 2	81 ± 4
	1,7 dimethylxantine	–	–	–
Analgaesics and metabolites	Morphine	68 ± 5	89 ± 2	98 ± 2
	Dihydromorphine	–	–	–
	Normorphine	–	–	–
	Methadone	26 ± 9	4 ± 5	−3 ± 10
	EDDP	30 ± 8	13 ± 6	12 ± 8
	Codeine	34 ± 9	64 ± 2	77 ± 5
	Norcodeine	–	–	–

(continued on next page)

Table 2 (*continued*)

Micropollutant class	Micropollutant	HSSF constructed wetland configuration		
		Steel slag substrate – 2 months operation	Gravel substrate – 2 months operation	Gravel substrate – 12 months operation
	Dihydrocodeine	14 ± 6	38 ± 3	67 ± 6
	Tramadol	7 ± 7	4 ± 6	4 ± 4
	N-Desmethyltramadol	12 ± 5	10 ± 11	12 ± 3
	O-Desmethyltramadol	15 ± 9	14 ± 5	4 ± 4
Stimulants and metabolites	Amphetamine	–	–	–
	Methamphetamine	–	–	–
	MDMA	−15 ± 64	2 ± 40	−14 ± 60
	MDA	22 ± 18	51 ± 35	59 ± 32
	Cocaine	88 ± 1	>92	81 ± 13
	Benzoylecgonine	39 ± 8	94 ± 1	93 ± 4
	Anhydroecgonine methylester	–	–	–
	Cocaethylene	40 ± 13	38 ± 14	27 ± 21
	Mephedrone	–	–	–
	MDPV	–	–	–
Opioid and metabolite	Heroin	–	–	–
	6-Acetylmorphine	–	–	–

Note: –, micropollutant not detected in receiving wastewater.
Key: E1, estrone; E2, 17β-estradiol; EE2, 17α-ethinylestradiol; CBZ, carbamazepine; CBZ 10,11 epoxide, carbamazepine 10,11 epoxide; EDDP, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; MDMA, 3,4-methylenedioxymethamphetamine; MDA, 3,4-methylenedioxyamphetamine.

the substrate is predominantly negatively charged (Zhang et al. 2014), electrostatic interactions are suggested as the sorption mechanism responsible for removal here. Other micropollutants which were found to be removed by sorption were E1, cocaine, benzoylecgonine and EDDP (data not shown). However, it must be considered that once equilibrium is achieved in a continuous process (i.e., the full-scale HSSF constructed wetlands), there will be no net removal by sorption unless accumulation can be demonstrated.

In contrast, notable bezafibrate removals were only found in biotic microcosm vessels which contained substrate. Here removals of 74.1–83.6% were observed over 50 h (see Fig. S4, Supplementary material). No removal was observed in abiotic microcosms or biotic vessels which did not contain substrate. This demonstrates removal of bezafibrate was biological in nature and governed by the active biofilm present on the substrate surface. The beta-blocker propranolol also showed evidence of being removed by biological degradation as it reduced in biotic vessels containing substrate, but not in equivalent abiotic vessels.

Finally, morphine was found to be removed by both sorption and biological transformation. Abiotic microcosms which contained substrate showed an initial reduction in morphine concentration of 28.3–33.7% within the first 4 h (see Fig. S4, Supplementary material). The concentration then remained stable for the rest of the study. This is typical for sorption as partitioning equilibrium is established almost immediately (Ternes et al. 2004). In biotic microcosms the concentration of morphine continued to reduce after 4 h due to biological transformation. After 26 h the initial concentration was reduced by 80.1–88.1%. Similar observations were noted for codeine. Little difference in micropollutant removal was observed for "light" and "dark" microcosms containing substrate under biotic and abiotic conditions (see Table S4, Supplementary material). This is to be expected as the substrate will shade the exposure of micropollutants to light.

Findings indicated both biological transformation and sorption contributed to micropollutant removal in microcosm studies, and is compound specific. This is in agreement with other researchers which have discussed the removal pathways of micropollutants in conventional substrates (e.g., gravel) (Ávila et al. 2010; Li et al. 2014). The remaining micropollutants (carbamazepine, ibuprofen, naproxen, cotinine, MDMA, venlafaxine and norketamine – see Table S4, Supplementary material) did not show any notable removals during microcosm studies and their concentrations remained largely unchanged. Conducting similar studies in the future as the bed (and biofilm) ages would further our understanding of micropollutant removal in such systems.

3.3. Micropollutants in HSSF constructed wetland sludge

In the full-scale beds, sludge accumulates over time which can act as a sink for micropollutants. Analysis of sludge collected from the longest operated wetland found 37 of the studied micropollutants to be present (Table 1). This suggests partitioning into sludge could contribute to their removal. Those sub-classes with the highest representation in sludge were anti-depressants and analgaesics. Nevertheless, a broad representation of micropollutant sub-classes was noted. To

Table 3
Concentration and enantiomeric fraction of chiral micropollutants in inlet wastewater (trickling filter effluent) and final effluent from the studied constructed wetlands.

Micropollutant class	Chiral micropollutant	Inlet – trickling filter effluent		Steel slag substrate effluent – 2 months operation		Gravel substrate effluent – 2 months operation		Gravel substrate effluent – 12 months operation	
		Conc. (ng L^{-1})	EF	Conc. (ng L^{-1})	EF	Conc. (ng L^{-1})	EF	Conc. (ng L^{-1})	EF
Illicit stimulant	MDMA	62.5 ± 42.4	0.23 ± 0.03	58.3 ± 21.7	0.20 ± 0.04	52.9 ± 22.6	0.11 ± 0.03	56.1 ± 16.6	0.12 ± 0.04
	MDA	28.4 ± 9.3	–[a]	21.3 ± 5.1	–[a]	15.0 ± 10.1	–[a]	12.3 ± 8.4	–[a]
	Methamphetamine	<MQL	–	<MQL	–	<MQL	–	<MQL	–
	Amphetamine	<MQL	–	<MQL	–	<MQL	–	<MQL	–
	Mephedrone	<MQL	–	<MQL	–	<MQL	–	<MQL	–
Beta-blocker	Atenolol	287 ± 27.6	0.48 ± 0.02	89.1 ± 7.7	0.47 ± 0.02	61.1 ± 7.4	0.46 ± 0.01	64.5 ± 7.4	0.41 ± 0.01
Analgaesic	Tramadol	1519 ± 42.6	0.67 ± 0.01	1406 ± 106.7	0.67 ± 0.01	1450 ± 66.2	0.68 ± 0.01	1456 ± 71.1	0.68 ± 0.01
Anti-depressant	Venlafaxine	553 ± 9.4	0.56 ± 0.01	502 ± 28.9	0.57 ± 0.01	533 ± 23.4	0.57 ± 0.01	539 ± 22.2	0.58 ± 0.01
	Desvenlafaxine	1173 ± 39.2	0.51 ± 0.00	1181 ± 96.7	0.51 ± 0.01	1245 ± 64.9	0.51 ± 0.01	1223 ± 86.2	0.50 ± 0.01

Key: conc., concentration; EF, enantiomeric fraction; MDMA, 3,4-methylenedioxy-methamphetamine; MDA, 3,4-methylenedioxy-amphetamine; MQL, method quantitation limit.
[a] <MQL of enantio-selective CBH method.

demonstrate, sludge concentrations of >100 μg kg^{-1} were found for methylparaben, bisphenol-A, propranolol, ranitidine, fluoxetine, sertraline, demethylcitalopram and carbamazepine. For partitioning into sludge to be an effective removal pathway, sludge micropolluant levels (e.g., mg bed^{-1}) need to be high relative to quantity removed from wastewater (mg d^{-1}). This would indicate accumulation within sludge over time (as no sludge is removed from the HSSF beds during operation).

The antidepressant sertraline was found at the greatest levels in sludge at 688 ± 127 μg kg^{-1} which equates to 4816 mg bed^{-1}. Sertraline was not found in wastewater at quantifiable levels throughout the sampling campaign (<4.7 mg d^{-1}). This indicates that sludge acts as a concentrating medium for sertraline. It is also likely that particulate bound sertraline in receiving wastewater also contributed to the levels found in sludge. This was not measured due to the low levels of suspended solids present (~30 mg L^{-1}). Furthermore, both fluoxetine (1197 mg bed^{-1}) and desmethylcitalopram (2100 mg bed^{-1}) were found at considerable concentrations in sludge despite showing no net removal from aqueous wastewater. Antidepressants are well-known for their partitioning in solid phases of environmental matrices (Lajeunesse et al., 2012). Both sertraline and fluoxetine are considered hydrophobic in nature with relatively high log K_{OW}'s of 5.29 and 4.65, respectively.

On the other hand, the estimated quantity of the preservative methylparaben (log K_{OW} 2.00) present in sludge was 2772 mg bed^{-1}. Considering the average removal of methylparaben achieved during treatment (3.4 mg d^{-1}), partitioning into the sludge bed is suggested to contribute to its overall removal. All other micropollutants that were found in sludge did not show such great discrepancies between sludge levels and removals from wastewater. It is postulated that the distribution of these micropollutants between wastewater and sludge is under equilibrium, similar to the observations of steroid estrogens in activated sludge plants (Andersen et al. 2005). As a result there is no net removal from wastewater by partitioning into sludge.

3.4. Presence of micropollutants in plant tissues of P. australis

It has been proposed that plant presence enhances micropollutant removal during constructed wetland treatment (Carvalho et al. 2014; Li et al. 2014; Verlicchi and Zambello 2014). Chen et al. (2015) reported that the presence of plants and plant litter increased diversity and abundance of bacterial communities in constructed wetlands. Nevertheless, little is known on the direct role of plants (e.g., uptake and metabolism) to micropollutant removal in HSSF constructed wetlands under field conditions. This is because there has been a lack of micropollutant analysis undertaken on emergent macrophytes grown in constructed wetlands due to the analytical challenges posed (Petrie et al. 2017).

In this study *P. australis* were collected and analysed from the gravel bed which had been operational for 12 months during the monitoring period to help understand the direct role of plants on micropollutant removal. A total of 17 micropollutants (12 parent compounds and 5 metabolites) were detected at least once in the studied plants at concentrations in the μg kg^{-1} range (Fig. 1). These were methylparaben, bisphenol-A, propranolol, ketamine and norketamine, venlafaxine and desmethylvenlafaxine, mirtazapine, carabamazepine and carbamazepine 10, 11 epoxide, diltiazem, caffeine, methadone and EDDP, tramadol and *N*-desmethyltramadol, and MDMA (Table 1). Methylparaben was found at the highest levels up to a maximum concentration of 6.7 μg $plant^{-1}$ found. All micropollutants found in *P. australis* were in the cationic or neutral form in wastewater (pH 8.0) which enables their movement across the root cell membrane. No negatively charged micropollutants were found within *P. australis* because charge repulsion with the negatively charged biomembrane of the plant root restricts uptake (Trapp 2009; Matamoros et al. 2012; Wu et al. 2013).

It has been previously reported that compounds with a log K_{OW} of between 0.5 and 3.0 can be taken up by plants (Pilon-Smits 2005; Zhang et al. 2014). This enables them to travel through the lipid membrane bilayer whilst being sufficiently water soluble to travel into cell fluids. They can then be transported within the plant by transpiration. This explained why micropollutants such as atenolol (log K_{OW} −0.03 and cationic – see Table S1, Supplementary material) were not found within *P. australis*. On the other hand, caffeine (log K_{OW} 0.16) was found within reeds. This is because it was present in the neutral form at pH 8, suggesting uptake of cationic and neutral species are governed by different mechanisms.

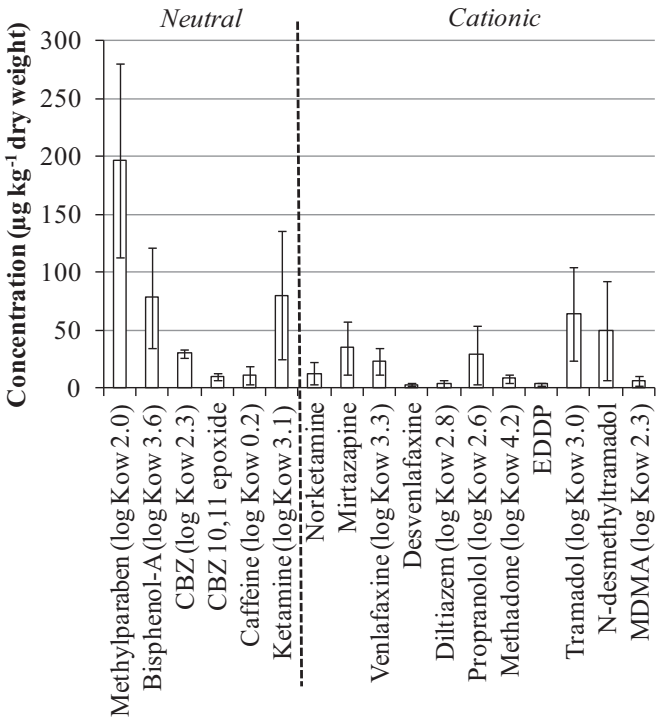

Fig. 1. Mean whole plant concentration of detected micropollutants in *P. australis* tissues ($n = 8$) Key: CBZ, carbamazepine; CBZ 10,11 epoxide, carbamazepine 10,11 epoxide; EDDP, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; MDMA, 3,4-methylene dioxymethamphetamine.

Process balances were used as a qualitative measure to help estimate the role *P. australis* plays on micropollutant removal during constructed wetland treatment. This was done using whole plant concentrations and accounted for the estimated number of plants within the bed. These process balances revealed the total quantity of micropollutants found in *P. australis* was small in comparison to the daily inlet load (see Fig. S5; Tables 1 and S5, Supplementary material). For example, the total quantity of propranolol found in *P. australis* was estimated to be 6.6 ± 7.4 mg bed^{-1} (using an estimated plant density of 10 plants per m^2) in comparison to the receiving load which was 109 ± 10.4 mg d^{-1} (see Fig. S5, Supplementary material). This suggests uptake by plants does not contribute significantly to total removals. However, further knowledge of plant metabolism is needed to better appreciate the direct role plants could play in micropollutant removal.

To better understand metabolism of micropollutants by *P. australis* under field conditions, sub-sections of *P. australis* were investigated for the presence of micropollutants. The different sections studied were roots, 4 × 30 cm sections of stem, and leaves. Notable differences were observed in the behaviour of different micropollutants. The illicit stimulant MDMA was found at similar concentrations throughout the plant ranging from 2.2 ± 0.3 μg kg^{-1} in roots to 5.2 ± 0.1 μg kg^{-1} in the first section of stem (Fig. 2). Interestingly the EF of MDMA was 0.25 ± 0.04 in roots which was similar to that of the inlet wastewater (EF = 0.23 ± 0.03 – Table 2). This demonstrates transport across the cell membrane was not a stereo-selective process in this instance.

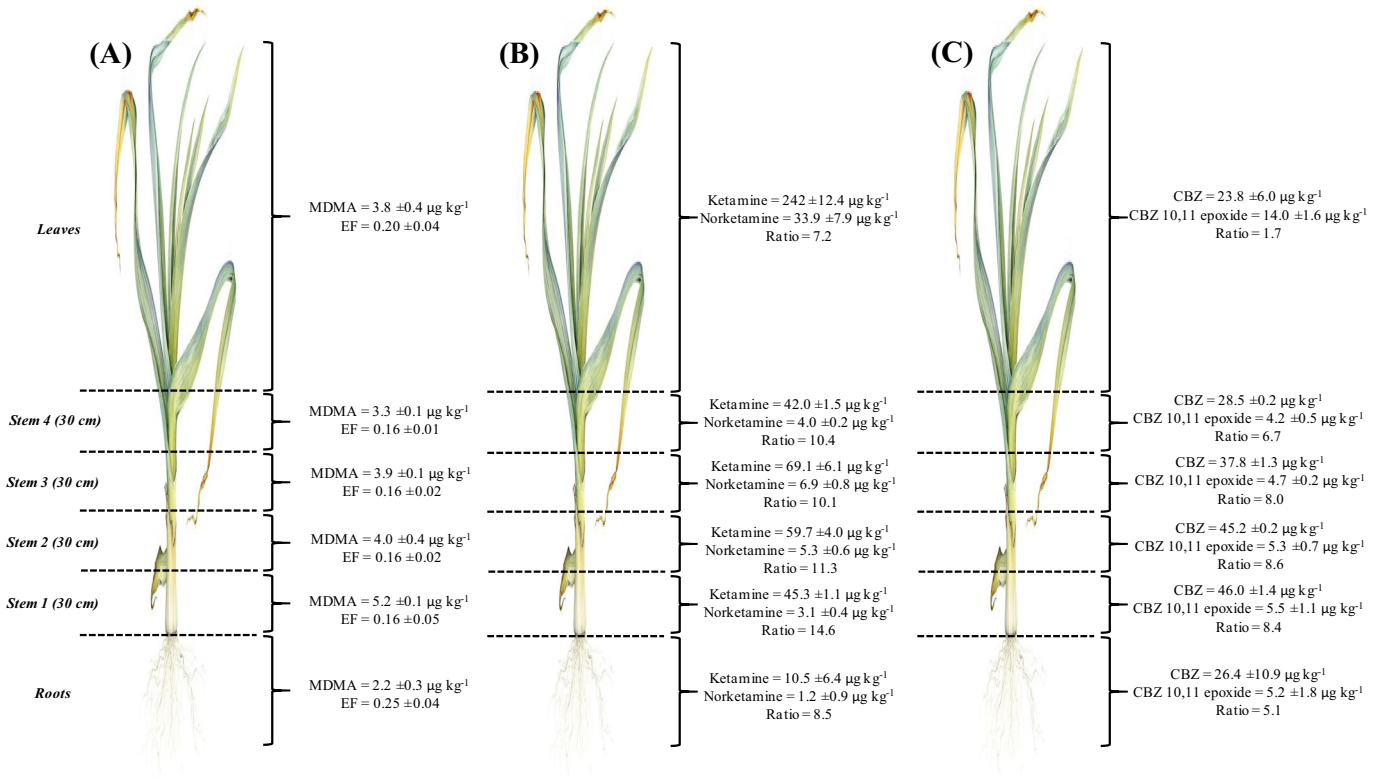

Fig. 2. Concentration of MDMA (A), ketamine and norketamine (B), and carbamazepine and carbamazepine 10,11 epoxide (C) in different plant sections (roots, stem and leaves). Concentrations of all detected micropollutants are in Table S6. Key: MDMA, 3,4-methylenedioxy-methamphetamine; EF, enantiomeric fraction; CBZ, carbamazepine; CBZ 10,11 epoxide, carbamazepine 10,11 epoxide.

However, stereo-selective changes were observed within the plant. In all sections of stem the EF was 0.16. This can be caused by stereo-selective metabolism within the plant or by stereo-selective transport between the roots and the stem. Stereo-selective metabolism of the chiral pesticide isofenphos-methyl has previously been observed in the vegetable pak choi (Gao et al. 2016).

Ketamine and its metabolite norketamine were present within the stem at concentrations ranging from 42.0–69.1 μg kg^{-1} and 3.1–6.9 μg kg^{-1}, respectively (Fig. 2). However, in leaves the concentration increased to 242 ± 12.4 μg kg^{-1} and 33.9 ± 7.9 μg kg^{-1}. This accounted for >50% of the total quantity found in *P. australis* (See Fig. S6, Supplementary material). Changes were observed to the ratio of ketamine to norketamine throughout the plant. In the first stem section the ratio was 14.6 which reduced throughout the plant to 7.2 in leaves (Fig. 2); suggesting ketamine was transformed to norketamine within the plant (assuming the rate of translocation within the plant is the same for ketamine and norketamine). Nevertheless, the rate of uptake and translocation of ketamine was greater than its rate of transformation resulting in its accumulation in leaves. A similar relationship was also observed for methylparaben, tramadol and venlafaxine (see Table S6, Supplementary material).

Carbamazepine and its metabolite carbamazepine 10,11 epoxide behaved similarly, although no parent compound accumulation (i.e., no increased concentration compared to the rest of the plant) was observed in leaves. Wu et al. (2013) has previously reported that carbamazepine is easily translocated from the roots of vegetables to their stems and leaves. In the stem the carbamazepine: carbamazepine 10,11 epoxide ratio ranged from 6.7 to 8.6. In leaves the ratio reduced to 1.7 due to an increased concentration of the metabolite. Again, this suggests metabolism of carbamazepine occurs within the plant. Metabolism of carbamazepine has been previously reported in vegetables (Goldstein et al. 2014; Malchi et al. 2014). Furthermore, Lv et al. (2017) found that the pesticides tebuconazole and imazalil were taken up from hydroponic water, translocated and degraded simultaneously by *P. australis*. Therefore to apportion the removal of micropollutants from the constructed wetland by phytoremediation, further knowledge of uptake and metabolism rates under field conditions are needed. However, it should be noted that there was no net removal of either carbamazepine or ketamine by the constructed wetland (Table 1), despite evidence of their metabolism. It is postulated that the rate of uptake and metabolism is too slow to have an impact on the overall removal of these micropollutants at the 14 h HRT which the constructed wetland operates. Nevertheless, wetland systems are often operated at longer HRTs (i.e., >5 days) which may enable plants to play a direct role in micropollutant removal.

4. Conclusion

The determination of 88 micropollutants in biotic phases of HSSF constructed wetlands revealed that biodegradation is the dominant mechanism of micropollutant removal in HSSF constructed wetlands. Although numerous micropollutants were susceptible to sorption onto the bed substrate as well as sludge, partitioning between aqueous and particulate phases is under equilibrium. Therefore the net removal of these micropollutants by sorption is minimal. Nevertheless, accumulation of the preservative methylparaben and antidepressents sertraline, fluoxetine and desmethylcitalopram in sludge suggested sorption contributes to their overall removal. Process balances indicated that *P. australis* did not directly contribute to micropollutant removal (i.e., through uptake and metabolism). However there was evidence of uptake and metabolism of recalcitrant micropollutants such as carbamazepine and ketamine.

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Appendix A. Supplementary data

Supplementary material contains photographs of the studied wetlands (Fig. S1), schematics of the wetlands showing sampling points (Figs. S2–3), behaviour of representative micropollutants during microcosm studies (Fig. S4), process balances (Fig. S5), percentage distribution of micropollutants within *P. australis* (Fig. S6), properties of studied micropollutants (Table S1), method detail (Tables S2–3), monitoring data (Tables S4–7) and additional references. Supplementary data to this article can be found online at doi:https://doi.org/10.1016/j.scitotenv.2018.02.242.

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