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

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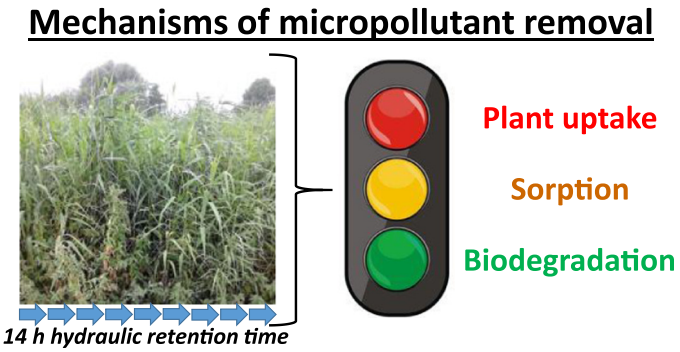
HIGHLIGHTS

- 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).