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The effect of loading frequency and plants on the degradation of sulfamethoxazole and diclofenac in vertical-flow constructed wetlands

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ABSTRACT

The aim of this study was to investigate the effect of loading frequency (1 pulse per day and 4 pulses per day) and vegetation (monoculture of *Phalaris arundinacea* 'Picta') on the removal and transformation of a mixture of pharmaceuticals diclofenac (DCF) and sulfamethoxazole (SMX) in lab-scale unsaturated vertical-flow constructed wetlands. Additionally, the system performance, the ecotoxicity of the wastewater (Microtox bioassay), and the microbial community structure and diversity in the top layer of the system were assessed. In the experiment, elevated concentrations of the pharmaceuticals (0.5 mg/L) were used to reflect the quality of wastewater from single houses or small settlements. The removal of DCF and SMX was deteriorated by the presence of *Phalaris arundinacea* 'Picta'. The lower loading frequency enhanced the removal of DCF and SMX only in the planted columns, suggesting combined effect of the two factors. For the unplanted systems no significant effect of the loading frequency was observed. The observed removal efficiency of SMX and DCF in the experiment was in the range 52.8–91.2% and 47.3–74.2%, respectively. The pharmaceuticals did not affect markedly the removal of DOC, TKN and $N\text{-}NH_4$, but they reduced the bacterial diversity within the rhizosphere and in the adjacent substrate of the systems. The main transformations of SMX were (mono-, di-, tri-)hydroxylation, demethylation, deamination and formation of glutathione conjugates, whereas for DCF only trihydroxylation was observed. The toxicity of the raw and treated wastewater containing the pharmaceuticals was at a level comparable with the control samples. This study showed that vertical-flow constructed wetlands can be efficient in the removal of DCF and SMX. Given that the constructed wetlands are inherently planted systems, it is suggested that the loading frequency is the operational variable that could be adjusted to enhance the removal of these pharmaceuticals.

1. Introduction

Pharmaceutical compounds (PhCs) are biologically active and persistent substances, which because of their widespread occurrence in the biosphere have been recognized as a threat to the environment (Santos et al., 2010). Many studies reported the presence of pharmaceuticals in the effluents from wastewater treatment plants (Ternes, 1998; Andreozzi et al., 2003; Miao et al., 2004; Castiglioni et al., 2006; Vieno et al., 2007). One of the most often detected PhCs in influents and effluents of wastewater treatment plants, surface water and groundwater are diclofenac (DCF) and sulfamethoxazole (SMX) (Gao et al., 2014; Vymazal et al., 2017; Sousa et al., 2018).

DCF belongs to the group of non-steroidal anti-inflammatory drugs (NSAIDs) administered either orally or topically and is characterised by high consumption (Vieno and Sillanpää, 2014). SMX is a prominent short-acting representative of sulfonamide antibiotics used in high amounts in human and veterinary applications (Baran et al., 2011). The concentration of DCF in raw municipal wastewaters reached up to 95 μg/L (Luo et al., 2014) and for SMX it was found to be up to 2 μg/L (Hirsch et al., 1999). For single-house effluents it was predicted that the

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concentrations of PhCs can be several-fold higher than in municipal wastewater (Abegglen et al., 2009). For SMX, the estimated concentration in the single-house wastewater can be as high as 4.4 mg/L (Abegglen et al., 2009). For DCF, no estimates are available, however for the other NSAIDs (naproxen and ibuprofen) a concentration of several mg/L was predicted (Abegglen et al., 2009).

The threat that is caused by DCF and SMX to the aquatic environment was assessed to be low, especially in terms of acute toxicity (Osorio et al., 2016), but the effect of their metabolites and mixtures of multiple pharmaceuticals is largely unknown (Lonappan et al., 2016; Osorio et al., 2016). Additionally, the environmental occurrence of SMX may create a threat by inducing antibiotic resistance in bacteria (Chen et al., 2016).

The wastewater treatment systems that were evaluated for the attenuation of the load of PhCs were mostly large conventional activated sludge plants (Margot et al., 2015). However, the elimination and transformation of pharmaceuticals were also studied in extensive systems such as constructed wetlands (CWs). Indeed, CWs apart from their important function in the removal of overall organic matter, nitrogen and phosphorus species have been indicated to be feasible to reduce the load of PhCs in the treated wastewater (Li et al., 2014).

SMX and DCF have been often studied in CWs (Gorito et al., 2017) and informally gained a status of model compounds in the studies on the fate of PhCs in CWs. The efficiency of SMX removal in CWs ranged from negative values (Rühmland et al., 2015) up to virtually complete elimination (Hijosa-Valsero et al., 2011). SMX removal was enhanced in the systems providing anoxic conditions (especially in horizontal-flow CWs) (Carranza-Diaz et al., 2014; Rühmland et al., 2015), but its removal in well-oxygenated systems such as vertical-flow CWs (VF-CWs) was low (Dan et al., 2013; Nowrotek et al., 2016). DCF removal efficiencies in CWs were reported to lie in the wide range from 0 to 96% (Gorito et al., 2017) and to be favoured by a combination of aerobic and anoxic microbial metabolic pathways (Zhang et al., 2012; Ávila et al., 2014a, Auvinen et al., 2017; Ghattas et al., 2017).

Usually, small CW treatment systems are composed of a single bed with vertical flow (VF-CW), which offers limited control over the treatment process. The VF-CWs are often implemented as unsaturated intermittently loaded systems to nitrify the wastewater from single households or small communities (Langergraber and Weissenbacher, 2017). Therefore, by design these systems support mostly aerobic processes with oxygen as the terminal electron acceptor, while anoxic processes play a minor role in these systems. It is especially the loading frequency in VF-CWs that could be controlled to slightly change the redox and oxygen conditions in the system to enhance the removal of organic matter and TKN. However, the effect of loading frequency on the removal of pharmaceuticals has been scantily discussed in the literature. In practice, loading frequencies vary from single pulse per day (large doses) to very frequent doses every 20 min (Nivala et al., 2013). More frequent doses potentially enhance oxygen transfer and increase redox potential (Nivala et al., 2013, Ávila et al., 2014b), however these conditions may vary in the course of a cycle. The conditions imposed by the feeding frequency depend on hydraulic properties of the bed and the presence and type of vegetation (Molle et al., 2006; Giraldi and Iannelli, 2009). Additionally, plants may also be responsible for direct uptake of some PhCs (Li et al., 2014) and may stimulate microbial activity in the system, thereby improving the elimination of PhCs (Zhu and Sikora, 1995, Gagnon et al., 2007, Faulwetter et al., 2009, Wu et al., 2011).

In general, the treatment of wastewater containing DCF and SMX in CWs does not provide complete mineralisation of the compounds but only transformation by various metabolic pathways. The knowledge on the transformation products (TPs) for DCF and SMX and many other PhCs occurring in CWs is still limited. This hinders advancement in understanding of transformation routes but also predicting environmental impact of the CW effluents taking into account that some TPs of DCF and SMX can be more toxic or can retransform to the parent compound (Göbel et al., 2007; Majewsky et al., 2014; Osorio et al., 2016).

The aim of this study was to investigate the effect of two loading frequencies and the presence of plants in microcosm unsaturated vertical-flow constructed wetlands on the removal efficiency of DCF and SMX and the quality of their transformation products. Additionally, to broaden the picture of the process it was aimed to assess the effect of DCF and SMX on the performance of the system, the toxicity of the effluents, and finally on the bacterial community structure and diversity in the upper substrate layer of the system.

2. Materials and methods

2.1. Experimental system

The laboratory system used in the experiment (Fig. S1, Supporting Information (SI)) was designed as a microcosm model of VF-CWs. The experimental system consisted of 24 columns (diameter 0.2 m, height 0.8 m each) filled up to 0.7 m with three layers of filtering media – bottom layer: gravel 3–8 mm (0–5 cm), main layer: quartz sand 0.5–1.0 mm (5–65 cm) and upper layer (65–70 cm): a mixture (1:1 by volume) of sand and organic soil (drawing of the column is given in Fig. SI, SI). The composition of the filtering media layers was the same in all the columns. Eight types of columns were used depending on: the loading frequency (1 pulse per day (hydraulic loading rate (HLR) 38 mm/pulse) or 4 pulses per day (HLR 10 mm/pulse)), the presence or absence of plants, and the presence or absence in the feed of the two pharmaceuticals (PhCs): DCF and SMX. Each type of column was operated in triplicate. The wastewater was fed on top of the columns at the specified loading frequencies. The intervals between the loadings were 24 h for 1 pulse per day and 6 h for 4 pulses per day, respectively. The columns were operated in the same manner on every day of the experiment. The loading duration was very short and lasted maximum 30 s. The daily batch volume was 1.2 L for each column. For the columns fed with 4 pulses per day this volume was divided equally into 4 batches of 0.3 L. The plants used in the experiment were *Phalaris arundinacea* Picta. The types of columns together with symbols used to denote them are listed in Table 1.

The influent was prepared in tap water by dissolving the following components (Felis et al., 2016): urea (208.76 mg/L), NH_4Cl (62.4 mg/L), yeast extract (264 mg/L), skim milk powder (118 mg/L), sodium acetate (510.4 mg/L), peptone (40 mg/L), KH_2PO_4 (41.37 mg/L), $KCr(SO_4)_2{\cdot}12H_2O$ (0.96 mg/L), $CuSO_4{\cdot}5H_2O$ (0.781 mg/L), $MnSO_4{\cdot}H_2O$ (0.108 mg/L), $NiSO_4{\cdot}7H_2O$ (0.359 mg/L), $PbCl_2$ (0.1 mg/L), $ZnCl_2$ (0.208 mg/L), $MgSO_4{\cdot}7H_2O$ (4.408 mg/L), $FeSO_4{\cdot}7H_2O$ (11.6 mg/L). The PhCs columns with and without plants were fed with an influent in which DCF sodium salt (Sigma-Aldrich) and SMX (Sigma-Aldrich) were dissolved to yield concentration of 0.5 mg/L of DCF and 0.5 mg/L of

Table 1
Types of columns used in the experiment and the symbols used to denote them.

	PhCs-1P-Plant	PhCs-1P-noPlant	PhCs-4P-Plant	PhCs-4P-noPlant	CTRL-1P-Plant	CTRL-1P-noPlant	CTRL-4P-Plant	CTRL-4P-noPlant
Feeding frequency	1 pulse/d		4 pulses/d		1 pulse/d		4 pulses/d	
Plants	Yes	No	Yes	No	Yes	No	Yes	No
Pharmaceuticals (PhCs)	Yes				No (control columns; CTRL)			