Impacts of soil organic matter, pH and exogenous copper on sorption behavior of norfloxacin in three soils

ZHANG Jie[1], LI Zhaojun[2], GE Gaofei[1], SUN Wanchun[2],
LIANG Yongchao[1,2,3,*], WU Laosheng[4]

1. College of Natural Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing 210095, China. E-mail: j_zhang101@163.com
2. Ministry of Agriculture Key Laboratory of Crop Nutrition and Fertilization, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, China
3. Key Laboratory of Oasis Eco-Agriculture and College of Agriculture, Shihezi University, Shihezi 832003, China
4. Department of Environmental Sciences, University of California, Riverside, Riverside, CA 92521, USA

Abstract

Norfloxacin sorption and the factors (soil organic matter (SOM), pH, and exogenous copper (Cu) influencing the sorption were investigated in a black soil (soil B), a fluvo-aquic soil (soil F), and a red soil (soil R). With increasing norfloxacin concentrations, sorption amount of norfloxacin increased in both the bulk soils and their SOM-removed soils, but the sorption capacity of SOM-removed soils was higher than that of their corresponding bulk soils, indicating that the process of norfloxacin sorption in soil was influenced by the soil properties including SOM. The sorption data in all bulk soils and SOM-removed soils were fitted to Freundlich and Langmuir models. The correlation coefficients suggested that the experimental data fitted better to Freundlich equation than to Langmuir equation. Furthermore, the data from soil F and SOM-removed F could not be described by Langmuir equation. The norfloxacin sorption amount decreased in soil B and soil F, whereas it increased in soil R as solution pH increased. The maximum K_D and K_{OC} were achieved in soil R when the equilibrium solution pH was 6. The norfloxacin sorption was also influenced by the exogenous Cu^{2+}, which depended on the soil types and Cu^{2+} concentrations. With increasing Cu^{2+} concentrations in solution, generally, sorption amount, K_D and K_{OC} for norfloxacin in soils increased and were up to a peak at 100 mg/L Cu^{2+}, and then the sorption amount decreased regardless of norfloxacin levels.

Key words: copper; norfloxacin; pH; soil organic matter; sorption

DOI: 10.1016/S1001-0742(08)62318-9

Introduction

Pharmaceutical antibiotics are widely used in human and veterinary medication to treat and prevent infectious bacterial diseases (Sarmah *et al*., 2006). In a number of situations where food-producing animals are intensively reared, some antimicrobials are added to food to control disease and improve food conversion efficiency. The pharmaceutical antibiotics excreted in urine or feces from the treated body, are either unaltered or as metabolites, some of which are still bioactive (Thiele-Bruhn, 2003). The amounts excreted can be up to 90% of an administered dose of antibiotics (Drillia *et al*., 2005). As a result, the unutilized portion of the antibiotics enters into the municipal sewage treatment systems where they can be adsorbed to sludge. Consequently, the antibiotics ingredients, including their metabolites and conjugates, can reach the terrestrial environment when the sewage sludge and manure are used as agricultural fertilizers. This, therefore, makes them potentially hazardous to other organisms

found in the environment, which may potentially affect the terrestrial and aquatic ecosystems (Migliore *et al*., 1996, 2003; Sarmah *et al*., 2006). Influences of antibiotics on terrestrial and aquatic ecosystems are dependent mainly on their fates in environment, especially soil environment (Rabølle and Spiild, 2000; Sarmah *et al*., 2006).

Sorption to solid surface is an important process for deciding the transport and fate of organic chemicals, such as antibiotics, in environment (Drillia *et al*., 2005; Lorphensri *et al*., 2006). Like other organic chemicals, the sorption behavior of antibiotics is also influenced not only by their own properties, including water solubility, acid-dissociation constant (pK_a) and octanol-water partition coefficient ($K_{o/w}$) (Chiou *et al*., 1979; Singh *et al*., 1990; Drillia *et al*., 2005; Lorphensri *et al*., 2006), but also by soil properties such as quality and quantity of organic matter, type and amount of clay minerals, cation exchange capacity (CEC) and pH value. Among them, soil organic matter (SOM) plays a major role in antibiotics sorption in soil. It is suggested that SOM can provide readily accessible sites and rate-limited sites to organic chemicals,

* Corresponding author. E-mail: ycliang@caas.ac.cn

but the distribution of antibiotics between soil and aqueous medium depends on the nature and degree to which the SOM humification occurs (Pu and Cutright, 2006; Bayard *et al.*, 1998). In addition, the solution pH can also strongly influence the sorption process through changing the electric charges of the soil and clay aggregates and the charge form of antibiotics in the solution (Sarmah *et al.*, 2006; Lorphensri *et al.*, 2006).

Norfloxacin (Nor) is widely used throughout the world for the treatment of urinary tract infections and belongs to a typical class of quinolone, a group of synthetic antibacterial agents being active against various microorganisms and containing 4-oxo-1,4-dihydroquinoline skeleton (Turel, 2002). The interactions of metal ions with quinolones were observed. The mechanism of the interaction among quinolones and metal was chelation among the metal cations and the 4-oxo and adjacent carboxyl groups, which is required for antibacterial activity. It is suggested that all quiolones can interact with metal ions (Efthimiadou *et al.*, 2007). The structures of quiolone complexes with metal ions, such as Co^{2+}, Ni^{2+}, Zn^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+} and Cu^{2+}, have been reported (Sadeek, 2005; Drevenšek *et al.*, 2006; Ruíz *et al.*, 2007; Efthimiadou *et al.*, 2007). As one member of quiolones, norfloxacin can also make complexes with metal ions, especially Cu^{2+} (Wallis *et al.*, 1996; Sadeek, 2005).

Copper, an industrial product and food additive, has been widely found in manure and sludge, and might finally enter the agricultural environment. It can bond to two norfloxacin molecules bidentately through carboxylic oxygen and ring carbonyl oxygen (Turel *et al.*, 1996). Thus it can be postulated that the sorption of norfloxacin on soils is affected by the presence of Cu^{2+} in soil environment. Therefore, the present study aims to: (1) investigate the sorption behavior of norfloxacin in three different soils; (2) evaluate the effects of selected soil properties on the sorption of norfloxacin; and (3) evaluate the influence of Cu^{2+} on the sorption behavior of norfloxacin in soil.

1 Materials and methods

1.1 Antibiotics and reagents

Norfloxacin (≥98.5%, standard grade) was purchased from China Institute of Veterinary Drug Control, Beijing. The physicochemical properties of norfloxacin are shown in Table 1 (Takács-Novák *et al.*, 1990; Ross and Riley, 1994). Cupric sulfate ($CuSO_4 \cdot 5H_2O$) was provided by Beijing Chemical Reagent Corporation with a purity of > 99%. Other reagents used in the experiments were of analytical grade. All solvents were of HPLC grade.

1.2 Soils

Three unpolluted field soil samples, with different soil properties and representing the major agricultural soils in East China, were used in this study. These are black soil (Aquic Haploborolls, soil B), collected from Hailun Experimental Station in Heilongjiang Province, fluvo-aquic soil (Typic Endoaquepts, soil F), collected from Chang-

Table 1 Physicochemical properties of norfloxacin

Molecular formula	Molecular weight	Water solubility (mg/L)	$\log K_{OW}$	pK_a
$C_{16}H_{18}FN_3O_3$	319.33	161000 (pH 5)	–1.7 (pH 5)	6.22 (pK_{a1})
		400 (pH 7)	–1.0 (pH 7)	8.51 (pK_{a2})
		910 (pH 9)	–1.63 (pH 9)	

ping Experimental Station in Beijing, and red soil (Typic Hapludults, soil R), collected from Qiyang Experimental Station in Hunan Province. The properties of tested soils were determined according to the methods described by Lu (2000) and are shown in Table 2. The values of pH in H_2O or in $CaCl_2$ were measured in extracts with water or $CaCl_2$ solution, and the ratio of soil to water or $CaCl_2$ was 1/5. All bulk soil samples were collected at the surface layer (0–20 cm).

1.3 Sorption experiments

In order to study the effect of SOM on the norfloxacin sorption in soil, three "SOM-removed soils" were obtained by removing SOM from bulk soils according to the methods described in literature (Kaiser, 2000; Pu and Cutright, 2006). They were named as SOM-removed B, SOM-removed F, and SOM-removed R. In order to remove SOM in soil, weighed bulk soil (50 g) was treated with 200 mL of 50°C H_2O_2 solution (30%), followed by washing with 1000 mL deionized H_2O. The soil samples were shaken and deposited. This cycle was repeated until the electric conductivity of the solution was < 50 μS. The soil was dried in an oven at 50°C and sieved through a 60-mesh, and sterilized at 120°C for 30 min. This process removed about 90% of the organic carbon of the bulk samples and the loss of clay minerals was less than 1%. Therefore, the treated samples can be considered as "organic matter-free" samples.

All norfloxacin sorption experiments were conducted in soil-water systems. The norfloxacin sorption in bulk soils and SOM-removed soils were conducted with a series of norfloxacin concentrations. Six treatments including 1.0, 5.0, 10.0, 50.0, 100.0, and 200.0 mg/L norfloxacin, were established with three replicates each. The soil samples were air-dried and sieved through a 60-mesh, and sterilized at 120°C for 30 min. To get the norfloxacin levels, different quantities of norfloxacin were added to the 50-mL glass centrifuge tubes with caps, containing 0.20 g of soil in 30 mL of 0.01 mol/L $CaCl_2$ solution. The tubes were shaken in the dark for 48 h at 180 r/min on a gyratory shaker, since the norfloxacin sorption takes about 48 h to reach equilibrium according to our preliminary test. The $CaCl_2$ solution was used to minimize the suspension of soil particles and to simulate natural soil water despite the fact that norfloxacin can form a complex with divalent cations, such as calcium ions (Rabølle and Spiild, 2000). A blank for each soil without norfloxacin and a control without soil for each norfloxacin concentration were made. Norfloxacin and media blanks were finally found to be

Table 2 Some basic properties of test soils used in the investigation

Sample	Classification	Organic matter (%)	pH in H_2O	pH in $CaCl_2$	CEC (cmol/kg)	Mainly clay minerals
Soil B	Aquic Haploborolls	3.28	7.28	6.46	29.78	Illite, vermiculite
Soil F	Typic Endoaquepts	1.73	8.42	7.69	24.29	Illite
Soil R	Typic Hapludults	1.91	4.60	4.15	14.57	Kaolinite, ferric

CEC: cation exchange capacity.

negligible. After centrifugating at 4500 ×*g* for 10 min, the amount of supernatant was removed and analyzed for norfloxacin concentration.

The effect of solution pH on norfloxacin sorption was evaluated in the soil-water system with three different pH values. The initial pH values of solutions containing norfloxacin 5.0 or 10.0 mg/L, respectively, were controlled at 4.00, 7.00, and 10.00 by adding dilute NaOH or HCl solutions. The solution equilibrium pH values were assayed and listed in Table 3. Norfloxacin and media blanks were conducted for each pH and its effect was found to be negligible.

An experiment was carried out to evaluate the effects of Cu^{2+} on norfloxacin sorption at different Cu^{2+} concentrations. The Cu^{2+} levels were 0, 25, 50, 100, 500, and 1000 mg/L in the solutions containing norfloxacin 5.0 or 10.0 mg/L. Norfloxacin and media blanks were conducted for each Cu^{2+} concentration and the effect was found to be negligible.

1.4 Norfloxacin assay

Norfloxacin was determined by a high-performance-liquid-chromatography (HPLC, Agilent 1100, USA) system with a XDB-C_{18} column and a fluorescence detector (Golet *et al.*, 2001). Fluorescence detection at an excitation wavelength of 283 nm and an emission wavelength of 447 nm was utilized. The HPLC mobile phase consisted of 83% solution A (0.025 mol/L H_3PO_4, pH 3.0 ± 0.1 adjusted with triethylamine) and 17% solution B (100% acetonitrile). The flow rate was 0.6 mL/min. The C_{18}-column was maintained at 35°C and the solution injection volume was 20 μL.

1.5 Data analysis

The amount of norfloxacin adsorbed in the soils after equilibration (C_s) was calculated from the difference between the initial and equilibrium concentration (C_e) in the aqueous phase. The experimental data were fitted to following equations.

Table 3 Equilibrium pH in various soils with different initial pH values

Soil type	Norfloxacin (mg/L)	Equilibrium pH		
		Initial pH 4	Initial pH 7	Initial pH 10
Soil B	5.0	6.93 ± 0.07	6.69 ±0.16	7.16 ±0.11
	10.0	6.12 ± 0.10	6.93 ± 0.07	7.39 ± 0.02
Soil F	5.0	7.44 ± 0.02	6.92 ± 0.10	7.46 ± 0.05
	10.0	6.63 ± 0.08	7.24 ± 0.05	7.42 ±0.08
Soil R	5.0	3.96 ± 0.06	4.54 ± 0.06	5.72 ± 0.11
	10.0	3.40 ± 0.06	5.05 ± 0.01	6.00 ± 0.04

The Freundlich equation can be expressed as Eq. (1):

$$C_s = K_F \times C_e^n \tag{1}$$

where, K_F is Freundlich distribution coefficient and n is Freundlich exponent. For $n = 1$, Eq. (1) is converted to the linear distribution equation (Eq. (2)):

$$C_s = K_D \times C_e \tag{2}$$

where, K_D (mL/g) is the distribution coefficient. K_{OC} is the distribution coefficient normalized to the percentage of organic carbon (f_{OC}) in soil, and it was calculated according to Eq. (3):

$$K_{OC} = K_D \times 100/f_{OC} \tag{3}$$

The Langmuir equation can be expressed as Eq. (4):

$$C_s = b \times C_e \times K_{max}/(1 + b \times C_e) \tag{4}$$

where, b (L/g) is Langmuir constant and K_{max} (mg/g) is the maximum sorption capacity. Rearranging Eq. (4), it can be expressed in the linear form (Eq. (5)):

$$C_e/C_s = 1/(b \times K_{max}) + C_e/K_{max} \tag{5}$$

1.6 Statistical analysis

The experimental data of the effects of pH and Cu^{2+} on norfloxacin sorption were subjected to variance analysis using the software SigmaStat 2.03, and statistical significance of means was judged by least significance difference (LSD) at the 0.05 or 0.01 probability level.

2 Results

2.1 Sorption isotherms of norfloxacin in bulk soils

The sorption isotherms of norfloxacin in the three tested bulk soils are shown in Fig. 1. As norfloxacin concentration increased, generally, the sorption amounts of norfloxacin increased in three bulk soils. The sorption amounts at 200.0 mg/L norfloxacin were 96, 128, and 71 times as high as those with 1.0 mg/L norfloxacin for soils B, F, and R, respectively. However, differences were observed in sorption amount of norfloxacin among the three tested soils (Fig. 1). At the low norfloxacin concentrations (1.0–50.0 mg/L), the sorption amount of norfloxacin in soil B was similar to that in soil R at the same initial norfloxacin concentration. At 100.0 and 200.0 mg/L norfloxacin, however, the sorption amounts of norfloxacin in soil B were 18%
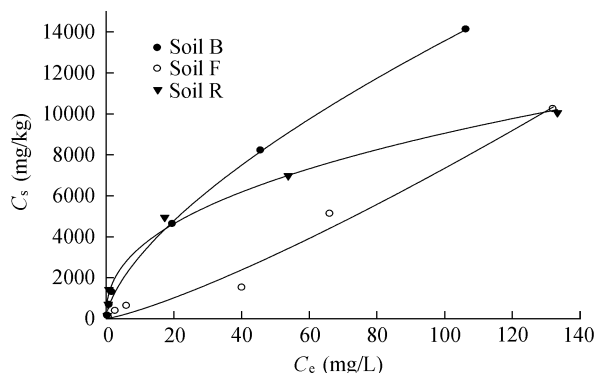
Fig. 1 Adsorbed (C_s) vs. equilibrium (C_e) concentrations of norfloxacin for bulk soils. Lines were calculated using Freundlich parameters obtained by non-linear regression.

and 40% higher than in soil R, respectively. Moreover, the sorption amounts of norfloxacin were significantly higher in soil B than in soil F in all the norfloxacin concentrations tested, in which the amounts of norfloxacin sorption in soil B were 1.4 (200.0 mg/L norfloxacin) and 3.1 (50.0 mg/L norfloxacin) times as high as those in soil F. An interesting result was found between soil F and soil R that with the increase of norfloxacin concentration, the sorption amounts of norfloxacin tended to be the same (Fig. 1). When norfloxacin concentration in solution was up to 200.0 mg/L there was a slight difference in the sorption capacity between soil R and soil F, in which the difference in sorption amount was only 178.5 mg/kg.

Freundlich equation (Eq. (1)) and Langmuir equation (Eq. (5)) were used to fit the sorption data and the parameters were listed in Table 4. The correlation coefficients (r) derived from Freundlich equation for soil B, F, and R were significant at 0.01 probability level, while those derived from Langmuir equation were very different among three soils. These results suggested that comparing with langmuir equation the Freundlich equation should be used to fit to the experimental data in the three tested soils.

2.2 Sorption of norfloxacin in SOM-removed soils

The sorption isotherms of norfloxacin in SOM-removed soils are shown in Fig. 2. The isotherms were similar to those of norfloxacin in bulk soils (Fig. 1). As norfloxacin

concentration increased, the sorption amounts of norfloxacin appeared to increase rapidly in all SOM-removed soils. When norfloxacin concentration ranged from 1.0 to 100.0 mg/L, the sorption amounts in SOM-removed F were significantly lower than those in SOM-removed B or SOM-removed R, which had similar sorption capacity. At 5.0 mg/L the sorption amounts of norfloxacin in soil SOM-removed B and SOM-removed R were 102% and 99% higher than that in soil SOM-removed F, compared to 76% and 79% at 50.0 mg/L. At 200.0 mg/L, however, the highest sorption amount was found in SOM-removed B, which was 1.7 and 1.6 times as high as that in SOM-removed F and SOM-removed R, respectively.

Again, both the Freundlich equation (Eq. (1)) and Langmuir equation (Eq. (5)) were used to fit the experimental data from the three SOM-removed soils (Table 5). The results, derived from Freundlich equation and Langmuir equation, were similar to those obtained from the experimental data in three bulk soils. The low correlation coefficient value from SOM-removed F suggests that Langmuir equation (Eq. (5)) could not be used to describe experimental data from this soil.

2.3 Effect of pH on norfloxacin sorption in bulk soils

The sorption amounts of norfloxacin in different soils at three pH values with two norfloxacin concentrations are listed in Table 6. The effects of solution pH on norfloxacin
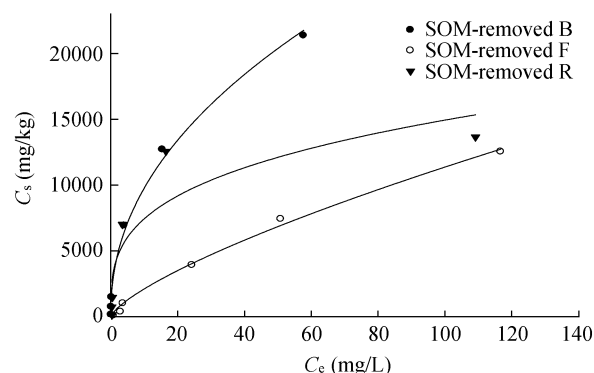

Fig. 2 Adsorbed (C_s) vs. equilibrium (C_e) concentrations of norfloxacin for SOM-removed soils. Lines were calculated using Freundlich parameters obtained by non-linear regression.

Table 4 Sorption coefficients of Freundlich and Langmuir sorption isotherms of norfloxacin in bulk soils

Soil type	Freundlich model			Langmuir model		
	K_F	n	r	K_{max} (mg/kg)	b (L/mg)	r
Soil B	503.74	0.74	0.97**	10826.16	0.03	0.93**
Soil F	118.63	0.86	0.98**	40158.95	0.00	0.25
Soil R	698.87	0.60	0.93**	9678.45	0.04	0.87*

* and ** mean significance at 0.05 and 0.01 levels, respectively.

Table 5 Sorption coefficients of Freundlich and Langmuir sorption isotherms of norfloxacin in SOM-removed soils

Soil type	Freundlich model			Langmuir model		
	K_F	n	r	K_{max} (mg/kg)	b (L/mg)	r
SOM-removed B	1833.30	0.69	0.94**	25298.36	0.09	0.96**
SOM-removed F	143.62	0.96	0.98**	65280.39	0.00	0.29
SOM-removed R	1213.00	0.73	0.86*	15484.52	0.08	0.93**

* and ** mean significance at 0.05 and 0.01 levels, respectively.

sorption were different among the tested soils. With the increase of initial solution pH, the sorption amounts decreased in soil B and soil F, while they increased in soil R regardless of norfloxacin level in the solution. The differences in the mean sorption amount of norfloxacin among the three soils were statistically significant ($P < 0.01$). Significant differences in sorption amounts of norfloxacin ($P < 0.01$) were also found among norfloxacin and initial solution pH values. There were statistically significant differences in terms of the interactive effects between any two factors (Table 6). Additionally, there was a statistically significant interaction among soil type, norfloxacin concentration, and pH level ($P < 0.01$).

Sorption at the initial norfloxacin concentration of 5.0 and 10.0 mg/L fell in the linear range of the sorption isotherm, thereby, the data could be used to calculate K_D and K_{OC} for norfloxacin at various solution pH values according to Eqs. (2) and (3). The effects of solution pH on K_D values are shown in Fig. 3. Similar to the tendency of sorption amount of norfloxacin in soils (Table 6), with increasing solution pH values, the K_D values decreased in soil B and soil F, but increased in soil R at both norfloxacin levels. In soil B, the K_D values at pH 7 and pH 10 were 10% and 37% lower than at pH 4 at 5.0 mg/L, compared to 9% and 28% at 10.0 mg/L. The same results were obtained in soil F. In soil R, however, the K_D values at pH 7 and pH 10 were 11% and 17% higher than that at pH 4 at 5.0 mg/L, compared to 2.1 times higher at 10.0 mg/L. Furthermore, at the same initial solution pH, which induced different equilibrium pH values in soil solution (Table 3), the differences in the K_D values were compared among three tested soils at both norfloxacin levels. At pH 4, the K_D values in soil F were 68% and 55% of those in soil B at 5.0 and 10.0 mg/L, compared to 45% and 48% at pH 7, and 34% and 40% at pH 10. By contrast, in soil R, the K_D values were 81%, 101%, and 153% of those in soil B at 5.0 mg/L, compared to 40%, 139%, and 174% at 10.0 mg/L (Fig. 3). Similar to K_D, K_{OC} values for norfloxacin also varied with solution pH and soil types (Fig. 4). Combined with the equilibrium pH of soil solution (Table 3), these results indicate that the equilibrium sorption trends based on equilibrium pH due to the buffering capacity of soils.

Table 6   Effects of pH on sorption amount of norfloxacin at 5.0 and 10.0 mg/L in bulk soils ($n = 54$)

Soil type	Norfloxacin (mg/L)	Sorption amount (mg/L)		
		Initial pH 4	Initial pH 7	Initial pH 10
Soil B	5.0	435.55	424.84	382.45
	10.0	868.18	850.49	809.39
Soil F	5.0	353.65	291.45	211.00
	10.0	706.48	660.26	564.48
Soil R	5.0	387.19	397.76	402.98
	10.0	653.90	871.03	870.89
Source of variation	d$f_{residual}$	LSD$_{0.05}$	LSD$_{0.01}$	
Soil	36	20.00	46.13	
Norfloxacin	36	48.22	241.60	
pH	36	20.00	46.13	
Soil × norfloxacin	36	28.29	65.24	
Soil × pH	36	22.35	37.07	
Nor × pH	36	28.29	65.24	
Soil × norfloxacin × pH	36	31.61	52.24	

LSD: least significant difference.

Thus, the relationships between the equilibrium pH of soil solution and K_D and K_{OC} values for norfloxacin were investigated at two norfloxacin levels and shown in Figs. 5 and 6. The K_D values for norfloxacin in soils were scanted in the range 0–600 mL/g in the equilibrium pH values ranging from 3.4 to 7.5, in which the maximum K_D appeared around pH 6 in soil R (Fig. 5, Table 3). Compared to the trend of the K_D for norfloxacin in soils, the K_D values in soil B tended towards convergence, while those in soil F and soil R hardly changed. The maximum of K_{OC} still appeared at pH around 6 in soil R (Fig. 6).

2.4 Effect of Cu ion on sorption of norfloxacin in bulk soils

The effects of exogenous Cu on norfloxacin sorption were investigated for different soils at norfloxacin level of 5.0 or 10.0 mg/L (Table 7). With increasing Cu^{2+} concentrations in solution, sorption amounts of norfloxacin in soil B and soil F increased and were up to a peak at 100 mg/L Cu^{2+}, and then the sorption amounts were decreased regardless of norfloxacin levels. As the norfloxacin concentration was 5.0 mg/L, in soil B, the sorption amounts of norfloxacin in the treatment with 25, 100, and 1000 mg/L Cu^{2+} were 1.1, 1.6, and 0.4 times as high as those in the control, compared to 1.7, 2.2, and 0.7 times as high as
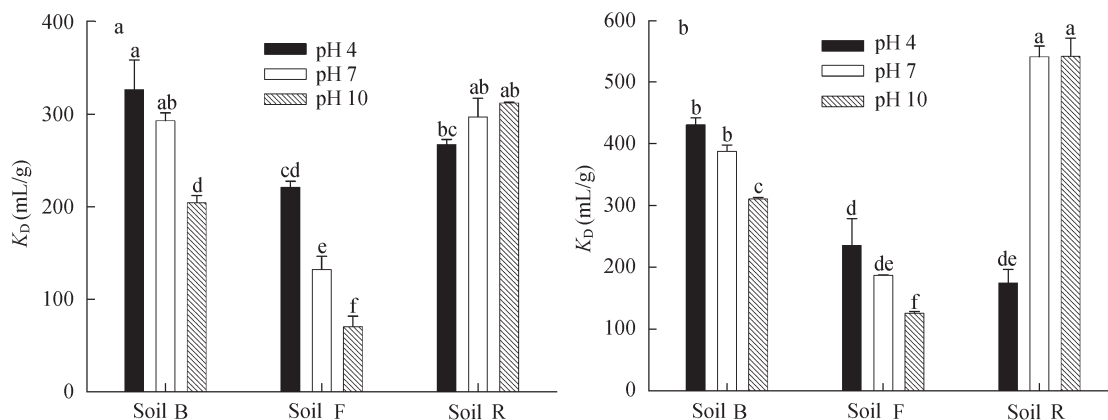

Fig. 3   Distribution coefficients, K_D at 5.0 (a) and 10.0 mg/L (b) norfloxacin solution at various pH values.
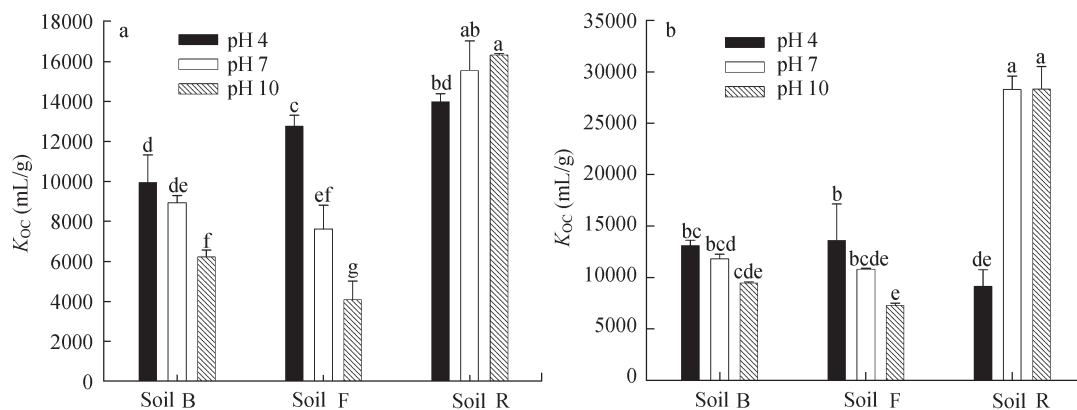
Fig. 4   Distribution coefficients, K_{OC} at 5.0 (a) and 10.0 mg/L (b) of norfloxacin solutions at various pH values.
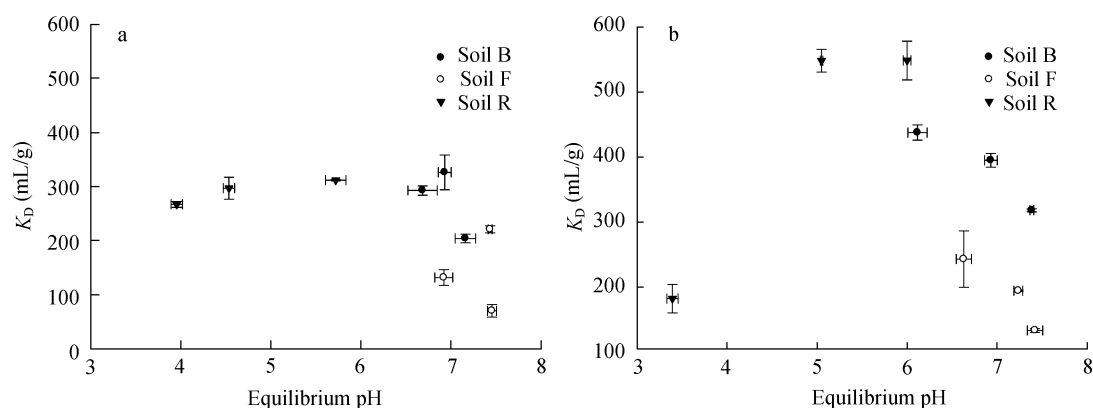

Fig. 5   Effect of solution equilibrium pH on the sorption coefficient K_D of norfloxacin at 5.0 (a) and 10.0 mg/L (b).
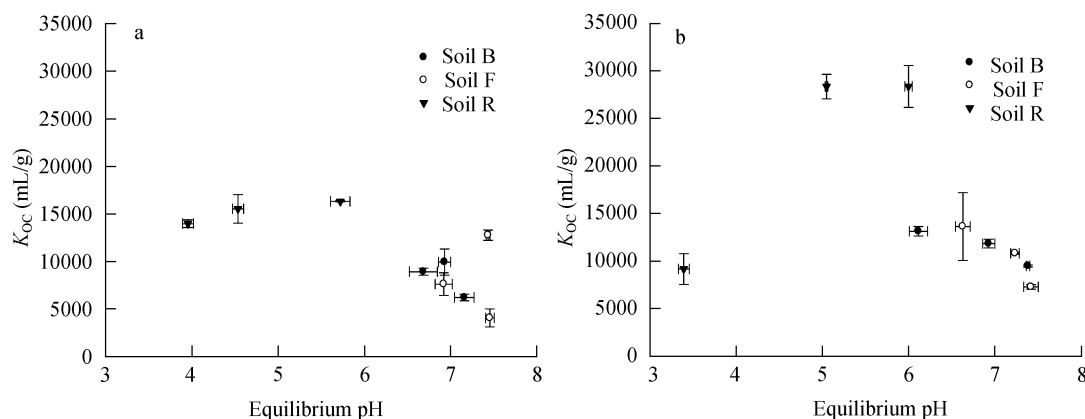

Fig. 6   Effect of solution equilibrium pH on the sorption coefficient K_{OC} of norfloxacin at 5.0 (a) and 10.0 mg/L (b).

those in the control treatment in soil F. Generally, with increasing Cu^{2+} concentrations in solution, the sorption amounts of norfloxacin in soil R showed similar tendency with those in soil B and soil F, and there was also a peak of sorption amount at 100 mg/L Cu^{2+}. However, compared to the control, the sorption amounts were decreased at 25 and 50 mg/L Cu^{2+}, which were 78% and 90% of that in the control. The similar results were obtained at 10.0 mg/L in the three tested soils (Table 7).

In order to understand the effects of soil type, norfloxacin concentration, and Cu level in solution on norfloxacin sorption in three tested soils, all data obtained were subjected to multi-way ANOVA (Table 7). There

was a statistically significant interaction between any two factors of soil type, norfloxacin concentration and Cu^{2+} level ($P < 0.01$). This indicates that the effect of one factor is not consistent at all combinations of the two other factors. The statistically significant differences were also found in the mean values among soil types ($P < 0.01$), norfloxacin concentrations ($P < 0.01$) and Cu levels ($P < 0.01$).

Because the norfloxacin concentrations (5 and 10 mg/L) in the study fell within the linear range of the sorption isotherm, the use of K_D was justified for the effect of Cu^{2+} on norfloxacin sorption. The effects of exogenous Cu^{2+} concentrations on the values of K_D for norfloxacin were

Table 7 Effect of Cu^{2+} on the sorption amount of norfloxacin at 5.0 and 10.0 mg/L in bulk soils ($n = 108$)

Soil type	Norfloxacin (mg/L)	Sorption amount (mg/L)					
		0 mg/L Cu^{2+}	25 mg/L Cu^{2+}	50 mg/L Cu^{2+}	100 mg/L Cu^{2+}	500 mg/L Cu^{2+}	1000 mg/L Cu^{2+}
Soil B	5.0	456.05	516.94	540.09	710.85	516.05	176.87
	10.0	849.11	992.07	1016.35	1466.80	1272.47	901.66
Soil F	5.0	319.95	535.00	549.45	697.55	538.97	232.33
	10.0	636.87	1012.03	1027.00	1454.89	1292.63	982.33
Soil R	5.0	490.16	387.15	441.18	650.80	410.17	27.00
	10.0	912.57	809.80	839.01	1384.30	991.50	554.70

Source of variation	$df_{residual}$	$LSD_{0.05}$	$LSD_{0.01}$
Soil	72	5.16	6.85
Norfloxacin	72	4.21	5.59
Cu^{2+}	72	7.29	9.68
Soil × Norfloxacin	72	7.29	9.68
Soil × Cu^{2+}	72	12.63	16.77
Nor × Cu^{2+}	72	10.31	13.70
Soil × Norfloxacin × Cu^{2+}	72	27.50	36.52

shown in Fig. 7. At low Cu^{2+} concentrations, the K_D and K_{OC} values increased with increasing Cu^{2+} concentrations, reached a peak at 100 mg/L Cu^{2+} and then decreased with high Cu^{2+} levels at both norfloxacin doses, which were similar to the tendency of sorption amounts in the range of Cu^{2+} levels in the study. However, the differences were found among the three tested soils. In the tubes treated with 100 mg/L Cu^{2+} at 5.0 mg/L norfloxacin, the K_D value for norfloxacin in soil B was 11.7 times as high as that of the control, compared to 18.3 and 3.5 times as high as that of corresponding control treatment in soil F and soil R. The

tendency of K_{OC} was similar to that of K_D in the same soil, which depended on solution Cu^{2+} concentrations (Fig. 8).

3 Discussion

The sorption behavior of pharmaceutical antibiotics in soil depended on the soil properties under different conditions (Sithole and Guy, 1987; Rabølle and Spiild, 2000; Jones *et al.*, 2005; Kolz *et al.*, 2005). A study by Tate *et al.* (1989) showed that organic matter and clay fractions had strong influences on the sorption of
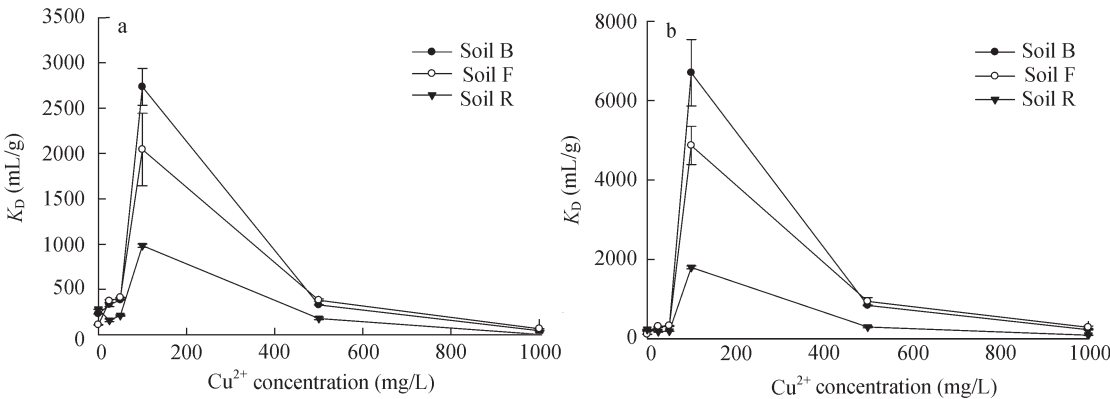

Fig. 7 Distribution coefficients, K_D at 5.0 (a) and 10.0 mg/L (b) solutions of norfloxacin at different Cu^{2+} concentrations.
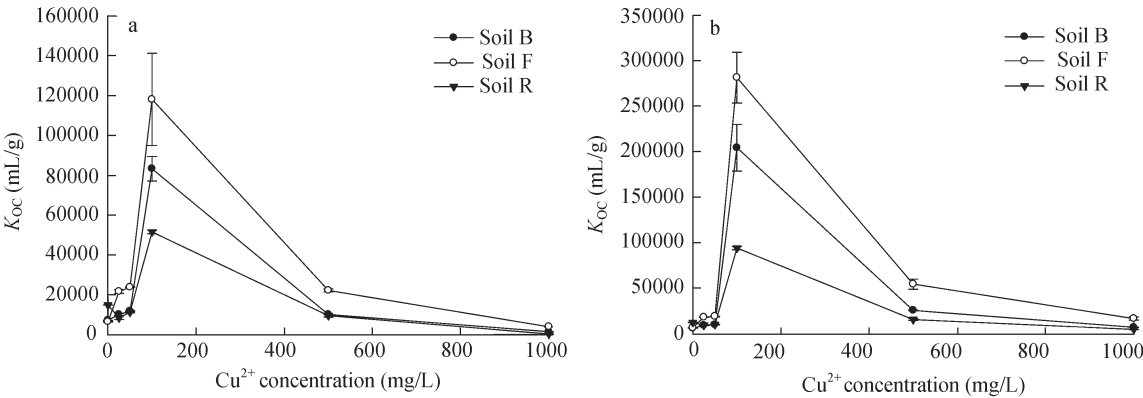

Fig. 8 Distribution coefficients, K_{OC} at 5.0 (a) and 10.0 mg/L (b) of norfloxacin solutions at various Cu^{2+} concentrations.

efrotomycin. In order to investigate the mechanisms of TCs (tetracycline (TC), oxytetracycline (OTC), and chlortetracycline (CTC)) sorption by soil and its constituents, batch studies were conducted in several soils varying in pH, cation exchange capacity (CEC), anion exchange capacity (AEC), clay content and type, and OC content under various background electrolyte concentrations (Sassman and Lee, 2005). Their results supported the hypothesis that pH and CEC play an important role in TC sorption. Soil organic matter also plays an important role in sorption of organic pollutant, but poor correlation between f_{OC} and OTC sorption on 30 soils was observed (Jones *et al*., 2005). In the present study, the results (Figs. 1 and 2) showed that norfloxacin sorption in bulk soils was lower than that in their corresponding SOM-removed soils at all the norfloxacin concentrations except for soil R and SOM-removed R at 1.0 mg/L norflocxacin. The ratios of sorption amount in bulk soil to the corresponding SOM-removed soil ranged from 0.90 (soil R at 1.0 mg/L) to 2.59 (soil F at 50.0 mg/L). Norfloxacin sorption in SOM-removed soil was higher than that in the corresponding bulk soil. The phenomena were possibly explained by the following two reasons: (1) more sorption sites in clay minerals were available for norfloxacin because of soil organic matter removal; (2) negative charges on the soil organic matter of the bulk soil could partially block the sorption of norfloxacin. The highest norfloxacin sorption among all SOM-removed was found in SOM-removed B, which may be attributed to the types and content of major clay minerals and proper pH value in soil B.

The sorption behavior of antibiotics in environmental materials depended not only on media properties but also on the properties of antibiotic. The effect of solution pH on norfloxacin sorption can be attributed to the following two aspects: (1) the dominant forms of norfloxacin in solution changed as a result of shifted solution pH; (2) the solution pH might change surface status of the soil clay minerals, such as surface charge, which can also be responsible for the interaction between antibiotics and soil clay minerals (Figueroa *et al*., 2004; Sassman and Lee, 2005). Norfloxacin has two proton-binding sites (carboxyl and piperazinyl group) with reported pK_a values of 6.22 and 8.51 (Table 1), therefore, norfloxacin can exist in four forms (neutral, zwitterionic, anionic, and cationic) (Takács-Novák *et al*., 1990; Ross and Riley, 1994). When pH is below 6.2 the positively charged form of norfloxacin is dominant. When pH is in the range of 6.20–8.50, the four forms exist with the zwitterionic form being dominant. When pH is higher than 8.5, the negatively charged form of norfloxacin is dominant (Lorphensri *et al*., 2006). In the present study, the equilibrium pH values were in the range of 3.40–7.50 (Table 3), in which the maximum values of K_D and K_{OC} occurred when the solution equilibrium pH was around 6 (Figs. 5 and 6). Under this pH value, most of the basic groups of norfloxacin are protonated and the positively charged norfloxacin molecules have cationic behavior with increasing net positive charge. Thus, as the norfloxacin became more cationic at pH around 6, the strong sorption through the electrical attraction of

countercharged norfloxacin and soil particles would occur in the solid matrix.

The effect of pH on soil properties is another reason for the maximum sorption in soil R. Combination of different soil solutions and clay surface status decides the sorption capacity. It was reported that the maximum sorption of norfloxacin to silica and alumina occurred at pH 6.8 and pH 8.2 respectively (Lorphensri *et al*., 2006), which was not in agreement with the results obtained from the present study. The difference may be caused mainly by the matrix types used in these studies. The study by Kulshrestha *et al*. (2004) showed that when OTC had a net positive charge at lower pH values, they tended to have greater sorption affinity with cation exchange as the dominant mechanism. For further insight to the effects of pH on drug sorption in soil and clay, similar conclusions were drawn using batch studies and empirical modeling (Colaizzi and Klink, 1969; Tolls, 2001; Sassman and Lee, 2005; Jones, 2005). The results from the present study (Figs. 3, 4, 5, and 6, Table 3) illustrated that the distribution of norfloxacin between soil phase and solution phase depended on the equilibrium pH, but not on the initial pH in solution.

In the present study, the results showed that Cu^{2+} could strongly influence the norfloxacin sorption in the solution, and higher Cu^{2+} levels inhibited the sorption of norfloxacin in all the three tested soils (Table 7, Figs. 7 and 8). These could be explained by the following three mechanisms. First, adding metallic ions can change the solution properties, such as ionic strength, and consequently influenced the drug sorption behavior. As was reported by McGinley *et al*. (1993), change of solution properties due to co-contaminants could apparently influence the sorption of the organic compounds. Secondly, metallic ions can change the combining model between drugs and media. It has been known that soil organic matter is a basic factor affecting sorption of organic compounds in soils, but metallic cations in soil may also affect sorption, as evidenced by batch studies and empirical modeling results (Gao *et al*., 2006). The results obtained by Sithole and Guy (1987) showed that the divalent cations could bridge the clay and drug. Finally, Cu^{2+} can interact with norfloxacin and form the complexes, which changed the forms of norfloxacin and then changed the sorption of norfloxacin (Sadeek, 2005).

4 Conclusions

In conclusion, the process of norfloxacin sorption in soil is influenced by SOM through cooperating with other soil properties such as, soil pH, and metallic cations. However, norfloxacin sorption depends heavily on soil types. The sorption behavior of norfloxacin in soil is caused by the interactions of soil and antibiotic, which is related to soil and antibiotic properties.

References

Bayard R, Barna L, Mahjoub B, Gourdon R, 1998. Investigation of naphthalene sorption in soils and soil fraction using bath and column studies. *Environmental Toxicology Chemistry*, 17: 2383–2390.

Chiou C T, Peter L J, Freed V H, 1979. A physical concept of soil-water equilibria for nonionic organic conpounds. *Science*, 206: 831–832.

Colaizzi J, Klink P, 1969. pH-partitioning behaviour of tetracyclines. *Pharmacology Science*, 58: 1184–1189.

Drevenšek P, Košmrlj J, Giester G, Skauge T, Sletten E, Sepcic K, Turel I, 2006. X-ray crystallographic, NMR and antimicrobial activity studies of magnesium complexes of fluoroquinolones – racemic ofloxacin and its S-form, levofloxacin. *Journal of Inorganic Biochemistry*, 100(11): 1755–1763.

Drillia P, Stamatelatou K, Lyberatos G, 2005. Fate and mobility of pharmaceuticals in solid matrices. *Chemosphere*, 60: 1034–1044.

Efthimiadou E K, Psomas G, Sanakis Y, Katsaros N, Karaliota A, 2007. Metal complexes with the quinolone antibacterial agent N-propyl-norfloxacin: Synthesis, structure and bioactivity. *Journal of Inorganic Biochemistry*, 101: 525–535.

Figueroa R A, Leonard A, Mackay A A, 2004. Modeling tetracycline antibiotic sorption to clays. *Environmental Science and Technology*, 38: 476–483.

Gao Y Z, Xiong W, Ling W T, Xu J M, 2006. Sorption of phenanthrene by soils contaminated with heavy metals. *Chemosphere*, 65: 1355–1361.

Golet E M, Alder A C, Hartmann A, Ternes T A, Giger W, 2001. Trance determination of fluoroquinolone antibacterial agents in urban wastewater by solid-phase extraction and liquid chromatography with fluorescence detection. *Analytical Chemistry*, 73: 3632–3638.

Jones A D, Bruland G L, Agrawal A G, Vasudevan D, 2005. Factors influencing the sorption of oxytetracycline to soil. *Environmental Science and Technology*, 24: 761–770.

Kaiser K, 2000. Dissolved organic matter sorption by mineral constituents of subsoil clay fraction. *Journal of Plant Nutrition and Soil Science*, 163: 531–535.

Kolz A C, Ong S K, Moorman T B, 2005. Sorption of tylosin onto swine manure. *Chemosphere*, 60: 284–289.

Kulshrestha P, Rossman Jr F G, Aga D, 2004. Investigating the molecular interactions of oxytetracycline in clay and organic matter: insights on factors affecting its mobility in soil. *Environmental Science and Technology*, 38: 4097–4105.

Lorphensri O, Intravijit J, Sabatini D A, Kibbey T C G, Osathaphan K, Saiwan C, 2006. Sorption of acetaminophen, 17-ethynyl estradiol, nalidixic acid, and Nor to silica, alumina, and a hydrophobic medium. *Water Research*, 2006: 1481–1491.

Lu R K, 2000. Analysis methods on soil and agricultural chemistry. Beijing: Agricultural Science and Technology Press.

McGinley P M, Katz L E, Weber Jr W J, 1993. A distributed reactivity model for sorption by soils and sediments. 2. Multicomponent systems and competitive effects. *Environmental Science and Technology*, 27: 1524–1532.

Migliore L, Brambilla G, Casoria P, Civitareale C, Cozzolino S, Gaudio L, 1996. Effect of sulphadimethoxine contamination on barley (*Hordeum distichum* L., Poaceae, Liliopsida). *Agriculture, Ecosystems and Environment*, 60: 121–128.

Migliore L, Cozzolino S, Fiori M, 2003. Phytotoxicity to and uptake of enrofloxacin in crop plants. *Chemosphere*, 52: 1233–1244.

Pu X C, Cutright T J, 2006. Sorption-desorption behavior of PCP on soil organic matter and clay minerals. *Chemosphere*, 64: 972–983.

Rabølle M, Spiild N H, 2000. Sorption and mobility of metronidazole, olaquindox, oxytetracycline and tylosin in soil. *Chemosphere*, 40: 715–722.

Ross D L, Riley C M, 1994. Dissociation and complexation of the fluoroquinolone antimicrobials – an update. *Journal of Pharmaceutical Biomedical Analysis*, 12: 1325–1331.

Ruíz P, Ortiz R, Perelló L, Alzuet G, González-Álvarez M, Liu-González M, Sanz-Ruíz F, 2007. Synthesis, structure, and nuclease properties of several binary and ternary complexes of Cu(II) with Nor and 1,10-phenantroline. *Journal of Inorganic Biochemistry*, 101: 831–840.

Sadeek A S, 2005. Synthesis, thermogravimetric analysis, infrared, electronic and mass spectra of Mn(II), Co(II) and Fe(III) Nor complexes. *Journal of Molecular Structure*, 753: 1–12.

Sarmah A K, Meyer M T, Boxall A B A, 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of vertinary antibiotics (VAs) in the environment. *Chemosphere*, 65: 725–759.

Sassman S A, Lee L S, 2005. Sorption of three tetracyclines by several soils: assessing the role of pH and cation exchange. *Environmental Science and Technology*, 39: 7452–7459.

Singh G, Spencer W F, Cliath M M, Genuchten M T, 1990. Sorption behavior of *s*-triazine and thiocarbmate herbicides on soil. *Journal of Environmental Quality*, 1: 520–525.

Sithole B B, Guy R G, 1987. Models for tetracycline in aquatic environments. I: Interaction with bentonite clay systems. *Water, Air, and Soil Pollution*, 32: 303–314.

Takács-Novák K, Noszál B, Hermecz I, Kereszturi G, 1990. Protonation equilibria of quinolone antibacterials. *Journal of Pharmacy*, 79: 89–96.

Tate R L, Halley B A, Taub R, Green-Erwin M L, 1989. Efrotomycin interaction with soil clay and organic matter fraction. *Journal of Agricultural Food Chemistry*, 37: 1165–1169.

Thiele-Bruhn S, 2003. Pharmaceutical antibiotic compounds in soils – a review. *Journal of Plant Nutrition and Soil Science*, 166: 145–167.

Tolls J, 2001. Sorption of veterinary pharmaceuticals in soils: a review. *Environmental Science and Technology*, 35: 3397–3406.

Turel I, 2002. The interactions of metal ions with quinolone antibacterial agents. *Coordination Chemistry Reviews*, 232: 27–47.

Turel I, Gruber K, Leban I, Bukovec N, 1996. Synthesis, crystal structure and characterization of three novel compounds of the quinolone family member (Nor). *Journal of Inorganic Biochemistry*, 61(3): 197–212.

Wallis S C, Gahan L R, Charles B G, Hambley T W, Duckworth P A, 1996. Cu(II) complexes of the fluoroquinolone antimicrobial ciprofloxacin. synthesis, X-ray structural characterization, and potentiometric study. *Journal of Inorganic Biochemistry*, 62: 1–16.