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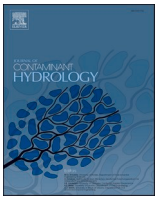

Sorption and biodegradation parameters of selected pharmaceuticals in laboratory column experiments

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ABSTRACT

Pharmaceutically active compounds have increasingly been detected in groundwater worldwide. Despite constituting a risk for human health and ecosystems, their fate in the environment has still not been exhaustively investigated. This study characterizes the transport behavior of five selected pharmaceutically active compounds (antipyrine, atenolol, caffeine, carbamazepine and sulfamethoxazole) in two sediments (coarse quartz sand and sandy loam) using column experiments with long-term injection of spiked groundwater. Transport parameters were estimated using an analytical reactive transport model. When five selected compounds were injected simultaneously, transport behavior of antipyrine, carbamazepine and the antibiotic sulfamethoxazole were similar to the conservative tracer in both sediments and under varying redox conditions. Atenolol and caffeine were retarded significantly stronger in the sandy loam sediment than in the coarse quartz sand. Biodegradation of caffeine was observed in both sediments after an adaption period and depended on dissolved oxygen. The identification of biodegradation processes was supported by monitoring of intracellular adenosine triphosphate (ATP_{itc}) as a measure for microbial activity. ATP_{itc} was present in varying concentrations in all sediments and was highest when biodegradation of pharmaceuticals, especially caffeine, was observed. When only caffeine and sulfamethoxazole were injected simultaneously, sulfamethoxazole was degraded while caffeine degradation was reduced. The latter seemed to be influenced by low concentrations in dissolved oxygen rather than the presence of the antibiotic sulfamethoxazole. Results of these experiments emphasize the impact on pharmaceutical sorption and (bio)degradation of sediment type and redox conditions, as well as available time for microbial adaption and the combination of pharmaceuticals that are released together into groundwater.

1. Introduction

Newly developed organic compounds and their metabolites have been increasingly observed in the environment (e.g. Bexfield et al. 2019; Lapworth et al. 2012; Öllers et al. 2001; Sui et al. 2015; Tang et al. 2019). Often referred to as EOCs (Emerging Organic Compounds), their fate in the environment remains largely unresolved (Kümmerer 2009; Lapworth et al. 2012). An important subset of EOCs are pharmaceutically active compounds (Scheytt et al. 2004). Stemming from untreated sewage effluents, septic tanks, hospital effluents as well as agriculture and livestock activities (Aus der Beek et al. 2016; Lapworth et al. 2012; Sui et al. 2015) or illegitimate disposal of expired medication via toilets and waste (Gauthier et al. 2008; Ternes et al. 2002), they enter aquatic systems in considerable concentrations (Löffler et al. 2005; Yamamoto et al. 2009), potentially ending up in groundwater (Bexfield et al. 2019; Löffler et al. 2005; Sui et al. 2015). Considering the use of groundwater for drinking water supply, its contamination with pharmaceuticals poses a risk to human health (Bexfield et al. 2019; Lapworth et al. 2012).

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Bioactive effects can arise even at concentration levels of few ng/L that have actually been detected in drinking water (Ericson et al. 2002; Wennmalm and Gunnarsson, 2005). Besides immediate health risks (e.g. elevated risk of cancer), the increasing presence of pharmaceutically active compounds in groundwater is also likely to lead to a multiplication of antibiotic resistant bacteria (Andrade et al. 2020; Haack et al. 2012; Szekeres et al. 2018).

In contrast to pesticides, pharmaceuticals have hardly been considered by legislators to date, and they have only recently come in the focus of research. Understanding the fate of pharmaceuticals in aquifers allows determining their potential for natural attenuation. This is of great interest in order to estimate their threat to ecosystems and human health as well as to set reasonable threshold values. The potential for natural attenuation of most pharmaceutically active compounds is not sufficiently investigated (Greenhagen et al. 2014; Regnery et al. 2017). For these reasons, it is absolutely required to conduct environmental studies on pharmaceutical transport behavior (Löffler et al. 2005; Williams et al. 2006). Especially interactions between compounds are inadequately understood (Regnery et al. 2017).

The systematic assessment of transport processes, however, is difficult at complex field sites where boundary conditions cannot be controlled. A proven alternative approach are laboratory investigations using flow-through column experiments (Banzhaf and Hebig 2016). They are supposed to reflect natural conditions of the saturated zone (Schaffer et al. 2012), while conditions are easily controllable and the complexity of the domain under investigation is reduced. So far, column experiments have been conducted to study pharmaceutical transport under varying physico-chemical conditions such as changes in pH (Schaffer et al. 2012), redox conditions and temperature (Alidina et al. 2015; Burke et al. 2014), and sediment properties (Greenhagen et al. 2014; Kodešová et al. 2015; Martínez-Hernández et al. 2014).

The present study is built on research by Kiecak et al. (2020) who conducted laboratory column experiments with sediments from different study sites to examine transport parameters for selected pharmaceuticals at different flow rates. One study site was the alluvial Vistrenque aquifer in France, where pharmaceutically active compounds occur in groundwater and surface water, mainly stemming from waste water treatment plant effluents (Kiecak et al. 2019). The sandy loam sediment from the Vistrenque aquifer was investigated in our present study. The selection of pharmaceuticals for this study was based on their occurrence at the field site in France and on their global relevance. It includes the antiepileptic and antipsychotic drug carbamazepine, the beta-blocker atenolol, the antibiotic sulfamethoxazole, the analgesic, non-steroidal anti-inflammatory drug phenazone (prior tradename antipyrine) as well as the stimulant caffeine. Carbamazepine, sulfamethoxazole and caffeine are worldwide among the most frequently reported pharmaceutical compounds in the environment; specifically in groundwater (Aus der Beek et al. 2016; Bexfield et al. 2019; Sui et al. 2015). The occurrence of atenolol and antipyrine is less frequent; however, both compounds have been detected in several environmental studies from countries all over the world (Aus der Beek et al. 2016).

Findings of Kiecak et al. (2020) showed that sorption processes in aquifer sediments are significant for the transport of several pharmaceutical compounds. Whereas flow rates turned out not to significantly influence transport behavior, the type of sediment was identified to play a major role. While their study provided important answers regarding the transport behavior of the listed pharmaceuticals, biodegradation could not clearly be identified which raised further questions. Previous research has found strong indications that biodegradation plays a key role in the natural attenuation of pharmaceutically active compounds (Lam et al. 2004; Ternes et al. 2002; Xu et al. 2009; Yamamoto et al. 2009). Especially caffeine and atenolol are known to be degraded by microorganisms (Dash and Gummadi 2010; Godfrey et al. 2007; Koroša et al. 2020).

A commonly applied method for the identification of biodegradation in column experiments is the use of an abiotic control in order to distinguish between biotic and abiotic processes. This can be achieved for example by adding sodium azide to the feed water of the abiotic control column (Burke et al. 2013; Kiecak et al. 2020; Maeng et al. 2011) or a combination of sodium azide and mercury chloride (de Wilt et al. 2018; Hamon et al. 2014; Martin et al. 2018) or sodium azide and copper sulphate (Hillebrand et al. 2013) and additionally by autoclaving of the sediment (e.g. Radke and Maier 2014). However, all of these methods threaten to have an undesired effect on sediment properties and experimental conditions: The addition of the above-mentioned biocidal compounds in too high concentrations might influence the sorption behavior of the organic compounds under investigation (Chefetz et al. 2006; Hillebrand et al. 2013; Martin et al. 2018; Vanderford et al. 2011). Other studies have shown that microbial activity might only be reduced instead of inhibited by application of sodium azide at a given concentration (Abel et al. 2013; Cabrol et al. 2017). Therefore, it needs to be monitored if sodium azide in concentrations reported to not affect sorption of other compounds and as applied previously (Kiecak et al. 2020) also is sufficient for creating abiotic conditions.

It further needs to be tested whether microbial communities in column experiments do in fact have enough time to adapt to the newly introduced compounds to start biodegradation when confronted with short pulse injection periods. Lag phases corresponding to adaption periods might be required for a change in the metabolic state of microorganisms which respond to changes in surrounding substrate conditions by activating new enzymatic pathways and repairing damaged cells. Especially microorganisms in nutrient poor conditions like in groundwater aquifers often suffer from cell damages when first encountering a new substrate (Barford et al. 1982; Wood et al. 1995). Lag phases for caffeine degradation of less than three days have been reported in several studies when degradation was actively enhanced (Li and Mclachlan 2019; Nayak et al. 2012; Pérez et al. 2009; Topp et al. 2006). In column experiments of De Wilt et al. (2018), under aerobic to nitrate reducing conditions, a lag phase of 27 days before caffeine degradation was observed. For atenolol degradation, Li and McLachlan (2019) observed adaption periods of 15–28 days in incubation tests. Antipyrine, carbamazepine and sulfamethoxazole have been found to be recalcitrant in many studies, where degradation did not occur within the timeframe of the experiments (Benotti and Brownawell 2009; Li and Mclachlan 2019). Baumgarten et al. (2011) found that the adaption period for sulfamethoxazole was not finished even after two years. Alidina et al. (2014), on the other hand, found that adaption of the microbial community did not play a significant role for the contaminant removal in their managed aquifer recharge setting. Overall, little is still known about the role and duration of adaption periods for microbial degradation of pharmaceuticals in aquifers.

In this study, long-term laboratory column experiments with continuous injection of pharmaceuticals were carried out in order to examine the transport behavior of selected pharmaceuticals with focus on the identification and quantification of biodegradation, providing more time for microorganisms to adapt to substrate conditions. Biological control parameters were measured in order to estimate microbial activity within the columns and to differentiate between sorption, biotic and abiotic degradation. Redox conditions within the columns were not actively controlled; instead the establishment of different redox conditions was monitored at the column outlets. To get insight into interaction effects of pharmaceutical compounds, an additional experiment was conducted to investigate the influence of the antibiotic sulfamethoxazole on the biodegradation of caffeine as supposedly easier degradable compound.

2. Material and methods

2.1. Experimental set up

Stainless steel columns were packed with sediments according to Kiecak et al. (2020). The two sediments selected for this study are in the

following referred to as V and G sediment. V sediment is a sandy loam from the Vistrenque alluvial aquifer in France with 29% grain size fraction <0.063 mm, cation exchange capacity of 2.48 cmolc/kg, a specific surface of 2.97 m^2/g (obtained from Kiecak et al. (2020)). TC and TOC fractions were 1.27 ± 0.42%, 0.07 ± 0.10%, respectively. Sediment V was packed in columns VA and VB. G sediment is coarse technical quartz sand (Dorsilit Nr. 5F, Quarzsande GmbH, Germany) with homogeneous grain size distribution of 1–2 mm, cation exchange capacity of 0.07 cmolc/kg, and a specific surface of 0.25 m^2/g (obtained from Kiecak et al. (2020)). Since we expected low sorption for the technical quartzsand, it was chosen as a reference to the V sediment. The total carbon and total organic carbon fraction (TC/TOC) were measured with the TOC-5050 *Shimadzu* (Kyoto, Japan) analyzer and are 0.07 ± 0.10% and 0.00 ± 0.00%, respectively. G sediment was packed in columns GA, GB, g1 and g2. After packing, the columns were saturated with local groundwater (HCO_3-Ca-Mg water facies) from Neuherberg, Germany. The used groundwater is geochemically similar to groundwater from the Vistrenque aquifer from where the V sediment was sampled. Properties of the Vistrenque groundwater (from the sampling point Caissargue) can be found in Kiecak et al. (2019). Local groundwater was chosen to be able to constantly provide fresh feed water for the columns. In order to obtain a chemical equilibrium between substrate and feed water, the sediment columns were flushed with pure groundwater for one month prior to the start of the experiments. The ionic composition of the inflow and outflow after equilibration are given in the Supplementary (S2). During both equilibration and the experiments, a peristaltic pump generated water flow from the bottom to the top of the columns at a constant flow rate. Although flow velocity had no impact on the transport behavior of pharmaceuticals in this sediment (Kiecak et al. 2020), flow velocities typically found in such porous aquifers were chosen (cf. Banzhaf and Hebig 2016). With the start of each column experiment, groundwater spiked with conservative tracers and pharmaceuticals as reactive tracers were continuously injected into the columns. The abiotic control columns VA and GA were additionally spiked with 50 mg/L sodium azide (according to Kiecak et al. (2019) and Kiecak et al. (2020)). Fraction samplers were used to obtain samples of the column outflow at defined time intervals. Hoses were sterile-packaged before being implemented in the fraction collector. The test tubes for the samples were steam sterilized before use. A polystyrene construction served to protect the samples in the fraction collectors from possible effects of photolysis. Properties of the columns and the experimental set up are summarized in Table 1.

2.2. Tracer solutions for long-term injection

Stock solutions were created for the injection of pharmaceutical spiked groundwater using distilled water as solvent for antipyrine, atenolol and caffeine, whereas methanol was used for the less water soluble compounds carbamazepine and sulfamethoxazole. A detailed description of the chemicals used in the experiments can be found in Kiecak et al. (2019). In order to eliminate residues of methanol in the feed water, the pipetted stock solution was evaporated from dry and clean 5 L Schott bottles under the fume hood. Next, groundwater was added to the bottles to create the feed water solutions. Fresh feed water solutions were regularly recreated to have constant supply for the long-term experiments. In order to establish water transport characteristics, sodium chloride was added to the first injection volume for each column as a conservative tracer. Target concentrations were 0.18 mg/L for pharmaceuticals (similarly to concentrations used by Banzhaf et al. (2012), ensuring that concentrations in our samples can be measured directly and quantified precisely without the need for preconcentration), and 80 mg/L for chloride (only in the first injection volume). To the GA, GB, VA, and VB columns, groundwater spiked with all 5 selected pharmaceuticals (antipyrine, atenolol, caffeine, carbamazepine, sulfamethoxazole) was continuously injected, where GA and VA were the abiotic controls. To g1 column, groundwater spiked with caffeine only was injected; to g2 column, feed water spiked with caffeine and sulfamethoxazole was injected. The total duration of the injections is given in Table 1.

2.3. Analytical methods

2.3.1. Pharmaceutical concentrations: UHPLC-MS/MS measurements

Pharmaceutical concentrations in inflow and outflow samples were analyzed using UHPLC-MS (Ultrahigh performance liquid chromatography coupled with mass-spectrometry). Instrumentation consisted of an Agilent 1290 Infinity LC system including an autosampler 1290 G4226A, a pump 1290 G4220A, a column thermostat 1290 G1316C (*Agilent Technologies*, Santa Clara, USA) and an API 4000 QTrap mass spectrometer from *Sciex* (Darmstadt, Germany). An Acquity HSS T3 column (1.8 µm, 50 × 2,1 mm I.D.) served for chromatographic separation. The flow rate was kept at 0.5 mL/min; the injection volume was 1 µL. Eluents were formic acid/ammonium formate (10 mmol/L) and methanol. The method description can be found in the Supplementary Material (Table S1). For further details (e.g. limit of detection), see Kiecak et al. (2019).

2.3.2. Chemical control parameters: Ions, DO, and DOC

Ion chromatography ICS-1100 (*Dionex*, Sunnyvale, USA) was used to analyze inflow and outflow samples for chloride, nitrite, bromide, nitrate, hydrogen phosphate and sulfate, lithium, sodium, ammonium, potassium, magnesium and calcium. Dissolved oxygen (DO) concentrations in water were measured using flow-through cells (FTC-SU-PST3-US *PreSens*, Regensburg, Germany) at the outlet of each column. The total concentrations of dissolved organic carbon (DOC) in groundwater, input solutions and samples were additionally measured employing a *Shimadzu* total organic carbon analyzer TOC-5000A (Kyoto, Japan). Redox conditions within the columns were not actively controlled or manipulated in this investigation in order to study conditions closer to natural conditions.

Table 1
Properties of the sediment packed columns and six experimental set-ups; V_{Water}: water volume, $V_{Sediment}$: sediment volume, n_{total}: porosity, Q: volumetric flow rate, v_s: water flux. VA and GA are the control columns spiked with sodium azide.

Sediment	Sandy loam (Vistrenque, France)		Coarse technical quartz sand (Dorsilit Nr. 5F, Quarzsande GmbH, Germany)			
Column	VB	VA	GB	GA	g1	g2
Column height [cm]	51	51	51	51	25	25
Column diam. [cm]	5.0	5.0	9.1	9.1	3.6	3.6
V_{Water} [mL]	438	434	1217	1244	99	100
$V_{Sediment}$ [mL]	563	567	2100	2073	156	154
n_{total} [−]	0.44	0.43	0.37	0.37	0.39	0.39
Q [mL/min]	0.51 ± 0.02	0.49 ± 0.02	0.48 ± 0.00	0.47 ± ±0.00	0.48 ± 0.01	0.45 ± 0.01
v_s [cm/h]	1.55 ± 0.06	1.49 ± 0.06	0.44 ± 0.00	0.43 ± 0.00	2.82 ± 0.06	2.66 ± 0.06
Duration [days]	50	50	61	61	33	33

2.3.3. Biological control parameter: Adenosine triphosphate (ATP)

ATP concentrations indirectly give information about the amount of cells in the water sample; intracellular ATP provides information on living cells (Crouch et al. 1993; Hammes et al. 2010). In order to obtain this measure, extracellular ATP that is suspended freely in water was centrifuged out of the suspension. Intracellular ATP concentrations were measured using the bioluminescent reaction of ATP with luciferin, catalyzed by the enzyme luciferase (Deluca and McElroy 1978; Lundin and Thore 1975; McElroy and DeLuca 1983). For this study, the Promega BacTiter-Glo™ Microbial Cell Viability Assay was applied. Bioluminescence was measured as relative light units (RLU) using the GloMax® luminometer (*Promega*, Madison, USA).

2.4. Numerical modeling

Numerical modeling of tracer breakthrough curves and estimation of transport parameters was performed using the STANMOD software CXTFIT 2.0. Deterministic equilibrium and two-site non-equilibrium transport models based on the convection dispersion equation (CDE) (Toride et al. 1999) were tested to fit the transport models to the measured data. The CDE one-dimensional steady-state flow in homogeneous subsoil (without a production term) is presented in Eq. 1, according to Toride et al. (1999), where $R_f[-]$ is the retardation coefficient, $C\ [M\,L^{-3}]$ is the averaged concentration of the compound in the liquid phase, $t\ [T]$ is the time, $D\ [L^2\,T^{-1}]$ is the dispersion coefficient, $x\ [L]$ is the distance, $v_p[L\,T^{-1}]$ is the average water flow velocity and $\mu\ [T^{-1}]$ is the first-order degradation coefficient resulting from both degradation in liquid and solid phase.

$$R_f\frac{\delta C}{\delta T} = D\frac{\delta^2 C}{\delta x^2} - v_p\frac{\delta C}{\delta x} - \mu C \tag{1}$$

Similarly, to Müller et al. (2013), we tested the deterministic non-equilbrium model provided by CXTFIT as two site non-equilibrium model with dimensional time and position and independent solution and adsorbed phase degradation rates. The CDE of the two-site deterministic non-equilibrium model according to Toride et al. (1999) (reduced to its dimensionless form and without production term) is given in Eqs. (2) and (3), where $\beta\ [-]$ is the partitioning coefficient, $\omega\ [-]$ is the mass transfer coefficient, T is the dimensionless time $[-]$, Z is a dimensionless distance $[-]$ and P [−] is the Peclet number $[-]$.

$$\beta R_f\frac{\delta C_1}{\delta T} = \frac{1}{P}\frac{\delta^2 C_1}{\delta Z^2} - \frac{\delta C_1}{\delta Z} - \omega(C_1 - C_2) - \mu_1 C_1 \tag{2}$$

$$(1-\beta)R_f\frac{\delta C_2}{\delta T} = \omega(C_1 - C_2) - \mu_2 C_2 \tag{3}$$

Fitting was achieved by least square inversion. The concentration mode was set to flux-averaged concentrations (C_f) (ratio of solute and water fluxes), input boundary conditions were set to step input and initial concentrations were set to zero, except for chloride where background concentrations of the groundwater were measured. Zero production was assumed for all compounds.

In the first step, chloride breakthrough concentrations were used for the estimation of water transport parameters (average water flow velocity v_p and the dispersion coefficient D) for each column by applying the deterministic equilibrium model, assuming zero retardation or degradation ($R_f = 1$, $\mu = 0\ d^{-1}$). In a second step, inverse estimation of transport parameters specific to pharmaceuticals (retardation and degradation coefficients, R_f and μ) was performed employing the beforehand estimated v_p and D. The deterministic two-site non-equilibrium model – when applied – required the estimation of two first-order degradation coefficients μ_1 and μ_2 (in liquid and in sorbed phase) and the additional estimation of the partitioning coefficient β, and the mass transfer coefficient ω (partitioning and mass transfer between two sorption sites). Both equilibrium and non-equilibrium models cannot account for temporal dynamics in degradation, e.g. as a result of changing redox conditions or occurring lag phases. In order to still get an approximated estimate in these cases, we temporally divided the experiment according to occurring redox conditions and adaption periods and successively fitted the degradation coefficient. This procedure was only used for the compound caffeine in GB and g1.

Further coefficients describing water and compound transport were calculated from the modeled parameters: The longitudinal dispersivity $\alpha_L[L]$ (Eq. (4)), effective porosity $n_{eff}[-]$ as a fraction of the mobile (V_{mobile}) and the total (V_{total}) water volume (Eq. (5), where v_s is the water flux $[L\,T^{-1}]$) and mean residence time $t_0[T]$ (Eq. (6), where Q is the flow rate)

$$\alpha_L = \frac{D}{v_p} \tag{4}$$

$$n_{eff} = \frac{V_{mobile}}{V_{total}} = \frac{v_s}{v_p} \tag{5}$$

$$t_0 = \frac{V_{mobile}}{Q} = n_{eff}\frac{V_{total}}{Q} \tag{6}$$

Pharmaceutical recoveries *Rec* were calculated at regular intervals as fraction of the outflow concentrations C_{out} at each new plateau in concentration and the inflow concentrations C_{in} in the respective injection volume (Eq. (7))

$$Rec = \frac{C_{out}(t_2)}{C_{in}(t_1)},\ t_2 - t_1 \gg t_0 \tag{7}$$

3. Results and discussion

3.1. Redox conditions and microbial activity

Redox conditions varied with time in all column experiments which also affected the fate of the tested compounds. DO concentrations ranged between 0.00 and 1.83 mg/L in VB, between 0.00 and 6.30 mg/L in VA, between 0.02 and 5.98 mg/L in GB, between 0.08 and 7.45 mg/L in GA, between 0.76 and 8.12 mg/L in g1, and between 0.00 and 5.62 mg/L in g2. The variations in nitrate and DO concentrations over time for all columns are displayed in the supplementary details (S3) (sulfate was not reduced at any point in any of the experiments).

Microbial activity in the columns was estimated via measurements of intracellular ATP (ATP_{itc}) in the outflow samples. ATP_{itc} concentrations in all samples were greater than the background concentration in pure groundwater of 3.6E-04 µg/L. In V columns, ATP_{itc} concentrations in the biotic setting were greater than in the abiotic setting (range of 1.5E-02–1.5E+00 µg/L and mean of 2.1E-01 µg/L in VB vs. a range of 1.4E-02–6.1E-01 and mean of 1.4E-01 µg/L in VA), but not on a statistically significant level (Welch two-sample t-test: df = 14.52, $p = 0.61$). In G columns, the concentrations in the biotic setting were significantly greater than in the abiotic setting (Welch two-sample t-test: df = 10.96, $p = 0.05$ with a range of 1.5E-02–5.0E-01 µg/L and a mean concentration of 1.4E-01 µg/L in GB and a range of 4.1E-03–1.1E-01 µg/L and mean of 3.2E-02 µg/L in GA). These results suggest that adding the indicated amount of sodium azide to the feed water was significantly (but not fully) inhibiting microbial activity in GA (coarse sand) while it was not sufficient in the more fine-grained sediment in VA. This corresponds to findings of Martin et al. (2018), according to which lower concentrations (< 50 mg/L) of sodium azide are effectively biocidal in a more sandy material. In our investigation however, this means we cannot fully consider the control columns VA and GA to be free of biodegradation processes. ATP_{itc} concentrations were generally lower in g1 and g2 columns, where only one or two compounds were injected, respectively. The range was 9.8E-03–7.8E-02 µg/L for g1 (only caffeine injected) and 1.8E-02–3.3E-01 µg/L for g2 (caffeine and sulfamethoxazole injected).

Table 2
Estimated transport parameters for water (average water flow velocity v_p, mean residence time t_0, and dispersivity α_L), effective porosity n_{eff} and pharmaceutical transport parameters (retardation coefficient R_f, degradation rates μ) for the six columns (non-equilibrium parameters, partitioning and mass transfer coefficients β and ω as well as the degradation rate for the second sorption site $\mu 2$ are given below whenever they were fitted).

	v_p [cm/h]	t_0 [h]	α_L [cm]	n_{eff} [−]		Antipyrine	Atenolol	Caffeine	Carbamazepine	Sulfamethoxazole
VB	3.85	13.24	3.78	0.40	R_f [−]	1.06E+00	6.15E+00	2.56E+00[c]	1.17E+00	9.58E-01
					μ [d^{-1}]	3.51E-02	6.66E-03[a]	3.18E-01	0.00E+00	8.36E-02
VA	3.50	14.59	7.63	0.43	R_f [−]	9.86E-01	5.63E+00	2.51E+00[d]	1.04E+00	8.89E-01
					μ [d^{-1}]	7.85E-03	5.64E-03[b]	1.62E-02	0.00E+00	1.57E-02
GB	1.29	39.41	0.27	0.34	R_f [−]	1.02E+00	1.04E+00	1.09E+00	1.02E+00	1.01E+00
					μ [d^{-1}]	3.21E-05	2.05E-01	3.00E-03[e] – 1.89E+00[f]	1.81E-03	1.23E-02
GA	1.20	42.41	0.14	0.36	R_f [−]	9.95E-01	1.06E+00	1.08E+00	1.01E+00	9.96E-01
					μ [d^{-1}]	0.00E+00	4.97E-02	0.00E+00	6.72E-03	0.00E+00
g1	7.16	3.49	0.18	0.39	R_f [−]	–	–	1.05E-01	–	–
					μ [d^{-1}]	–	–	4.22E+01[f]	–	–
g2	6.83	3.66	0.24	0.39	R_f [−]	–	–	1.17E+00	–	9.89E-01
					μ [d^{-1}]	–	–	3.37E-01	–	6.94E-01

[a] $\beta = 5.23E\text{-}01$; $\omega = 2.08E\text{-}02$; $\mu 2 = 1.00E\text{-}07$.
[b] $\beta = 4.51E\text{-}01$; $\omega = 1.90E\text{-}02$; $\mu 2 = 1.00E\text{-}07$.
[c] Fit to plateau:1.63E+00.
[d] Fit to plateau: 1.14E+00.
[e] Nitrate reducing conditions.
[f] Oxic conditions, after adaption period.

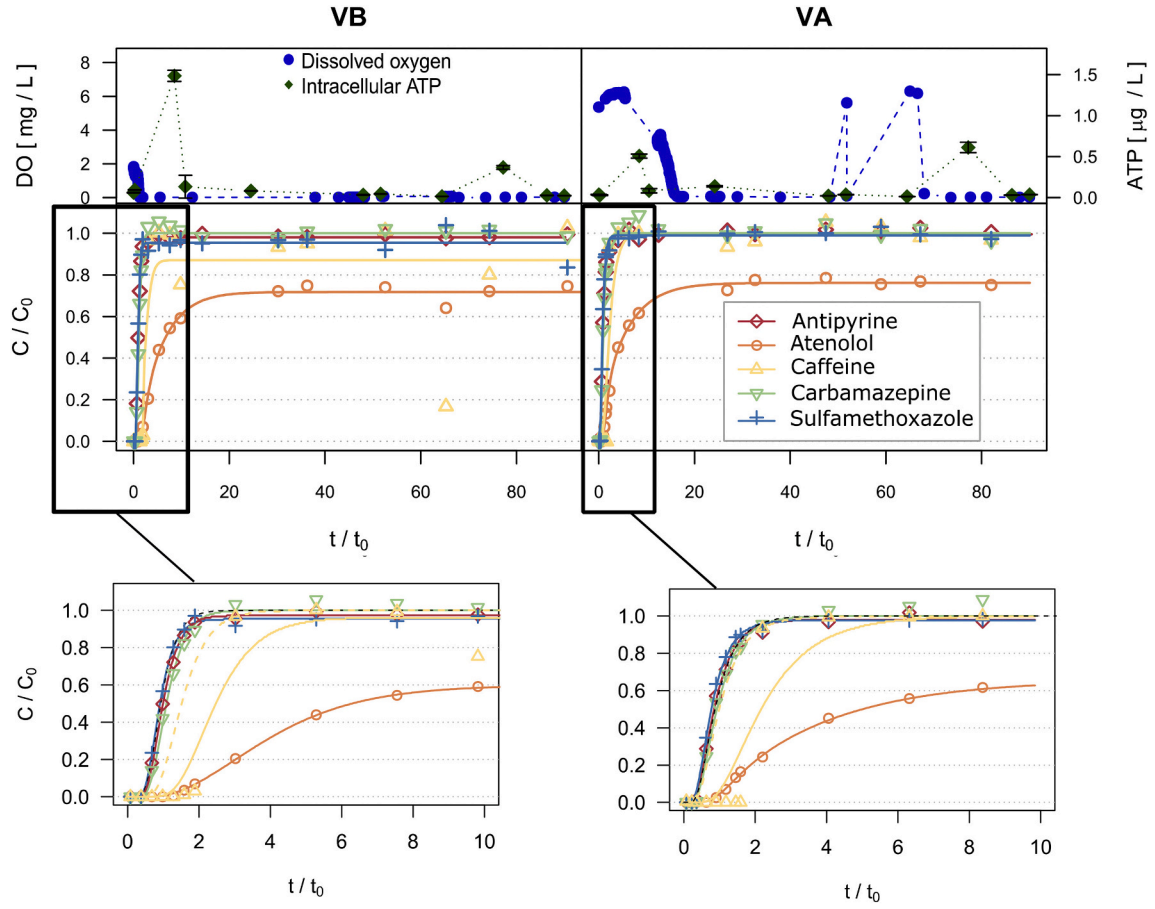

Fig. 1. Breakthrough curves and long-term transport behavior of 5 selected pharmaceuticals in the sandy loam sediment from Vistrenque: biotic setting VB, abiotic setting VA (spiked with sodium azide). The top panels depict the changes of DO and ATP_{itc} concentrations as indication for redox conditions and microbial activity. Pharmaceutical concentrations are normalized with corresponding input concentrations C_0; the time is normalized by the mean residence time t_0 of water in the columns. The dashed black line indicates the breakthrough of the conservative chloride tracer. The dashed yellow line corresponds to the alternative modeling scenario for caffeine transport. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. Breakthrough curves and long-term transport parameters of pharmaceuticals

The modeling results of all experiments are given in Table 2. Overall tendencies of the five selected compounds and two selected sediments for retardation were atenolol > caffeine > carbamazepine > antipyrine > sulfamethoxazole. Retardation was overall stronger in sandy loam sediment (V) than in coarse quartz sand (G). The general tendency for degradation of the compounds was atenolol > sulfamethoxazole > antipyrine > carbamazepine. Caffeine was either almost completely removed under oxic conditions or hardly degraded under anoxic conditions.

Since pharmaceutical concentrations detected in groundwater bodies are usually significantly lower than concentrations used in this laboratory study, the modeled values, as presented above, might not be directly transferrable to field conditions. However, many laboratory tests for the exploration of biodegradation and sorption of organic contaminants in water-sediment systems work with concentrations at the µg/L to mg/L level (Kodešová et al. 2015; Shrestha et al. 2016). We can assume that our results still provide valuable information for the comparison of the behavior of the different compounds in the environment. Furthermore, biodegradation rates of some compounds in this study are assumed to be redox dependent. A range is given for caffeine biodegradation rates in GB, where the lowest value was estimated for nitrate reducing conditions and the highest value for oxic conditions. Estimated first-order degradation rates cannot exactly reflect the biodegradation process over time and distance as we do not know the spatial heterogeneity of microbial degradation activity or the occurrence of degradation hotspots within the columns. The necessary simplification made here, however, still gives a valid estimation for the whole system under investigation and is similar to field studies where degradation coefficients are derived from measurements in wells. The so-estimated first-order degradation coefficients are thus useful for describing and comparing a compound's biodegradability (see Regnery et al. 2015).

The transport behavior of the individual compounds in this investigation will be further described in the following sections. The breakthrough and long-term concentration curves for all five pharmaceuticals are displayed together with the variations in DO and ATP_{itc} concentrations in Fig. 1 for VB and VA columns and in Fig. 2 for GB and GA columns.

3.2.1. Atenolol

Atenolol was subject to sorption and retardation in both sediments but stronger in the V sediment (Figs. 1 and 2). Over the entire duration of the experiments, the overall lowest recovery was found for atenolol in the V sediment (Fig. 3). This sediment has a significant amount of clay and a greater specific surface of 2.97 m^2/g compared to the G sediment (0.25 m^2/g). Ionic sorption of the cationic compound atenolol to the negatively charged surface of clay minerals was assumed by Martínez-Hernández et al. (2014). Besides the clay fraction, the higher carbon content in the V sediment likely enhanced sorption as compared to the G sediment. Kiecak et al. (2019) found a correlation of carbon content in sediment with atenolol sorption. A generally high sorption affinity of

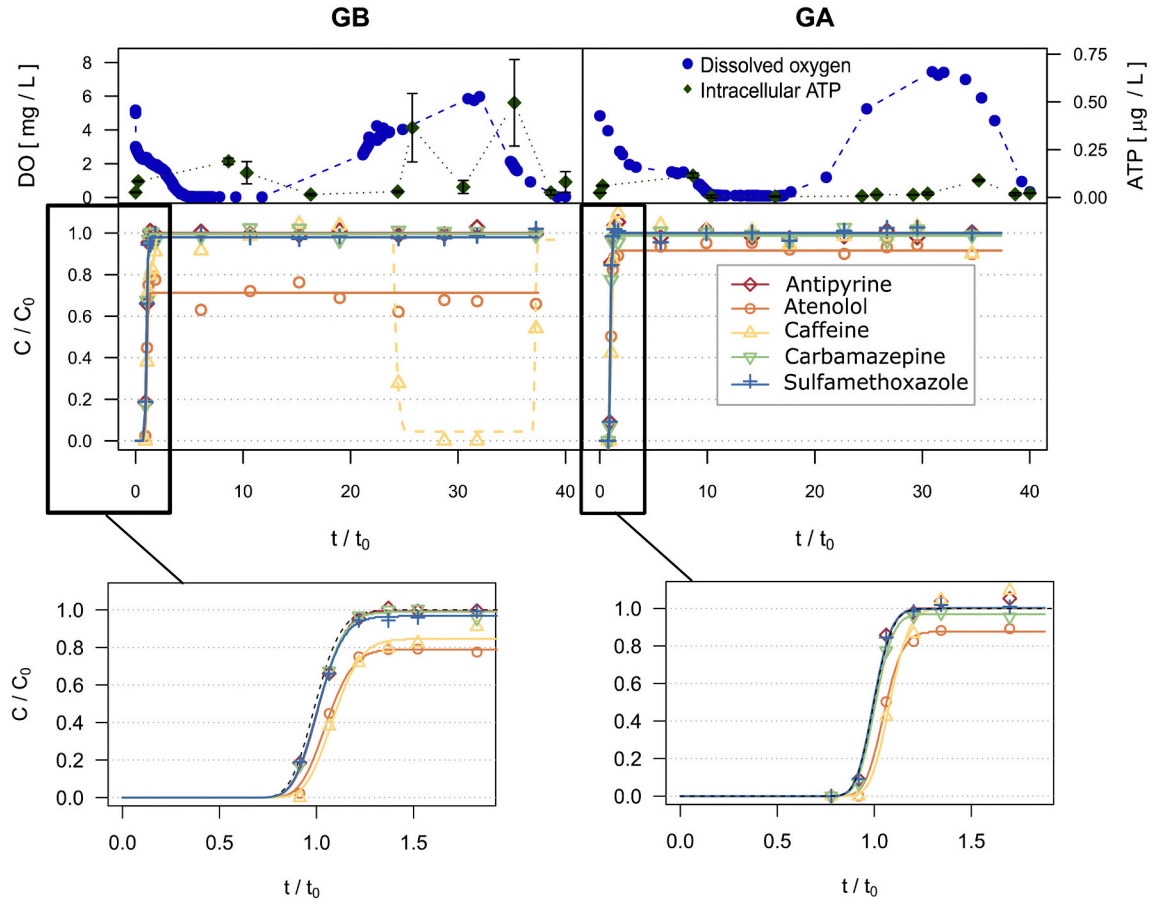

Fig. 2. Breakthrough curves and long-term transport behavior of 5 selected pharmaceuticals in quartz sand: biotic setting GB, abiotic setting GA (spiked with sodium azide). The top panels depict the changes of DO and ATP_{itc} concentrations as indication for redox conditions and microbial activity. Pharmaceutical concentrations are normalized with corresponding input concentrations C_0; the time is normalized by the mean residence time t_0 of water in the columns. The dashed black line indicates the breakthrough of the conservative chloride tracer.

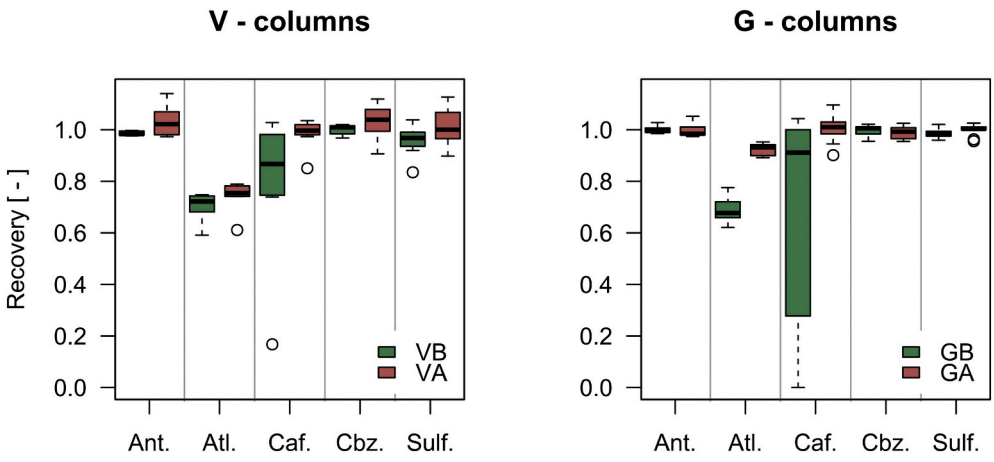

Fig. 3. Recovery rates of each compound calculated as fraction of the injected concentrations for weekly samples after reaching the plateau concentrations. VB and GB (left boxplots, green) are the biotic settings for sandy loam (Vistrenque) and coarse sand, respectively. VA and GA (right boxplots, red) are the abiotic controls spiked with sodium azide. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

atenolol was observed by Burke et al. (2013).

Atenolol transport behavior in the V sediment was best simulated using a two-site non-equilibrium model, which allowed accounting for two different sorption sites. This resulted in a partitioning coefficient β (describing the partitioning of the compound between the two sorption sites as a fraction) of 0.52 and 0.45 in VB and VA, respectively, and a mass transfer coefficient $\omega = 0.02$ in both VB and VA. This suggests that atenolol was adsorbed to more than one (ionic) sorption site in the V sediment with a minor exchange between the sorption sites. Besides sorption, atenolol was also subject to degradation in the V sediment. However, results from the VB and VA column experiments did not allow to clearly determine whether this was in fact a result of biodegradation or rather abiotic degradation processes.

Atenolol removal due to biodegradation was observed in the G sediment. Microbial activity was significantly higher in GB than in the control column GA. This presumably resulted in the higher degradation rates implying that more biotransformation was observed in GB. However, irreversible sorption of atenolol taking place at the same time cannot be ruled out and might to some extent also be the reason for atenolol removal here. In both sediments, atenolol removal seemed to be independent from DO concentrations.

3.2.2. *Caffeine*

Caffeine was subject to retardation in both sediments. Comparable to atenolol, it was sorbed stronger in the V sediment. The breakthrough curve of caffeine in V sediment was not captured properly by measurements (and consequently by the model), as slowly increasing caffeine concentrations in the beginning were followed by an abrupt increase to the plateau. Martínez-Hernández et al. (2014) assumed predominantly irreversible ionic sorption of the cationic compound caffeine to negatively charged surfaces of clay minerals which might have occurred in in the V sediment of our study, too. The dynamics of this process, however, could not be captured adequately by transport models implemented in CXTFIT 2.0. Therefore, two (equilibrium) fitting scenarios were carried out, a) to all available data and b) with improved fit to the plateau concentrations. This resulted in two different retardation coefficients (2.6/1.6 in VB; 2.5/1.1 in VA for scenario a)/b) respectively), possibly referring to different sorption processes (Fig. 1, solid and dashed yellow lines, respectively).

We presumably observed biodegradation of caffeine in all biotic settings. In the biotic settings VB and GB, caffeine transport behavior was in strong contrast to the control columns VA and GA (Figs. 1 and 2). Dropping caffeine concentrations were coinciding with an increase in ATP_{itc} concentrations indicating biological degradation. Biodegradation of caffeine is strongly depending on redox conditions. The first enzymatic steps in the biological conversion of caffeine are the successful removal of three methyl groups which is catalyzed by oxidative demethylase enzymes (Glück and Lingens 1987; Middelhoven and Lommen 1984; Yamaoka-Yano and Mazzafera 1999). A full caffeine degradation pathway by the common bacteria *Pseudomonas putida* has been presented by Yamaoka-Yano and Mazzafera (1999). Dash and Gummadi (2010) found the process to be driven by a variety of microorganisms (including *Klebsiella*, *Rhodococcus* and *Algaligenes* sp.) using *N*-demethylase enzymes, either as hydrolysis or, as described before, as an oxidation process. Rauch-Williams et al. (2010); Wood et al. (1995); Alidina et al. (2014); Godfrey et al. (2007); and Topp et al. (2006) all observed enhanced biodegradation of caffeine with increased oxygen supply in soil and aquatic environments. In contrast, de Wilt et al. (2018) found in their column experiments that caffeine was degraded under all tested redox conditions (including nitrate and sulfate reducing conditions).

As displayed in Figs. 1 and 2, caffeine degradation in our experiments clearly coincided with the occurrence of oxic conditions. However, the inset of caffeine biodegradation did not occur immediately with rising DO concentrations, but after a lag time. The duration of the lag time was estimated to be around 5–7 days in VB, 8 days in GB and 17–18 days in g1. De Wilt et al. (2018) found that under aerobic and nitrate reducing conditions (comparable to our study), the lag phase for caffeine degradation lasted 27 days. In contrast, Hebig et al. (2017) observed an almost immediate removal of caffeine in their column experiments, when organic carbon was present in the substrate.

As described in section 2.4, we divided the GB column experiment into periods of similar redox conditions (presence of oxygen and/or nitrate) in order to capture the temporally varying caffeine biodegradation. Degradation coefficients were then estimated by successively fitting them for each period. The range of estimated caffeine biodegradation rates in GB is given in Table 2. For the g1 experiment, the estimated degradation coefficient was readjusted for the period after the adaption phase. In general, it was a challenge to capture the temporal dynamics of caffeine sorption and biodegradation using CXTFIT as a transport model. Other more refined modeling approaches might be recommendable for future studies, taking into account variable chemical and biological boundary conditions.

3.2.3. *Antipyrine, carbamazepine, and sulfamethoxazole*

Transport behavior of antipyrine, carbamazepine and sulfamethoxazole hardly differed from the conservative tracer in both sediments when five compounds were injected simultaneously (retardation

coefficients around 1, degradation coefficients close to 0), regardless of occurring redox conditions. A low affinity to sorption of carbamazepine and sulfamethoxazole was confirmed by Alidina et al. (2014) and Kodešová et al. (2015); a low potential for degradation of these two compounds was found by a variety of studies (Amy and Drewes 2007; Gauthier et al. 2008; Löffler et al. 2005; Radke and Maier 2014; Regnery et al. 2015). Zero degradation was observed for antipyrine by Pieper et al. (2010) in a bioreactor experiment. Overall, those three compounds exhibit a low potential for natural attenuation, and thus an increased contamination risk.

3.3. Comparison of long-term and pulse injection experiments

Experiments of Kiecak et al. (2020) comprised mostly the same sediments, compounds and column set-up as presented here. Instead of steady long-term injection, pulse injections were conducted at different flow-rates with injection durations between 9.7 and 23 h (G sediment) and 16 to 51.3 h (V sediment). Transport parameters obtained from both pulse and long-term injection experiments are compared in Fig. 4.

There is general agreement in the low retardation of all compounds in G sediment as well as of antipyrine, carbamazepine, and sulfamethoxazole in G and V sediment. Low degradation rates of antipyrine in both sediments as well as low degradation of atenolol and carbamazepine in the G sediment where found in both studies.

However, differences were observed in caffeine degradation in G sediment with biotic conditions, where degradation rates varied across a large range in the long-term experiments due to the occurrence of different redox conditions from oxic to nitrate reducing. Under oxic conditions, caffeine degradation was estimated to be stronger in the long-term experiments but it was found to be equal (or even smaller) under nitrate reducing conditions. The pulse injection experiments also suggested considerably greater degradation rates for atenolol in V sediment, whereas atenolol retardation coefficients were lower than obtained from the corresponding long-term experiment. Thus, atenolol removal was estimated to be more a sorption process in the long-term experiments where it had been classified as degradation process in the pulse-injection experiment. Similarly, caffeine retardation was estimated to be larger in the long-term injection.

experiments than in the pulse injection experiments. It is possible that the duration of pulse injection experiments might not be long enough to capture the plateau concentrations of compounds in a sediment where they are subject to strong retardation.

3.4. Interaction effects on sulfamethoxazole and caffeine

In g1, where caffeine was the only injected compound, it was degraded after approx. 120 t/t_0 (420 h) (Fig. 5) while redox conditions remained oxic. Caffeine was entirely depleted from this point on until the end of the experiment. In the complementary g2 experiment, where caffeine was injected simultaneously with sulfamethoxazole, the latter was degraded ($\mu = 0.69\ d^{-1}$). This was in contrast to the GB column experiment with five compounds. Sulfamethoxazole degradation was thus enhanced when fewer (degradable) compounds were present. At the same time, caffeine degradation was strongly impaired in g2. We may hypothesize that caffeine degradation was affected in the presence of the antibiotic sulfamethoxazole. For instance, Liu et al. (2009) observed low toxic effects of certain antibiotics, including sulfamethoxazole, on soil microbial activity. Similarly, Näslund et al. (2008) found that the biodegradation of organic pollutants in marine sediments was inhibited in the presence of antibiotics. In our study, however, results of the GB and VB column experiments suggested that sulfamethoxazole did not suppress the biodegradation of caffeine. In g2, anoxic conditions established early in the experiment (see Fig. S3 in the supplementary details) which might be the cause for reduced caffeine degradation in comparison to g1. Therefore, we conclude that low concentrations in DO, rather than inhibition by the antibiotic, led to the strongly reduced caffeine degradation.

Overall, from the results of this study we cannot clearly determine to what extent redox conditions, required adaption time, or the presence of sulfamethoxazole contributed to the reduction of caffeine degradation in g2 as compared to g1. Further research would be required to shed more light on the effects of antibiotics on the degradation of groundwater pollutants.

3.5. Methodological notes

Strong variations in dissolved oxygen (DO) and nitrate concentrations could partly be traced back to varying concentrations of total dissolved organic carbon (DOC) monitored in the column inflow and outflow. Since these variations were unrelated to pharmaceutical loads, they were assumed to be due to varying amounts of residual methanol (which was used as solvent for 2 out of 5 compounds). Methanol was

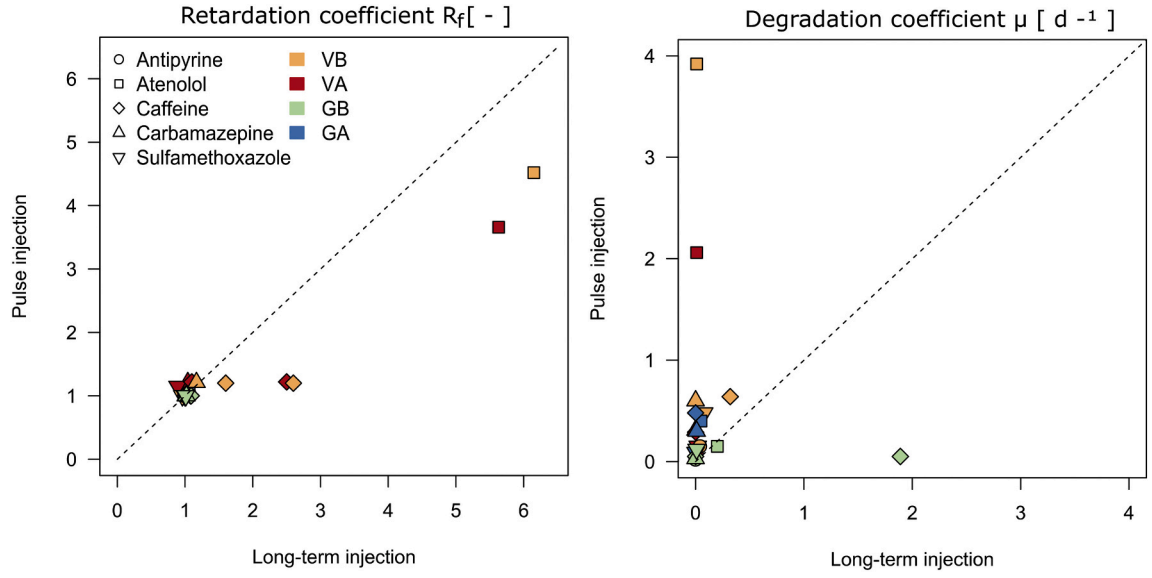

Fig. 4. Comparison of R_f and μ coefficients for VB, VA, GB, and GA columns estimated from pulse injection by Kiecak et al. (2020) and long-term injection experiments of this study. Coefficients obtained by Kiecak et al. (2020) for different flow rates have been averaged in this figure.

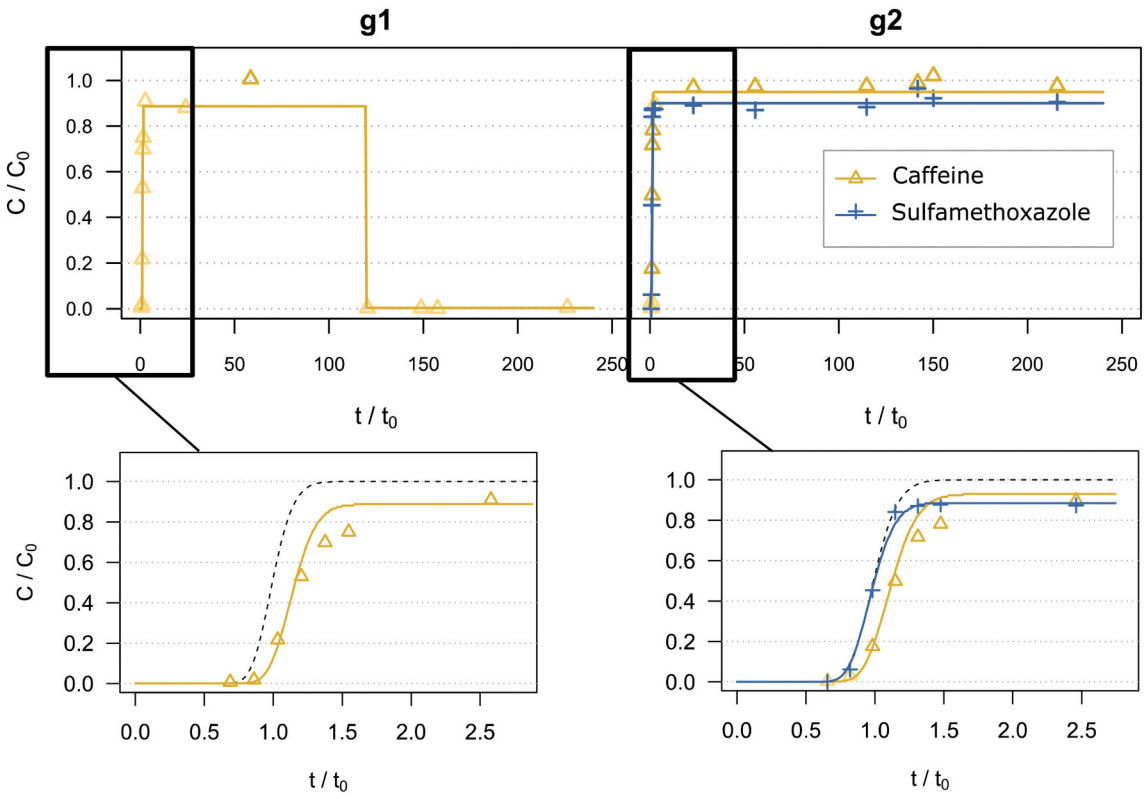

Fig. 5. Breakthrough curves and long-term transport behavior of caffeine alone (g1) and caffeine and sulfamethoxazole combined (g2) in coarse sand. Concentrations are normalized with corresponding input concentrations C_0; the mean residence time t_0 of water in the columns. The dashed black line indicates the breakthrough of the conservative chloride tracer.

attempted to be evaporated from a dry and clean 5 L Schott® bottle within 30 min under the fume hood, before adding fresh groundwater. However, based on a follow up test, we suggest an evaporation time of 4–5 h in order to make sure that methanol concentrations are negligible. In this study, an absolute maximum of 0.018 Vol% methanol was estimated to have remained in the solution if it was not evaporated at all. The metabolization of methanol-bound carbon possibly caused biological depletion of dissolved oxygen and nitrate (cf. Anthony 1986). DO was temporally depleted in all columns except for g1 where only caffeine was injected and which constituted the only experiment without the use of methanol as solvent. Therefore, we suspect residual methanol in the feed water of the columns to be the main reason for strong variations in redox conditions in our experiments.

4. Conclusions

The aim of our study was to investigate the long-term transport behavior of five selected pharmaceuticals in different sediments, discern biodegradation from abiotic processes and elucidate the effect of an antibiotic compound on an easier degradable compound. When five selected compounds were injected simultaneously in the column experiments, antipyrine, carbamazepine and the antibiotic sulfamethoxazole showed similar transport behavior as the conservative tracer in both sediments and under varying redox conditions. During the entire duration of the experiments, very low to no degradation or sorption were observed, thus showing the low potential for natural attenuation and conservative transport for those compounds. Therefore, we conclude that these compounds pose the highest contamination threat of the five compounds tested in this study.

Atenolol and caffeine were depleted to varying extents. Atenolol was degraded independently of oxygen concentrations in G sediment, where a clear difference in atenolol transport could be observed between the biotic and the control setting with inhibited microbial activity. The removal process was therefore likely due to biodegradation rather than abiotic processes. In V sediment, sorption seemed to be the dominant atenolol removal process.

Biodegradation of caffeine was found in the biotic settings of both sediments after a lag time of 120 to 420 h when enough dissolved oxygen was present.

When only caffeine and sulfamethoxazole were injected simultaneously, sulfamethoxazole was degraded while caffeine degradation was reduced. We assume that biodegradation of sulfamethoxazole was enhanced when less other compounds were available. The lower caffeine degradation in this experiment seemed to be influenced by low concentrations of dissolved oxygen rather than the presence of the antibiotic sulfamethoxazole. The effect of an antibiotic compound on the transport behavior of other pharmaceuticals remained unclear and requires further investigation.

Intracellular ATP concentrations (ATP_{itc}) were measured in outflow samples for estimating the amount of living cells and thus microbial activity in the columns. The results suggested that entirely sterile conditions were not achieved in the abiotic control columns by adding the indicated amount of sodium azide. However, microbial activity was significantly reduced in the coarse sand sediment and was lowered in the sandy loam sediment. This resulted in pronounced differences between biotic and control columns regarding caffeine degradation (in both sediments) and atenolol degradation (in G sediment). The correlation of caffeine degradation and enhanced ATP_{itc} concentrations further strengthened the assumption that caffeine was biologically degraded. Overall, results of this study emphasize the effect on transport behavior of sediment properties and redox conditions, as well as available time for microbial adaption and the variety of pharmaceuticals that are released together into groundwater.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author Statement.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jconhyd.2020.103738.

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