Effects of Soil/Solution Ratios and Cation Types on Adsorption and Desorption of Tetracycline in Soils

Yanyu Bao
    Key Lab. of Pollution Processes and
        Environmental Criteria
    Tianjin Key Lab. of Environmental
    Remediation and Pollution Control
    College of Environmental Science
        and Engineering
    Nankai Univ.
    Tianjin 300071, China

Qixing Zhou*
    Key Lab. of Pollution Processes
        and Environmental Criteria
    Tianjin Key Lab. of Environmental
    Remediation and Pollution Control
    College of Environmental Science
        and Engineering
    Nankai Univ.
    Tianjin 300071, China

    and
    Key Lab. of Terrestrial Ecological Process
    Inst. of Applied Ecology
    Chinese Academy of Sciences
    Shenyang 110016, China

Ying Wan
Qiang Yu
Xiujie Xie
    Key Lab. of Pollution Processes
        and Environmental Criteria
    Tianjin Key Lab. of Environmental
    Remediation and Pollution Control
    College of Environmental Science
        and Engineering
    Nankai Univ.
    Tianjin 300071, China

The adsorption and desorption behavior of tetracycline on an Alfisol and an Ultisol was investigated at varying soil/solution ratios (1:10, 1:50, and 1:100) and different cation types ($CaCl_2$, KCl, and NaCl) and H_2O using a batch equilibration experiment. Kinetic studies revealed that the adsorption of tetracycline on the two soils was rate limited, and the adsorption kinetics could be well described by the Elovich equation and exponent equation. Changes in the soil/solution ratio affected the relative equilibration time in the Ultisol, but there was no effect of the initial tetracycline concentration. The adsorption rate in the soils decreased as the soil/solution ratio increased from 1:50 to 1:10. The adsorption and desorption data on tetracycline in the two soils could be described by the Freundlich isotherm equation, with r values >0.952 under all experimental treatments. The Ultisol always had stronger adsorption (higher distribution coefficient [K_d] value) than the Alfisol under all experimental conditions. The K_d values decreased with increasing soil/solution ratios in the two tested soils at 1 or 1.25 mg L^{-1} of tetracycline; however, this trend did not occur at 25 mg L^{-1} of tetracycline. The adsorption isotherm had an S-type curve that changed into an L-type curve with decreasing soil/solution ratios from 1:10 to 1:100. The presence of cations in the soil solution, especially $CaCl_2$, can significantly reduce the adsorption of tetracycline in soils. The presence of cations also significantly reduced the nonlinearity of the adsorption isotherms.

Abbreviations: CEC, cation exchange capacity; HI, hysteresis index; HPLC, high-performance liquid chromatography.

Tetracycline has been extensively used as a human and veterinary antibiotic and as an animal growth promoter (Blackwell et al., 2007; Kim et al., 2007; Sanderson et al., 2005). Tetracycline antibiotics are currently ranked second in terms of antibiotic production and use worldwide, and in first place in China (Gu and Karthikeyan, 2005). It has been reported that 25 to 75% (Kulshrestha et al., 2004) or even 70 to 90% (Jjemba, 2002; Halling-Sørensen, 2000) of tetracyclines administered to animals are excreted in an antimicrobially active form in urine or feces. It is well known that animal manure is a low-cost fertilizer for agricultural use in soils; however, this will cause ecotoxicological concern due to tetracycline contamination (Bao et al., 2009a; Kay et al., 2005; Loke et al., 2002), which will become more serious with frequent application of waste discharges containing high-tetracycline residues. At present, tetracyclines have been detected in surface soils with concentration from micrograms to milligrams per kilogram (Aga et al., 2006; Hamscher et al., 2000). For example, tetracycline was detected in topsoil at a high concentration (20 mg kg^{-1} soil; Hamscher et al., 2000). For this reason, the fate and behavior of antibiotics, as an emerging important pollutant in the soil environment, are a growing concern due to their ecological risk (Lin et al., 2008; Batt et al., 2007).

Tetracycline may be transported from soil surfaces through mineral horizons to the aquifer, causing surface and groundwater contamination (Sarmah et al., 2006). Adsorption and desorption are major processes influencing various behaviors of antibiotics in the soil, for example, bioavailability, transport, and mobility in the soil environment. There is a need to understand the interactions of tetracycline with soils

Table 1. Selected soil properties†.

Soil	pH	SOM	CEC	Extractable Ca²⁺, K⁺ and Na⁺	Fe and Al oxides	Texture		
						Clay (<0.001 mm)	Silt (0.001–0.01 mm)	Sand (>0.01 mm)
				——— cmol kg⁻¹ ———	g kg⁻¹	——————— % ———————		
Alfisol	6.23	3.86	19.88	3.13, 1.18, 1.97	61.09	48.90	36.20	14.90
Ultisol	5.52	1.06	13.69	1.23, 0.52, 0.49	499.8	25.60	45.20	29.20

† SOM, soil organic matter; CEC, cation exchange capacity.

so that robust adsorption and desorption predictors can be developed for tetracycline. Bioassay studies have revealed that antibiotics lose their antibacterial activity when they are adsorbed on soils (Koschorreck et al., 2002). Adsorption of tetracycline could be affected by a number of environmental conditions including soil/solution ratios, cations in solution, and the soil's exchange capacity. Thus, quantitative analysis of the impact of these factors on tetracycline adsorption in soils is an important strategy for understanding and predicting the fate of tetracycline in soils. Compared with other well-known xenobiotics such as pesticides, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls (Zhou, 1995; Zhou et al., 2006, 2008), however, there is very limited information available on the fate and transformation of antibiotics in soil–water environments (Gu and Karthikeyan, 2005). As one of the most important antibiotics, tetracycline adsorption in soils is more complicated than other xenobiotics due to its physiochemical properties.

Several investigations had shown that there is a high adsorption of tetracycline by reference soil components, such as clays (Kulshrestha et al., 2004) and hydrous oxides (Gu and Karthikeyan, 2005). Several investigations also revealed the adsorption and desorption characteristics of tetracycline and the effect of different conditions. These investigations showed that the adsorption of antibiotics is dependent on the pH and cation exchange capacity (CEC) of the soil (Sassman and Lee, 2005). Furthermore, different soil components such as Al and Fe hydrous oxides (Gu and Karthikeyan, 2005), clay (Kulshrestha et al., 2004), and organic matter (Kulshrestha et al., 2004; Gu et al., 2007) in soils could strongly adsorb tetracycline. The effect of cations was mentioned in Mackay and Canterbury (2005), Sassman and Lee (2005), Ter Laak et al. (2006), Martin (1979), and Porubcan et al. (1978); however, the effect of different cations in different soils was not fully elucidated. The soil/solution ratio is also an important environmental condition in soils, and relevant investigations are scarce. Accordingly, the objectives of the present investigation were to: (i) determine tetracycline adsorption kinetics; and (ii) investigate the influence of the soil/solution ratio and cation types on the adsorption and desorption of tetracycline.

MATERIALS AND METHODS
Tetracycline

Tetracycline hydrochloride (99% purity, analytical grade) was bought from Sigma Chemical Co. (St. Louis, MO) and the standard reference material (99.4% purity) was obtained from the Institute of Veterinary Drug Control (Beijing, China). The materials were stored at 4°C.

The molecular structure of tetracycline contains tricarbonyl-amide, phenolic diketone, and dimethylamine groups that contribute to a marked pH-dependent speciation impacting aqueous solubility and lipophilicity. Tetracycline has three values of the negative logarithm of the acid dissociation constant, pK_a (3.30, 7.68, and 9.69), and can thus occur as a cationic, zwitterionic, and anionic species under acidic, moderately acidic to neutral, and alkaline conditions, respectively (Gu and Karthikeyan, 2005). Therefore, in the pH regime of environmental interest (pH 4.0–8.0), tetracycline would be dominated by zwitterionic species, especially at pH 5.5 (Sarmah et al., 2006).

Soil Samples

Two surface (0–20 cm) soils, an Alfisol and an Ultisol, were used. The Alfisol samples were collected from a forest park in the Tianjin Economic-Technological Development Area in northern China. Its clay mineral was hydromica, and it was a forest soil. The Ultisol samples were collected in the suburb of Guilin, Guangxi Municipality, in southwestern China. Its clay mineral was kaolinite, and it was a virgin soil. The two soils were analyzed for tetracycline by high-performance liquid chromatography (HPLC) before this experiment, and no tetracycline residue was found from either addition to the environment or formation by organisms in the environment. The samples were air dried, gently crushed to pass through a 2-mm sieve, thoroughly mixed, and stored in closed containers at room temperature before use. Selected properties of the soils are listed in Table 1. The pH values in H_2O were determined at a 1:2.5 (w/w) soil/water ratio using a combination electrode. Soil organic matter was determined by oxidation with $K_2Cr_2O_7$ and titration of the excess dichromate with $(NH_4)_2FeSO_4$. The CEC was determined using 1 mol L⁻¹ NH_4OAc. Extractable Ca²⁺, K⁺, and Na⁺ (cmol kg⁻¹) contents were determined using water at a 1:2.5 (w/w) soil/water ratio. Texture was determined by the pipette method (Gee and Bauder, 1986). Iron and Al oxides were determined by citrate–bicarbonate–dithionite extraction.

Adsorption Kinetics

To investigate the interaction of tetracycline with the soils, the rate of tetracycline adsorption vs. time was measured in darkness at 25 ± 1°C for 24 h. The initial concentration of tetracycline was 25.0 mg L⁻¹ and the suspension was kept at constant pH (7.0) and an ionic strength of 0.01 mol L⁻¹ $CaCl_2$. About 0.5 g (±0.0001 g) of air-dried soil was mixed with 20 mL of 0.01 mol L⁻¹ $CaCl_2$ and 5 mL of tetracycline solution in a 50-mL glass test tube and agitated in a rotary shaker for 24 h. We added azide (0.01 mol L⁻¹ NaN_3) to prevent biotic transformation of tetracycline during the adsorption kinetic and batch equilibration experiments. Samples were withdrawn at specified time intervals (3, 8, 15, 30, and 45 min, and 1, 2.5, 5, 7, 11, 18, and 24 h) and centrifuged immediately at 2500 × g for 20 min. The concentration of tetracycline

in the supernatant was directly measured by HPLC. All experiments were performed in triplicate. The quantity of tetracycline adsorbed by the soils was calculated by subtraction.

Similar tests were performed for a 12.5 mg L^{-1} initial concentration of tetracycline and a soil/solution ratio of 1:10. All experiments were also conducted in triplicate.

Adsorption and Desorption Experiments

Adsorption and desorption experiments were performed according to a standard batch equilibration method (Organization for Economic Co-operation and Development, 2000). The glass centrifuge tubes were covered with aluminum foil to prevent exposure to light. Adsorption experiments included samples, blanks, and controls. For samples, 0.5 g of soil sample was placed in a 25-mL glass test tube containing 5 mL of 0.01 mol L^{-1} CaCl$_2$ with different tetracycline concentrations (1 or 1.25, 2.5, 5, 12.5, and 25 mg L^{-1}) and 20 mL of CaCl$_2$ solution. Blanks contained 25 mL of CaCl$_2$ solution and 0.5 g of each soil, and the control contained 20 mL of CaCl$_2$ solution and 5 mL of tetracycline solution without any soil. The suspension was first shaken at 200 rpm at 25 ± 1°C for 24 h and then centrifuged at 2500 × g (25 ± 1°C) for 10 min. Aliquots including the bandwidth of supernatant volumes were removed, and the concentration of tetracycline in the supernatant was measured by HPLC by calculating the amount of tetracycline sorbed by the soils. Desorption experiments were performed immediately after the adsorption experiments. Shaking and separation of the soils and the aqueous phase were conducted as described above after the addition of fresh CaCl$_2$ solution (0.01 mol L^{-1}). Before analysis, the pH of each sample solution was adjusted to 2.0 to 3.0 with 6 mol L^{-1} HCl solution to minimize degradation and epimerization of tetracycline and adsorption to silanol groups that might be present on the glassware (Sassman and Lee, 2005).

The adsorption and desorption isotherms of tetracycline in different soil/solution ratios (1:10, 1:50 and 1:100) and different cations (CaCl$_2$, KCl, and NaCl) and H$_2$O were obtained using the same procedure. All experiments were conducted in triplicate.

Analytical Methods

Supernatant solutions were filtered through a syringe filter of 0.45-μm pore size (polypropylene; Tianjin Mei Varitek Co. Ltd., Tianjin, China). Then the concentration of tetracycline was measured by reverse-phase HPLC on a Waters ODS-C18 column (5 μm, 46 by 255 mm; Waters Corp., Milford, MA) with ultraviolet detection at 360 nm. The mobile phase was a mixture of 0.01 mol L^{-1} oxalic acid and acetonitrile (75:25 v/v) at a flow rate of 1.0 mL min^{-1}. The retention time for tetracycline was 4.56 min. The limit of detection for tetracycline was 0.01 mg L^{-1} (Bao et al., 2009b) and the limit of quantification was 0.018 mg L^{-1}.

Data Evaluation and Processing

The adsorption rate of tetracycline (mg kg^{-1} h^{-1}) was calculated as

$$\text{Adsorption rate} = \frac{\text{amount adsorbed in soil}(\text{mg kg}^{-1})}{\text{time}(\text{h})}$$

Sampling times were selected as 0 to 0.25, 0.25 to 1, 1 to 5, 5 to 11 and 11 to 24 h. Laboratory adsorption isotherm data were estimated using the linearized Freundlich equation:

$$\log C_s = \log K_f + \frac{1}{n}\log C_e \qquad [1]$$

where C_s is the adsorbed concentration (mg kg^{-1}), K_f is the Freundlich adsorption coefficient, $1/n$ is the Freundlich exponent, and C_e is the concentration in the solution phase (mg L^{-1}). A similar procedure was followed for the desorption isotherms.

Models describing the adsorption of tetracycline on soils are necessary and useful tools. The Elovich equation, exponential equation, first-order equation, and diffusion equation are often used to simulate the chemical kinetics process of compound adsorption:

Elovich equation:

$$y = a + b\ln t \qquad [2]$$

Exponential equation:

$$y = at^b \qquad [3]$$

First-order equation:

$$y = a\left[1 - \exp(-bt)\right] \qquad [4]$$

Diffusion equation:

$$y = a + bt^{1/2} \qquad [5]$$

where t is time (h), y is the concentration of tetracycline absorbed (mg kg^{-1}), and a and b are the model constants.

The hysteresis index (HI) was calculated as the ratio of the Freundlich desorption and adsorption exponents (Barriuso et al., 1994):

$$\text{HI} = \frac{1/n \text{ desorption}}{1/n \text{ adsorption}} \qquad [6]$$

Significant difference tests at P = 0.05 or 0.01 were performed by ANOVA using SPSS 13.0 for Windows (SPSS Inc., Chicago). Adsorption and desorption data were fitted by SigmaPlot 8.02 (Systat Software, San Jose, CA).

RESULTS AND DISCUSSION
Adsorption Kinetics

To evaluate adsorption affinity, adsorption kinetic studies were performed for different tetracycline concentrations (12.5 and 25 mg L^{-1}) and soil/solution ratios (1:50 and 1:10) at 298 K and pH 7.0 (Fig. 1). The adsorption kinetics exhibited two distinct stages: very rapid adsorption (<1 h) at the initial stage, followed by relatively slow adsorption, which is an indication of nonequilibrium adsorption for tetracycline on these soils. Kulshrestha et al. (2004) observed similar results for oxytetracycline adsorption on clay. The time to reach relative equilibrium was slightly longer (5 h) at the low tetracycline concentration (12.5 mg L^{-1}) than at the high concentration (25 mg L^{-1}; 2.5 h) for a soil/solution ratio of 1:50 for both soils. The relative equilibrium time was longer for a soil/solution ratio of 1:10 (11 h), as shown in Fig. 1c, than for a ratio of 1:50 (5 h), as shown in
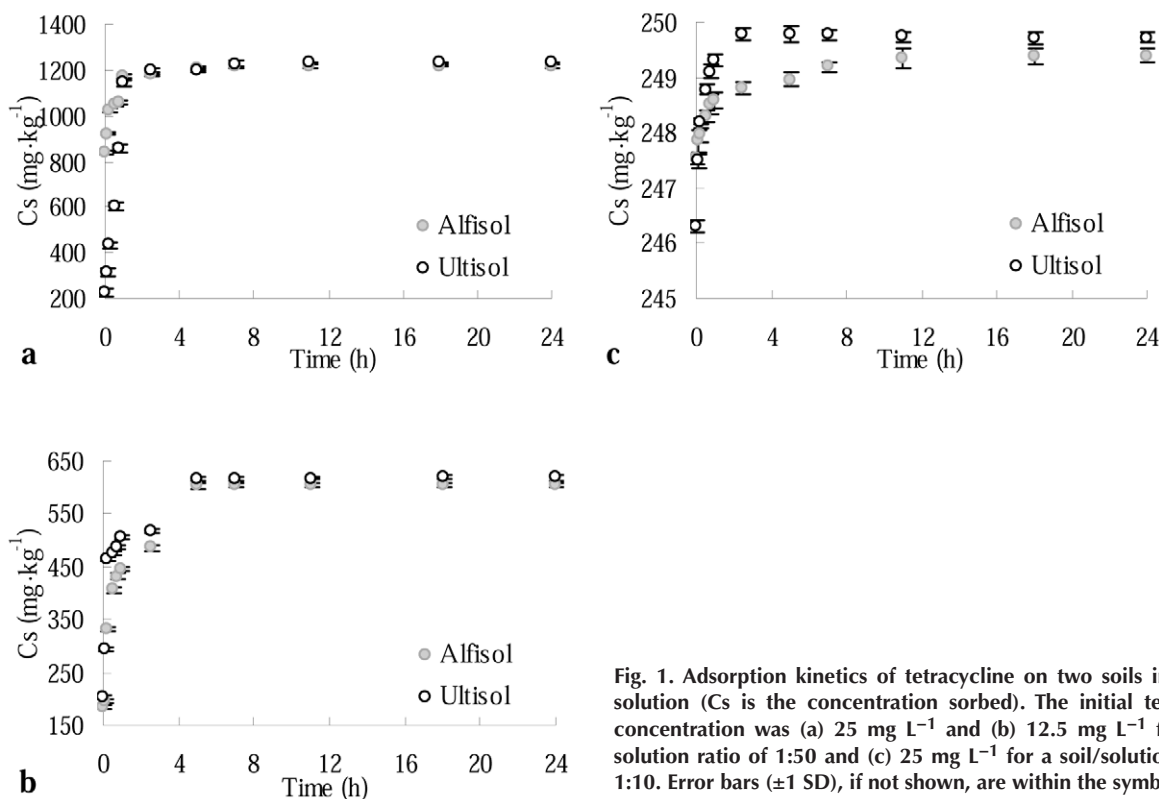
Fig. 1. Adsorption kinetics of tetracycline on two soils in a CaCl$_2$ solution (Cs is the concentration sorbed). The initial tetracycline concentration was (a) 25 mg L^{-1} and (b) 12.5 mg L^{-1} for a soil/solution ratio of 1:50 and (c) 25 mg L^{-1} for a soil/solution ratio of 1:10. Error bars (±1 SD), if not shown, are within the symbols.

Fig. 1a, for a tetracycline concentration of 25 mg L^{-1} in the Ultisol. The soil/solution ratio had almost no effect, however, on the relative equilibrium time in the Alfisol.

Prediction of the adsorption rate provides important information about antibiotic availability in the liquid phase. The results in Table 2 indicate that the adsorption rate, calculated using Eq. [1], was the highest in the first 0.25 h of the whole experiment and decreased thereafter with time. The kinetic studies revealed that the adsorption rate decreased with time, which shows that the adsorption of tetracycline on the two soils was rate limited (Bao et al., 2009b). The adsorption kinetics of tetracycline could be affected by its initial concentration and soil/solution ratio, so the adsorption rate was calculated to investigate their effect (Table 2). When the concentration of tetracycline was decreased from 25 to 12.5 mg L^{-1}, the adsorption rate decreased significantly ($P < 0.01$) within 0.25 h in the Alfisol but slightly increased in the Ultisol. The adsorption rate decreased significantly with an increase in the soil/solution ratio in both soils.

Because there was only a minor difference between the amount of tetracycline adsorbed after 5 (or 11) and 24 h, 24 h was considered to be a sufficient equilibration time, which was consistent with Organization for Economic Co-operation and Development guidelines. The results also showed that >97% of the tetracycline was adsorbed on the soils when the adsorption equilibrium was reached.

The four models describing the adsorption of tetracycline in the two soils with different initial tetracycline concentrations and soil/solution ratios are listed in Table 3. We observed that the four equations can all simulate the kinetic process between C_s and time (h) in the two tested soils because all correlation coefficients reached a significance level of 0.05. Usually, the more optimal the model is, the higher the r value is. Thus the fitting equations were in the sequence Elovich equation > exponential equation > first-order equation > diffusion equation, according to the order of average r values from 0.803 to 0.942. The Elovich equation and the exponential equation were the best fitted kinetics describing C_s changes with time in the two soils because all r

Table 2. Tetracycline adsorption rate of two soils under different conditions and at different sampling times.

Soil	Soil/solution ratio	Tetracycline conc.	Tetracycline adsorption rate				
			0–0.25 h	0.25–1 h	1–5 h	5–11 h	11–24 h
		mg L^{-1}			mg kg^{-1} h^{-1}		
	1:50	25.0	4099 ± 34.0†	200 ± 23.6	9.04 ± 4.33	0.79 ± 2.38	0.08 ± 0.89
Alfisol	1:50	12.5	1328 ± 14.2	152 ± 10.5	39.5 ± 2.70	0.10 ± 1.84	0.07 ± 0.62
	1:10	25.0	992 ± 0.60	0.82 ± 0.40	0.01 ± 0.07	0.07 ± 0.05	0 ± 0.02
	1:50	25.0	1736 ± 59.6	944 ± 40.9	13.6 ± 6.48	5.56 ± 2.87	0 ± 1.07
Ultisol	1:50	12.5	1856 ± 11.9	55.9 ± 8.55	27.5 ± 1.50	0.21 ± 0.85	0.14 ± 0.60
	1:10	25.0	993 ± 0.32	1.50 ± 0.25	0.12 ± 0.07	0 ± 0.04	0 ± 0.02

† Mean ± standard error.

**Table 3. Parameters for kinetic models of tetracycline adsorption on soils.**

Soil	Soil/solution ratio	Tetracycline conc.	a	b	r	a	b	r
		mg L⁻¹						
			Elovich equation $y = a + b\ln t$			Exponential equation $y = at^b$		
Alfisol	1:50	25	1082 ± 14.07†	60.53 ± 0.711	0.937**	1077 ± 16.05	0.053 ± 0.007	0.923**
	1:50	12.5	423.6 ± 12.64	75.53 ± 6.392	0.966**	409.2 ± 19.76	0.158 ± 0.022	0.933**
	1:10	25	248.5 ± 0.022	0.316 ± 0.011	0.994**	248.5 ± 0.022	0.0013 ± 0.00	0.993**
Ultisol	1:50	25	821.5 ± 47.43	184.9 ± 23.98	0.925**	789.9 ± 70.47	0.186 ± 0.040	0.872**
	1:50	12.5	473.8 ± 15.37	63.02 ± 7.768	0.932**	465.0 ± 20.32	0.119 ± 0.020	0.897**
	1:10	25	248.7 ± 0.155	0.498 ± 0.079	0.895**	248.7 ± 0.156	0.0020 ± 0.0003	0.894**
		Avg.	–	–	0.942**	–	–	0.919**
			First-order equation $y = a[1 - \exp(-bt)]$			Diffusion equation $y = a + bt^{1/2}$		
Alfisol	1:50	25	1147 ± 29.70	21.31 ± 0.425	0.719**	977.5 ± 14.62	67.22 ± 6.352	0.777**
	1:50	12.5	570.4 ± 25.15	2.621 ± 0.489	0.923**	251.5 ± 7.957	99.42 ± 4.046	0.846**
	1:10	25	248.8 ± 0.168	106.1 ± 9.399	0.558*	248.0 ± 0.133	0.3636 ± 0.055	0.902**
Ultisol	1:50	25	1226 ± 29.22	1.763 ± 0.170	0.986**	347.4 ± 9.790	254.7 ± 5.494	0.831**
	1:50	12.5	572.5 ± 19.86	5.737 ± 1.049	0.919**	326.3 ± 8.770	82.91 ± 4.209	0.782**
	1:10	25	249.2 ± 0.233	88.95 ± 5.527	0.752**	248.1 ± 0.395	0.4804 ± 0.163	0.681*
		Avg.	–	–	0.810**	–	–	0.803**

* Correlation coefficient is significant at the 0.05 level, $r_{0.05} = 0.553$.

** Correlation coefficient is significant at the 0.01 level, $r_{0.01} = 0.684$.

† Mean ± standard error ($n = 12$).

values under different conditions reached a significance level of 0.01 ($r_{0.01} = 0.684, r_{0.05} = 0.553$). For the Elovich equation, r values were from 0.895 to 0.994, and for the exponential equation, r values were from 0.872 to 0.993. The results indicated that the adsorption kinetics of tetracycline by the two tested soils could be well described by the Elovich and exponential equations.

Adsorption and Desorption Isotherms

The adsorption isotherms of tetracycline are depicted in Fig. 2 and 3, and the fitting parameters of tetracycline adsorption and desorption using the linearized Freundlich equation are summarized in Table 4. The results demonstrated that the Freundlich equation can be fitted to the adsorption and desorption data, with r values exceeding 0.952 ($P < 0.01$), similar to the results reported by Kulshrestha et al. (2004). All adsorption isotherms were nonlin-
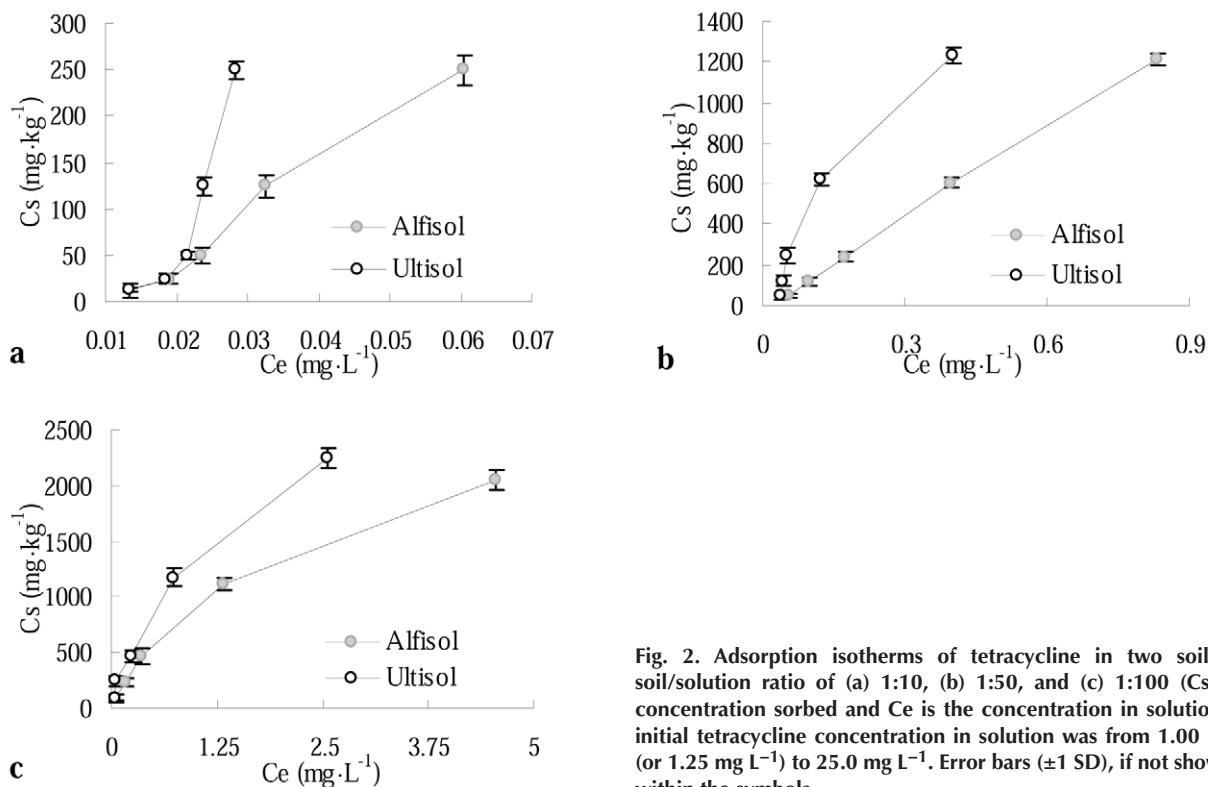
Fig. 2. Adsorption isotherms of tetracycline in two soils at a soil/solution ratio of (a) 1:10, (b) 1:50, and (c) 1:100 (Cs is the concentration sorbed and Ce is the concentration in solution). The initial tetracycline concentration in solution was from 1.00 mg L⁻¹ (or 1.25 mg L⁻¹) to 25.0 mg L⁻¹. Error bars (±1 SD), if not shown, are within the symbols.

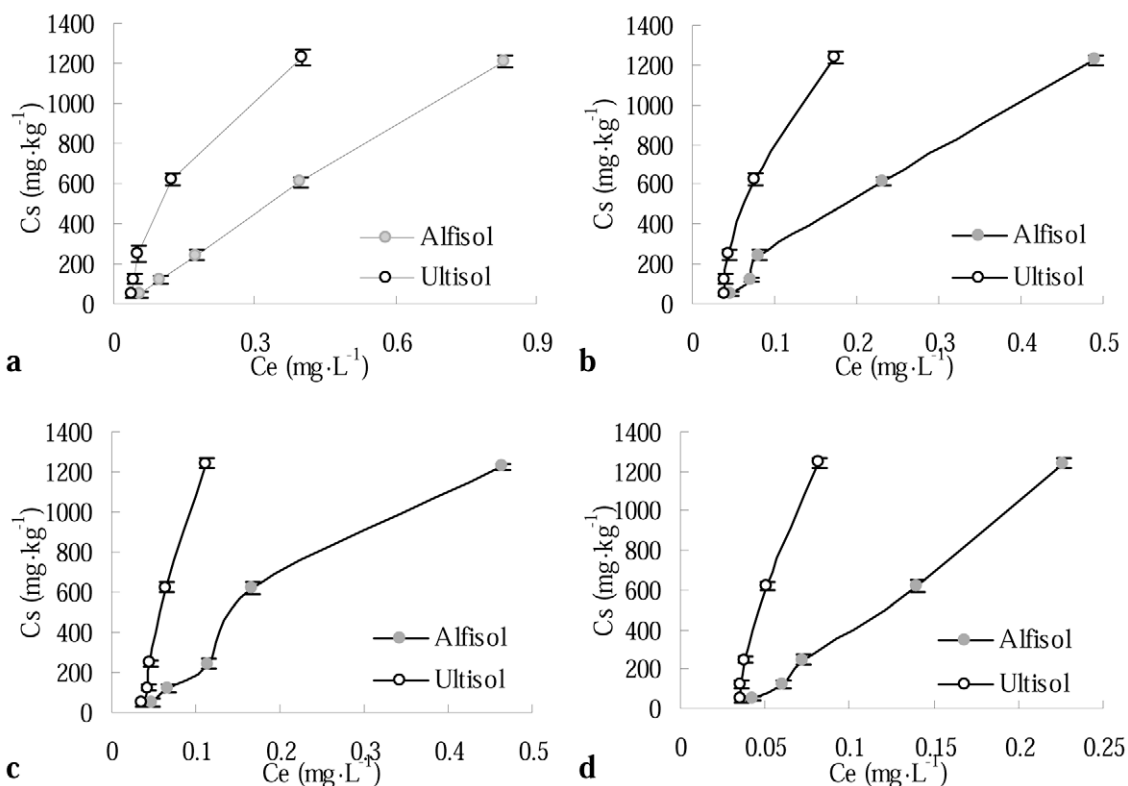**Fig. 3. Adsorption isotherms of tetracycline in two soils in a solution of (a) CaCl₂, (b) KCl, (c) NaCl, and (d) H₂O (Cs is the concentration sorbed and Ce is the concentration in solution). The initial tetracycline concentration in solution was from 1.00 to 25.0 mg L⁻¹. Error bars (±1 SD), if not shown, are within the symbols.**

ear, with $1/n$ values ranging from 0.578 to 4.805. Different parameters ($\log K_f$ and $1/n$) showed that the adsorption of tetracycline was dependent on the soil type and environmental conditions. Calvet (1989) and Singh (2002) reported that the adsorption isotherm was an S-type curve for $1/n > 1$, implying competition between water molecules and tetracycline for adsorption sites at the low concentration of tetracycline and an increasing adsorption tendency with an increase in the equilibrium concentration. An L-type isotherm was observed for $1/n < 1$, suggesting strong interaction between the soil and tetracycline molecules and a decreas-

ing adsorption tendency with an increase in the equilibrium concentration (Calvet, 1989; Singh, 2002). As shown in Fig. 2, the adsorption isotherm of an S-type curve changed into an L-type curve with decreasing soil/solution ratios from 1:10 to 1:100. The results show that the change in soil/solution ratio affected the adsorption mechanism of tetracycline. As shown in Fig. 3, the adsorption isotherms were line types, with $1/n$ from 0.986 to 1.102 with different cations, including Ca^{2+}, K^+, and Na^+, in the Alfisol because $1/n$ tended to 1.0. The adsorption isotherm was the S-type with $1/n = 1.552$ for no cations in the experimental control (H_2O).

Table 4. Parameters for the Freundlich equations (K_f, Freundlich adsorption coefficient; $K_{f,des}$, Freundlich desorption coefficient; $1/n$, Freundlich exponent; HI, hysteresis index) associated with adsorption and desorption of tetracycline in two soils for different soil/solution ratios and cations.

Soil	Soil/solution ratio	Cation	Adsorption			Desorption			HI
			$\log K_f$	$1/n$	r	$\log K_{f,\,des}$	$1/n$	r	
Alfisol	1:10	CaCl₂	4.312 ± 4.327†	1.564 ± 0.227	0.983**	4.130 ± 3.917	1.873 ± 0.271	0.984**	1.198
	1:50	CaCl₂	3.166 ± 1.598	1.102 ± 0.046	0.999**	3.259 ± 1.898	0.972 ± 0.054	0.998**	0.882
	1:100	CaCl₂	2.937 ± 1.695	0.578 ± 0.042	0.996**	3.264 ± 1.769	0.871 ± 0.058	0.997**	1.507
	1:50	KCl	3.409 ± 2.382	1.021 ± 0.091	0.995**	4.174 ± 3.607	1.483 ± 0.146	0.993**	1.452
	1:50	NaCl	3.425 ± 2.692	0.986 ± 0.164	0.978**	4.356 ± 3.777	1.621 ± 0.135	0.995**	1.644
	1:50	H₂O	4.098 ± 3.318	1.552 ± 0.101	0.997**	3.986 ± 3.381	1.337 ± 0.143	0.992**	0.861
Ultisol	1:10	CaCl₂	9.834 ± 10.80	4.805 ± 0.562	0.991**	10.43 ± 7.320	5.957 ± 2.408	0.989**	1.240
	1:50	CaCl₂	3.423 ± 2.693	0.817 ± 0.138	0.976**	3.605 ± 2.137	0.883 ± 0.198	0.955**	1.081
	1:100	CaCl₂	3.107 ± 1.761	0.612 ± 0.048	0.996**	3.486 ± 2.394	0.749 ± 0.083	0.991**	1.224
	1:50	KCl	4.040 ± 3.708	1.232 ± 0.234	0.969**	4.134 ± 3.925	1.249 ± 0.289	0.952**	1.014
	1:50	NaCl	4.773 ± 4.642	1.763 ± 0.317	0.973**	4.807 ± 3.768	1.724 ± 0.376	0.958**	0.978
	1:50	H₂O	5.561 ± 5.617	2.256 ± 0.435	0.968**	7.446 ± 6.727	3.585 ± 0.669	0.971**	1.589

** Significant at the 0.01 level; $n = 5$, $r_{0.01} = 0.874$, $r_{0.05} = 0.754$.

† Mean ± standard error.

In the Ultisol, the adsorption isotherm was the S-type with $1/n$ from 1.232 to 2.256 under K^+, Na^+, and H_2O; however, it was the L-type with $1/n = 0.817$ under Ca^{2+}. The $1/n$ values (1.552 and 2.256) in H_2O were higher than in Ca^{2+}, K^+, or Na^+ for the two soils, which shows strong competition between water molecules and tetracycline for adsorption sites when no cations were added. The results also show that adding different cations can affect the curve of the adsorption isotherms in soils and the effect is different in different soils. This conclusion indicates that the adsorption mechanism changed with the soil type and environmental conditions.

As shown in Table 5, the adsorption of tetracycline on the soils was very strong, with high K_d under all conditions. In the Alfisol, the minimum and maximum K_d values were 838.3 and 5474 L kg^{-1}, respectively. In the Ultisol, the minimum and maximum K_d values were 876.2 and 15,278 L kg^{-1}, respectively. There was a high K_d for excess adsorption sites in the soils, and K_d increased with an increase in the tested concentration (1 or 1.25–25.0 mg L^{-1}) of tetracycline in solution except for the 1:100 soil/solution ratio in the two tested soils. Higher K_d (or K_f) values from 5706 to 164,973 L kg^{-1} were also found by Sassman and Lee (2005) in three soils (Drummer-1, Toronto-4, and EPA-14) because tetracycline has multiple ionizable functional groups. There were always much higher K_d values in the Ultisol under the same environmental conditions, especially at the higher tetracycline concentration (25.0 mg L^{-1}), with a significant difference ($P < 0.01$) between the Alfisol and the Ultisol. As shown in Table 1, the soil organic matter content, CEC, and clay content in the Alfisol were high; however, there were high Al and Fe hydrous oxides in the Ultisol. This suggests that Al and Fe hydrous oxides played major roles in regulating the adsorption of tetracycline because the other environmental conditions were the same for the two soils. Gu and Karthikeyan (2005) reported that Al and Fe hydrous oxides had a great influence on the adsorption of tetracycline. Aluminum and Fe hydrous oxides could have a more important role in tetracycline adsorption than soil organic matter or CEC.

Effect of Soil/Solution Ratios on Adsorption and Desorption

From the results in Tables 4 and 5, it is clear that the soil/solution ratio exerted a strong effect on the adsorption of tetracycline by the soils. As shown in Table 5, the K_d values basically decreased with increasing soil/solution ratios in the two tested soils at the low tetracycline concentration (1 or 1.25 mg L^{-1}). It was reported by Chang and Wang (2002) that the sorbed amounts of four contaminants (Cu, Cd, butachlor, and deltamethrin) decreased with increasing soil/solution ratios at the same initial concentration, which was similar to our results. The decreasing K_d indicated that the adsorption ability of tetracycline on soil decreased. This phenomenon occurs because the higher sorbent (soil) concentration dilutes the sorbate (tetracycline) in the solid phase at higher soil/solution ratios. This trend did not occur, however, at the high tetracycline concentration (25 mg L^{-1}). The K_d value was always higher in the Ultisol than in the Alfisol for the same soil/solution

ratio. For example, the difference in K_d between the Ultisol and the Alfisol was 0.060, 453.7, and 658.0 L kg^{-1} for soil/solution ratios of 1:10, 1:50, and 1:100, respectively, at the low tetracycline concentration, indicating that K_d differences increased with a decrease in the soil/solution ratio. As shown in Table 4, the $1/n$ values decreased when the soil/solution ratio decreased from 1:10 to 1:100 in the two soils. The Freundlich exponent ($1/n$) was higher for the Ultisol than for the Alfisol at soil/solution ratios of 1:10 and 1:100, with differences of 3.241 and 0.034, respectively; however, $1/n$ was lower for the Ultisol than for the Alfisol at a soil/ solution ratio of 1:50. These results demonstrate that nonlinear adsorption was stronger for soil/solution ratios of 1:10 and 1:100 because $1/n$ was not close to unity for the two soils; however, $1/n$ was closer to unity for a soil/solution ratio of 1:50 for both soils and especially for the Alfisol.

The $logK_f$ values were higher for tetracycline desorption than for its adsorption, indicating that desorption hysteresis occurred due to irreversible adsorption of a proportion of the tetracycline molecules, so the HI was calculated in this study. In this study, desorption and adsorption experiments were performed under the same conditions, and it is highly unlikely that experimental artifacts caused adsorption–desorption hysteresis. As shown in Table 4, different degrees of hysteresis were observed for different soil/solution ratios. This hysteresis can probably be attributed to little desorption and entrapment of adsorbed tetracycline within the soil matrix and irreversible binding to specific adsorption sites, which was also found with the same phenomena for tetracycline by Pils and Laird (2007). The HI in the Alfisol was 1.198 and 0.882 at soil/solution ratios of 1:10 and 1:50, respectively, and the HI in the Ultisol was 1.240 and 1.081, respectively. The results showed that the HI was lower in the Alfisol than in the Ultisol at soil/solution ratios of 1:10 and 1:50. As shown in Table 5, the K_d values suggested that tetracycline always adsorbed more strongly in the Ultisol than in the Alfisol at the same soil/solution ratio. Our results indicated that the Ultisol exhibited maximum adsorption and minimum desorption at soil/solution ratios of 1:10 and 1:50 and also that the Ultisol might pose a lower risk of tetracycline remobilization than the Alfisol for these soil/solution ratios.

Table 5. Adsorption distribution coefficients (K_d) for tetracycline in the tested soils.

Soil	Soil/solution ratio	Cation	Tetracycline conc.	K_d	Tetracycline conc.	K_d
			mg L^{-1}	L kg^{-1}	mg L^{-1}	L kg^{-1}
Alfisol	1:10	$CaCl_2$	1.25	931.3	25.0	4,129
	1:50	$CaCl_2$	1	838.3	25.0	1,450
	1:100	$CaCl_2$	1	1813	25.0	450.3
	1:50	KCl	1	1020	25.0	2,500
	1:50	NaCl	1	1013	25.0	2,636
	1:50	H_2O	1	1123	25.0	5,474
Ultisol	1:10	$CaCl_2$	1.25	931.9	25.0	8,793
	1:50	$CaCl_2$	1	1292	25.0	3,060
	1:100	$CaCl_2$	1	2471	25.0	876.2
	1:50	KCl	1	1270	25.0	7,153
	1:50	NaCl	1	1335	25.0	11,010
	1:50	H_2O	1	1335	25.0	15,278

The HI was at its minimum at a soil/solution ratio of 1:50 for the soils, indicating that a ratio higher or lower than 1:50 might slow down desorption of tetracycline from soils.

Effect of Cation Types on Adsorption and Desorption

The presence of different cations could alter the soil–solution distribution of tetracycline by competing for charge sites on soils. Sassman and Lee (2005) reported that the occurrence of Ca^{2+} and K^+ in soil solution caused different K_d values when the concentration of tetracycline was the same but they did not discuss the effect of different cations on the desorption of tetracycline, so we investigated the role played by different cations and H_2O. In our experiment, Ca^{2+}, K^+, and Na^+ were used as buffer cations and the results were compared with those obtained for no cation in the experimental control (H_2O). As shown in Table 5, the K_d values increased in the order $CaCl_2$ < KCl < NaCl < H_2O for the two soils when the concentration of tetracycline was 25 mg L^{-1}. When the concentration of tetracycline was 1 mg L^{-1}, the K_d values increased in the order $CaCl_2$ < NaCl < KCl < H_2O in the Alfisol; however, the sequence was KCl < $CaCl_2$ < NaCl = H_2O in the Ultisol. It has been reported that tetracycline forms strong 2:1 metal–ligand complexes with multivalent cations (Wessels et al., 1998; Schmitt and Schneider, 2000; Gu and Karthikeyan, 2005). In our study, divalent Ca^{2+} and monovalent K^+ and Na^+ were used. It can be predicted that the complexation of tetracycline would be stronger with Ca^{2+} than with K^+ or Na^+, in agreement with the experimental results except for the Ultisol at 1 mg L^{-1} (Table 5). As shown in Table 5, the K_d values for Ca^{2+}, K^+, Na^+, and H_2O were 1292, 1270, 1335, and 1335 L kg^{-1}, respectively, which shows that the K_d values differ nonsignificantly ($P > 0.05$). Previous researchers also reported strong complexation of tetracycline with Ca^{2+} (Gu and Karthikeyan, 2005). Adsorption was the greatest in the absence of a cation, also indicating that cations can significantly reduce the adsorption of tetracycline on soils. The decrease in the adsorption of ionizable tetracycline following the addition of cations in solution might be attributable to several factors, such as the formation of neutral ion pairs, screening of negative charges on the soil surface, a decrease in tetracycline solubility due to the salting effect, or inhibition of the release of organic matter. As shown in Table 4, the nonlinearity exponent ($1/n$) increased in the order NaCl < KCl < $CaCl_2$ < H_2O in the Alfisol, but the order was $CaCl_2$ < KCl < NaCl < H_2O for the Ultisol. The value of $1/n$ was close to unity, ranging from 0.986 to 1.102 for the three cations in the Alfisol, and $1/n$ was 0.817 and 1.232 in $CaCl_2$ and KCl, respectively, for the Ultisol. So the adsorption isotherms were line types under different cations including Ca^{2+}, K^+ and Na^+ in the two soils. The $1/n$ value was the maximum in H_2O, with values of 1.552 and 2.256 for the Alfisol and the Ultisol, respectively, confirming that the presence of cations can significantly reduce adsorption nonlinearity.

The HI increased in the order H_2O < $CaCl_2$ < KCl < NaCl in the Alfisol, but the order for the Ultisol was NaCl < KCl < $CaCl_2$ < H_2O (Table 4). These results could be caused by different soil properties in the two soils. The K_d values suggest that tetracycline was adsorbed more strongly by the Ultisol (Table 5), and the HI was also the greatest in H_2O solution (Table 4). This indicates that the maximum adsorption and minimum desorption occurred for the Ultisol in a H_2O solution. There was little risk of tetracycline remobilization in the Ultisol in the H_2O solution but this was not true for the Alfisol.

CONCLUSIONS

The adsorption kinetics of tetracycline on the two tested soils could be well described by the Elovich equation and the exponential equation. High K_d values and a low HI in this study indicated strong adsorption of tetracycline on the tested soils; however, the adsorption behavior of tetracycline was considerably altered by a change in soil types or environmental conditions. The Ultisol had stronger adsorption than the Alfisol under all conditions. The $1/n$ values decreased with an increase in the soil/solution ratio. Cations significantly decreased the adsorption of tetracycline and K_d increased in the order $CaCl_2$ < KCl < NaCl < H_2O in the two soils when the initial concentration of tetracycline was 25 mg L^{-1}. There was little risk of tetracycline remobilization in the Ultisol in a H_2O solution because of strong adsorption and a hysteresis effect. A change in the soil/solution ratio or cation in soil solution could alter the adsorption and desorption behavior of tetracycline. These conditions should be taken into consideration when determining how to affect the environmental behavior of tetracycline in a real soil environment.

REFERENCES

Aga, D.S., S. O'Connor, S. Ensley, J.O. Payero, D. Snow, and D. Tarkalson. 2006. Determination of the persistence of tetracycline antibiotics and their degradates in manure- amended soil using enzyme-linked immunosorbent assay and liquid chromatography–mass spectrometry. J. Agric. Food Chem. 53:7165–7171.

Bao, Y.Y., Q.X. Zhou, L.Z. Guan, and Y.Y. Wang. 2009a. Depletion of chlortetracycline during composting of aged and spiked manures. Waste Manage. 29:1416–1423.

Bao, Y.Y., Q.X. Zhou, and Y.Y. Wang. 2009b. Adsorption characteristics of tetracycline by two soils: Assessing role of soil organic matter. Aust. J. Soil Res. 47:286–295.

Barriuso, E., A. Laird, W.C. Koskinen, and R.H. Dowdy. 1994. Atrazine desorption from smectites. Soil Sci. Soc. Am. J. 58:1632–1638.

Batt, A.L., S. Kim, and D.S. Aga. 2007. Comparison of the occurrence of antibiotics in four full-scale wastewater treatment plants with varying designs and operations. Chemosphere 68:428–435.

Blackwell, P.A., P. Kay, and A.B.A. Boxall. 2007. The dissipation and transport of veterinary antibiotics in a sandy loam soil. Chemosphere 67:292–299.

Calvet, R. 1989. Adsorption of organic chemicals in soil. Environ. Health Perspect. 83:145–177.

Chang, T.W., and M.K. Wang. 2002. Assessment of sorbent/water ratio effect on adsorption using dimensional analysis and batch experiments. Chemosphere 48:419–426.

Gee, G.W., and J.W. Bauder. 1986. Particle-size analysis. p. 383–412. *In* A. Klute (ed.) Methods of soil analysis. Part 1. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

Gu, C., and K.G. Karthikeyan. 2005. Interaction of tetracycline with aluminum and iron hydrous oxides. Environ. Sci. Technol. 39:2660–2667.

Gu, C., K.G. Karthikeyan, S.D. Sibley, and J.A. Pedersen. 2007. Complexation of the antibiotic tetracycline with humic acid. Chemosphere 66:1494–1501.

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

Hamscher, G., S. Sczesny, A. Abu-Qare, H. Höper, and H. Nau. 2000. Substances with pharmacological effects including hormonally active substances in the environment: Identification of tetracyclines in soil fertilized with animal slurry. Dtsch. Tierarztl. Wochenschr. 107:332–334.

Jjemba, P.K. 2002. The potential impact of veterinary and human therapeutic agents in manure and biosolids on plants grown on arable land: A review. Agric. Ecosyst. Environ. 1918:1–12.

Kay, P., P.A. Blackwell, and A.B.A. Boxall. 2005. Column studies to investigate the fate of veterinary antibiotics in clay soils following slurry application to agricultural land. Chemosphere 60:497–507.

Kim, S., J.N. Jensen, D.S. Aga, and A.S. Weber. 2007. Tetracycline as a selector for resistant bacteria in activated sludge. Chemosphere 66:1643–1651.

Koschorreck, J., C. Koch, and I. Ronnefahrt. 2002. Environmental risk assessment of veterinary medicinal products in the EU: A regulatory perspective. Toxicol. Lett. 131:117–124.

Kulshrestha, P., R.F. Giese, and D.S. Aga. 2004. Investigating the molecular interactions of oxytetracycline in clay and organic matter: Insights on factors affecting its mobility in soil. Environ. Sci. Technol. 38:4097–4105.

Lin, A.Y.C., T.H. Yu, and C.F. Lin. 2008. Pharmaceutical contamination in residential, industrial, and agricultural waste streams: Risk to aqueous environments in Taiwan. Chemosphere 74:131–141.

Loke, M.L., J. Tjørnelund, and B. Halling-Sørensen. 2002. Determination of the distribution coefficient ($\log K_d$) of oxytetracycline, tylosin A, olaquindox and metronidazole in manure. Chemosphere 48:351–361.

Mackay, A.A., and B. Canterbury. 2005. Oxytetracycline sorption to organic matter by metal-bridging. J. Environ. Qual. 34:1964–1971.

Martin, S.R. 1979. Equilibrium and kinetic studies on the interaction of tetracyclines with calcium and magnesium. Biophys. Chem. 10:319–326.

Organization for Economic Co-operation and Development. 2000. Adsorption/desorption using a batch equilibrium method. Method no. 106. *In* OECD guidelines for the testing of chemicals. OECD Publ., Paris.

Pils, J.R.V., and D.A. Laird. 2007. Sorption of tetracycline and chlortetracycline on K- and Ca-saturated soil clays, humic substances, and clay–humic complexes. Environ. Sci. Technol. 41:1928–1933.

Porubcan, L.S., C.J. Serna, J.L. White, and S.L. Hem. 1978. Mechanism of adsorption of clindamycin and tetracycline by montmorillonite. J. Pharm. Sci. 67:1081–1087.

Sanderson, H., F. Ingerslev, R.A. Brain, B. Halling-Sørensen, J.K. Bestari, C.J. Wilson, D.J. Johnson, and K.R. Solomon. 2005. Dissipation of oxytetracycline, chlortetracycline, tetracycline and doxycycline using HPLC-UV and LC/MS/MS under aquatic semi-field microcosm conditions. Chemosphere 60:619–629.

Sarmah, A.K., M.T. Meyer, and A.B.A. Boxall. 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.

Sassman, S.A., and L.S. Lee. 2005. Sorption of three tetracyclines by several soils: Assessing the role of pH and cation exchange. Environ. Sci. Technol. 39:7452–7459.

Schmitt, M.O., and S. Schneider. 2000. Spectroscopic investigation of complexation between various tetracyclines and Mg^{2+} or Ca^{2+}. PhysChemComm 9:1–14.

Singh, N. 2002. Sorption behavior of triazole fungicides in Indian soils and its correlation with soil properties. J. Agric. Food Chem. 38:138–141.

Ter Laak, T.L., H. Schmitt, W.A. Gebbink, and J. Tolls. 2006. The sorption of two antimicrobial agents to 13 different soils. Environ. Toxicol. Chem. 25:933–941.

Wessels, J.M., W.E. Ford, W. Szymczak, and S. Schneider. 1998. The complexation of tetracycline and anhydrotetracycline with Mg^{2+} and Ca^{2+}: A spectroscopic study. J. Phys. Chem. B 102:9323–9331.

Zhou, Q.X. 1995. Ecology of combined pollution. China Environ. Sci. Press, Beijing.

Zhou, Q.X., M.E. Wang, and J.D. Liang. 2008. Ecological detoxification of methamidophos by earthworms in phaeozem co-contaminated with acetochlor and copper. Appl. Soil Ecol. 40:138–145.

Zhou, Q.X., Q.R. Zhang, and T.H. Sun. 2006. Technical innovation of land treatment systems for municipal wastewater in northeast China. Pedosphere 16:297–303.