Photodegradation of pharmaceutical antibiotics on slurry and soil surfaces[1]

Sören Thiele-Bruhn[2,3] and Dirk Peters[2]

Abstract

Pharmaceutical antibiotics are emerging soil environmental contaminants while knowledge about their fate and degradation is insufficient. Thus, the dissipation of selected antibiotics and its kinetics through photodegradation and other abiotic processes on substrate surfaces was investigated. Oxytetracycline, chlorotetracycline, sulfanilamide, sulfadimidine, sulfadiazine, sulfadimethoxine, sulfapyridine, fenbendazole, and *p*-aminobenzoic acid were either spread on sterilized layers (≤ 0.8 mm) of quartz sand, two topsoil samples, and pig slurry or dissolved in water. Samples were either irradiated using arc light or, in parallel, kept in the dark for 28 d. All antibiotics were directly photodegraded in water with first order rate coefficients (k_p) from 0.005 to 0.12 d^{-1}. Without irradiation, the proportions recovered after incubation from soils, sand and slurry ranged from 98 % to a complete decrease. Photodegradation further decreased the recovered concentration by a median factor of 2. However, for the sulfonamides and fenbendazole average k_p in soils and sand was 0.01 d^{-1}, and thus 2.4 times smaller compared to water. Tetracycline photodegradation followed biphasic kinetics indicating two fractions of different photodegradability. Enhanced photodegradation of the first fraction ($k_{p,1}$ 0.19 - 1.43 d^{-1}) was attributed to additional indirect photodegradation processes. The second fraction was practically unavailable for photodegradation ($k_{p,2} \leq 0.0003$ d^{-1}); between 55 to 94 % of the initial tetracycline concentration were not photodegraded. In pig slurry, photodegradation of most antibiotics was considerably increased with k_p of 0.10 to 3.33 d^{-1} and went along with a photobleaching of the slurry itself.

Keywords: abiotic dissipation, kinetics, pig slurry, fenbendazole, sulfonamides, tetracyclines

Zusammenfassung

Photodegradation von pharmazeutischen Antibiotika auf Gülle- und Bodenoberflächen

Pharmazeutische Antibiotika wurden als Umweltschadstoffe in Böden neu erkannt. Die Kenntnisse über das Verhalten und den Abbau dieser Substanzen sind jedoch noch unzureichend. Daher wurden die Photodegradation ausgewählter Antibiotika und ihre Kinetik sowie weitere abiotische Abnahmeprozesse auf Substratoberflächen untersucht. Oxytetracyclin, Chlortetracyclin, Sulfanilamid, Sulfadimidin, Sulfadiazin, Sulfadimethoxin, Sulfapyridin, Fenbendazol und *p*-Aminobenzoesäure wurden entweder auf sterilisierte Schichten (≤ 0,8 mm) von Quarz-Sand, zwei Oberbodenproben und Schweinegülle aufgesprüht oder in Wasser gelöst. Die Proben wurden über 28 d mit einer Bogenlichtlampe beleuchtet bzw. parallel im Dunkeln gelagert. Alle Antibiotika wurden in Wasser durch direkte Photodegradation mit Ratenkoeffizienten erster Ordnung (k_p) zwischen 0,005 und 0,12 d^{-1} abgebaut. In Böden, Sand und Gülle variierten die nachweisbaren Anteile der Antibiotika von 98 % bis zu einer vollständigen Abnahme. Durch Photodegradation wurden die nachweisbaren Konzentrationen zusätzlich vermindert; die Abnahme war im Median um den Faktor 2 stärker. In den Böden und im Sand lag k_p jedoch für die Sulfonamide und Fenbendazol im Mittel bei 0,01 d^{-1} und war damit 2,4 mal kleiner als in Wasser. Die Photodegradation der Tetracycline folgte einer zweiphasigen Kinetik, was auf zwei Fraktionen unterschiedlicher Abbaubarkeit durch Licht hindeutet. Eine verstärkte Photodegradation der ersten Fraktion ($k_{p,1}$ 0,19 - 1,43 d^{-1}) wurde durch zusätzliche indirekte Photodegradations-Prozesse erklärt. Die zweite Fraktion war einer Photodegradation praktisch unzugänglich ($k_{p,2} \leq 0{,}0003$ d^{-1}); zwischen 55 und 94 % der anfänglichen Tetracyclin-Konzentration wurde nicht durch Licht abgebaut. In Schweinegülle war die Photodegradation der meisten Antibiotika mit k_p zwischen 0,10 und 3,33 d^{-1} wesentlich verstärkt und ging mit einer Photobleichung der Gülle einher.

Schlüsselworte: Abiotische Abnahme, Kinetik, Schweinegülle, Fenbendazol, Sulfonamide, Tetracycline

---

1 Contribution to the round table „Antibiotics in manures“ organized by the Institute of Plant Nutrition and Soil Science of FAL on December 8, 2005

2 University of Rostock, Institute for Land Use/Soil Science, Justus-von-Liebig-Weg 6, 18051 Rostock/Germany

3 University of Trier, Soil Science, FB VI Environmental Sciences, 54286 Trier/Germany; Email: soeren.thiele-bruhn@uni-trier.de

1 Introduction

Pharmaceutical antibiotics from human and veterinary medicinal use have received increasing attention as soil environmental contaminants. Pharmaceuticals are excreted from the treated body and subsequently reach agricultural soils by fertilization with contaminated manure and sewage sludge (Jørgensen and Halling-Sørensen, 2000; Hirsch et al., 1999), where they might provoke adverse effects (Thiele-Bruhn 2003). It has been estimated that amounts of up to kilograms of pharmaceutical antibiotics per hectare may annually enter agricultural soils and a concentration level similar to that of pesticides is nearly reached (van Gool 1993; Winckler and Grafe, 2001). Accordingly, pharmaceutical antibiotics have been determined in soils, groundwater, and drainage water (Boxall et al., 2002; Campagnolo et al., 2002; Hamscher et al., 2002; Kolpin et al., 2002). Some antibiotics were shown to persist in soil (Hamscher et al., 2002; Gavalchin and Katz, 1994; Winckler and Grafe, 2001) while knowledge about the mechanisms of their degradation is insufficient.

Among different processes, photodegradation possibly contributes to the decomposition of antibiotics on soil surfaces. Organic fertilizers are often spread on soil surfaces, and thus the antibiotics therein are exposed to sunlight and may photodegrade. Substances that are photodegradable, water-soluble and non-volatile are especially susceptible to photodegradation on soil surfaces (Miller and Donaldson, 1994). These three properties are found for most antibiotics (Thiele-Bruhn, 2003). Many antibiotic compounds such as tetracyclines, sulfonamides, and fluoroquinolones have been shown to be photodegradable in liquids (Oka et al., 1989; Lunestad et al., 1995; Burhenne et al., 1997; Halling-Sørensen et al., 2003; Boreen et al., 2004; Boreen et al., 2005). However, information about the photodegradation of xenobiotics on soil surfaces in general, and especially about the effects of photodegradation on pharmaceutical antibiotics, is scarce. A first study on sulfadiazine photodegradation using air-dry soil dust samples (< 0.1 mm) was recently published (Wolters and Steffens, 2005).

Therefore, our aim was to investigate the photodegradation of selected, widely administered pharmaceutical antibiotics on surfaces of different substrates. Specific objectives were a) to quantify photodegradation in thin layers of field moist soils, quartz sand and pig slurry and to determine the kinetics of photodegradation on these substrates, b) to relate soil photodegradation results to those obtained in pure water as a standard of comparison, and c) to separate photodegradation from other abiotic dissipation processes in substrates. To allow for the unequivocal quantification of photodegradation and of other abiotic processes, biodegradative activity was explicitly excluded by sterilization of the substrates.

2 Materials and methods

2.1 Substrates

Samples from two long-term unfertilized topsoils of a sandy Cambisol (Rostock, Germany) and a loess Chernozem (Bad Lauchstädt, Germany), typical for agricultural soils in Central Europe, pure quartz sand (Merck, Darmstadt, Germany), and pig slurry from fattening pigs (agricultural experimental station, University of Rostock, Germany) were used as substrates. Selected general properties of the substrates are listed in Table 1. All substrates were analyzed to be free from background concentrations of antibiotics (data not shown). Substrates were air-dried or in the case of pig slurry freeze-dried and homogenized.

2.2 Chemicals

Standard substances of the tetracyclines chlorotetracycline (CTC) and oxytetracycline (OTC), the sulfonamides sulfanilamide (SAA), sulfadimidine (sulfamethazine; SDM), sulfadiazine (SDZ), sulfadimethoxine (SDT), sulfapyridine (SPY), *p*-aminobenzoic acid (*p*ABA), and the benzimidazole fenbendazole (FBZ) with a purity of > 99 %

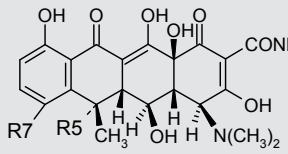
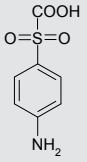
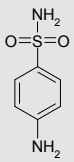
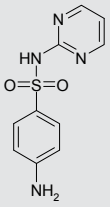
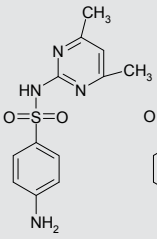
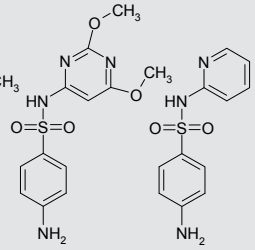
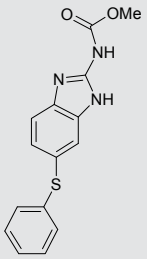
Table 1:
General properties of the selected substrates

Substrate	pH	OC [a]	C/N	Texture	Clay	$Fe_{oxalate}$	$Mn_{oxalate}$	$Al_{oxalate}$
		[g kg^{-1}]	ratio	class		[g kg^{-1}]		
Pig slurry	4.8 [b]	17.5 [b]	12.6 [b]		6.0 [b]	0.18 [c]	0.03 [c]	0.03 [c]
Quartz sand	6.0 [d]	< 0.5		sand		< 0.001	< 0.001	< 0.001
Sandy Cambisol	6.6 [d]	7.9	10.6	loamy sand	31	1.25	0.05	0.72
Loess Chernozem	7.0 [d]	16.1	10.3	silt loam	231	1.27	0.31	1.13

[a] Organic carbon; [b] Determined in the pig slurry with 4.0 % dry matter content;
[c] Average from data reported for animal manure by Japenga and Harmsen (199); [d] Determined in 0.01 M $CaCl_2$ solution

Table 2:

Selected physicochemical properties (from literature cited in Thiele-Bruhn (2003)) and molecular structures of the pharmaceutical antibiotics, and detection limits of the analytical method

	CTC	OTC	*p*ABA	SAA	SDM	SDZ	SDT	SPY	FBZ
CAS-No	57-62-5	79-57-2	150-13-0	63-74-1	57-68-1	68-35-9	122-11-2	144-83-2	43210-67-9
MW [a]	478.9	460.4	137.1	172.2	278.3	250.3	310.3	249.3	299.4
pK_a [b]	3.3/7.8/9.3	3.3/7.3/9.1	2.4/4.9	1.9/10.6	2.8/7.6	1.6/6.4	2.4/6.0	2.9/8.4	5.5/10.3
log K_{ow} [b]	-0.62	-0.90	0.83	-0.62	0.89	-0.09	1.63	0.35	3.85
S [c]	630	310	6110	7500	278	77	343	249	> 10
LOD [d]	20	15	18	50	10	10	10	9	50
LOQ [d]	5	3	5	10	2	2	2	2	5
molecular structure	 R5: H R7: Cl R5: OH R7: H								

[a] Molecular weight [g mol^{-1}]; [b] At 25 °C
[c] Water solubility at 25 °C [mg L^{-1}]; [d] Limit of detection and limit of quantification by HPLC-DAD [µg L^{-1}]

Table 3:

Spiked concentrations [µg g^{-1}] and proportions thereof [%] recovered after 1 h and 28 d in samples with and without irradiation by a halide arc lamp, respectively; mean values of 2 - 4 repetitions and maximum deviations therefrom

	CTC	OTC	*p*ABA	SAA	SDM	SDZ	SDT	SPY	FBZ
	Spiked concentration [µg g^{-1}]								
	500	500	500	500	250	500	500	250	100
	1 h incubation [%]								
Pig slurry	54 ± 9.4	72 ± 12	51 ± 0.6	79 ± 0.8	82 ± 1.0	73 ± 0.3	75 ± 0.3	82 ± 0.5	
Quartz sand	58 ± 4.0		72 ± 7.7					79 ± 5.2	60 ± 17
Cambisol	66 ± 2.9	101 ± 1.2	70 ± 0.1	91 ± 4.4	91 ± 1.2	90 ± 0.9	78 ± 2.3	88 ± 4.3	
Chernozem	19 ± 5.6		17 ± 1.3					61 ± 3.9	77 ± 5.1
	28 d incubation of non-irradiated samples [%]								
Pig slurry	50 ± 0.5	53 ± 0.9	2 ± 0.3	43 ± 0.2	48 ± 1.2	50 ± 0.6	49 ± 0.1	69 ± 1.0	
Quartz sand	22 ± 2.3		83 ± 3.9					70 ± 17	59 ± 8.7
Cambisol	5 ± 2.1	18 ± 2.3	< LOD[a]	91 ± 2.8	91 ± 2.7	98 ± 2.5	84 ± 7.9	92 ± 9.1	
Chernozem	10 ± 2.3		21 ± 2.2					69 ± 3.7	77 ± 5.2
	28 d incubation of irradiated samples [%]								
Pig slurry	3 ± 0.1	7 ± 0.2	3 ± 0.3	8 ± 0.9	20 ± 0.7	9 ± 0.5	5 ± 0.1	15 ± 0.2	
Quartz sand	0.3 ± 0.5		27 ± 2.7					39 ± 12	57 ± 5.9
Cambisol	0.5 ± 0.2	5 ± 0.6	< LOD	46 ± 3.0	65 ± 9.1	84 ± 1.0	54 ± 6.7	64 ± 5.7	
Chernozem	3 ± 0.9		10 ± 3.4					50 ± 9.7	62 ± 6.9

[a] <LOD = concentration below limit of detection

were obtained from Riedel de Haën and Sigma-Aldrich (Taufkirchen, Germany). Standard solutions were prepared in methanol. All solvents used were of HPLC grade and obtained from Riedel de Haën (Taufkirchen, Germany). The molecular structures and physicochemical properties of the antibiotics are shown in Table 2.

2.3 Sample preparation and incubation

To prepare substrate layers, 5 g soil and quartz sand and 0.29 g freeze-dried pig slurry, respectively, were uniformly distributed in glass petri dishes (inner diameter 9 cm). Substrates were moistened with autoclaved water (HPLC grade, Roth, Karlsruhe, Germany) to 60 % of their water holding capacity while the freeze-dried pig slurry was readjusted to the original water content (96 %). Water content was controlled throughout the incubation time. The resulting layers had a thickness of 0.5 mm for soils, corresponding to the thickness of the photic zone outlined by Miller and Donaldson (1994), and a thickness of 0.8 mm for pig slurry. All substrate layers were sterilized using hard UV radiation (254 nm) for 2 h prior to spiking with antibiotics in order to exclude microbial transformation. The effectiveness of the sterilization was proven by determining the microbial activity in the samples using the dehydrogenase activity test from Thalmann, cited in Schinner et al. (1993). During incubation, the substrates exhibited no dehydrogenase activity (data not shown). In preliminary experiments it was demonstrated that UV-sterilization did not alter the soil sorption of the antibiotics (Thiele-Bruhn et al., 2004a).

A 2-mL volume of antibiotic standard solution in methanol was evenly sprayed onto the substrate layers. For complete evaporation of methanol, the samples were subsequently exposed to an air flow for 30 minutes. The resulting spiked concentrations are listed in Table 3 and were selected to ensure detectable concentrations over the whole incubation time. In total, 24 different combinations of antibiotics and substrates were investigated. The petri dishes were covered with polystyrene lids and sealed to inhibit water evaporation. All samples were prepared in duplicate. Eight selected samples were investigated in four repetitions to ensure experimental reproducibility. The coefficients of variance were 2.7 % and less for antibiotic concentrations of quadruplicate repeated samples.

The samples were incubated at 25 °C, measured at the substrate surface, for up to 28 d. They were either irradiated or, in parallel, stored in the dark. For irradiation a metal halide arc lamp (HPI-T 400W, Philips, Hamburg, Germany) was used for 22 h per day. The effective light spectrum at the substrate surface under the polystyrene lid was determined using an optical spectrum analyzer with photodiode (Spectro 320 D, Instrument Systems, Munich, Germany). The relative spectral intensity (%) of the arc light in the photodegradative range of 290 to 400 nm is shown in Figure 1. Light absorption spectra of all investigated antibiotics were determined and representative spectra are depicted in Figure 1. Similar to the spectrum of sunlight on earth at sea level, the spectral intensity of arc light declined at lower wavelengths. At a wavelength < 320 nm the light flux was very small and declined to zero at < 290 nm irrespective of the use of polystyrene lids. Polystyrene is known to have

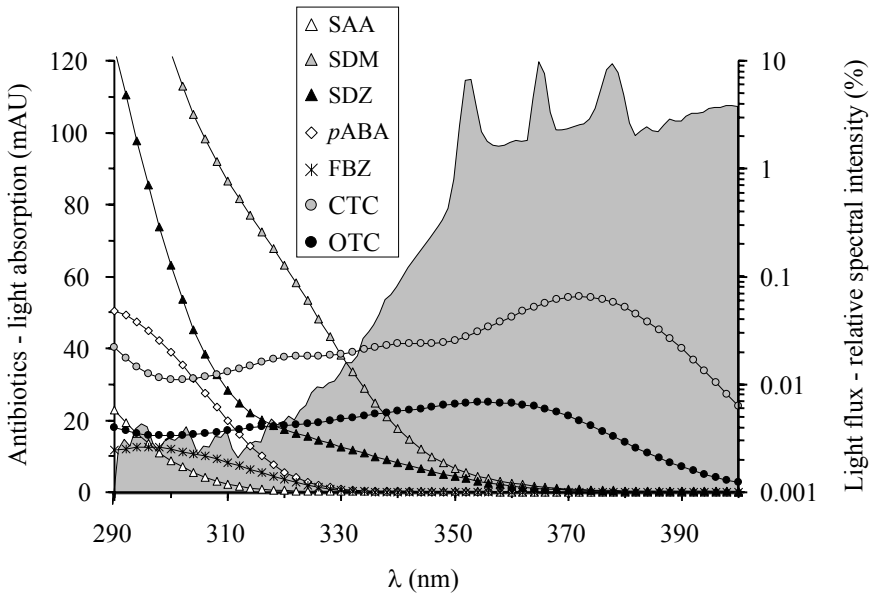


Figure 1:

Absorption spectra of selected pharmaceutical antibiotics and effective light spectrum of the metal halide arc lamp used for the irradiation of the samples (shaded area)

high absorbance for UVB light (290 - 315 nm). The total light transmission through the polystyrene lids amounted to 84 % of the irradiated light flux.

To investigate the antibiotic photodegradation in aqueous solution, the standard substances were dissolved at 100 µg mL^{-1}, SDZ at 50 µg mL^{-1}, and FBZ at 10 µg mL^{-1} in HPLC grade water. Irradiation was carried out for 14 d, using the above-mentioned experimental set-up and arc light. In parallel, aqueous standard solutions in quartz glass round flasks were exposed to the natural sunlight. The latter was done in Rostock, Germany (54° latitude) on clear days between March 22 and April 12 from 9:00 am to 5:00 pm, with the samples thermostated at 20 °C. The aqueous solutions were directly analyzed for the residual concentrations of antibiotics.

2.4 Extraction and determination of antibiotics

Substrate samples were incubated for 1 h and 1, 3, 7, 14, and 28 d, respectively, subsequently air-dried at 35 °C for 2 h and homogenized. From 2 g soil and 0.25 g dry matter of pig slurry, respectively, tetracyclines were extracted according to the modified total extraction method of Hamscher et al., (2002) using citric ethylacetate and ultra-sonication. Total extraction of sulfonamides, *p*ABA, and FBZ was done using methanol (Thiele, 2000). After centrifugation, aliquots of the supernatants were evaporated and redissolved in 1 mL of methanol. Detection was done with a Hewlett Packard 1050 HPLC system (Palo Alto, CA), equipped with a diode array detector (Agilent 1100, Böblingen, Germany) operated at 265 nm. A C18 reversed phase, 250 × 4.6 mm, 100 - 5 column (Macherey-Nagel, Düren, Germany) served as the stationary phase while methanol and 0.01 M H_3PO_4 were delivered in a gradient program at 1 mL min^{-1} as the mobile phase. All compounds were quantified using external standard mixtures. Detection limits are listed in Table 2.

2.5 Data analysis

Kinetics of the decrease in antibiotic concentration over the time course was mathematically described. To this end, single and coupled first order rate equations were applied using the best-fit method and the CFIT software for non-linear regression (Helfrich, 1996):

$$C = C_0 \times \exp(-k \times t) \quad (1)$$

$$C = C_{0,1} \times \exp(-k_1 \times t) + C_{0,2} \times \exp(-k_2 \times t) \quad (2)$$

The concentration C [µmol g^{-1}] is recovered after the incubation time t [d] and the initial concentration C_0 [µmol g^{-1}] is degraded at the rate coefficient k [d^{-1}]. In the biphasic model (Eq. [2]), two different fractions ($C_{0,1}$; $C_{0,2}$) of fast (k_1) and slow (k_2) concentration decrease are distinguished. In various cases, no decrease of the second fraction ($C_{0,2}$) was observed and the rate coefficient was zero. Thus, Eq. 2 simplified to

$$C = C_{0,1} \times \exp(-k_1 \times t) + C_{0,2} \quad (3)$$

For the sterile samples incubated in the dark, rate coefficients of abiotic decrease (k_a) were calculated. To differentiate the contribution of photodegradation quantitatively from that of the other abiotic processes, data determined from irradiated samples were pair-wise corrected by subtracting the corresponding data obtained from non-irradiated samples. Resulting differences between the concentrations of antibiotics in non-irradiated and irradiated samples were attributed to photodegradation. The calculated photodegradation rate coefficients (k_p) were also defined by the single and coupled first order rate equations (Eq. 1, 2 and 3).

3 Results and discussion

3.1 Abiotic dissipation of antibiotics in non-irradiated substrates

In the different non-irradiated substrates, a decrease of the total extractable concentration of all antibiotics was determined. Although abiotic dissipation proceeded over several days in the solid substrates, i.e. quartz sand and the topsoil samples, most of the decrease in extractable concentrations was complete after 1 h. The recovered fraction ranged from 17 to 101 % of the spiked concentration (Table 3). In the two topsoil samples, the extent of the initial decrease was stronger in Chernozem compared to Cambisol, the former exhibiting a larger sorptive capacity from soil organic matter, clay, and pedogenic oxides (Table 1). Furthermore, pH affects the speciation, and thus the soil sorption of amphoteric antibiotics (Boxall et al., 2002; Thiele-Bruhn et al., 2004a) as they were investigated in this study. However, the antibiotic speciation was not substantially different among the solid substrates with pH ranging from 6 to 7 (Table 1) but could in part explain the small extractable concentrations of the tested antibiotics in pig slurry (pH 4.8). Yet, the small number of investigated substrates did not allow for statistical testing.

The fractions of the tetracyclines and sulfonamides that were extractable after 1 h corresponded to the recovery rates of 38 to 69 % for tetracyclines (Hamscher et al., 2002) and of 90 to 103 % for sulfonamides (Thiele-Bruhn et al., 2004a), which were determined for moist soil samples. Thus, the results from extraction after 1 h might be termed as the recovery rate of the extraction method and these fractions were not considered for the mathematical

Table 4:

Rate coefficients [d^{-1}] of the abiotic decrease of pharmaceutical antibiotics in different substrates following single (k_a) or coupled first order kinetics ($k_{a,1}$/$k_{a,2}$), reducing one (C_0) or two ($C_{0,1}$/$C_{0,2}$) different fractions [%] of the concentration recovered after 1 h; determination coefficient (R^2) and standard deviation (*SD*) of the curve fit; level of significance $p < 0.01$

		Pig	Solid substrates		
		slurry	Quartz sand	Cambisol	Chernozem
CTC	k_a; $k_{a,1}$/$k_{a,2}$	0.003	0.04	10/0.01	0.14/0.01
	C_0; $C_{0,1}$/$C_{0,2}$	99	98	86/14	36/64
	R^2; *SD*	0.13[a]; 4.4	0.98; 9.14	0.98; 18.6	0.85; 8.76
OTC	k_a; $k_{a,1}$/$k_{a,2}$	0.002		23/0.002	
	C_0; $C_{0,1}$/$C_{0,2}$	106		77/23	
	R^2; *SD*	0.01[a]; 47.7		0.98; 1.6	
*p*ABA	k_a; $k_{a,1}$/$k_{a,2}$	0.28	no	24/0.26	no
	C_0; $C_{0,1}$/$C_{0,2}$	99	decrease[c]	63/37	decrease
	R^2; *SD*	0.98; 10.3		0.93; 21.5	
SAA	k_a; $k_{a,1}$/$k_{a,2}$	0.02		no	
	C_0; $C_{0,1}$/$C_{0,2}$	96		decrease	
	R^2; *SD*	0.95; 14.4			
SDM	k_a; $k_{a,1}$/$k_{a,2}$	0.89/ 0		no	
	C_0; $C_{0,1}$/$C_{0,2}$	50/50		decrease	
	R^2; *SD*	0.69; 111			
SDZ	k_a; $k_{a,1}$/$k_{a,2}$	0.01		no	
	C_0; $C_{0,1}$/$C_{0,2}$	92		decrease	
	R^2; *SD*	0.47[b]; 33.4			
SDT	k_a; $k_{a,1}$/$k_{a,2}$	21/0.01		no	
	C_0; $C_{0,1}$/$C_{0,2}$	5.9/94		decrease	
	R^2; *SD*	0.72; 27.7			
SPY	k_a; $k_{a,1}$/$k_{a,2}$	21/0.003	0.005	no	no
	C_0; $C_{0,1}$/$C_{0,2}$	6.1/94	104	decrease	decrease
	R^2; *SD*	0.52[b]; 11.1	0.62[b]; 18.2		
FBZ	k_a; $k_{a,1}$/$k_{a,2}$		no		no
	C_0; $C_{0,1}$/$C_{0,2}$		decrease		decrease
	R^2; *SD*				

[a] curve-fit not significant; [b] level of significance $p < 0.05$
[c] no decrease = no significant decrease in concentration from 1 h to 28 d of incubation

modeling of the degradation kinetics. To this end, not the spiked concentration (C_{sp}) but the concentration recovered after 1 h was set as the initial concentration (C_0; Table 4 and Table 5).

During the 28-d incubation, following the first hour, no significant overall decrease in concentration was determined for FBZ, *p*ABA and the sulfonamides in most solid substrates. Between 21 and 98 % of the spiked concentration of these antibiotics was recovered after 28 d (Table 3), which corresponded on average (minimum, maximum in parentheses) to 105 % (89 - 124 %) of the concentration recovered after 1 h. Only the reduction of SPY in quartz sand and of *p*ABA in Cambisol to 70 % and below the limit of detection, respectively, was significant (Table 3), while the concentration of the tetracyclines significantly decreased in all non-irradiated substrates (Table 3). The decrease of CTC and SPY in quartz sand followed single first order kinetics (k_a 0.04 and 0.005 d 1, Table 4). Coupled processes were determined for the two tetracyclines and *p*ABA in the topsoil samples with proportions of 36 to 88 % decreasing

at larger rate coefficients ($k_{a,1}$ 0.14 - 24 d^{-1}; Table 4). In pig slurry, though, the decrease in concentration was very pronounced for most antibiotics and the residual antibiotic fraction ranged from 2 to 69 % of the initial spiking level (Table 3). Calculated rate coefficients ranged from 0.002 (k_a) to 21 ($k_{a,1}$) plus 0.01 ($k_{a,2}$) [d^{-1}]. Except for the tetracyclines, this decrease was significant at $p < 0.05$.

Non photo-induced abiotic dissipation processes of the antibiotics were not further characterized in this study. In the literature comparable findings were assigned to a) complexation of tetracyclines and specific adsorption (Wessels et al., 1998; Thiele-Bruhn, 2003), b) a slow diffusion into and sequestration in pores and interlayers of aggregates and particles (Pinck et al., 1962; Nowara et al., 1997), c) chemical degradation e.g., through hydrolysis (Halling-Sørensen 2000), and d) the formation of non-extractable residues (Kreuzig and Höltge, 2005), while volatilization can be neglected (Wolters and Steffens, 2005). From the presented results it is deduced that processes a) through d) might have been especially relevant in pig slurry.

3.2 Photodegradation of antibiotics in water

In pure water, a monotonic decrease in the concentration of all antibiotics was observed during irradiation. In contrast, no degradation was determined under light exclusion. When glass-vials with aqueous solutions were wrapped in aluminum-foil and exposed to sunlight, recovery rates of the antibiotics ranged from 90 to 105 % (data not shown). For both sunlight and arc light, kinetics of direct photolysis was best-fit using a single first order equation (Eq. 1). Determination coefficients (R^2) ranged from 0.57 to 0.99 and the standard deviation of the curve-fit ranged from 0.49 to 9.48 (Table 5). Rate coefficients of photodegradation by sunlight ranged from 0.02 to 0.11 d^{-1}. They were about the same for CTC, *p*ABA, SDT, SPY, and FBZ when arc light was used (Table 5). Arc light thus resulted in photodegradation similar to that under sunlight conditions found in Central Europe at the start of the vegetation period, which is, along with midsummer, a peak season of manure field application. However, rate coefficients were considerably larger for OTC (factor 1.7) and smaller for SDM, SDZ (factor 3.3) and SAA (factor 8.0). Boreen et al. (2005) investigated the sunlight photodegradation of sulfonamides in deionized water and reported rate coefficients that ranged for SDM, SDZ, and SDT from 0.48 to 0.78 [d^{-1}]. Recalculating their data obtained in May and June at 45° latitude to the conditions found in late March at 54° (our study; data not shown) gave a conformance within a factor of 2 (SDT) to 7 (SDZ). The differences between arc light and sunlight did not follow the overlap of the spectral light intensity of the halide arc lamp and the antibiotic light absorption (see

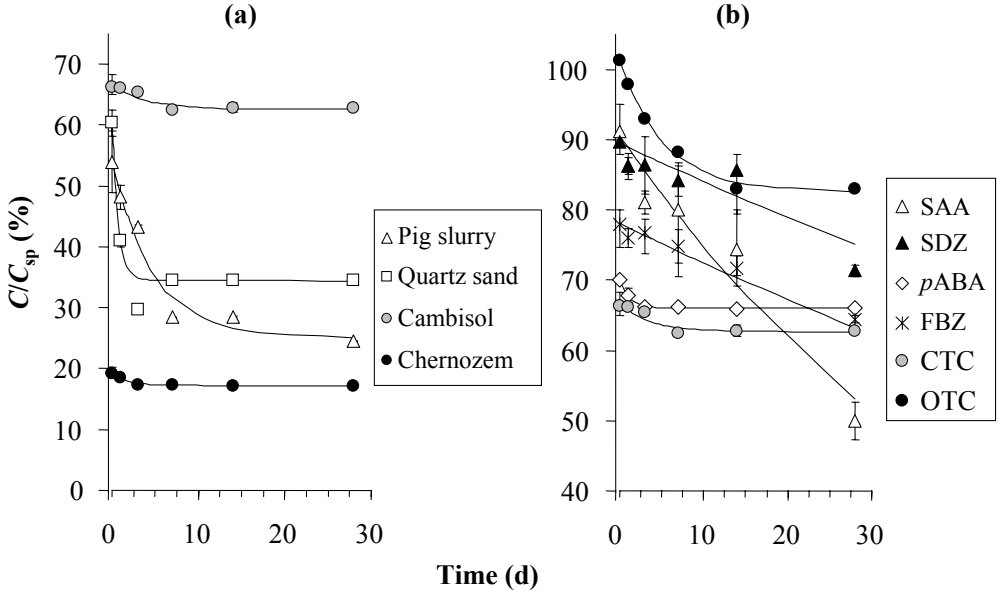


Figure 2:

Determined (symbols) and fitted (lines) proportions of the spiked concentration (C_{sp}) decreases due to photodegradation of a) chlorotetracycline in four different, irradiated, sterile substrates and b) of selected pharmaceutical antibiotics in an irradiated, sterile topsoil sample of a sandy Cambisol; FBZ was spiked to a topsoil of a loess Chernozem. Parameters of the curve-fit are listed in Table 5. Error bars of maximum and minimum values not shown are smaller than symbols

Table 5:

Rate coefficients [d^{-1}] of the photodegradation of pharmaceutical antibiotics in different substrates following single (k_p) or coupled first order kinetics ($k_{p,1}/k_{p,2}$), reducing one (C_0) or two ($C_{0,1}/C_{0,2}$) different fractions [%] of the concentration recovered after 1 h; determination coefficient (R^2) and standard deviation (SD) of the curve fit; level of significance $p < 0.01$

		Water		Pig	Solid substrates		
		Sunlight	Arc light	slurry	Quartz sand	Cambisol	Chernozem
CTC	k_p; $k_{p,1}/k_{p,2}$	0.11	0.10	0.22/0.001	1.43/0.0002	0.25/ 0	0.56/0.0003
	C_0; $C_{0.1}/C_{0.2}$	102	99	53/47	45/57	6.0/94	10/90
	R^2; SD	0.90; 2.64	0.98; 2.89	0.94; 6.31	0.99; 2.03	0.79; 1.38	0.84; 1.98
OTC	k_p; $k_{p,1}/k_{p,2}$	0.07	0.12	0.30/ 0		0.19/ 0	
	C_0; $C_{0.1}/C_{0.2}$	103	95	64/38		19/81	
	R^2; SD	0.87; 2.03	0.99; 3.64	0.95; 6.52		0.96; 1.61	
*p*ABA	k_p; $k_{p,1}/k_{p,2}$	0.07	0.04	3.33/ 0	0.02	0.86/ 0	0.004
	C_0; $C_{0.1}/C_{0.2}$	100	100	15/86	95	5.8/94	102
	R^2; SD	0.97; 1.02	0.83; 3.58	0.92; 4.50	0.99; 0.82	0.92; 0.60	0.77; 2.26
SAA	k_p; $k_{p,1}/k_{p,2}$	0.04	0.005	0.26/ 0		0.02	
	C_0; $C_{0.1}/C_{0.2}$	100	102	45/56		99	
	R^2; SD	0.93; 0.97	0.73; 1.41	1.00; 1.04		0.90; 5.32	
SDM	k_p; $k_{p,1}/k_{p,2}$	0.03	0.009	0.10/ 0		0.01	
	C_0; $C_{0.1}/C_{0.2}$	100	95	45/53		97	
	R^2; SD	0.96; 0.96	0.73; 2.21	0.79; 9.12		0.70; 6.74	
SDZ	k_p; $k_{p,1}/k_{p,2}$	0.02	0.006	0.21/ 0		0.006	
	C_0; $C_{0.1}/C_{0.2}$	100	100	49/52		100	
	R^2; SD	0.98; 0.98	0.94; 0.65	0.99; 1.94		0.63; 4.07	
SDT	k_p; $k_{p,1}/k_{p,2}$	0.04	0.04	0.44/0.003		0.01	
	C_0; $C_{0.1}/C_{0.2}$	101	95	44/58		101	
	R^2; SD	0.94; 0.95	0.81; 9.48	0.96; 4.61		0.75; 7.40	
SPY	k_p; $k_{p,1}/k_{p,2}$	0.04	0.04	0.37/0.006	0.02	0.01	0.01
	C_0; $C_{0.1}/C_{0.2}$	100	99	45/56	99	100	101
	R^2; SD	0.98; 0.49	0.92; 4.13	0.99; 2.31	0.72; 8.08	0.87; 4.10	0.85; 5.14
FBZ	k_p; $k_{p,1}/k_{p,2}$	0.05	0.03		0.02		0.008
	C_0; $C_{0.1}/C_{0.2}$	102	100		92		101
	R^2; SD	0.57; 1.90	0.89; 3.49		0.68; 9.27		0.82; 3.30

Figure. 1). The differences might be attributed to differences in the geometry of the reaction vessels and differing relative intensities of wavelengths between the two light sources. It can not be excluded that the latter was possibly aggravated by a screening of irradiation by the polystyrene lids.

Photodegradation was strongest for the tetracyclines, while degradation of the sulfonamides, *p*ABA, and FBZ was much less pronounced (Table 5). This confirmed the findings of Lunestad et al. (1995) and Halling-Sørensen (2000), who investigated antibiotic photodegradation in seawater. For SAA, SPY, and the tetracyclines, evolving metabolites were observed by HPLC (data not shown) but were not further identified as it was not the aim of this study.

3.3 Photodegradation of antibiotics on irradiated solid substrates

Photodegradation was observed for all investigated antibiotics and substrates. The extractable antibiotic concentration, residing in the irradiated samples after the 28-d

incubation period, was by a median factor of 2 smaller than in non-irradiated samples (Table 3). Photodegradation was separated from the other abiotic processes by pair-wise correction of the data from irradiated and non-irradiated samples. The curves obtained showed a monotonic decrease (Figure 2 a, b). For the sulfonamides, FBZ, and in parts *p*ABA, photodegradation on soils and quartz sand was best described by a single first order equation (Eq. 1). The resulting rate coefficients of photodegradation (k_p) ranged from 0.006 to 0.02 d^{-1} (Table 5) and were similar or slightly smaller compared to water.

For all other samples photodegradation was not sufficiently described by a single first order equation (Eq. 2). Instead, the biphasic model (Eq. 3) was best-fit for the tetracyclines in all solid substrates and for *p*ABA in Cambisol (Table 5). For these five samples, photodegradation rate coefficients of the first phase ($k_{p,1}$) ranged from 0.19 to 1.43 d^{-1} and were on average by a factor of 9 larger than those obtained for photodegradation in water that was irradiated with arc light. The rate coefficients of the second phase ($k_{p,2}$) were zero or considerably small (≤ 0.0003 d 1). This indicated that a concentration level was reached within 28 d, which was not or virtually not affected by photodegradation.

The different rate coefficients of photodegradation in water and in the solid substrates confirmed that additional to direct photolysis other photodegradative processes are relevant in solid substrates. The accelerated photodegradation especially of the tetracyclines corresponded to findings reported by Boreen et al. (2005) and Wolters and Steffens (2005), who determined enhanced photodegradation of sulfonamides though. They explained the enhancement by a bathochromic shift of the absorption bands of adsorbed chemicals (Leermakers et al., 1965; Parlar, 1990) and a sensitized photodegradation through an energy transfer from excited soil substrates (Konstantinou et al., 2001). Additionally, indirect photodegradation through triplet excited-state dissolved organic matter (Boreen et al., 2005), •OH radicals and •ROO-radicals from soil organic matter (Wolters and Steffens, 2005) was reported. Singlet oxygen ($^1O_2$) that is produced on soil surfaces (Miller and Donaldson, 1994) might further enhance the photochemical degradation of antibiotics but did not affect sulfonamides with six-membered heterocyclic substituents (Boreen et al., 2004; Boreen et al., 2005). In this context it must be stated that the investigated antibiotic concentrations by far exceeded the concentrations found in the environment. Due to the non-linear sorption of the antibiotics tested (Figueroa et al., 2004; Thiele-Bruhn et al., 2004b), the dissolved fraction was much larger than typically found in the environment (e.g., Campagnolo et al., 2002; Hamscher et al., 2002), which might have affected photodegradation.

Photodegradation of the tetracyclines in solid substrates and of *p*ABA in Cambisol, though accelerated, was limited to a fraction of $C_{0,1}$ ranging from 5.8 to 45 %, while photodegradation of the sulfonamides and of FBZ was in part slower in soil than in water. In total, photodegradation of antibiotics was thus less in soils as opposed to water. This is explained by the shielding of compounds from direct photolysis by soil (Miller and Donaldson., 1994), while the role of soil humic substances is controversially discussed. According to Aguer et al. (2002) and Miller and Donaldson (1994) soil humic and fulvic acids exhibit no photoinductive activity or even reduce the photoinductive activity of other chromophores. In contrast, Konstantinou et al. (2001) and Wolters and Steffens (2005) reported a promoting effect through radical formation and alteration of absorption spectra of adsorbed compounds which overcompensates for the stabilizing effect through sorption. The latter might be reflected by in part enhanced rates of photodegradation in soil as opposed to water but not by the smaller degraded fractions presented here.

It must be stated that the different abiotic processes including photodegradation probably competed for the same available antibiotic fraction as it was reported by Miller and Donaldson (1994) and Balmer et al. (2000). The simple pair-wise correction of the data from irradiated and non-irradiated samples was possibly not sufficient to exactly separate the different processes.

3.4 Photodegradation of antibiotics in irradiated pig slurry

Photodegradation in pig slurry led to strikingly smaller residual concentrations of all antibiotics, despite CTC, than in topsoils (Table 3, Figure 2 a) and even than in water. Additionally, photodegradation in pig slurry was faster than in pure water and significantly faster ($p < 0.05$) than in the other solid substrates (Table 5). The disappearance times (DT_{50} [d]) in the substrates increased in the sequence (median for all antibiotics; minimum-maximum): pig slurry (13.9; 1.0 - 39.1) < water (17.3; 5.8 - 139) < Cambisol (50.2; 2.8 - 116). During incubation, the color of the irradiated pig slurry changed from dark brown to a light reddish brown.

The strong impact of photodegradation can be attributed to different causes. A better light penetration into the thin layers of pig slurry compared to soil was experimentally observed. Additionally, light scattering on suspended particles might have increased the spatial distribution of the radiation (Miller and Zepp, 1979). Also, it is assumed that various pig slurry constituents might have served as photoinductive chromophores and enhanced the indirect photodegradation of antibiotics. Despite reactive metal(hydr)oxides, pig slurry contains numerous dissolved organic compounds in high concentration (Bril and Salomons, 1990; Liang et al., 1996). Dissolved organic compounds act as sensitiz-

ers and enhance the photodegradation of organic chemicals such as sulfonamides (Parks, 1985; Boreen et al., 2005). When metal ions are complexed by such dissolved organic matter, they effectively mediate photosensitized reactions, resulting in a photobleaching of the organic matter itself (Zepp 1998), as the latter was observed in this study. Furthermore, the investigated pig slurry was characterized by a strongly acidic pH that resulted in an antibiotic speciation different from the other substrates (data not shown) and promoted antibiotic adsorption (Thiele-Bruhn et al., 2004a). It can be speculated whether this further enhanced sensitized photodegradation and altered absorption spectra of the antibiotics.

4 Conclusions

Photodegradation was studied trying to mimic light conditions found in Central Europe in late winter. The nine investigated pharmaceutical antibiotics from the structural classes of the tetracyclines, sulfonamides, and benzimidazoles were photodegraded in water with different first order rate coefficients (k_p). In non-irradiated sterile soil, the decrease in concentration was governed by abiotic processes that led to a decrease of the extractable fraction. The extent of this abiotic decrease varied with the soil sorptive properties and reduced the antibiotic fraction available for photodegradation. Consequently, the total photodegraded concentration of antibiotics was mostly smaller on soil surfaces compared to water. In contrast, photodegradation was considerably enhanced in pig slurry. It is concluded that under field conditions, photodegradation may contribute to the overall reduction of the concentration, and thus the biotic activity of antibiotics. Photodegradation will be relevant, when antibiotics are spread on soil surfaces in thin layers of manure, applied on days with large light intensity and water evaporation. However, these conditions are not fulfilled when the antibiotics are quickly transferred into the soil depth e.g., through infiltrating water, or when manure is mixed into the soil. Further field investigations are needed to elucidate the role of photodegradation of antibiotics on arable soils and the significance of evolving metabolites

Acknowledgements

The authors gratefully acknowledge G. Hamscher and H. Pawelzick (Zentrumsabteilung für Lebensmitteltoxikologie, Tierärztliche Hochschule Hannover, Germany) for LC-MS/MS analysis of background concentrations of antibiotics in pig slurry. Optical spectrum analysis was performed at the Leibniz-Institute of Atmospheric Physics (Kühlungsborn, Germany) and we especially thank J. Höffner and M. Gerding. Proof reading and language editing was generously done by S.L. Foran (Lethbridge, Canada) and G. Jandl (Rostock, Germany). Parts of this work were funded by the German Research Foundation, DFG (Th 678/3-1)

References

Aguer J-P, Richard C, Trubetskaya O, Trubetskoj O, Lévèque J, Andreux F (2002) Photoinductive efficiency of soil extracted humic and fulvic acids. Chemosphere 49:259-262

Balmer ME, Goss K-U, Schwarzenbach RP (2000) Photolytic transformation of organic pollutants on soil surfaces - an experimental approach. Environ Sci Technol 34:1240-1245

Boreen AL, Arnold WA, McNeill K (2005) Triplet-sensitized photodegradation of sulfa drugs containing six-membered heterocyclic groups: Identification of an SO2 extrusion photoproduct. Environ Sci Technol 39:3630-3638

Boreen AL, Arnold XA, McNeill K (2004) Photochemical fate of sulfa drugs in the aquatic environment: Sulfa drugs containing five-membered heterocyclic groups. Environ Sci Technol 38:3933-3940

Boxall ABA, Blackwell P, Cavallo R, Kay P, Tolls J (2002) The sorption and transport of a sulphonamide antibiotic in soil systems. Toxicol Lett 131:19-28

Bril J, Salomons W (1990) Chemical composition of animal manure: a modelling approach. Neth J Agric Sci 38:333-351

Burhenne J, Ludwig M, Nikoloudis P, Spiteller M (1997) Photolytic degradation of fluoroquinolone carboxylic acids in aqueous solution .1. Primary photoproducts and half-lives. Environ Sci Pollut Res 4:10-15

Campagnolo ER, Johnson KR, Karpati A, Rubin CS, Kolpin DW, Meyer MT, Esteban JE, Currier RW, Smith K, Thug KM, McGeehin M (2002) Antimicrobial residues in animal waste and water resources proximal to large-scale swine and poultry feeding operations. Sci Total Environ 299:89-95

Figueroa RA, Leonard A, Mackay AA (2004) Modeling tetracycline antibiotic sorption to clays. Environ Sci Technol 38:476-483

Gavalchin J, Katz SE (1994) The persistence of fecal-borne antibiotics in soil. J Assoc Off Anal Chem Int 77:481-485

Halling-Sørensen B (2000) Algal toxicity of antibacterial agents used in intensive farming. Chemosphere 40:731-739

Halling-Sørensen B, Sengeløv G, Ingerslev F, Jensen LB (2003) Reduced antimicrobial potencies of oxytetracycline, tylosin, sulfadiazin, streptomycin, ciprofloxacin, and olaquindox due to environmental processes. Arch Environ Contam Toxicol 44:7-16

Hamscher G, Sczesny S, Höper H, Nau H (2002) Determination of persistent tetracycline residues in soil fertilized with liquid manure by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. Anal Chem 74:1509-1518

Helfrich H-P (1996) CFIT: Software for non-linear regression. Mathematical Seminar of the Faculty of Agriculture, University of Bonn

Hirsch R, Ternes T, Haberer K, Kratz KL (1999) Occurrence of antibiotics in the aquatic environment. Sci Total Environ 225:109-118

Japenga J, Harmsen K (1990) Determination of mass balances and ionic balances in animal manure. Neth J Agric Sci 38:353-367

Jørgensen SE, Halling-Sørensen B (2000) Drugs in the environment. Chemosphere 40:691-699

Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT (2002) Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999-2000: A national reconnaissance. Environ Sci Technol 36:1202-1211

Konstantinou IK, Zarkadis AK, Albanis TA (2001) Photodegradation of selected herbicides in various natural waters and soils under environmental conditions. J Environ Qual 30:121-130

Kreuzig R, Höltge S (2005) Investigations on the fate of sulfadiazine in manured soil: Laboratory experiments and test plot studies. Environ Toxicol Chem 24:771-776

Leermakers PA, Weis LD, Thomas HT (1965) Photochemistry of adsorbed organic molecules.2. Cage effects in photolysis of azobisisobutyronitrile and tetramethyl-1,3-cyclobutanedione on silica gel. J Am Chem Soc 87:4403-4404

Liang BC, Gregorich EG, Schnitzer M, Schulten HR (1996) Characterization of water extracts of two manures and their adsorption on soils. Soil Sci Soc Am J 60:1758-1763

Lunestad BT, Tore B, Samuelsen OB, Fjelde S, Ervik A (1995) Photostability of eight antibacterial agents in seawater. Aquaculture 134:217-225

Miller GC, Donaldson SG (1994) Factors affecting photolysis of organic compounds on soils. In: Helz GR, Zepp RG, Currier RW (eds) Aquatic and Surface Photochemistry; (6). Boca Raton, FL : Lewis Publishers, pp 97-109

Miller GC, Zepp RG (1979) Effects of suspended sediments on photolysis rates of dissolved pollutants. Water Res 13:453-459

Nowara A, Burhenne J, Spiteller M (1997) Binding of fluoroquinolone carboxylic acid derivatives to clay minerals. J Agric Food Chem 45:1459-1463

Oka H, Ikai Y, Kawamura N, Yamada M, Harada K, Ito M, Suzuki M (1989) Photodecomposition products of tetracyclines in aqueous solution. J Agric Food Chem 37:226-231

Parks OW (1985) Photodegradation of sulfa drugs by fluorescent light. J Assoc Off Anal Chem Int 68:1232-1234

Parlar H (1990) The role of photolysis in the fate of pesticides. In: Hutson DH, Roberts TR (eds) Environmental fate of pesticides. New York, NY : Wiley, pp 245-276

Pinck LA, Soulides DA, Allison FE (1962) Antibiotics in soils: 4. Polypeptides and macrolides. Soil Sci 94:129-131

Schinner F, Ähllinger R, Kandeler E, Margesin R (1993) Bodenbiologische Arbeitsmethoden. Berlin : Springer Verlag, 389 p

Thiele S (2000) Adsorption of the antibiotic pharmaceutical compound sulfapyridine by a long-term differently fertilized loess Chernozem. J Plant Nutr Soil Sci 163:589-594

Thiele-Bruhn S (2003) Pharmaceutical antibiotic compounds in soils - a review. J Plant Nutr Soil Sci 166:145-167

Thiele-Bruhn S, Aust MO (2004a) Effects of pig slurry on the sorption of sulfonamide antibiotics in soil. Arch Environ Contam Toxicol 47:31-39

Thiele-Bruhn S, Seibicke T, Schulten HR, Leinweber P (2004b) Sorption of sulfonamide pharmaceutical antibiotics on whole soils and particle-size fractions. J Environ Qual 33:1331-1342

van Gool S (1993) Possible effects on the environment of antibiotic residues in animal manure. Tijdschr Diergeneeskd 118:8-10

Wessels JM, Ford WE, Szymczak W, Schneider S (1998) The complexation of tetracycline and anhydrotetracycline with Mg^{2+} and Ca^{2+}: a spectroscopic study. J Phys Chem B 102:9323-9331

Winckler C, Grafe A (2001) Use of veterinary drugs in intensive animal production: evidence for persistence of tetracycline in pig slurry. J Soils Sed 1:66-70

Wolters A, Steffens N (2005) Photodegradation of antibiotics on soil surfaces: Laboratory studies on sulfadiazine in an ozone-controlled environment. Environ Sci Technol 39:6071-6078

Zepp RG (1998) Photoreactivity of dissolved organic matter in freshwater environments. In: 216th ACS National Meeting, Boston, 23-27 August 1998, Book of Abstracts. American Chemical Society : Washington, D.C.