Article:
Al-Khazrajy, Omar S A, Bergström, Ed and Boxall, Alistair B A orcid.org/0000-0003-3823-7516 (2017) FACTORS AFFECTING THE DISSIPATION OF PHARMACEUTICALS IN FRESHWATER SEDIMENTS. Environmental Toxicology and Chemistry. pp. 1-33. ISSN 1552-8618

https://doi.org/10.1002/etc.4015

Factors Affecting the Dissipation of Pharmaceuticals in Freshwater Sediments

Omar S. A. Al-Khazrajy [a,c], Ed Bergström[b], Alistair B.A. Boxall[a*]

[a] Environment Department, University of York, Wentworth Way, Heslington, York, YO10 5NG, UK.

[b] Centre of Excellence in Mass Spectrometry and Department of Chemistry, University of York, Heslington, York, YO10 5DD, UK.

[c] Department of Chemistry, College of Ibn al-Haitham, University of Baghdad, Baghdad, Iraq

*Corresponding author: Alistair B. A. Boxall, Telephone +44 (0)1904 324791; email- alistair.boxall@york.ac.uk

Abstract

Degradation is one of the key processes governing the impact of pharmaceuticals in the aquatic environment. Most studies on the degradation of pharmaceuticals have focused on soil and sludge with fewer exploring persistence in aquatic sediments. Here we investigate the dissipation of six pharmaceuticals from different therapeutic classes in a range of sediment types. Dissipation of each pharmaceutical was found to follow first-order exponential decay. Half-lives in the sediments ranged from 9.5 d (atenolol) to 78.8 d (amitriptyline). Under sterile conditions, the persistence of pharmaceuticals was considerably longer. Stepwise multiple linear regression analysis was performed to explore the relationships between half-lives of the pharmaceuticals, sediment physicochemical properties and the sorption coefficients for the compounds. Sediment clay, silt and organic carbon content and microbial activity were the predominant factors related to the degradation rates of diltiazem, cimetidine and ranitidine. Regression analysis failed to highlight a key property which may be responsible for observed differences in the degradation of the other pharmaceuticals. The present study results suggest degradation rate of pharmaceuticals in sediments is determined by different factors and processes and does not exclusively depend on a single sediment parameter.

Keywords: Pharmaceuticals, Dissipation, Sediment, Physicochemical properties

INTRODUCTION

Thousands of tonnes of pharmaceuticals are produced and consumed annually for human and veterinary medicine (Zuccato et al. 2000; Castiglioni et al. 2005; Sarmah et al. 2006). Generally, following use, pharmaceuticals are discharged as either the parent compound or metabolites to the sewerage system (Boxall et al. 2012). The incomplete removal of pharmaceuticals in wastewater treatment plants (WWTPs) and the relative persistence of these compounds explains their widespread occurrence in the natural environment (Kolpin et al. 2002; Benotti and Brownawell 2009; Yan et al. 2013; Chen and Zhou 2014; Shi et al. 2014). Industrial and hospital emissions, land application of sewage sludge and direct disposal are other routes of entry of pharmaceuticals to the environment (Christensen 1998; Daughton and Ternes 1999; Kümmerer 2009). With the increasing recognition that pharmaceuticals occur in the environment, there is a need for a better understanding of their environmental fate and risks to non- target organisms (Daughton and Ternes 1999; Loffler et al. 2005; Mompelat et al. 2009).

Once pharmaceuticals are introduced into the aquatic environment, their fate and behaviour will be determined by processes such as biodegradation, hydrolysis or photodegradation, as well as sorption to natural solid matter including suspended solids and bed sediments (Yamamoto et al. 2009; Liang et al. 2013). Degradation is one of the key processes governing the fate and impacts of organic compounds in the environment. Consequently, an assessment of the persistence of a pharmaceutical is required as part of the environmental risk assessment process during the marketing authorisation of new pharmaceutical active ingredients (EMEA 2006).

Many studies have explored the degradation of pharmaceuticals in wastewaters (e.g. Quintana et al. 2005; Joss et al. 2006), sludge (e.g. Carballa et al. 2007; Radjenović et al. 2009; Li and Zhang 2010) and soils (Monteiro and Boxall 2009; Xu et al. 2009; Lin et al. 2011). The most important dissipation pathway for pharmaceuticals in the environment is microbial degradation (Fang et al. 2012). The rate and degree of degradation of pharmaceuticals are affected by the environmental conditions such as pH, temperature, the abundance of microorganisms and the presence of biosolids as well as the physicochemical properties of the compound such as the degree of dissociation and lipophilicity of the pharmaceutical (Monteiro and Boxall 2009). For example, soil texture was found to affect the degradation rates of caffeine, with faster degradation being observed in loam and sandy loam soils compared to a silt loam soil (Topp et al. 2006).

Amitriptyline also showed faster dissipation in a loam soil compared to clay and sandy soils (Li et al. 2013). Benotti and Brownawell (2009) reported variations in degradation rates for 19 pharmaceuticals in estuarine and coastal surface water samples and suggested that faster rates of pharmaceutical degradation occurred in waters with a greater abundance of total bacteria or the presence of microbial communities that are better able to transform these compounds. While data are available on the persistence of pharmaceuticals in soils and surface waters, few studies have investigated the behaviour and degradation of pharmaceuticals in sediments, even though this compartment is considered as a sink for many pharmaceuticals, especially cationic pharmaceuticals (Löffler and Ternes 2003; Loffler et al. 2005; Boxall and Ericson 2012).

The studies that have been done on sediments have focused on anti-inflammatory compounds, antibiotics, and lipid regulators. Conkle et al. (2012) focused on the degradation of selected pharmaceuticals under aerobic and anaerobic conditions in wetland sediments. Carbamazepine was found to be highly persistent in sediment with half-lives between 165-264 d under aerobic conditions and these were increased by factors of 1.5-2.5 under anaerobic conditions. Ibuprofen and gemfibrozil showed relatively short half-lives of around 20 d under aerobic conditions and these increased by factors of 11-34 under anaerobic conditions. Ibuprofen showed low half-lives ranging from 1.2 to 2.5 d depending on the flow rate of overlying water (Kunkel and Radke 2008). These findings show that hydraulic conditions can drive the rate of pharmaceutical degradation in sediment. Thuy and Loan (2014) studied the fate of antibiotics (ciprofloxacin, griseofulvin, and rifampicin) in water only and a water-sediment system. The half-lives of the antibiotics in the water system (8-20 d) were shorter than in the water-sediment system (23-39 d).

Although previous studies have reported the degradation of selected pharmaceuticals in sediments, limited information is available on the effects of sediment characteristics on the persistence of these molecules. A better understanding of the factors that influence degradation rate of pharmaceuticals in sediment could help to better inform the environmental risk assessment of pharmaceutical active ingredients. Therefore, here we present the findings of a study to characterise the dissipation of six human-use pharmaceuticals with different chemical properties and therapeutic usages in ten freshwater sediments with a wide range of physicochemical properties.

MATERIALS AND METHODS

Chemicals and solvents

Analytical grade (≥98% purity) amitriptyline hydrochloride, atenolol, cimetidine, diltiazem hydrochloride, mefenamic acid and ranitidine were purchased from Sigma-Aldrich (UK). Chemical structures, physicochemical properties and therapeutic uses of the selected pharmaceuticals are given in the Supporting Information (Table S1). The study pharmaceuticals were selected based on their usage amounts and based on risk-based prioritisation studies, performed by our group, to identify the pharmaceuticals of most concern in the environment in the UK (Guo et al. 2016) and Iraq (Al-Khazrajy and Boxall 2016a). For example, amitriptyline and mefenamic acid were ranked as high priority compounds based on their potential to elicit pharmacological mechanism-related effects in aquatic organisms in the UK and Iraq. Atenolol and ranitidine were ranked as high priority based on their potential to elicit chronic effects at lower trophic levels in the UK. Solvents (acetonitrile, methanol, acetone, ethyl acetate and water) were of high-performance liquid chromatography (HPLC) grade (Fisher Scientific). Ammonium hydroxide solution (35%) was purchased from Fisher Scientific. Formic acid (96 %), 2,3,5-Triphenyl-tetrazolium chloride (TTC) solution, tris (hydroxymethyl) ammoniomethane and triphenylformazane (TPF) were obtained from Sigma-Aldrich (UK). Stock solutions of the reference compounds were prepared in methanol and stored at −20 °C until use.

Sediment collection and characterisation

Eight freshwater sediments, from small rivers and streams in England (located in Yorkshire and Leicestershire) and two from Iraq (located in Baghdad and Karbala), were sampled from the top 0-5 cm surface layer. Sediments were collected from remote and sparsely urbanized areas which were expected to be less affected by pharmaceutical contamination sources (e.g. WWTPs and hospitals). Since the main purpose of the current study was to investigate the effect of sediment properties on the degradation of pharmaceuticals, sediments were chosen to cover a diverse range of properties (e.g. clay content which ranged from 0.35 to 37.25% and organic carbon content (OC) which ranged from 0.98 to 9.90%).

Selected physicochemical properties of the sediments are given in Table 1. Sediment samples were collected using a pre-cleaned stainless steel spade and placed along with overlying water into 1 L amber glass bottles which had been cleaned with acetone, deionized water and then dried. Following collection, sediments were transferred to the laboratory, where plant residues and debris were removed manually. The wet slurry was then sieved to 2 mm, homogenized and stored at 4^{o}C for less than a month prior to the study. For characterisation, the sediments were subjected to granulometric analysis to determine the texture using a Malvern laser granulometer (Hydro 2000MU, UK); the OC in the sediments was measured according to the ISO10694 protocol using a total carbon content analyzer (Viro Macro Elemental (CN) Analyser, Germany). Sediment pH values, in 0.01 M $CaCl_2$, were determined using a sediment to solution ratio of 1:5. Cation exchange capacity and exchangeable metals were analysed by Forest Research UK following the ISO 11260 & 14254 protocols using a dual view ICP-OES (Thermo iCAP 6500 duo). Prior to use of sediments in the degradation study, each sediment type was analysed, using the analytical procedures described below, to determine the background levels of the pharmaceuticals of interest. For all sediments, background concentrations of the study compounds were found to be below the analytical limits of detection.

Table 1 Sampling locations and measured properties of the study sediments used in the degradation studies

Sediment	Coordinates	Texture	Silt %	Clay %	Sand %	OC %	pH $CaCl_2$	CEC (cmol+ /kg)	Bioactivity (mg TPF /kg sediment)
BTC	54.017012, -0.881074	Sandy loam	35.48	34.25	62.92	2.83	6.88	13.45	5161.1
BW	53.982712, -0.790092	Loam	45.92	4.73	49.35	9.9	8.1	35.58	5521.0
MIL	53.964920, -0.719305	Sandy clay	0.88	37.25	61.87	8.02	7.15	37.08	280.2
GER	53.935850, -1.054470	Sandy clay loam	1.22	30.97	67.81	5.69	7.1	24.26	2630.8
HLM	54.242978, -1.055166	Sandy	10.08	0.12	89.8	0.98	6.65	5.85	825.3
MOR	54.371324, -0.965524	Loamy sand	21.05	0.35	78.6	3.52	6.35	11.26	4767.2
HAB	52.626226, -0.890155	Loamy sand	26.7	1.12	72.18	1.12	7.45	11.34	404.1
SKF	52.620847, -0.905779	Sandy clay loam	0.38	36.52	63.1	7.92	7.02	28.39	4256.0
BGD	33.361904, 44.370943	Silt loam	58.15	2.04	39.81	3.42	7.1	12.99	258.1
HUS	32.623024, 44.027632	Silt loam	71.15	2.91	25.94	3.51	7.3	19.07	428.4

Sediments from England were collected from Buttercrambe (BTC), Bishop Wilton (BW), Millington (MIL), German beck (GER), Helmsley (HLM) and North Yorkshire Moors National Park (MOR), all in North Yorkshire; and Harborough (HAB) and Skeffington (SKF) in Leicestershire. The sediments from Iraq were collected from the Tigris River in Baghdad (BGD) and the Alhussainya River (HUS) in Karbala city, TPF= triphenylformazane.

Degradation of pharmaceuticals

Aerobic degradation studies were performed using sterilised and non-sterilised sediments following the method of Ying and Kookana (2003). The persistence of the pharmaceuticals in all sediment types was investigated under non-sterile conditions, while four sediments, selected to give a range of extremes of sediment characteristics, were used for the sterile studies. In brief, 5 g (dry weight equivalent) of sediment were weighed into 40-mL screw capped amber glass vials. The ratio of sediment to solution was 1:1. Samples were pre-incubated for 6 d, in the dark. The samples (three replicates) were then spiked with 50 µL of the standard solution (containing 20 mg/L of each pharmaceutical) to give a nominal concentration of 200 ng/g (dry weight) for each pharmaceutical. Vials were thoroughly shaken for 30 seconds and placed in an incubator in the dark at 20 ± 2 ^{o}C. Vials were loosely capped to avoid contamination while

allowing air exchange. A headspace of about 60% of the vials height was used to provide sufficient headspace to ensure aerobic conditions.

Sterilisation of the sediments was achieved by autoclaving (Prestige medical, UK) sediments at 120 ^{o}C under 300 kPa for 30 min three times over three consecutive days. To confirm the sterility of the autoclaved sediments, the microbial activity of the sediments was tested following the method described in the next section. Due to possible water loss during the incubation, sample vials were regularly weighed to monitor the water content of the water-sediment system and, if necessary, water content was adjusted with HPLC grade water. After each addition, samples were gently shaken (without resuspension of sediment fines to avoid the disturbance of sediment-borne microorganisms (Abia et al. 2017)) to ensure aerobic conditions. Triplicate subsamples of the sterilised and non-sterilised sediment were withdrawn at 0, 3, 7, 14, 28, 56 and 90 d following the start of the study and immediately stored in a freezer at – 22 ^{o}C until analysis (analysis occurred within one week).

Sediment bioactivity

The bioactivity of sediments in the degradation experiments was measured by using TTC solution (0.1 g, distilled water: 10 ml) to measure dehydrogenase activity in living organisms which is an indicator of sediment microbial activity (Monteiro and Boxall 2009). Subsamples of each sediment type (from day 90 of the incubation) were incubated with 5 ml (0.5% by weight) of colourless TTC at 30 ^{o}C in 0.1M tris buffer (tris (hydroxymethyl) ammoniomethane) adjusted to pH 7.6. The colourless TTC is reduced to red water-insoluble TPF by the dehydrogenase enzyme in bacteria. After incubation for 24 hours, the TPF was extracted with 25 mL of acetone. The samples were then agitated for 1 hour at 250 oscillations min^{-1} and centrifuged at 2500 g for 10 min. The absorbance of the supernatant was then measured at 485 nm using an Ultraviolet-visible (UV-Vis) spectrophotometer (160 Spectrophotometer, Shimadzu, Japan). The absorbance measurements were converted to bioactivity (mg TPF/kg) based on a calibration curve developed from a set of TPF standards.

Extraction of pharmaceuticals and SPE clean-up

The study pharmaceuticals were extracted from the test sediments using sonication-assisted extraction and extracts were then cleaned-up using solid phase extraction (SPE) according to the procedures previously developed by our group (Al-Khazrajy and Boxall, 2017). In brief, frozen samples were thawed at room temperature then 10 ml of 2% NH_4OH in methanol was added. Samples were then vortexed for 15 seconds and ultra-sonicated for 15 min. The slurry was then agitated at 250 rpm for 10 minutes. The slurry samples were then centrifuged at 4500 rpm for 10 min. The resulting supernatant was filtered through a 0.45 μm nylon filter and then decanted into a 500-ml Erlenmeyer flask. The extraction was repeated using 2% formic acid in methanol while in a third extraction cycle only 5 ml of methanol was used. The supernatants from the three steps were combined and diluted with Milli-Q water (ELGA purelab, UK) to produce a total volume of 400 ml (methanol < 5%).

The aqueous sediment extracts were then adjusted to pH=10 using NH4OH solution prior to a solid phase extraction (SPE) clean up step. The SPE was conducted on 6-ml (200 mg) Oasis hydrophilic–lipophilic balance (HLB) SPE cartridges (Waters, USA). The preconditioning step was done by passing through 5 ml of methanol followed by 10 ml of Milli-Q water. The diluted aqueous extract samples were then loaded onto the SPE cartridge at a rate of 10-20 ml/min using a vacuum manifold (Supelco VisiprepTM, UK) for extraction of 12 samples in parallel, after which the cartridges were rinsed with 10 ml of 5% methanol in Milli-Q water and then dried under air for 30 minutes. The cartridges were then eluted with 2 x 2.5 ml methanol followed by 1.0 ml of 2% NH4OH in methanol. The eluates were dried under a gentle nitrogen stream using a concentrator (DB-3A, TECHNE, UK) at 30oC. The dried extract was reconstituted into 1.0 ml of methanol: water (20:80) and sonicated for 5 minutes. The reconstituted extracts were then stored in a freezer at -22 oC prior to analysis.

Analytical method

Cleaned-up extracts were analysed by liquid chromatography tandem mass spectrometry (LC-MS/MS) using an Applied Biosystems/MDS Sciex API 3000 triple quadrupole mass spectrometer interfaced with a Dionex UltiMate® 3000 HPLC. A reversed phase C18 analytical column of 150 mm × 4.6 mm and 5.0

μm particle size (Zorbax Eclipse XDB-C18) was used for separation and quantification. A Zorbax Eclipse XDB-C18 4.6 mm x 12.5 mm 5.0 μm guard column was also used. The column temperature was maintained at 35 °C and the autosampler temperature was set at 4 °C. The injected sample volume was 10 μL. Mobile phases A and B were 10 mM ammonium acetate/acetic acid buffer (pH 4.8) and acetonitrile and the flow rate was 1.0 mL min^{-1}. The gradient elution program was: 90% of mobile phase A for equilibration and holding steps for 1 min each, mobile phase B was then increased linearly to 25% over 10 min, and then rapidly increased to 90 % over 2 min, this composition was held for a further 5 min before returning to the initial condition of 10% mobile phase A over 1 min. The column was re-equilibrated for 6 min at the initial mobile phase composition until 25 min, which was also the total run time.

The tandem mass (MS/MS) analysis was performed using a triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source. For the MS analysis, standard solutions (1.0 mg/mL) of each analyte were directly infused along with the mobile phase into the mass spectrometer. The positive ion mode was selected on the basis of the presence of a basic functionality in the chemical structure of all the pharmaceuticals except mefenamic acid which contains acidic functionality. Even so, mefenamic acid exhibited very low limit of detections (LODs) and limit of quantifications (LOQs) so there was no need to switch to negative ionization mode. ESI source temperature, capillary and cone voltage and flow rate of the desolvation gas were optimized to obtain the highest intensity of precursor molecules of the six analytes. The collision gas pressure and collision energy of collision were optimized for a maximum response of the fragment ions obtained. Precursor ions and product ions for MS/MS detection and their respective collision energies are listed in the supporting information (Table S2) together with typical retention times of all target analytes. For MS/MS detection, the instrument was operated in Multiple Reactions Monitoring (MRM) mode and identifications were made by comparing retention times and substance specific mass spectra. All data were acquired and processed using Analyst 1.4.2 software.

The performance of the analytical method (precision, accuracy, LOD and LOQs) are described in Al-Khazrajy and Boxall (Al-Khazrajy and Boxall 2017). Recoveries for the test chemicals ranged from 40.1% (ranitidine in BW sediment) to 120.6% (amitriptyline in HAB sediment) and LODs ranged from

0.02 (diltiazem) to 2.5 ng/g (mefenamic acid). The relative standard deviations (RSDs) were less than 16% (see Table S3 in the supporting information). The stability of the study pharmaceuticals during storage was also checked. Recoveries from freshly spiked high microbial activity sediment (BTC sediment) were compared to equivalent spiked sediments at day 0 that had been stored in a freezer for 90 days. The recoveries obtained for all pharmaceuticals from the frozen samples were approximately the same as those of the freshly spiked samples showing negligible concentration changes (Table S4, supporting information).

Data analysis

The concentrations of pharmaceuticals in the sediment systems were plotted against time of incubation using Microsoft Excel 2010 software. The degradation rate constant k (per day) was then estimated by fitting a first-order exponential decay model to the data. The time for 50% (DT50) and 90% (DT90) dissipation were then estimated.

Statistical analysis

Statistical analyses (ANOVA and Multiple linear regression (MLR)) were performed using the SPSS 23.0 statistical software package with the significance level being $p<0.05$. Prior to the statistical analyses, the normality of the data was first evaluated using the Kolmogorov-Smirnov and Shapiro-Wilk methods. All variables were found to be normally distributed ($p>0.05$), except microbial activity and sorption coefficients (Kds) for diltiazem and mefenamic acid in the MLR analysis, these were therefore normalized using logarithmic transformations. Two way- ANOVA was used for each sediment and pharmaceutical to explore differences between concentrations in sediment over time. Stepwise MLR analysis was employed to find relationships between degradation rate as the dependent variable and combinations of sediment physical-chemical property parameters as the explanatory variables. Model adequacy was measured by the squared correlation coefficient (R^2), the Fisher criterion (F) and the significance level (p). The general form of the regression equations is described in Equation 1:

$$Y=b_0 + b_1X_1 + b_2X_2 + \ldots\ldots + b_5X_5 + \ldots\ldots + b_nX_n \quad (1)$$

Where Y is the dependent variable representing degradation rate (k), b_0 is the intercept, b_1. . .b_n are regression coefficients, and X_1–X_n are independent variables referring to the chosen predictors.

RESULTS AND DISCUSSION

Degradation of pharmaceuticals in sediment

The dissipation of pharmaceutical concentrations in sediments over time is plotted in Figure 1 for the non-sterile treatments and Figure 2 for the sterile treatments. Calculated times for half of the compound to dissipate (DT50) for sterile and non-sterile treatments are summarised in Table 2. Associated first-order degradation rate constants (k) and DT90 values are given in the supporting information in Tables S5 and S6. In the non-sterile sediments, no lag phase was observed for the pharmaceuticals, and the degradation of pharmaceuticals in the ten sediments was well described by the first-order exponential decay model. Relatively poor fits of the dissipation curves were seen for amitriptyline and atenolol in SKF sediment and for mefenamic acid in BGD sediment. This may be related to the rapid dissipation during the first 7-14 d of incubation. There were some marked differences between sediments in their ability to degrade different pharmaceuticals even where test sediments had similar characteristics. All six pharmaceuticals showed moderate persistence, with DT50 values ranging from 9.5 d (atenolol) to 78.8 d (amitriptyline) (Table S5). In general, the degradation half-lives of pharmaceuticals decreased in the order amitriptyline > mefenamic acid > diltiazem > cimetidine > ranitidine > atenolol in each individual sediment. The dissipation of all six compounds in the four sediments tested under sterilised conditions was also found to follow the first-order exponential decay kinetics (Figure 2). Generally, the dissipation of pharmaceuticals in the sterile sediments was slower than in the non-sterile systems indicating that biodegradation was mainly responsible for the observed dissipation of the study pharmaceuticals. Findings for the individual pharmaceuticals are discussed below.

Table 2 Calculated half-lives (in days) for the study pharmaceuticals in sediments under non-sterilized (based on ten sediments) and sterilised (based on four sediments) conditions

Compound	Kinetics	Non-sterilised			Sterilised		
		Median	Min.	Max.	Median	Min.	Max.
Amitriptyline	First order	62.2	44.4	78.8	90.6	76.2	106.6
Atenolol	First order	13.0	9.5	17.4	23.5	20.5	28.8
Cimetidine	First order	27.6	18.5	36.5	42.5	28.2	54.1
Diltiazem	First order	26.7	21.7	35.4	65.2	57.7	78.7
Mefenamic acid	First order	29.3	19.7	35.0	54.7	44.7	60.9
Ranitidine	First order	16.7	10.1	37.5	40.9	35.9	46.8

Amitriptyline

Under non-sterilised conditions, the dissipation of amitriptyline was described well by the first order kinetic model and the dissipation was slow compared to the other pharmaceuticals (Figure 1). DT50 values for the compound ranged from 44.4 to 78.8 d and DT90 values from 147.6 to 261.4 d (supporting information Table S5). Significant differences in dissipation were observed across the sediment types ($F = 45.3$; $P<0.001$). Under sterile conditions, half-lives for amitriptyline were higher (supporting information Table S5). The half-lives of amitriptyline in BGD and HLM sediment increased from 66.6 to 106.6 d and from 78.8 to 105.0 d, respectively, indicating the importance of microorganisms for amitriptyline dissipation in these two sediments. While, to the best of our knowledge, no literature data are available on the degradation of amitriptyline in sediment, the persistence of the compound has been explored in soil. Our half-lives are similar to those reported in soils with different textures where DT50s ranging from 34.1to 85.3 d were observed (Li et al. 2013). In this study, the dissipation of amitriptyline was suggested to result from the formation of non-extractable residues and the degradation of the parent compound to nortriptyline (N-desmethyl amitriptyline) and amitriptyline-N-oxide.

Atenolol

Under non-sterilised conditions, atenolol degraded more quickly than the other pharmaceuticals in all sediments over time. Dissipation differed significantly between sediment types ($F= 4.2$ and $P<0.001$). In sterilised treatments, atenolol showed DT50s almost 2-3 times higher than the non-sterile treatments in BTC and GER sediments while in BGD and HLM sediment atenolol exhibited only a small increase in DT50 values (Table S5). This behaviour suggests that microbial activity may have contributed less to the observed dissipation in these sediments and that the observed loss was caused by abiotic processes. The observed half-lives for atenolol are greater than the DT50s of between 2.8 and 10.3 d observed by Kodešová et al [43] in soil. They showed that dissipation of the compound is slow in soil with a higher adsorption affinity to atenolol. For many of the compounds, data from other studies suggest that the observed dissipation is due to conversion into transformation products. It is important to recognise that these transformation products could be more stable than the parent compound and also may pose a risk to the environment (Boxall et al. 2004). For atenolol, based on previous work, the observed dissipation may be explained by the conversion of the parent molecule to metoprolol acid which comparatively more stable in water-sediment systems than atenolol (Svan et al. 2016).

Cimetidine

Under non-sterile conditions, sediments showed significant differences in their ability to degrade cimetidine ($F = 9.6$; $p<0.001$) with half-lives ranging from 18.5 d in the HUS sediment to 36.5 d for both the MOR and MIL sediments. With the exception of the HUS sediment, cimetidine was found at measurable concentrations after 90 d of incubation. Dissipation half times under sterile conditions ranged from 28.2 to 54.1 d. The differences in half-lives between sterilised and non-sterilised conditions indicate that microorganisms play a role in the dissipation of the molecule. The degradation of the cimetidine in solid phase environment has not been studied and reported half-lives are only available for seawater with DT50 values ranging from 9.8 to >100 d (Benotti and Brownawell 2009). Degradation of cimetidine in aqueous solutions is believed to be via photo oxidation or chlorination (Latch et al. 2003; Buth et al. 2007).

Diltiazem

Diltiazem showed moderate dissipation in the study sediments with DT50 values of up to 35.4 d being obtained. Significant differences in diltiazem dissipation were seen across sediment types ($F= 16.0$; $p<0.01$). Slow dissipation was observed for diltiazem under sterilised conditions with half-lives ranging from 57.7 to 78.7 d. Previously reported half-lives of diltiazem were only found for surface water and soil. Benotti and Brownawell, (2009) reported half-lives of diltiazem ranging from 5.5 to 36 d in coastal seawater under non-sterile conditions. Wu et al. (2010) reported half-lives for diltiazem of 11- 44 and 14-84 d in soils amended with biosolids under aerobic and anaerobic conditions, respectively. In this same study, biodegradation and soil texture were reported as the main drivers for the observed dissipation of diltiazem.

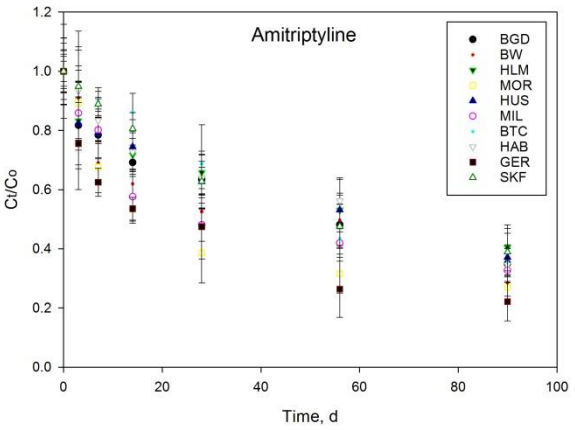
Mefenamic acid

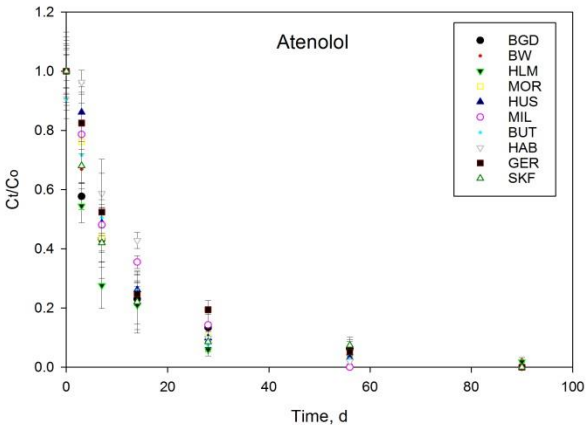
Mefenamic acid displayed significant differences in dissipation between the non-sterile sediments ($F= 11.5$; $p<0.001$) with DT50 values ranging from 19.7 to 35.0 d. Unlike in the non-sterilised studies, degradation curves for the mefenamic acid were characterized by an initial lag phase (day 0–7) in BTC sediment (Figure 2). This would most likely be attributed to adaptation of the microbial population. Nevertheless, this lag phase was not observed for the other sediments. Half-lives obtained here agree well with those obtained in lake water under different experimental conditions (filtered and non-filtered water, sunlight and dark) which ranged from 15.5 to 66.6 d (Araujo et al. 2011) and are to the lower end of the range (12.5 to 104 d) found by Yamamoto et al. (2009) in river water sampled from two different urban streams. These higher DT50s, previously observed, are probably explained by the lower abundance of microbial activity found in surface water in comparison to sediment (Boxall and Ericson 2012).

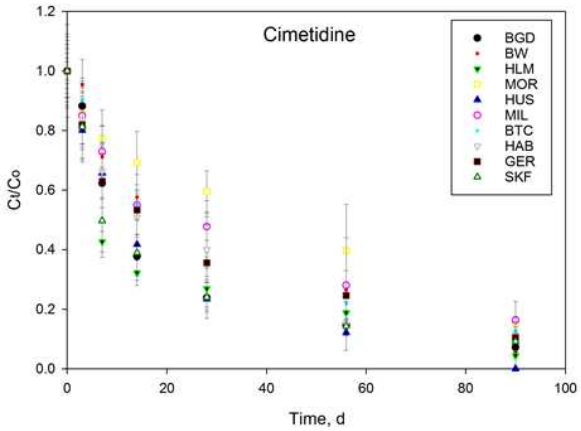
Ranitidine

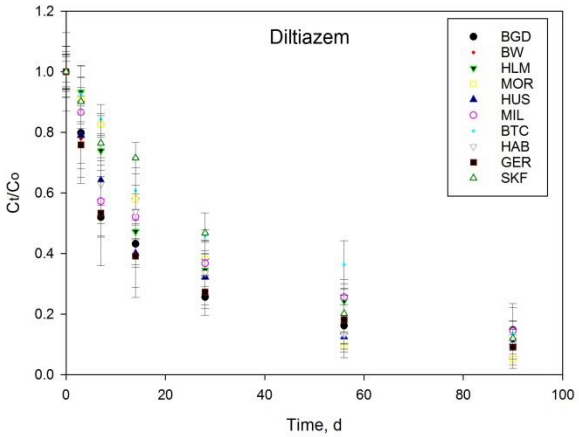
For ranitidine, the maximum half-life observed in non-sterilised treatments was in the HAB sediment (37.5 d) and the minimum was 10.1 d in the BW sediment. Significant differences were seen in

dissipation across the sediment types ($F=5.8$ and $p<0.001$). In sterilised sediments, with the exception of the BGD sediment, an initial lag phase in the degradation of ranitidine was observed. Half-lives were found to be 1.6 (HLM sediment) to 3.1 (GER sediment) times greater than the non-sterilised treatments (supporting information Tables S5, S6) suggesting that biodegradation is the main dissipation mechanism. Half-lives ranging from 15 to 100 d for ranitidine have been reported in the literature for seawater (Benotti and Brownawell 2009). Ranitidine has also been characterized as not readily biodegradable in a Zahn–Wellens inherent biodegradability test (OECD 302 B) (Bergheim et al. 2012).









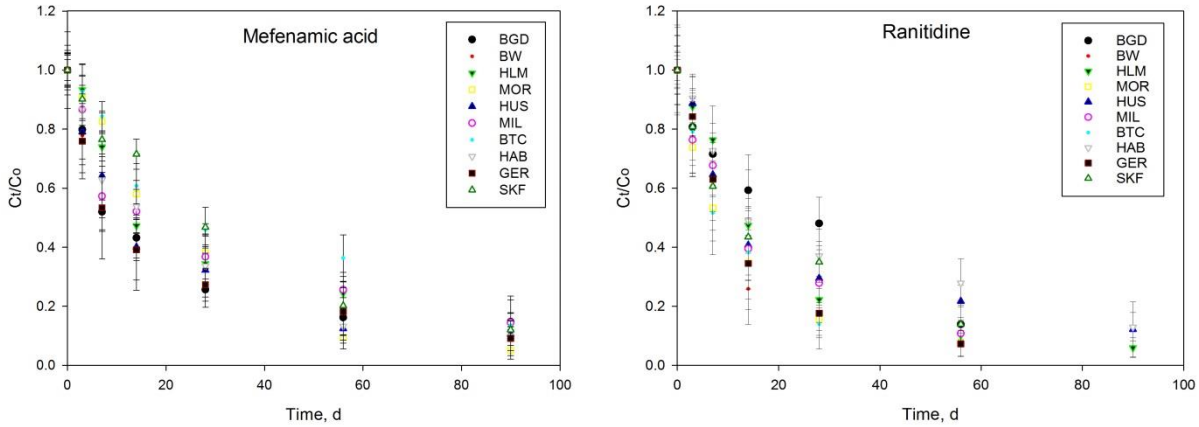
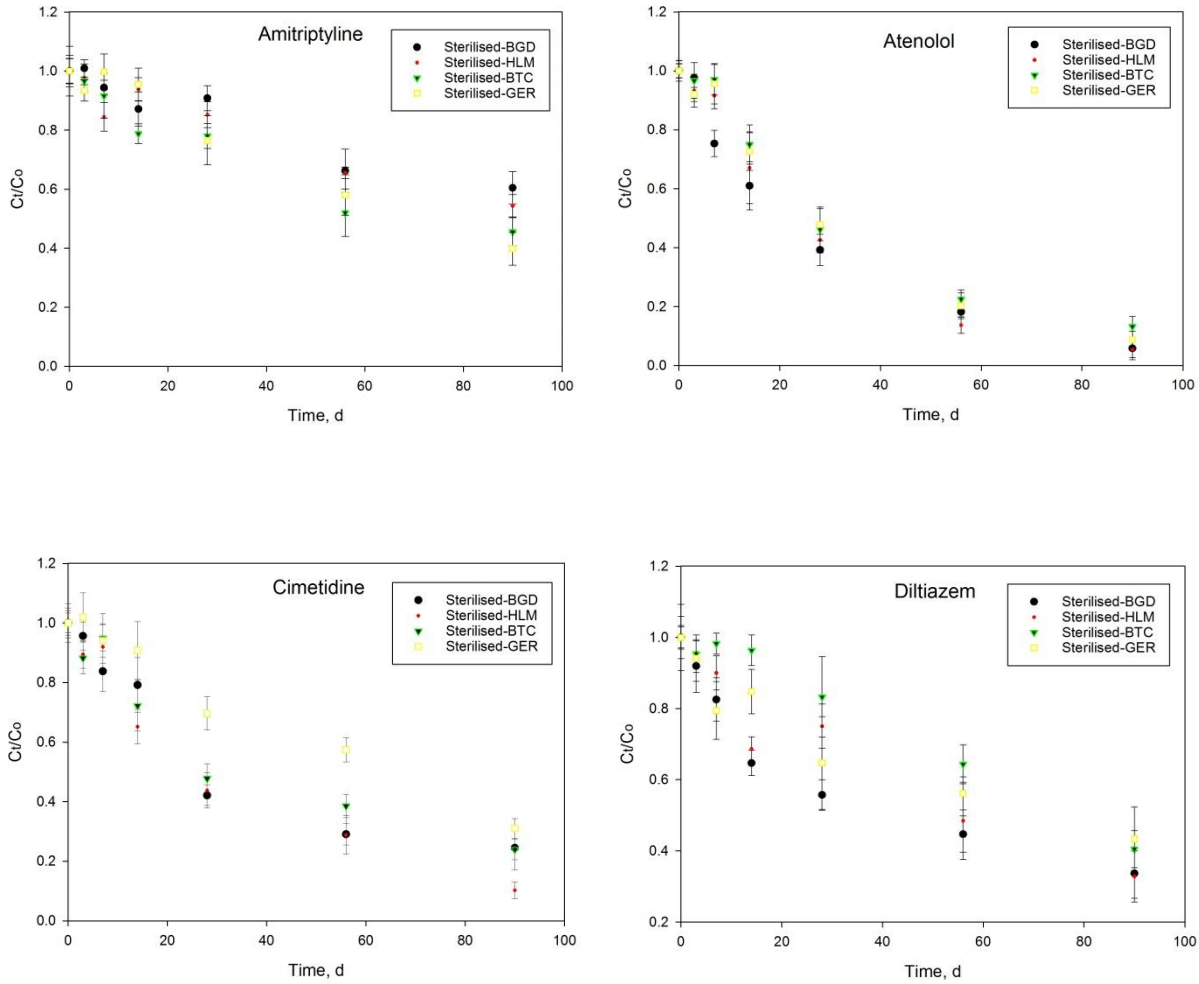


Figure 1 Mean concentration (±S.D.) for pharmaceuticals in the test sediments over time, corrected for day 0 concentrations, in ten non-sterilised sediments.



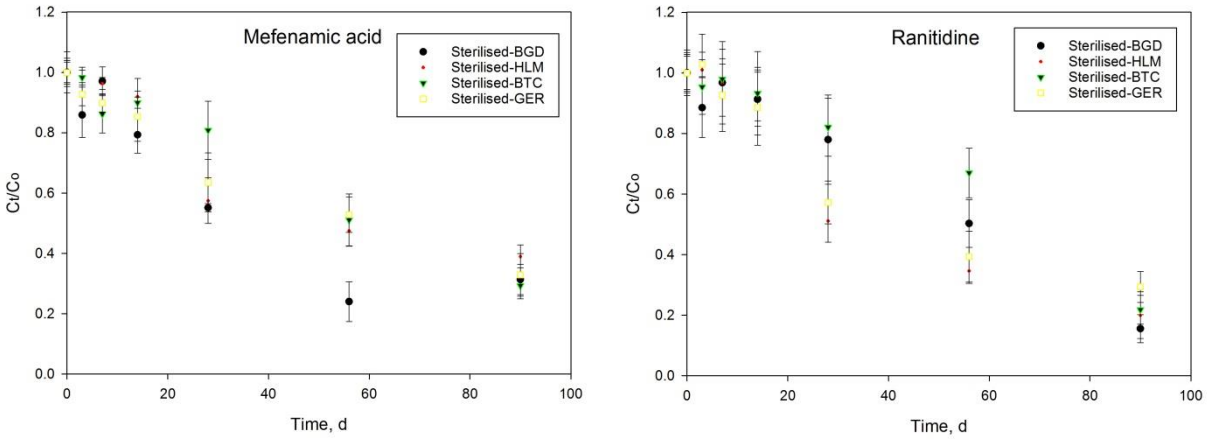


Figure 2 Mean concentrations (± S.D.) for pharmaceuticals over time, corrected for day 0 concentrations, in four sterilised sediments.

Multiple linear regression analysis

To better understand the drivers of the observed degradation of pharmaceuticals across sediments, relationships between sediment physico-chemical properties, microbial activity and adsorption coefficients and degradation rate were explored using multiple linear regression analysis. Lipophilicity of the pharmaceutical corrected for the sediment pH (Log Dow) was also included. Factors such as the OC content of the matrix, pH and the level of microbial activity have previously been shown to be important parameters determining degradation rates of ionisable compounds (Kah et al. 2007; Xu et al. 2009). The adsorption coefficient (Kd) was also included (obtained from Al-Khazrajy and Boxall (2016b) except ranitidine) since adsorption may modify the bioavailability of chemicals (Maqueda et al. 2009). Each pharmaceutical and sediment was considered individually. The best performing regression models for each study compound are shown in Table 3.

For diltiazem, the first proposed model only included clay % as the main variable describing degradation (R^2 =0.534; $p<0.05$). The inclusion of the sediment microbial activity (in log form) in the equation improved the fit (R^2 = 0.812; $p<0.01$; Table 3). This suggests that biodegradation is a key process in diltiazem dissipation in the tested sediments. The decreasing DT50 of diltiazem with increasing clay content is supported by findings of degradation studies of pesticides and pharmaceuticals in other

matrices like soil (Xu et al. 2009; Ghafoor et al. 2011; Wu et al. 2012). Silt % (R^2= 0.461, p<0.05) was selected as the only descriptor for cimetidine. The result observed in the present study for diltiazem and cimetidine regarding the involvement of clay and silt in the final regression models is expected since in our previous study (Al-Khazrajy and Boxall, 2016b) we found that the sorption affinity of the compounds is highly dependent on the log Dow (diltiazem) and OC% and clay% (cimetidine) so the identification of these parameters may be a reflection of the fact that they provide information on the bioavailability of the molecules to the microbes. For ranitidine the first descriptor chosen by the model was microbial activity (R^2= 0.631; p<0.01) but when OC% was included, the fit improved (R^2 of 0.869; p<0.001). These two descriptors are normally found to dominate the degradation of chemicals since microbial activity would be higher in an OC rich matrix (Villaverde et al. 2008; Maqueda et al. 2009). None of the sediment parameters was identified by the model to clearly describe the degradation of amitriptyline, atenolol and mefenamic acid. This may be explained by the fact that degradation of these molecules is driven by factors other than those evaluated in this study. For example, factors such as the diversity structure of the microbial communities in the different sediments and the chemistry of the sediment pore water could be important in determining rates of degradation of the molecules (Boxall and Ericson 2012).

Table 3 Multiple linear regression equations for predicting degradation rates of the study pharmaceuticals based on sediment properties. No relationships were obtained for amitriptyline, atenolol and mefenamic acid

Compound	Predictor	R^2	Multiple regression function
Diltiazem	Clay %Microbial activity	0.821**	k= 0.01+ 0.00017 (clay %)+ 0.005 log (microbial activity)
Ranitidine	Microbial activity OC %	0.869 **	k= 0.16 log (microbial activity)+ 0.02 (OC%)- 0.021
Cimetidine	Silt %	0.461 *	k= 0.022 + 0.00015 (silt %)

** p < 0.01, *p<0.05.

CONCLUSION

The study focused on primary degradation of the study compounds. Results showed some marked differences between the sediments in their ability to degrade different pharmaceuticals. The most persistent compound amongst the pharmaceuticals studied was amitriptyline while atenolol was found to degrade the most quickly. The present study also investigated the effects of a range of variables on the dissipation of targeted pharmaceuticals in environmental freshwater sediment. Results indicated that some pharmaceuticals are amenable to microbial degradation while for others, the dissipation was probably driven by abiotic processes or the formation of nonextractable residues. MLR demonstrated that degradation of pharmaceuticals in sediment is a very complex process and cannot be explained by a single mechanism due to different interactions between different processes that influence the breakdown of pharmaceuticals. Pharmaceuticals with similar structures may also behave differently as shown in the current study for the two antihistamines. Microbial degradation appeared to dominate the dissipation of diltiazem and ranitidine while abiotic breakdown was found to explain the breakdown of cimetidine. The factors governing the other pharmaceuticals in sediments were unclear. In the future, we recommend that work is done using a wider range of well characterized pharmaceuticals and sediments. Such work could lead to the development of new models that would allow the prediction of degradation of pharmaceuticals at high spatial resolutions. These models will be invaluable for better characterizing the environmental fate of pharmaceuticals in natural systems.

REFERENCES

Abia ALK, James C, Ubomba-Jaswa E, Momba MNB. 2017. Microbial remobilisation on riverbed sediment disturbance in experimental flumes and a human-impacted river: Implication for water resource management and public health in developing sub-saharan African countries. Int. J. Environ. Res. Public Health 14:1–18. doi:10.3390/ijerph14030306.

Al-Khazrajy OSA, Boxall AB. 2017. Determination of pharmaceuticals in freshwater sediments using ultrasonic-assisted extraction with SPE clean-up and HPLC–DAD or LC- ESI-MS/MS detection. Anal. Methods. doi:10.1039/C7AY00650K.

Al-Khazrajy OSA, Boxall ABA. 2016a. Risk-based prioritization of pharmaceuticals in the natural environment in Iraq. Environ. Sci. Pollut. Res. 23:15712–15726. doi:10.1007/s11356-016-6679-0.

Al-Khazrajy OSA, Boxall ABA. 2016b. Impacts of compound properties and sediment characteristics on the sorption behaviour of pharmaceuticals in aquatic systems. J. Hazard. Mater. 317:198–209. doi:10.1016/j.jhazmat.2016.05.065.

Araujo L, Villa N, Camargo N, Bustos M, García T, de Jesus Prieto a. 2011. Persistence of gemfibrozil, naproxen and mefenamic acid in natural waters. Environ. Chem. Lett. 9:13–18. doi:10.1007/s10311-009-0239-5.

Benotti MJ, Brownawell BJ. 2009. Microbial degradation of pharmaceuticals in estuarine and coastal seawater. Environ. Pollut. 157:994–1002. doi:10.1016/j.envpol.2008.10.009.

Bergheim M, Gieré R, Kümmerer K. 2012. Biodegradability and ecotoxicitiy of tramadol, ranitidine, and their photoderivatives in the aquatic environment. Environ. Sci. Pollut. Res. 19:72–85. doi:10.1007/s11356-011-0536-y.

Boxall A, Rudd M. 2012. Pharmaceuticals and personal care products in the environment: what are the big questions? Environ. Health Perspect. 9:1221–1229.

Boxall AB a, Sinclair CJ, Fenner K, Kolpin D, Maund SJ. 2004. When synthetic chemicals degrade in the environment. Environ. Sci. Technol. 38:368A–375A. doi:10.1021/es040624v.

Boxall ABA, Ericson JF. 2012. Environmental fate of human pharmaceuticals. In: Human Pharmaceuticals in the Environment. Springer. p. 63–83.

Buth JM, Arnold W a., Mcneill K. 2007. Unexpected products and reaction mechanisms of the aqueous chlorination of cimetidine. Environ. Sci. Technol. 41:6228–6233. doi:10.1021/es070606o.

Carballa M, Omil F, Ternes T, Lema JM. 2007. Fate of pharmaceutical and personal care products (PPCPs) during anaerobic digestion of sewage sludge. Water Res. 41:2139–2150. doi:10.1016/j.watres.2007.02.012.

Castiglioni S, Bagnati R, Calamari D, Fanelli R, Zuccato E. 2005. A multiresidue analytical method using solid-phase extraction and high-pressure liquid chromatography tandem mass spectrometry to measure pharmaceuticals of different therapeutic classes in urban wastewaters. J. Chromatogr. A 1092:206–215. doi:10.1016/j.chroma.2005.07.012.

Chen K, Zhou JL. 2014. Occurrence and behavior of antibiotics in water and sediments from the Huangpu River, Shanghai, China. Chemosphere 95:604–612. doi:10.1016/j.chemosphere.2013.09.119.

Christensen FM. 1998. Pharmaceuticals in the environment--a human risk? Regul. Toxicol. Pharmacol. 28:212–221. doi:10.1006/rtph.1998.1253.

Conkle JL, Gan J, Anderson M a. 2012. Degradation and sorption of commonly detected PPCPs in wetland sediments under aerobic and anaerobic conditions. J. Soils Sediments 12:1164–1173. doi:10.1007/s11368-012-0535-8.

Daughton CG, Ternes T a. 1999. Pharmaceuticals and personal care products in the environment: Agents of subtle change? Environ. Health Perspect. 107:907–938. doi:10.1289/ehp.99107s6907.

Emea. 2006. Guideline on the environmental risk assessment of medicinal products for human use. Comm. Med. Prod. Hum. Use. EMEA/CHMP/SWP/4447/00:1–12.

Fang Y, Karnjanapiboonwong A, Chase DA, Wang J, Morse AN, Anderson TA. 2012. Occurrence, fate, and persistence of gemfibrozil in water and soil. Environ. Toxicol. Chem. 31:550–555. doi:10.1002/etc.1725.

Ghafoor A, Jarvis NJ, Thierfelder T, Stenström J. 2011. Measurements and modeling of pesticide persistence in soil at the catchment scale. Sci. Total Environ. 409:1900–1908. doi:10.1016/j.scitotenv.2011.01.049.

Guo J, Sinclair CJ, Selby K, Boxall ABA. 2016. Toxicological and ecotoxicological risk-based prioritization of pharmaceuticals in the natural environment. Environ. Toxicol. Chem. 9999:n/a-n/a. doi:10.1002/etc.3319.

Joss A, Zabczynski S, Göbel A, Hoffmann B, Löffler D, McArdell CS, Ternes T a., Thomsen A, Siegrist H. 2006. Biological degradation of pharmaceuticals in municipal wastewater treatment: Proposing a classification scheme. Water Res. 40:1686–1696. doi:10.1016/j.watres.2006.02.014.

Kah M, Beulke S, Brown CD. 2007. Factors influencing degradation of pesticides in soil. J. Agric. Food Chem. 55:4487–4492. doi:10.1021/jf0635356.

Kodešová R, Kočárek M, Klement A, Golovko O, Koba O, Fér M, Nikodem A, Vondráčková L, Jakšík O, Grabic R. 2016. An analysis of the dissipation of pharmaceuticals under thirteen different soil conditions. Sci. Total Environ. 544:369–381. doi:10.1016/j.scitotenv.2015.11.085.

Kolpin DW, Kolpin DW, Furlong ET, Furlong ET, Meyer MT, Meyer MT. 2002. Environ. Sci. Technol. 2002, 36, 1202-1211. Environ. Sci. Technol. 36:1202–1211. doi:10.1021/es011055j.

Kümmerer K. 2009. The presence of pharmaceuticals in the environment due to human use - present knowledge and future challenges. J. Environ. Manage. 90:2354–2366. doi:10.1016/j.jenvman.2009.01.023.

Kunkel U, Radke M. 2008. Biodegradation of acidic pharmaceuticals in bed sediments: Insight from a laboratory experiment. Environ. Sci. Technol. 42:7273–7279. doi:10.1021/es801562j.

Latch DE, Packer J, Arnold WA, McNeill K. 2003. The photochemical fate of cimetidine and ranitidine hydrochloride. Prepr. Ext. Abstr. ACS Natl. Meet., Am. Chem. Soc., Div. Environ. Chem. 43:266–271.

Li B, Zhang T. 2010. Biodegradation and adsorption of antibiotics in the activated sludge process. Environ. Sci. Technol. 44:3468–3473. doi:10.1021/es903490h.

Li H, Sumarah MW, Topp E. 2013. Persistence of the tricyclic antidepressant drugs amitriptyline and nortriptyline in agriculture soils. Environ. Toxicol. Chem. 32:509–516. doi:10.1002/etc.2112.

Liang X, Chen B, Nie X, Shi Z, Huang X, Li X. 2013. The distribution and partitioning of common antibiotics in water and sediment of the Pearl River Estuary, South China. Chemosphere 92:1410–1416. doi:10.1016/j.chemosphere.2013.03.044.

Lin K, Bondarenko S, Gan J. 2011. Sorption and persistence of wastewater-borne psychoactive and antilipidemic drugs in soils. J. Soils Sediments 11:1363–1372. doi:10.1007/s11368-011-0421-9.

Loffler D, Rombke J, Meller M, Ternes T a. 2005. Environmental fate of pharmaceuticals in water/ sediment systems. Environ. Sci. Technol. 39:5209–5218.

Löffler D, Ternes T a. 2003. Determination of acidic pharmaceuticals, antibiotics and ivermectin in river sediment using liquid chromatography-tandem mass spectrometry. J. Chromatogr. A 1021:133–144.

doi:10.1016/j.chroma.2003.08.089.

Maqueda C, Villaverde J, Sope??a F, Undabeytia T, Morillo E. 2009. Effects of soil characteristics on metribuzin dissipation using clay-gel-based formulations. J. Agric. Food Chem. 57:3273–3278. doi:10.1021/jf803819q.

Mompelat S, Le Bot B, Thomas O. 2009. Occurrence and fate of pharmaceutical products and by-products, from resource to drinking water. Environ. Int. 35:803–814. doi:10.1016/j.envint.2008.10.008.

Monteiro SC, Boxall AB a. 2009. Factors affecting the degradation of pharmaceuticals in agricultural soils. Environ. Toxicol. Chem. 28:2546–2554. doi:10.1897/08-657.1.

Quintana JB, Weiss S, Reemtsma T. 2005. Pathways and metabolites of microbial degradation of selected acidic pharmaceutical and their occurrence in municipal wastewater treated by a membrane bioreactor. Water Res. 39:2654–2664. doi:10.1016/j.watres.2005.04.068.

Radjenović J, Petrović M, Barceló D. 2009. Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment. Water Res. 43:831–841. doi:10.1016/j.watres.2008.11.043.

Sarmah AK, Meyer MT, Boxall AB a. 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere 65:725–759. doi:10.1016/j.chemosphere.2006.03.026.

Shi H, Yang Y, Liu M, Yan C, Yue H, Zhou J. 2014. Occurrence and distribution of antibiotics in the surface sediments of the Yangtze Estuary and nearby coastal areas. Mar. Pollut. Bull. 83:317–323. doi:10.1016/j.marpolbul.2014.04.034.

Svan A, Hedeland M, Arvidsson T, Jasper JT, Sedlak DL, Pettersson CE. 2016. Identification of transformation products from β-blocking agents formed in wetland microcosms using LC-Q-ToF. J. Mass Spectrom. 51:207–218. doi:10.1002/jms.3737.

Thuy HTT, Loan TTC. 2014. Degradation of selected pharmaceuticals in coastal wetland water and sediments. Water. Air. Soil Pollut. 225. doi:10.1007/s11270-014-1940-y.

Topp E, Hendel JG, ZEXUN LU, Chapman R. 2006. Biodegradation of caffeine in agricultural soils. Can. J. soil Sci. 86:533–544.

Villaverde J, Kah M, Brown CD. 2008. Adsorption and degradation of four acidic herbicides in soils from southern Spain. Pest Manag. Sci. 64:703–710. doi:10.1002/ps.1545.

Wu C, Spongberg AL, Witter JD, Fang M, Czajkowski KP, Ames A. 2010. Dissipation and leaching potential of selected pharmaceutically active compounds in soils amended with biosolids. Arch. Environ. Contam. Toxicol. 59:343–351. doi:10.1007/s00244-010-9500-y.

Wu X, Li M, Long Y, Liu R, Yu Y, Fang H. 2012. Adsorption, mobility and degradation of diphenamid in chinese soils. KSCE J. Civ. Eng. 16:547–553. doi:10.1007/s12205-012-1393-z.

Xu J, Wu L, Chang AC. 2009. Degradation and adsorption of selected pharmaceuticals and personal care products (PPCPs) in agricultural soils. Chemosphere 77:1299–1305. doi:10.1016/j.chemosphere.2009.09.063.

Yamamoto H, Nakamura Y, Moriguchi S, Nakamura Y, Honda Y, Tamura I, Hirata Y, Hayashi A, Sekizawa J. 2009. Persistence and partitioning of eight selected pharmaceuticals in the aquatic environment: Laboratory photolysis, biodegradation, and sorption experiments. Water Res. 43:351–362. doi:10.1016/j.watres.2008.10.039.

Yan C, Yang Y, Zhou J, Liu M, Nie M, Shi H, Gu L. 2013. Antibiotics in the surface water of the Yangtze Estuary: Occurrence, distribution and risk assessment. Environ. Pollut. 175:22–29. doi:10.1016/j.envpol.2012.12.008.

Ying GG, Kookana RS. 2003. Degradation of five selected endocrine-disrupting chemicals in seawater and marine sediment. Environ. Sci. Technol. 37:1256–1260. doi:10.1021/es0262232.

Zuccato E, Calamari D, Natangelo M, Fanelli R. 2000. Presence of therapeutic drugs in the environment. Lancet 355:1789–1790. doi:10.1016/S0140-6736(00)02270-4.