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Article

Tracing the Influence of Manure as a Complex Mixture on the Soil Sorption of Pharmaceuticals Back to Selected Chemical Constituents

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Abstract: Pharmaceutically active compounds (PhACs) enter soil with organic substrates such as manure. Such complex substrates differently affect PhACs' soil sorption. To elucidate the effects, batch experiments were conducted using five selected chemicals as model constituents. Urea, KH_2PO_4, acetic acid, phenol and nonadecanoic acid (C:19) altered the sorption strength and/or nonlinearity of sulfadiazine, caffeine and atenolol in a topsoil. The nonlinear Freundlich model best described sorption. Overall, the PhACs' sorption coefficients increased in the sequence urea<phosphate<phenol<C:19<acetic acid. The sorption nonlinearity also increased in most cases. The effects on sulfadiazine and caffeine were rather similar, but in many cases different from atenolol. Phosphate mobilized sulfadiazine and caffeine and urea mobilized sulfadiazine, which was explained by sorption competition resulting from specific preference of similar sorption sites. Soil sorbed phenol strongly increased the sorption of all three PhACs; phenolic functional groups are preferred sorption sites of PhACs in soil. The large increase in sorption of all PhACs by acetic acid was attributed to a loosening of the soil organic matter and thus the creation of additional sorption sites. The effect of C:19 fatty acid, however, was inconsistent. These results help to better understand the sorption of PhACs in mixtures of soil and manure.

Keywords: Atenolol; caffeine; sulfadiazine; dissolved organic matter; sewage; specific sorption; sorption competition

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1. Introduction

Pharmaceutical active compounds (PhACs) such as antibiotics, analgesics, and beta blockers, to name a few, are highly potent chemicals [1]. Since the early work on PhACs in the soil environment around the turn of the millennium [2-6] it has become clear that PhACs can no longer be called emerging contaminants, but residual contamination of the soil environment with PhACs is a ubiquitous problem [7-9]. Residual concentrations in soil are typically in the order of micrograms per kilogram of soil [9-12], and hence in the same range as those of pesticides. Similar to "pesticide", PhAC is a collective term for many different classes of small- to medium-sized molecular compounds. However, one of the main differences is that PhACs, except for a few agents against bacterial pathogens in plants, are not intentionally released into the soil environment. Consequently, the adverse effects of PhACs are unintended but can be significant, such as the impact on soil antibiotic resistance [13, 14], which, in the sense of the One Health approach, also endangers human health [15, 16]. In addition, the vast majority of PhAC inputs come from livestock manure, as well as sewage (biosolids) and wastewater from humans (for simplicity all these substrates are referred to as "slurry" in the following) [17-20]. This is because PhACs are predominantly and rapidly excreted from the treated body [21, 22] and the excreta are subsequently used for fertilization or irrigation of agricultural soils. Concentrations in slurry range from a few up to several thousand micrograms per kilogram [11], with mixed multi-component contaminations occurring in most cases [23].

Manure and similar substrates are highly complex mixtures of diverse constituents. These constituents range a) in size from particulate tissues, e.g. residual feed and bedding material, to truly dissolved single molecules, b) in chemical identity from components such as salts to organic macromolecules, and c) from highly polar to very non-polar compounds, while d) the overall composition varies depending on the organism type, age, diet, and storage conditions of the manure [24-27]. Adding slurry to soil alters the composition of the soil organic matter (SOM), resulting in enrichment of manure-specific compound classes [28, 29] and a significant increase in the amount of dissolved organic matter (DOM) [30]. This result greatly influences the fate and especially the sorption of PhACs in the soil, the latter being one of the most important soil properties to mitigate environmental pollution.

The complex interaction between slurry, PhACs and soil is reflected in contrasting reports in the literature on the effects of slurry on the soil sorption of PhACs, ranging from increased immobilization [31-33] to increased mobilization and transport [34-36]. These results indicate that the overall effect depends largely on the individual combination of the ternary mixture of soil - PAHs - slurry and especially on the composition of the slurry. This was investigated in respective research, focusing on PhACs' soil sorption in the presence of slurry from different sources and of fractions of slurry such as the dissolved organic matter (DOM) fraction and polarity fractions (e.g. [37-40]). In contrast, the impact of different chemical components of manure has, to the best of our knowledge, not yet been investigated.

Accordingly, it was the aim of this study to investigate the effect of selected pure chemicals, representing relevant constituents of manure, on the soil sorption of PhACs. This was done by using urea and potassium dihydrogen phosphate (KH_2PO_4) as major nutrient components, acetic acid and phenol as a representatives of volatile organic compounds, and nonadecanoic acid (C:19) as non-polar organic constituent of manure. The presence of these selected chemical compounds in manure had been previously reported [41-45]. The antibiotic sulfadiazine, the beta-blocker atenolol and the psychotropic drug caffeine were tested as PhACs, which had been previously shown to differently interact with manure fractions [46]. Thus, this study followed up on previous research to further elucidate sorption in ternary soil-fertilizer-PhAC mixtures [46]. It was hypothesized that the selected model compounds affect the sorption of the PhACs differently.

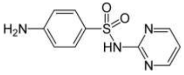
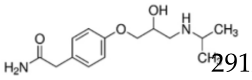
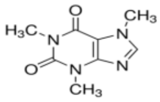
2. Materials and Methods

The sorption experiments were performed with a soil sample from an arable field. Briefly, the topsoil of a Haplic Cambisol was sampled near Ferschweiler in the region of Trier (N 49° 51,77'; E 6° 49,26') from a depth of 0–15 cm. The soil sample (sample II) was also used in our previous study [46]. It was selected because it showed good sorption of the tested PhACs and a clear effect of manure DOM on the sorption. Most importantly, the acidic pH of the soil ensured that pH changes due to the addition of the selected model compounds would not alter the speciation of the three PhACs tested to such an extent that this effect alone would explain the altered sorption. Instead, the goal was to also capture the effect of chemical interaction between the sorbates on sorption.

Soil pH and electrical conductivity (EC) were determined by a pH meter (electrode SenTix 21, WTW, Germany) and conductivity meter (Cond 340i, WTW, Germany), respectively, in 0.01 M $CaCl_2$ at a soil to solution ratio of 1:2.5. Organic carbon (OC) and nitrogen content of the soil were measured by elemental analysis (EA 3000, Hekatech, Wegberg, Germany). Oxalate extractable iron oxides (Fe_o) were determined using the modified method from Schwertmann [47] and using graphite furnace atomic absorption spectrometry (ContrAA 700 High Resolution Continuum Source, Varian, Palo Alto, CA). The investigated soil properties were as follows: pH_{CaCl2} 4.98; EC ($mS \cdot cm^{-1}$) 2.43; OC ($mg \cdot g^{-1}$) 11.57; C/N ratio 11.25; Fe_o (%) 0.18; CEC ($mmol_c \cdot kg^{-1}$) 37.18; clay (%) 6.0; texture class loamy sand.

Analytical grade standard chemicals of sulfadiazine, caffeine and atenolol were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Selected physicochemical properties of the tested PhACs are listed in Table 1. Methanolic stock solutions of the PhACs were prepared at a concentration of 1 $mg \cdot mL^{-1}$ and stored at 4 °C in the dark.

Table 1. Molecular structure and chemical properties of the three selected PhACs sulfadiazine, atenolol and caffeine as well as of the five chemicals used as model compounds of manure.

Compound	Molecular formula	CAS number	Molar mass ($g \cdot mol^{-1}$)	pK_a 1	pK_a 2	K_{OW} [a]	Water solubility ($mg \cdot L^{-1}$)
			Pharmaceutical active compounds (PhACs)				
Sulfadiazine		68-35-9	250.30	1.57 [b]	6.50 [b]	0.812 [b]	2000
Atenolol		29122-68-7	266.34		9.60 [c]	1.445 [d]	429
Caffeine		58-08-2	194.19	0.40 [c]	10.4 [c]	0.851 [c]	21600
			Model compounds (manure constituents) [e]				
Urea	$CO(NH_2)_2$	57-13-6	60.06	0.18		0.008	5.45e+5
Monopotassium phosphate	KH_2PO_4	7778-77-0	136.09	2.15	6.82	–	2.22e+5
Acetic acid	CH_3COOH	64-19-7	60.05	4.76		-0.17	4.76e+5
Phenol	C_6H_5OH	108-95-2	94.11	9.99		28.84	8.28e+4
Nonadecanoic acid C:19	$C_{18}H_{37}COOH$	646-30-0	298.50	4.78		2.75e+8	0.002

[a] K_{OW} of the neutral species; [b] data from [48]; [c] http://www.drugbank.ca; [d] http://www.toxnet.nlm.nih.gov; [e] US-EPA EPISuite

In addition, five pure model compounds i.e., urea, KH_2PO_4 (phosphate), acetic acid, phenol and nonadecanoic acid (C:19) were investigated regarding their influence on the soil sorption of sulfadiazine, caffeine and atenolol. These model compounds resemble different components of manure. The chemicals were obtained from Merck (Darmstadt, Germany) and C:19 was obtained from Supelco (Darmstadt, Germany) and all were of analytical grade or higher purity.

To investigate the sorption of the three PhACs in the presence of manure constituents, the aforementioned five pure model compounds were added to soil at a spiking level of 5 $mmol \cdot g^{-1}$. The spiking level was roughly estimated based on the composition of manure and the typical addition rates of manure to soil. It is admitted that the concentrations of the model substances investigated are not uniform in manure; but this was left out in order to obtain comparable results instead. All these samples were combined with one of the three PhACs added in two different spiking concentrations, i.e. 20 and 70 $\mu g \cdot g^{-1}$. Furthermore, control samples without addition of the model compounds and samples without both, model compounds and PhACs were investigated. All samples were prepared in triplicate.

The sorption of sulfadiazine, atenolol and caffeine to the soil sample in the presence and absence of pure model compounds was investigated according to OECD guideline 106 [49]. For each sample, 5 g of soil were suspended in 0.01 M $CaCl_2$ (soil-to-liquid ratio = 1:2.5 *w*/*w*) and equilibrated for 12 h to fully remoisten the soil. Then the model compounds and PhACs were added and the samples were agitated on an end-over-end shaker at 22 °C at 15 rpm for 72 h (the equilibrium time was determined in a preliminary kinetic experiment; data not shown). Afterwards, samples were immediately centrifuged at 2000 *g* for 30 min. In order to analyze the PhACs, the supernatants were solid phase extracted using an HR-X cartridge (Macherey–Nagel, Düren, Germany) preconditioned with 6 mL of methanol followed by 6 mL of HPLC grade water. After the supernatant was passed through the cartridge, it was rinsed with 6 mL of a methanol:water mixture (2:8 *v*/*v*) and subsequently dried in a

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nitrogen gas stream for 30 min. Finally, the target PhACs were eluted from the cartridge using 6 mL methanol. The eluted volume was evaporated to about 0.5 mL in a rotary evaporator (Rotavapor R-114, Switzerland) and redissolved in 1.0 mL methanol. Samples were spiked with 0.5 µg sulfadimidine as internal standard for quantification and transferred to amber LC autosampler vials. The recovery rates of sulfadiazine, atenolol and caffeine were 84.03%, 79.49% and 90.15%, respectively.

Analytical determination of the pharmaceuticals was done by using LC-ESI-MS/MS. Chromatographic separation was achieved with a Hypersil Gold C18 HPLC column (50×2.1 mm, 3.0 µm, Thermo Electron, USA) as stationary phase and HPLC water with 0.1% formic acid (*v*/*v*; eluent A) and methanol with 0.1% formic acid (*v*/*v*; eluent B) as mobile phases. A gradient program was used to deliver the mobile phases at a flow rate of 0.2 mL min^{-1} with 98% of eluent A as initial condition, linearly increased within 10 min to 100% of eluent B, 4-min isocratic elution, and 1-min linear gradient back to 98% of eluent A, the latter held for 2 min to equilibrate the column. The sample injection volume was 10 µL. The chromatographic system consisted of a Shimadzu LC-20 HPLC (Shimadzu, Duisburg, Germany) coupled to an API 3200 LC–ESI–MS/MS (Applied Biosystems/MDS Sciex Instruments, Toronto, Canada) operated in positive ion mode. The settings of the ion-source were as follows: ion spray voltage 5000 V; source temperature 400 °C; collision gas 7 psi; curtain gas 25 psi. The Analyst 1.4.2 software (Applied Biosystems/MDS Sciex Instruments, USA) was used for peak integration and data assessment. The evaluation of chromatograms was done as reported by Ngigi, et al. [50]. The analytical method yielded limits of detection of 5 µg·L^{-1} and limits of quantification (LOQ) of 10 µg·L^{-1}. All the data reported in this study were higher than the LOQ.

The Freundlich, Langmuir, and linear isotherm models were tested to describe the sorption data. All three models were selected based on the results of numerous previous studies showing that these models are best suited to describe soil sorption of polar PhACs (e.g., [46, 51-53]). Of the three equations selected, the Freundlich model was clearly the best to describe the data. Therefore, only the results of the sorption isotherm fit using the Freundlich model according to Equation (1) are presented.

$$q_e = K_f \times c_e^n \quad , \tag{1}$$

where c_e (µmol·mL^{-1}) is the equilibrium solution concentration of the pharmaceutical, and q_e (µmol·g^{-1}) is the equilibrium adsorbed concentration. The parameters K_f (µmol$^{(1-n)}$·mL^{n}·g^{-1}) and n denote the Freundlich sorption coefficient and the Freundlich exponent as measure of the isotherm's nonlinearity. In order to better compare the sorption coefficients of isotherms with different nonlinearity, the linear sorption coefficient (K_d) was additionally calculated from K_f using Equation (2) [32].

$$K_d = K_f \times c_e^{n-1} \ . \tag{2}$$

For this purpose, K_d (mL·g^{-1}) was calculated in this study based on an equilibrium concentration (c_e) of 10 µmol·mL^{-1}. From the K_d the K_{OC} was calculated according to Equation (3).

$$K_{OC} = K_d \ / \ \mathrm{OC}\ (\%) \times 100 \ . \tag{3}$$

Curve fitting by nonlinear regression was done using the SigmaPlot 14 software (Systat GmbH, Frankfurt/Main, Germany).

To determine differences between isotherms, graphs were described by their confidence intervals [3]. The half width *A* of a confidence interval at a solution concentration c_W (mg L^{-1}) and a probability of error of α was calculated (Equation 4).

$$A = SE \times t_{N-2,\alpha/2} \times \sqrt{\frac{1}{N} + \frac{\left(lgc_w - lgc_{wq}\right)^2}{\left(\sum lgc_{wi}{}^2\right) - \left(\sum lgc_{wi}\right)^2 \times 1/N}} \tag{4.}$$

SE was the standard error of the regression, *t* the *t*-quantile, *N* the number of observations, c_{wi} the measured solution concentration of i = 1-N and c_{wq} the mean solution concentration. Differences between isotherms were significant, when the difference of two isotherms at a given solution concentration c_W exceeded the sum of half the confidence intervals. The confidence interval was (Equation 5)

$$\left[lgK_f + n \times lgc_w - A;\ lgK_f + n \times lgc_w + A\right] \tag{5.}$$

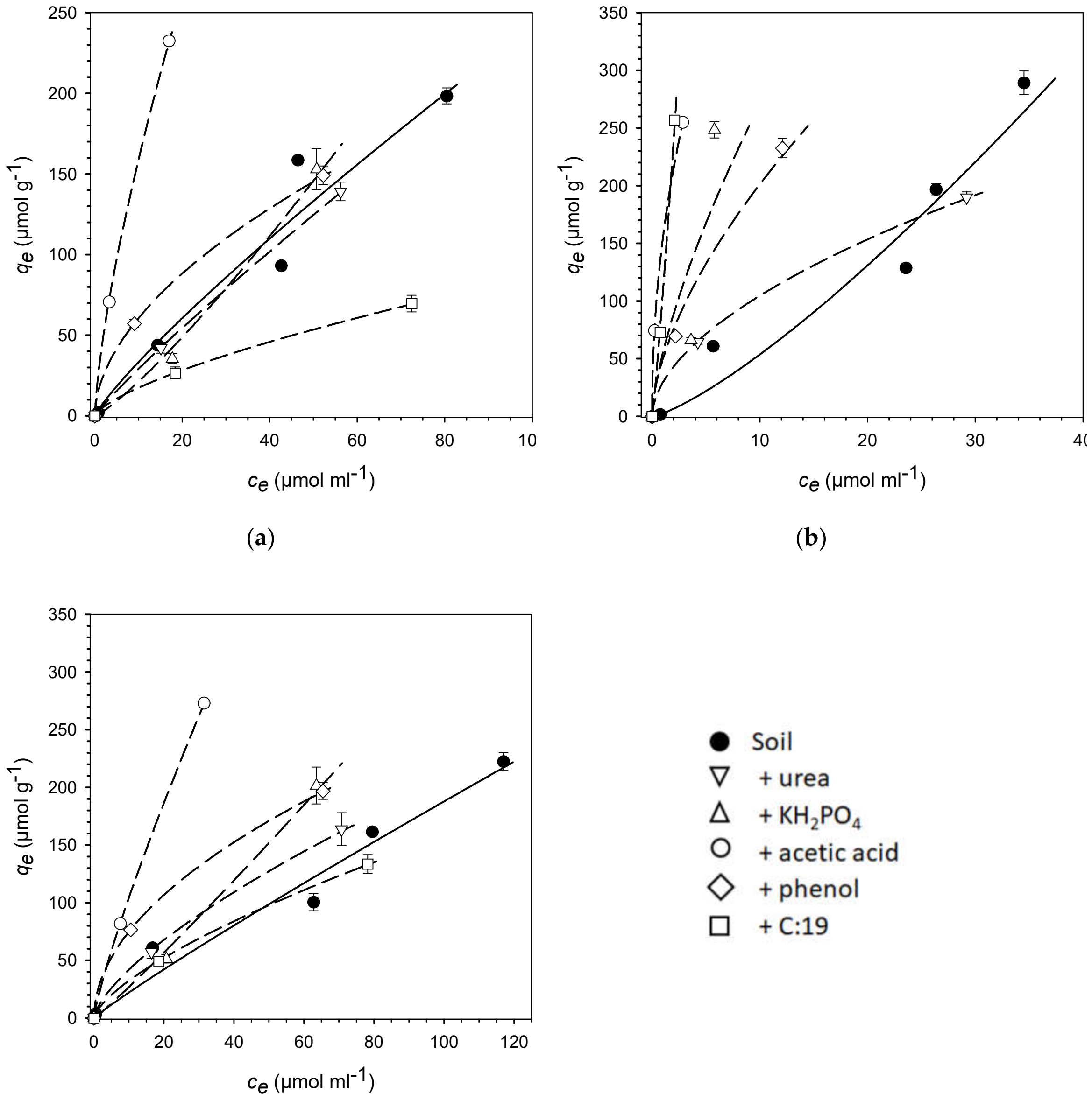
3. Results

The soil pH and OC content as relevant soil parameters were changed in the presence of the model substances (Table 2). Adding the organic chemicals urea, acetic acid and phenol slightly increased the total OC content, while the C:19 fatty acid with the highest relative C content in the molecular structure (Table 1) increased the OC content by almost 10%. The effect on soil pH was by far the greatest in the presence of acetic acid with a decrease from pH 4.98 to 2.56, whereas all other compounds decreased pH by 0.6 pH units or less within an equilibrium time of 72 h (Table 2).

Table 2. Soil organic carbon content (OC) and pH ($CaCl_2$) of samples without (soil) or equilibrated with 5 mmol·g^{-1} of five different model compounds representing relevant chemical constituents of manure.

	Soil	+urea	+KH_2PO_4	+acetic acid	+phenol	+C:19
OC (mg·g^{-1})	11.57	11.63	11.57	11.78	11.93	12.71
pH	4.98	4.90	4.40	2.56	4.49	4.46

The slightly nonlinear sorption of the three PhACs in soil was well described by the Freundlich sorption isotherm model (Figure 1). The goodness of the model fit was reflected in the statistical parameters in question, e.g., a coefficient of determination (R^2) of 0.92 and higher and *SE* of 4.28 and less (Table 3). However, the fit by the model was significantly weaker for the combination of atenolol and KH_2PO_4 with R^2 of 0.48 and a standard error (SE) of 24.3. It should be noted that the sorption of the PhACs to the unmodified soil (without the addition of manure model compounds) was investigated in a previous study by testing six concentration levels [46]. Based on these results and the performance of the model fit, it was inferred that this was also valid for the smaller data sets with only three concentration levels obtained in this study.



(**a**) (**b**)

(**c**)

Figure 1. Sorption of PhACs ((**a**) sulfadiazine, (**b**) atenolol, (**c**) caffeine) to soil (topsoil of a Haplic Cambisol) in the absence (Soil) or presence of different model compounds representing chemical constituents of manure, added to soil at 5 mmol·g^{-1}. Dissolved and adsorbed equilibrium concentrations are displayed (mean values with standard deviation as error bars) and were fitted using the Freundlich isotherm model (Equation 1; lines). Note the different scaling of the axes in the sub-figures. Data for the control (Soil) were taken from [46].

Soil sorption of all three PhACs was largely affected by the addition of the different manure model compounds. Overall, the effects were highly variable, ranging from decreased to increased mobility for sulfadiazine, while mobilization was observed in all cases for atenolol and mobilization or no significant effect for caffeine (derived from confidence intervals, see Equations 4 and 5; data not shown.). On average of all three PhACs, the sorption coefficients (K_f and K_d) increased in the presence of the model compounds in the sequence urea < phosphate < phenol < C:19 < acetic acid. However, clear differences were observed between the individual combinations of model substances and PhACs. Similarly, the Freundlich exponents (n) as a measure of the sorption nonlinearity were altered differently in the different combinations with the model substances. In most cases, the exponents were reduced and the nonlinearity of the isotherms increased. The reduction of the Freundlich exponent *n* by the model substances was as follows: Phosphate reduced *n* the least and even increased *n* for the sorption of sulfadiazine and caffeine, while *n* decreased more and more with the addition of C:19, urea, acetic acid and phenol (Table 3). In general, the effects on the sorption of sulfadiazine and caffeine were rather similar, while the effects on the sorption of atenolol were very different in many cases.

Table 3. Freundlich model parameters (K_f and n) and derived linear sorption coefficients (K_d and K_{OC}) for soil sorption of PhACs without (Soil) or with the addition of five different model compounds representing relevant chemical constituents of manure.

PhAC	Isotherm parameter	Soil	+urea	+KH_2PO_4	+acetic acid	+phenol	+C:19
Sulfadiazine	K_f	4.66	3.71	1.39	31.1	17.5	3.56
	n	0.86	0.90	1.19	0.71	0.54	0.69
	R^2	0.94	0.99	0.92	0.99	0.99	0.97
	SE	2.41	1.15	1.43	3.85	2.55	1.26
	K_d	3.36	2.94	2.15	15.9	6.08	1.76
	K_{OC}	290	253	186	1,350	510	138
Atenolol	K_f	2.79	29.6	55.1	155	50.0	107
	n	1.28	0.55	0.69	0.48	0.61	1.16
	R^2	0.92	0.99	0.48	1.00	0.94	0.97
	SE	2.47	4.28	24.3	2.47	2.37	0.18
	K_d	5.38	10.5	27.1	46.6	20.1	154
	K_{OC}	465	902	2,340	3,950	1,690	12,100
Caffeine	K_f	2.65	8.63	2.29	15.3	23.0	6.71
	n	0.93	0.69	1.07	0.84	0.51	0.69
	R^2	0.96	0.95	0.93	1.00	0.99	0.99
	SE	1.37	3.98	2.05	1.99	3.17	1.76
	K_d	2.23	4.21	2.71	10.5	7.50	3.25
	K_{OC}	193	362	234	888	629	256

Phosphate decreased the Freundlich sorption coefficient (K_f) of sulfadiazine and caffeine (and K_d of sulfadiazine). At the same time, and only in these two cases, the sorption exponents (*n*) were increased, reaching values >1. The antibiotic SDZ was also slightly mobilized in the presence of urea and C:19 as was indicated by reduced sorption coefficients. In comparison, caffeine was not mobilized by urea and C:19. Instead, sorption coefficients increased, although the enhanced immobilization was weakest compared to the other model substances. In contrast, the sorption coefficient of atenolol strongly increased by a factor of 38.4 in the presence of the C:19 fatty acid, while the Freundlich exponent slightly declined but remained at a value >1. Both phenol and acetic acid strongly increased the sorption coefficients of all three PhACs; K_d increased by factors of 1.8 to 3.6 for phenol and 4.6 to 8.5 for acetic acid.

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Table 4. Pearson correlation coefficient (*r*) and significance level (*p*) of correlations between the OC content and pH of the soil samples equilibrated with the five model compounds and the resulting linear sorption coefficients (K_d) of the three tested PhACs. Significant correlations are highlighted in bold.

		sulfadiazine	atenolol	caffeine
OC [a]	*r*	-0.164	**0.942**	-0.049
	p	0.756	**0.005**	0.927
OC [b]	*r*	0.518	0.366	0.785
	p	0.371	0.544	0.116
pH [a]	*r*	**-0.918**	-0.181	**-0.827**
	p	**0.010**	0.732	**0.042**
pH [c]	*r*	0.034	-0.501	-0.274
	p	0.957	0.390	0.655

[a] All data; [b] without data for samples with addition of C:19; [c] without data for samples with addition of acetic acid.

Apparently, the OC content highly and significantly (p<0.05) explained the soil sorption of atenolol with a Pearson correlation coefficient (*r*) of 0.942, while this was not the case for sulfadiazine and caffeine (Table 4). However, the correlation of K_d values of atenolol with OC was very much governed by the K_d in the presence of C:19, since C:19 provided the most additional OC to the soil sample (Table 2) and resulted in by far the strongest sorption of atenolol (Figure 1b; Table 3). Removing this extreme data point from the correlations resulted in a non-significant relationship between the OC content and soil sorption, also for atenolol (Table 4). Accordingly, normalization of K_d to K_{OC} (Table 3) did not result in a closer relationship between the sorption coefficient and the OC content of soil (data not shown). Contrary to theoretical expectations, normalizing the K_{OC} to the OC content of the soil sample did not result in an alignment of the sorption coefficients (Table 3).

The pH was closely and significantly (p<0.05), negatively correlated with the sorption coefficients of sulfadiazine and caffeine in the soils amended with the different model compounds (Table 4). No such correlation, however, was found for atenolol. When the values for K_d in the presence of acetic acid, which had lowered the pH of the samples the most, were removed, all correlations became non-significant. Furthermore, the speciation of the three PhACs remained almost unaffected despite the pH changes. Both atenolol and caffeine occurred solely as cationic species throughout the range from pH 2.56 to 4.98. The distribution of sulfadiazine between cationic, neutral and anionic species started from pH 4.98 with 0.04%, 97.03% and 2.93%, respectively, reached a maximum of neutral species of 99.07% at pH 4.40 and decreased substantially to 90.71% neutral species but 9.28% cationic species at the lowest pH of 2.56.

4. Discussion

The mostly minor effects of the five model compounds on the soil pH and on the OC content of the soil sample (Table 2) were expected. The very slight pH decrease in the presence of otherwise alkaline urea indicated that some hydrolyzation to ammonium and further oxidation to nitrate had occurred within the equilibrium time of 72 hours [54]. Hence, it must be expected that not only the intact urea molecule but also ammonium and nitrate ions influenced the sorption of the three PhACs.

Although OC content and pH are well-known to be among the soil properties that dominate the sorption of polar PhACs [55, 56], weak or non-significant correlations indicated that the effect of the model compounds on the soil sorption of the PhACs was not simply related to the changes in these two soil properties (Table 4). The inability of OC content alone to explain soil sorption of PhACs was already reported in the early work of Tolls [4] and subsequently confirmed many times (e.g. [33]. For example, in addition to SOM, mineral soil colloids are highly relevant as sorbents for polar compounds [55, 57]. Furthermore, the nonlinearity of PhACs' sorption isotherms (Figure 1) showed that soil sorption was not based on partitioning into SOM (Table 2). This was also confirmed by the increase in the nonlinearity of the sorption isotherms in the presence of the model compounds, which can be read from the decreasing Freundlich exponents (n). It is well known that a decline of n can be interpreted as an increase in sorption heterogeneity [58]. Accordingly, it is presumed that the added model compounds, when sorbed in soil, resulted in an increase in specific sorption sites for PhACs or, in some cases, competition for specific sorption sites with PhACs.

Sorption competition occurred when sulfadiazine or caffeine were combined with phosphate as was previously observed [59]. Phosphate ions especially occupy the sorption sites at metal oxide minerals through ligand-exchange [60], which are thus no longer available to the PhACs as likewise preferred sorption sites [52]. The increase of the Freundlich exponent n to values >1 indicated such sorption competition, which is particularly effective at low spiking concentrations.

The result that only sulfadiazine was mobilized by urea, whereas caffeine and atenolol were stronger sorbed (Table 3), indicated a very specific interaction. Sulfonamides such as sulfadiazine are known to bind to sorption sites via the amino group [55, 61]. Urea and ammonium formed from it compete for the same sorption sites. The slight increase in sorption of cationic caffeine and atenolol instead may be due to the small effect of urea on soil pH. It may also indicate that atenolol with an amino group adjacent to an electronegative oxo group in the molecular structure (Table 1) does not sorb in the same way as sulfadiazine.

Similar to urea, also the C:19 fatty acid slightly mobilized sulfadiazine, while the soil sorption of caffeine was slightly and that of atenolol was strongly increased (Table 3). This was surprising given the speciation of the three PhACs; an association of the neutral sulfadiazine with the largely nonpolar fatty acid was hypothesized. Atenolol may have sorbed more strongly with the fatty acid because of its theoretically slightly lower polarity (higher K_{OW}, Table 1). It should be noted that different humic substances in combination with different PhACs may alter their sorption in the soil in very different ways [62]. The exact causes, as with this result, require further mechanistic studies.

Phenol strongly increased the sorption coefficients of all three PhACs and had even the strongest effect on the sorption heterogeneity of sulfadiazine and caffeine (Figure 1; Table 3). The sorption of phenol itself is largely governed by the association with organic matter and is rather weak [63], with reported soil sorption coefficients being a factor of ten smaller than the soil sorption coefficients determined for the three PhACs in this study. As a result, phenol did not compete with PhACs for sorption sites, but instead provided new and more heterogeneous sorption sites. Accordingly, naturally occurring phenolic compounds and phenolic functional groups contribute substantially to the sorption of PhACs in soil [61, 64].

A very strong increase in sorption coefficients of the PhACs was found in the presence of acetic acid (Figure 1). In contrast, the effect on sorption nonlinearity was rather weak for sulfadiazine and caffeine. This may indicate that acetic acid increased the sorption without altering the sorption mechanisms. This is only partly explained by the strong effect on pH (Table 2), which hardly led to a change in the speciation of the three PhACs (see results section), but impedes the sorption of the tested PhACs' cationic species. It seems more significant that acetic acid leads to a loosening and partly dissolution of the supramolecular structure of SOM [65]. This would create and open additional, but not different, sorption sites for the PhACs. The partial dissolution of the supramolecular structure will also have led to the formation of additional dissolved organic matter (DOM). PhACs form mobile associations with DOM [34, 66]. However, such associations could not be explicitly investigated in this study due to the solid phase extraction method used, which also captures DOM associates as part of the dissolved fraction. Since a decrease in dissolved fractions was even observed in the presence of acetic acid, there is no evidence of mobilization by additional DOM.

In most cases, the tested manure constituents led to an increased soil sorption of the three PhACs. This is consistent with the effect of adding manure and sewage sludge to soils. These substrates, with their high solid phase content, often result in greater immobilization of PhACs [33, 67]. However, it was expected that the pure chemicals tested would act more like DOM from manure and have a mobilizing effect [34, 66, 68]. Instead, the contrasting results of this study showed that the colloidal properties of DOM result in a different effect on the sorption of PhACs compared to pure chemicals. In addition, the effects of complex mixtures of compounds, such as those found in manure DOM, are very much defined by the different constituents and their proportions in the mixture. Accordingly, also opposing effects of manure DOM on the sorption of PhACs have been reported [69, 70].

5. Conclusions

Batch experiments with selected pure chemicals serving as model compounds for manure constituents successfully demonstrated the differential effects on the mobility and sorption of PhACs in soil. It became clear that it is not the effects on soil properties, i.e., organic matter content and pH, but rather the specific interactions of these individual model compounds with sorption sites in the soil that in turn create new sorption sites for the PhACs or, less often found in this study, hinder the sorption of the PhACs. Manure, sewage sludge and wastewater, however, are complex mixtures of myriads of different individual compounds. Additive, synergistic or antagonistic interactions of these components with respect to soil sorption must be anticipated. Therefore, the effects of manure on soil sorption of PhACs or even other agrochemicals are likely to be more complex than these initial experiments to elucidate the effects of different material components of manure have been able to show. Further research is needed on this. The results of this study using dissolved model compounds differ from the findings on the predominantly mobilizing effect of manure-derived DOM on the sorption of PhACs in soil. In contrast, this study found a predominantly immobilizing effect of most of the model compounds tested. It is suggested that it is the colloidal fractions of DOM, rather than the dissolved individual chemical constituents of DOM, that are relevant to this divergent effect on soil sorption of PhACs.

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