



## Competitive sorption and desorption behavior for three fluoroquinolone antibiotics in a wastewater treatment wetland soil

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### ABSTRACT

Significant amounts of pharmaceuticals are discharged into the environment through wastewater effluent. Sorption has been shown to be a significant aqueous removal pathway for many of these compounds. Competition between ciprofloxacin (CIP), ofloxacin (OFL) and norfloxacin (NOR) and their sorption to, and desorption from, a surrogate Louisiana wastewater treatment wetland soil were investigated to gain insight into the fate and transport of the pollutants within wastewater treatment wetlands. This study was undertaken in the context of a treatment wetland that continuously receives pharmaceuticals. Therefore it is important to understand the total capacity of this soil to sorb these compounds. Sorption to this treatment wetland soil was found to provide a major and potentially long-term removal pathway for these antibiotics from wastewater. Log  $K_F$  values for all three compounds were between 4.09 and 3.90 for sorption and 4.24 and 4.05  $\mu\text{g}^{1-1/n} (\text{cm}^3)^{1/n} \text{g}^{-1}$  for desorption. The compounds were sorbed in amounts ranging from 60% to 90% for high and low loading, respectively. The majority of the compounds were sorbed to the soil within the first 20 h, indicating that treatment wetland may not need long retention times (weeks to months) in order to remove these compounds. Sorption  $K_D$  values for competition (20 ppm of each compound for 60 ppm of total fluoroquinolones) ranged from 2300 to 3800  $\text{cm}^3 \text{g}^{-1}$  which is between both the 20 (4300–5800  $\text{cm}^3 \text{g}^{-1}$ ) and 60 (1300–3000  $\text{cm}^3 \text{g}^{-1}$ ) ppm single compound  $K_D$  values, indicating that there is competition between these three compound for sorption sites. Sorption and desorption data (single component and mixture) collectively provide the following evidence: (1) NOR and, to a lesser extent, CIP outcompete OFL for sorption sites, (2) OFL sorbes to its share of “quality” sorption sites, and (3) competition only occurs for lesser “quality” binding sites.

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### 1. Introduction

Many wastewater treatment facilities discharge effluent into wetlands for tertiary treatment to further reduce nutrient concentrations before discharge to surface water bodies (Verhoeven and Meuleman, 1999; Day et al., 2006; Wang et al., 2006). Pharmaceutically active compounds (PhAC) are consistently found in wastewater treatment plant (WWTP) effluent and are loaded to treatment wetlands (White et al., 2006; Conkle et al., 2008). Wetlands have been shown to be effective at reducing PhAC concentrations (Matamoros and Bayona, 2006; Conkle et al., 2008). However, little research has addressed specific removal mechanisms of PhACs within these wetland systems. Therefore, there is a great need for research addressing the chemical interactions, fluxes and system mitigation capacities of PhACs in the environment.

One family of PhACs that is of concern is broad spectrum antibiotics, such as fluoroquinolones (FQs) (Batt et al., 2006; Castiglioni et al., 2006). Fluoroquinolones are used in human, veterinary and agricultural applications with CIP, OFL and NOR being three of the most widely used (Pico and Andreu, 2007). Fluoroquinolones have been detected at levels of up to 0.036 and 0.45  $\mu\text{g L}^{-1}$  in surface water and wastewater effluent, respectively (Batt et al., 2007; Vieno et al., 2007). This raises concerns about potential ecotoxicity of these compounds, individually or as a mixture (Isidori et al., 2005; Robinson et al., 2005) and the evolution of resistance to frontline antibiotics by bacterial strains (Kern, 2007). Fluoroquinolones enter the environment by passing through WWTPs and in land application of WWTP biosolids (Golet et al., 2003; Pico and Andreu, 2007).

Unlike the more traditional pollutants, such as polyaromatic hydrocarbons, FQs are polar compounds which, depending on pH, can either be anionic, zwitterionic or cationic. This means that sorption coefficient ( $K_D$ ) values vary with soil type and physico-chemical properties, making partitioning predictions difficult

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(Gu and Karthikeyan, 2005; Pico and Andreu, 2007). Research on pharmaceuticals in the environment has shown that pH plays a significant role in sorption capacity (Sassman and Lee, 2005; Gu et al., 2007). While the pH range of many freshwater wetland soils in Louisiana ranges from 6 to 7 (Jeng and Hong, 2005; Visser and Sasser, 2009), we recognize that wetland pH can vary even over 24 h as well as between systems. While we chose an environmentally relevant pH, Vasudevan et al. (2009) demonstrated that difference in pH can affect the overall sorption of ciprofloxacin.

Previous research in loamy soils suggests that FQ antibiotics may have high partitioning, low mobility, and low biodegradation rates (Golet et al., 2003). Fluoroquinolones have been shown to have  $K_D$  values, ranging widely from 70 to 353 000 L kg<sup>-1</sup>, indicating that sorption to solid materials can be a major pathway for removal of FQs from water (Batt et al., 2006; Castiglioni et al., 2006; Lee et al., 2007). In particular, sorption of FQs to solid materials may constitute a significant removal mechanism of FQs in wetlands (Fytianos et al., 2000; Gu and Karthikeyan, 2005). Within a “real world” system, the situation is even more complex due to competition (Li and Werth, 2001; Bonin and Simpson, 2007). This may occur when one or more compounds outcompete another for binding sites, resulting in blocked sorption or physical displacement of a compound from a binding site, reduced sorption, and a greater release downstream.

The sorption of any compound to a fixed amount of solid matrix is not infinite. Thus, it is vital to understand the sorption of such compounds to the soil within a treatment wetland over the long term. Consequently, the goals of this study are to determine: (1) sorption capacities of the soils for CIP, NOR and OFL at high concentrations to mimic long term loading, (2) the binding constants for CIP, NOR and OFL to organic-rich wetland soils, and (3) whether there is competition between CIP, NOR and OFL for sorption sites in soils.

## 2. Methods

### 2.1. Soil

Bayou Castine (15R 784949E, 3361530N) serves as the control wetland for the Mandeville, LA WWTP and soil was collected from this site for this study (Conkle et al., 2008). It is classified as an Arat Silty Clay Loam, which is a fine silty, siliceous, non-acid, thermic Typic Hydraquent (Trahan et al., 1990). The surface soil (0–10 cm) was collected by pushcore, homogenized, rid of woody debris and plant material and stored at 4 °C. Soil characterization included total and extractable metals (DeLaune et al., 2008), total carbon and nitrogen (Malecki-Brown and White, 2009), organic matter (White and Reddy, 2000) and total phosphorus (Malecki-Brown et al., 2007); data are reported in Tables S1 and S2.

### 2.2. Stock and spike solutions

CIP, NOR and OFL (Fig. S1) were obtained from Sigma–Aldrich (St. Louis, MO) and used to create the 100 mg L<sup>-1</sup> aqueous stock solution, from which a 6-point standard curve and spike concentrations were created. All solutions contained 0.01 M CaCl<sub>2</sub> background electrolyte and 100 ppm sodium azide (NaN<sub>3</sub>), with NaN<sub>3</sub> acting as a biocide to prevent microbial degradation.

### 2.3. Sorption and desorption

The Organization for Economic Cooperation and Development (OECD) method 106, adsorption–desorption batch equilibrium method was followed (European Union, 2000); the only deviations being a longer equilibrium time (>24 h) and the addition of NaN<sub>3</sub>.

Each vial was spiked with one antibiotic at one of the following concentration levels: 20, 30, 40, 50, 60 or 80 ppm. Samples were prepared with 100 mg (dry basis) field moist soil in glass scintillation vials in quadruplicate and incubated at 24 °C while being orbitally shaken at 150 rpm for 3–5 d in the dark. Immediately after incubation, samples were centrifuged at 3000 rpm (604g) for 15 min and the supernatant was extracted. Desorption incubations were run using the same sorption samples after removal of the supernatant, the remaining solution was determined and accounted for by mass. Subsequently, the original volume of the matrix solution was added to each vial. From the concentration of the solution at pseudo-equilibrium, and knowing the volume remaining in the vial prior to matrix solution addition, the concentration of the antibiotic concentration at desorption time 0 (zero) was determined. The samples were then incubated, extracted and analyzed in the same manner as in the sorption experiment. Controls showed that, except for sorption to the soil, the losses of the FQ antibiotic amounts were negligible (<1%), validating the experimental procedure.

All samples were analyzed following Conkle et al. (2009) using an Agilent 1100 (Santa Clara, CA) series HPLC with UV and fluorescence detectors fitted with a Zorbax eclipse XDB C18 (4.6 × 150 mm × 5 μm) column. Only UV detection was used during this experiment. For NOR and CIP analyses, a 60:40 mobile phase consisting of acetate buffer (pH = 3) with triethylammoniumphosphate buffer (TEA); acetonitrile (ACN) was used. For OFL analysis, the mobile phase remained the same but the ratio was adjusted to 70:30. The simultaneous detection method for all three FQs used a mobile phase solvent system containing citric acid buffer (pH 2.5):ACN:methanol with the 82:8:10 ratio. Analytical parameters are provided in Table S3 (Conkle et al., 2009). The ideal soil to solution ratio was determined to be four parts of solution to nine parts dry soil equivalent and the equilibrium was achieved within 3 d.

### 2.4. Simultaneous compound analysis

Sorption and desorption of soil samples spiked with 20 and 60 ppm FQs were examined along with an additional set of samples spiked with a solution containing 20 ppm of each compound, for a total of 60 ppm of antibiotics in the mixture. The 60 ppm multiple compound samples were used to determine effects on sorption or desorption as a result of competition between compounds for binding sites. Sorption and desorption  $K_D$  values at 20 and 60 ppm for CIP, NOR and OFL were compared to determine any difference in sorption potential at these two concentration levels. The individual 20 and 60 ppm  $K_D$  values were then compared to the  $K_D$  values determined for multiple compound analyses, and a comparative matrix is provided in Fig. S2.

### 2.5. Data analysis

Percent and total amount sorbed was determined using the OECD guidelines. The amount sorbed ( $C_s^{ads}$ ) (Eq. (1)) was calculated using mass balance between the compound concentration initially in solution ( $C_0$ ) and the amount remaining in solution at equilibrium ( $C_{aq}^{ads}$ ), which accounts for sorption to the glass vials. The calculations also account for the initial solution volume ( $V_0$ ) and dry soil mass ( $m_{soil}$ ).

$$C_s^{ads}(eq) = \frac{[C_0 - C_{aq}^{ads}(eq)] \times V_0}{m_{soil}} \quad (\mu\text{g g}^{-1}) \quad (1)$$

The sorption distribution coefficient ( $K_D$ ) (Eq. (2)) for a particular concentration was calculated by dividing the  $C_s^{ads}$  at equilibrium by the concentration in solution ( $C_{aq}^{ads}$ ) at equilibrium.

$$K_D = \frac{C_s^{ads}(eq)}{C_{aq}^{ads}(eq)} \text{ (cm}^3 \text{ g}^{-1}) \quad (2)$$

Freundlich isotherms were determined by graphing the logarithm of the compound concentration in soil on the y-axis and the logarithm of the aqueous compound concentration on the x-axis. The equation of the best-fit line was determined, with the y-intercept being the Freundlich coefficient ( $K_F$ ), and the 1/slope being the regression coefficient. It should be noted that although  $n \neq 1$  ( $n$  = Freundlich constant), the use of  $K_D$  to compare the sorption affinity of these individual FQs to its mixture may be allowed due to similarity in  $N$ , or by the use of the equation  $K_D = K_F C_e^{(N-1)}$  (Carmo et al., 2000).

A one-way ANOVA was run using SPSS 15 (SPSS Inc.) to determine the difference between the  $K_D$  means for sorption and desorption of 60 ppm, 20 ppm and competition (20 ppm each of CIP, NOR, OFL in solution). If the homogeneity of variance (HOV) was  $>0.05$ , a Latin Square Design (LSD) was used to determine differences between the  $K_D$  means. If the HOV was  $<0.05$ , the Dunnett's T3 test was used.

### 3. Results and discussion

#### 3.1. Single compound sorption and desorption

Steady state was achieved within 2–3 d at 20 ppm of each compound, with 50–60% being sorbed within the first 16 h (Table S3). While the total sorbed mass of each compound increased from ~10 g in a 20 ppm solution to 28–35 g per 1 kg of dry soil in an 80 ppm solution (Fig. S3), the percent of the sorbed FQs decreased from ~90% for all three FQs at 20 ppm to  $76.7 \pm 5.1\%$  for NOR, to  $72.1 \pm .06\%$  for OFL, and to  $62.3 \pm 3.2\%$  for CIP at 80 ppm. The data in Table 1 show that  $K_D$  decreased as a function of sorbate concentration in the order: NOR > OFL > CIP. The trend shows the soil has the ability to sorb a higher% of the total compound at lower concentrations. Therefore, at environmentally relevant concentrations, it is likely this soil has the capacity to sorb >90% of any of the three compounds and indicates sorption is a dominant removal pathway from the aqueous phase.

In regards to desorption, the  $K_D$  values for all concentrations yielded the following trend: NOR > CIP > OFL. For OFL, CIP, and NOR the percentage desorbed (20 ppm:80 ppm) were  $4.07 \pm 0.76$ :  $11.83 \pm 0.97$ ;  $3.76 \pm 0.75$ :  $6.09 \pm 1.18$  and  $2.04 \pm 0.25$ :  $5.31 \pm 1.44$ ,

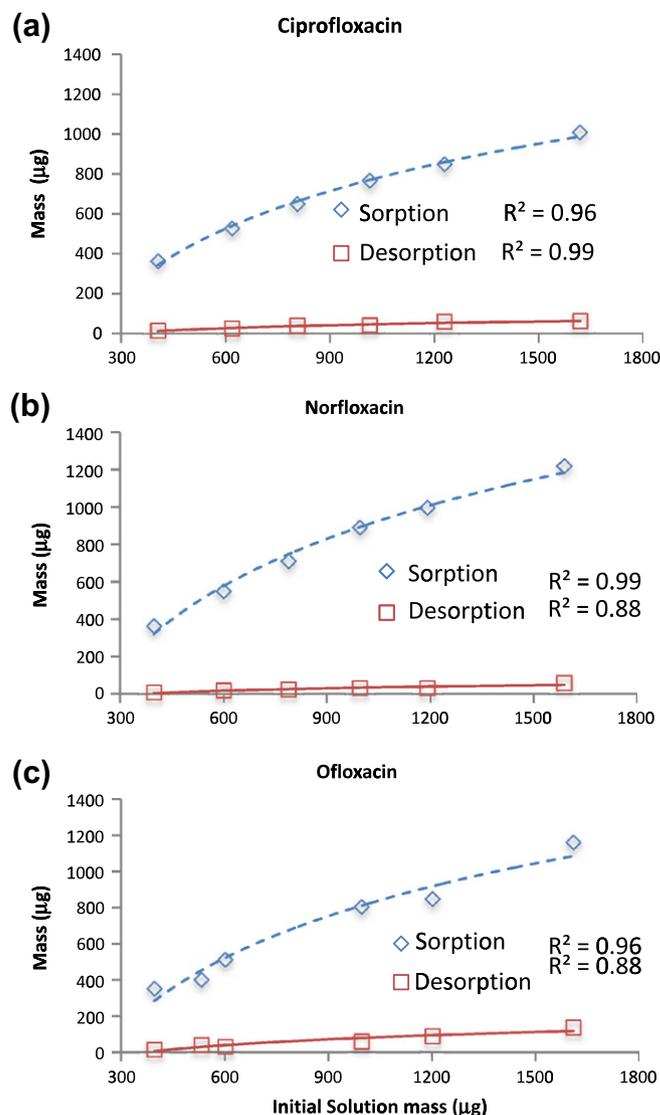


Fig. 1. Sorption and desorption of each compound. The x-axis represents the initial compound mass in solution. The y-axis represents either the compound mass on the soil (sorption) or the compound mass in solution (desorption) at pseudo-equilibrium after 3 d for: (a) CIP, (b) NOR, and (c) OFL.

Table 1

Sorption and desorption data for mass of soil and percent of three antibiotic compound sorbed along with the  $K_D$  values at each loading concentration.

Initial concentration (ppm)	Dry soil (g kg <sup>-1</sup> )			Sorbed (%)			$K_D$ (cm <sup>3</sup> g <sup>-1</sup> )		
	CIP	NOR	OFL	CIP	NOR	OFL	CIP	NOR	OFL
<i>Sorption</i>									
20	10.5 ± 0.4	10.1 ± 0.13	10.1 ± 0.3	89.2 ± 1.3	91.0 ± 1.9	88.3 ± 1.3	4844 ± 628	5791 ± 1280	4325 ± 634
30	14.7 ± 0.8	15.8 ± 0.64	14.5 ± 0.8	85.2 ± 4.7	91.5 ± 0.8	85.0 ± 5.5	3587 ± 1730	6234 ± 727	3541 ± 1291
40	18.5 ± 3.2	20.5 ± 0.4	16.5 ± 1.5	80.5 ± 9.5	90.0 ± 2.8	72.8 ± 5.9	2798 ± 1405	5382 ± 1422	1576 ± 530
50	22.3 ± 0.6	25.2 ± 0.2	22.4 ± 1.0	75.6 ± 2.6	89.4 ± 2.5	80.7 ± 3.9	1835 ± 267	4984 ± 1177	2387 ± 587
60	23.6 ± 2.6	28.1 ± 1.6	24.0 ± 3.0	69.0 ± 6.7	83.6 ± 4.4	70.5 ± 8.0	1338 ± 520	3073 ± 1035	1471 ± 630
80	28.2 ± 1.3	34.8 ± 2.4	33.7 ± 1.1	62.3 ± 3.3	76.7 ± 5.2	72.1 ± 0.6	934 ± 127	1942 ± 450	1496 ± 82
<i>Desorption</i>									
20	10.1 ± 0.3	9.9 ± 0.1	9.7 ± 0.3	3.8 ± 0.8	2.0 ± 0.3	4.1 ± 0.76	5431 ± 1029	10 005 ± 1308	5042 ± 1044
30	14.0 ± 0.7	15.3 ± 0.5	13.6 ± 0.7	4.8 ± 0.7	3.1 ± 0.6	5.9 ± 1.2	4021 ± 659	6664 ± 1530	3376 ± 762
40	17.7 ± 2.7	19.6 ± 0.4	15.1 ± 1.4	6.3 ± 1.3	3.3 ± 0.2	7.6 ± 2.0	3168 ± 765	6281 ± 375	2519 ± 644
50	21.1 ± 0.6	24.3 ± 0.3	20.8 ± 1.1	5.4 ± 1.4	3.7 ± 0.7	7.2 ± 0.4	4071 ± 1390	5407 ± 972	2569 ± 166
60	22.0 ± 2.6	27.1 ± 1.4	21.8 ± 2.9	6.9 ± 1.3	3.6 ± 0.9	10.4 ± 2.1	2788 ± 621	5733 ± 1257	1796 ± 395
80	26.5 ± 1.0	32.9 ± 1.8	29.7 ± 0.9	6.1 ± 1.2	5.3 ± 1.4	11.8 ± 1.0	3224 ± 717	4017 ± 1449	1573 ± 135

ppm = parts per million; CIP = ciprofloxacin; NOR = norfloxacin; OFL = ofloxacin; NOR.

respectively) (Fig. 1a–c). The overall trend is that FQs bind less tightly at higher concentration, with OFL being most loosely bound to this soil. Results lead to three questions: (1) where does the studied soil's sorption capacity come from, (2) what is the mechanism of sorption and, (3) are there different sorption sites?

### 3.1.1. Sorption capacity

In order to answer the first question, one must study the sorbent. The soil contained  $1830 \text{ mg kg}^{-1}$  Ca ( $1756 \text{ mg kg}^{-1}$  exchangeable),  $29284 \text{ mg kg}^{-1}$  Al ( $11 \text{ mg kg}^{-1}$  exchangeable) and  $11072 \text{ mg kg}^{-1}$  Fe ( $170 \text{ mg kg}^{-1}$  exchangeable), an overall cation exchange capacity (CEC) of  $19.8 \text{ cmol}_c \text{ kg}^{-1}$  with a clay content of 31.3%, and organic matter content of  $18.5 \pm 1.0\%$  (Tables S1 and S2). Ca, Al, and Fe are known to complex with fluoroquinolones and have been found to play an important role in a soil's FQs sorption capacity (Gu and Karthikeyan, 2005; Zhang and Huang, 2007; Zhang and Dong, 2008). In addition, cation exchange is also an important mechanism in the sorption of FQs (Lee et al., 2007). In addition, the soil has a high percentage (18%) of organic matter, which has been shown to have a high sorption affinity for FQs (Golet et al., 2003) and a number of mechanisms can regulate sorption of FQs (further discussion of these mechanisms is provided in Supporting information). From an applied point of view, the results show that: (i) this wetland soil has a high sorption capacity for the FQs, (ii) FQs are rapidly sorbed, and (iii) other removal mechanisms (e.g. photodegradation) need only play a minor role to reduce the remaining concentration in the aqueous phase.

### 3.1.2. Sorption mechanism

Insight into sorption mechanisms comes from batch sorption data fitted with the Freundlich isotherm. Log-transformed sorption  $K_F$  values for FQs in this study give the following trend: NOR ( $4.09$ ) > CIP ( $4.01$ ) > OFL ( $3.9$ ) (Fig. 2a–b). Desorption  $K_F$  values are higher but show a similar behavior: NOR ( $4.24$ ) > CIP ( $4.12$ ) > OFL ( $4.05$ ). In addition, sorption–desorption isotherms are hysteretic and highly non-linear. In another study on a soil with pH = 4.3, 7.1% OC, 57% sand, 27% silt and 16% clay, a value of  $\text{Log } K_{F,\text{des}}$  of 3.75 and  $\text{Log } K_{F,\text{sorp}}$  of 3.50 were reported for OFL, which are only slightly lower than our values (Drillia et al., 2005). In contrast, our  $K_F$  values for NOR sorption are somewhat lower than 4.32 and 4.45 obtained for two of the three mineral soils (Zhang and Dong, 2008). Our Bayou Castine wetland soil has a pH of 6.87, ~10% OC, 31.31% clay and  $19 \text{ cmol kg}^{-1}$  CEC. Thus, it is likely that sorption of the FQs is not only due to association with minerals present in soil, but also due to associations with soil organic matter (SOM). Zhang and Dong (2008) found that the presence of low molecular weight organic acids decreased the sorption capacity of NOR in a mineral soil. Therefore, presence of organic acids may have contributed to a decrease in the total FQ sorption capacity.

These FQs have two different  $\text{p}K_a$  values: carboxylic ( $\text{p}K_{a1} = 5.90\text{--}6.23$ ) and amino ( $\text{p}K_{a2} = 8.28\text{--}8.89$ ) (Tolls, 2001; Pico and Andreu, 2007). Since the pH of Bayou Castine soil is 6.87, a greater fraction of FQs (~80%) exist in zwitterionic (or neutral) form, whereas the remaining portion is cationic. The presence of charged groups in an organic molecule enables ion-ion as well as ion-dipole interactions, which greatly influence sorption to mineral/clay surfaces as well as to SOM. Neutral molecules can also be sorbed via various retention mechanisms, such as hydrophobic forces, hydrogen-bonding, ion-dipole, and aromatic electron donor-acceptor processes. For this reason, high potential for sorption to the mineral phase and SOM within this soil is expected.

### 3.1.3. Different sorption sites

The highly non-linear nature of Freundlich isotherms of FQs in this study suggests that site-specific interactions of varying ener-

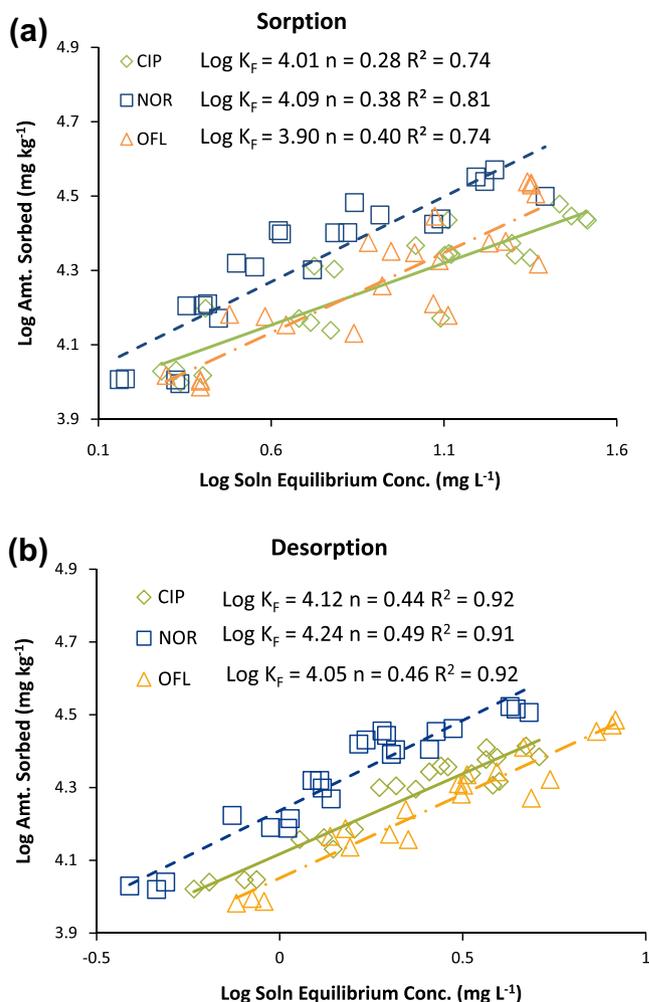


Fig. 2. Freundlich isotherms for sorption (a) and desorption (b) for three antibiotic compounds.

gies contribute to FQ sorption aside from nonspecific hydrophobic forces. Non-linearity in sorption of organic compounds with charged groups, and even of unionized polar organic compounds, is expected (Chiou et al., 2000; Schwarzenbach et al., 2003). Sorption isotherm non-linearity of NOR and OFL ( $N = 0.76$  and  $0.61$ , respectively) to highly mineral soils with OC < 0.5% have been observed (Drillia et al., 2005; Zhang and Dong, 2008). Hysteresis is evident by desorption  $K_F$  > sorption  $K_F$  (see supporting information for background on FQs sorption to soil and data interpretation). The experimental design makes artificial hysteresis very unlikely, thus the observed hysteresis can be considered as real and attributable to mineral hydrophobic nanosites, sorbate-induced rearrangement of the SOM matrix during sorption, and the creation of rigid and dilated pores, which may not fully relax back during the desorption process (Xia and Pignatello, 2001; Sander et al., 2006). The zwitterionic nature for the FQs studied, as well as the zwitterionic and heterogeneous nature of the organic matter, can explain the non-linearity and hysteresis noted above. Organic matter within the soil may compete with the FQs for available binding sites. For example, it has been found there was a marked decrease in the sorption capacity of NOR to a mineral soil in China in the presence of low molecular weight organic acids (Zhang and Dong, 2008). Due to the high organic matter content of the soil, it can be argued that any binding site within the mineral phase would be coated with organic matter and the vast majority of the observed sorption is due to the presence of organic matter rather than the mineral phase.

Data indicate there is a distribution of sites capable of sorbing FQs within the wetland soil. This comes from the: (1) difference in binding constants at different concentrations, (2) change in binding constant order among FQs as a function of concentration, and (3) observed hysteresis. These points also lead to the possibility of competition between FQs for sorption sites within the wetland soil.

### 3.2. Multi-compound (competitive) sorption and desorption

In order to address competition between the FQs for sorption sites within the soil, three scenarios were explored. Each individual FQ was sorbed at 20 ppm. Additionally, all three compounds—each at 20 ppm—were simultaneously sorbed for a cumulative 60 ppm FQ concentration. Finally, each FQ was studied in separate at 60 ppm (Fig. 3a). Individual compound sorption  $K_D$  values at 20 ppm showed no significant difference (CIP and NOR,  $p = 0.523$ ; CIP and OFL,  $p = 0.601$ ; NOR and OFL,  $p = 0.243$ ), while at 60 ppm, NOR had a statically greater  $K_D$  value compared to CIP ( $p = 0.01$ ) and OFL ( $p = 0.016$ ), with a general trend of NOR > OFL = CIP. The  $K_F$  from the Freundlich fits also show preferential sorption for NOR, followed by CIP. Therefore, the  $K_D$  values are consistent with what would be predicted based on the isotherm data.

The multi-compound sorption comparison show that the 20 ppm individual  $K_D$  values are significantly higher than the 60 ppm mixture  $K_D$  values for CIP ( $p = 0.048$ ) and OFL ( $p = 0.001$ ). NOR  $K_D$  values for 20 ppm individual analysis are not significantly different ( $p = 0.093$ ) than the mixture  $K_D$  (Fig. 3a). The 60 ppm individual  $K_D$  for NOR is not statistically different from the  $K_D$  for NOR in the 60 ppm mixture. Both of these findings reinforce the hypothesis that NOR outcompetes the other two compounds for binding

sites. In fact, preferential sorption of NOR occurs when OFL is also present. The same analysis shows that CIP lies between NOR and OFL. These findings suggest that: (1) NOR competes for OFL sites only and CIP has unique sites and/or (2) NOR and CIP compete with OFL for sorption sites.

In terms of desorption, the  $K_D$  values are as expected from the isotherm analysis at both 20 and 60 ppm. No significant difference was observed between the mixture and 20 ppm results for CIP and OFL ( $p = 0.095$  and 0.267, respectively) (Fig. 3b). For NOR, the 20 ppm individual  $K_D$  value was significantly greater than that in the mixture  $K_D$  ( $p = 0.01$ ). Only OFL ( $p < 0.01$ ) had a mixture  $K_D$  that was significantly greater than in the 60 ppm individual solution. In fact, the  $K_D$  for OFL in the mixture was the same as for NOR, which was unexpected, given the sorption data discussed above. This indicates that while OFL is outcompeted in terms of total sorption site occupation, the site “quality”, determined by sorption strength or irreversibility, partially reverses that effect.

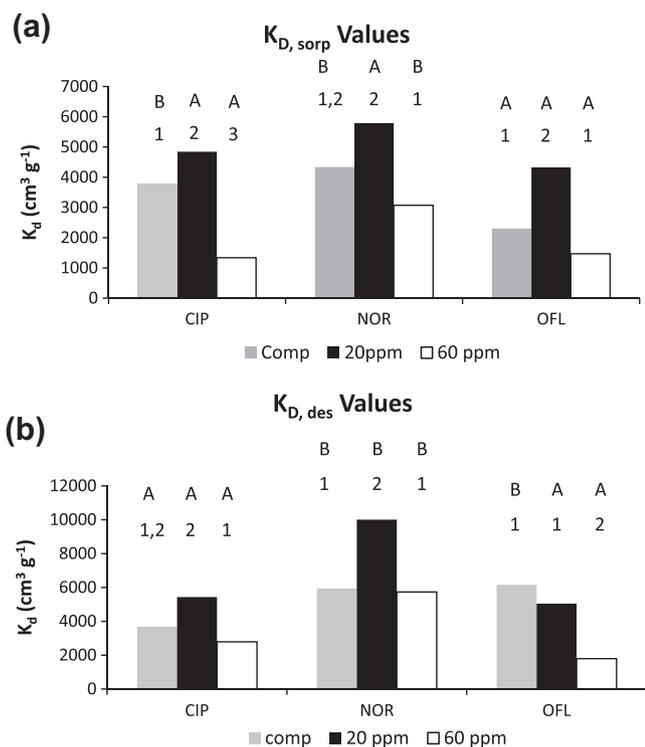
The results demonstrate that competition and 20 ppm values of OFL are significantly higher than the 60 ppm desorption  $K_D$  values. On the other hand, the 20 ppm and competition  $K_D$  values are not significantly different (Fig. 3b), demonstrating that while OFL sorbs less than NOR and CIP, it sorbs to higher quality sites in the presence of the other two compounds. While each of the three antibiotic FQ compounds are similar, there are some structural differences which lead to OFL preferentially sorbing to high quality binding sites. The electrostatic surface potential models of each compound (Fig. S4a–c) shows a highly negative surface potential exists on the carboxylic group while a positive electrostatic surface potential is situated on the amino group, localizing the charges in the zwitterionic (or neutral) form of FQs. OFL, however, has a more positive surface potential than CIP and NOR on the opposite end of the carboxylic group. Moreover, the aromatic moieties in FQ display neutral to minimal negative charges on its surface, whereas the different substituents attached to the nitroaromatic moiety exhibit the greatest positive charge concentration along the surface of the molecule. This parallels previous suggestion of Carrasquillo et al. (2008) that the distribution of charges in the FQ molecule permits an orientation that would allow optimal interactions with sorption sites. Hence, the carboxylate group in FQs are capable of electrostatic interactions with the multivalent cations within the mineral phase and/or hydrogen-bonding with natural organic matter, which is enhanced by a greater charge separation within the molecule. The net dipole moments (debye) OFL (8.63) > CIP (8.38) > NOR (8.09) suggests that the neutral OFL molecule is the most polarized hydrophobic compound among the three FQs. Aside from other interactions, hydrophobic forces are also higher for OFL because of its larger hydrophobic surface, which also contributes to the observed trend. In addition, Zhu and Pignatello (2005) found that polarizability contributes between ~15% and 40% of sorption free energy. Therefore, compounds that are more polarizable are more likely to bind. In this case, charge–dipole interactions are probable between the carboxylate and phenolate groups of the natural organic matter and the positively charged surface of the FQs.

Sorption and desorption data (Fig. 3a–b) collectively provide the following evidence: (1) overall NOR and, to a lesser extent, CIP out-compete OFL for sorption sites, (2) OFL sorbs to its share of “quality” sorption sites, and (3) competition only occurs for lesser “quality” binding sites.

## 4. Environmental implications

### 4.1. Loadings

Treatment wetlands continually receive wastewater containing a suite of pharmaceuticals and personal care products. These



**Fig. 3.** Sorption and desorption  $K_D$  values showing competition compared to individual values at 20 and 60 ppm. The 20 and 60 ppm values represent one single compound in solution at that concentration. Competition data represents all three compounds (CIP, NOR, OFL) in solution at 20 ppm each, for a cumulative concentration of 60 ppm. Letters represent subsets of comparisons between treatment levels (i.e. 60 ppm CIP vs. 60 ppm NOR vs. 60 ppm OFL, etc.). Numbers represent subsets of comparisons between treatments for each compound (i.e. 20 ppm CIP vs. Competition CIP vs. 60 ppm CIP, etc.).

wetland soils are responsible for significant reduction in the concentration of aqueous pharmaceutical compounds through sorption. Previous research has shown that soils have capacity to sorb pharmaceuticals at these low concentrations, however, these studies do not address long term aggregate loading. We set out to simulate longer-term WWTP operation, where, for example, a daily loading of  $7.5 \times 10^6$  L of  $2 \mu\text{g L}^{-1}$  FQ concentration, would roughly equate to 15 g by mass removed from the water. Therefore sorption incubation concentrations were set to mimic cumulative effects of longer-term soil exposure. It should be noted that this wetland receives a consistent daily load of municipal wastewater, with no inputs from surface runoff.

White et al. (2006) also determined that some treatment wetlands may receive a significant mass of these compounds annually (27 kg). Therefore, if sorption is the major removal pathway of these compounds from the aqueous phase, soils would need to be able to sorb large amounts of compounds without reaching saturation. The soils here appear to be capable of sorbing a higher amount of these antibiotic compounds than would come from daily loading.

#### 4.2. Sorption

The treatment wetland soil used in this study sorbed 60–90% of each compound within 2–3 d. The rapid sorption to the soil indicates that treatment wetlands do not need extended retention times in order to be effective at reducing aqueous concentrations of these compounds. If loading of PhACs were halted, which is unlikely in a wetland receiving wastewater, the wetland soil would only release a small percentage of the amount sorbed. Freundlich isotherm values were  $\sim 4 \text{ L kg}^{-1}$  (Fig. 1), which also points to sorption as is a major removal pathway. These data indicate that this soil has a significant ability to sorb and immobilize these FQs, mitigating any downstream releases during wastewater treatment. However, further studies need to establish whether the release of low amounts of these FQs would continue over the long term. Treatment wetlands in LA have a pH between 6 and 7, therefore the small pH range will not alter sorption when comparing different treatment wetlands.

#### 4.3. Competition

Sorption of compounds in wastewater to a wetland soil is complicated by the presence of a suite of pharmaceutical compounds and other wastewater contaminants. There is competition between the compounds studied for preferred binding sites. Therefore, one compound may decrease the sorption capacity of another compound, which may cause greater migration of compounds downstream. The competition effect is of concern in any treatment wetland where 10s–100s of compounds have been detected (White et al., 2006).

Further study is needed to address the fact that treatment wetlands, over the course of decades, are continually loaded with a wide range of drug compounds. Competition between the three compounds in this study demonstrates the need for expanded studies that examine sorption behavior of multiple pharmaceuticals and personal care products.

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#### Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemosphere.2010.06.012.

#### References

- Batt, A.L., Bruce, I.B., Aga, D.S., 2006. Evaluating the vulnerability of surface waters to antibiotic contamination from varying wastewater treatment plant discharges. *Environ. Pollut.* 142, 295–302.
- Batt, A.L., Kim, S., Aga, D.S., 2007. Comparison of the occurrence of antibiotics in four full-scale wastewater treatment plants with varying designs and operations. *Chemosphere* 68, 428–435.
- Bonin, J.L., Simpson, M.J., 2007. Sorption of steroid estrogens to soil and soil constituents in single- and multi-sorbate systems. *Environ. Toxicol. Chem.* 26, 2604–2610.
- Carmo, A.M., Hundal, L.S., Thompson, M.L., 2000. Sorption of hydrophobic organic compounds by soil materials: application of unit equivalent Freundlich coefficients. *Environ. Sci. Technol.* 34, 4363–4369.
- Carrasquillo, A.J., Bruland, G.L., Mackay, A.A., Vasudevan, D., 2008. Sorption of ciprofloxacin and oxytetracycline zwitterions to soils and soil minerals: influence of compound structure. *Environ. Sci. Technol.* 42, 7634–7642.
- Castiglioni, S., Bagnati, R., Fanelli, R., Pomati, F., Calamari, D., Zuccato, E., 2006. Removal of pharmaceuticals in sewage treatment plants in Italy. *Environ. Sci. Technol.* 40, 357–363.
- Chiou, C.T., Kile, D.E., Rutherford, D.W., Sheng, G.Y., Boyd, S.A., 2000. Sorption of selected organic compounds from water to a peat soil and its humic-acid and humin fractions: potential sources of the sorption nonlinearity. *Environ. Sci. Technol.* 34, 1254–1258.
- Conkle, J.L., White, J.R., Metcalfe, C.D., 2008. Reduction of pharmaceutically active compounds by a lagoon wetland wastewater treatment system in Southeast Louisiana. *Chemosphere* 73, 1741–1748.
- Conkle, J.L., Lattao, C.V., White, J.R., Cook, R.L., 2009. Pharmaceutical analysis for environmental samples: individual and simultaneous determination of ciprofloxacin, ofloxacin and norfloxacin using an HPLC with fluorescence and UV detection with a wetland soil matrix. *Anal. Lett.* 42, 2937–2950.
- Day, J.W., Westphal, A., Pratt, R., Hyfield, E., Rybczyk, J., Kemp, G.P., Day, J.N., Marx, B., 2006. Effects of long-term municipal effluent discharge on the nutrient dynamics, productivity, and benthic community structure of a tidal freshwater forested wetland in Louisiana. *Ecol. Eng.* 27, 242–257.
- DeLaune, R.D., Jugsujinda, A., Gambrell, P., Miao, S., 2008. Metal Concentrations and Trace Metal Al and Fe Ratios in Soil of the Chenier Plain, Southwest Louisiana Coastal Zone. Taylor & Francis Inc. pp. 300–312.
- Drillia, P., Stamatelatou, K., Lyberatos, G., 2005. Fate and mobility of pharmaceuticals in solid matrices. *Chemosphere* 60, 1034–1044.
- European Union, 2000. OECD: 106. Adsorption-Desorption Using a Batch Equilibrium Method. OECD Guideline for the Testing of Chemicals, 45.
- Fytianos, K., Voudrias, E., Kokkalis, E., 2000. Sorption-desorption behaviour of 2,4-dichlorophenol by marine sediments. *Chemosphere* 40, 3–6.
- Golet, E.M., Xifra, I., Siegrist, H., Alder, A.C., Giger, W., 2003. Environmental exposure assessment of fluoroquinolone antibacterial agents from sewage to soil. *Environ. Sci. Technol.* 37, 3243–3249.
- Gu, C., Karthikeyan, K.G., 2005. Sorption of the antimicrobial ciprofloxacin to aluminum and iron hydroxides. *Environ. Sci. Technol.* 39, 9166–9173.
- Gu, C., Karthikeyan, K.G., Sibley, S.D., Pedersen, J.A., 2007. Complexation of the antibiotic tetracycline with humic acid. *Chemosphere* 66, 1494–1501.
- Isidori, M., Lavorgna, M., Nardelli, A., Pascarella, L., Parrella, A., 2005. Toxic and genotoxic evaluation of six antibiotics on non-target organisms. *Sci. Total Environ.* 346, 87–98.
- Jeng, H.W., Hong, Y.J., 2005. Assessment of a natural wetland for use in wastewater remediation. *Environ. Monit. Assess.* 111, 113–131.
- Kern, W.V., 2007. New plasmid-borne quinolone-resistance determinant in *Escherichia coli*. *Future Microbiol.* 2, 473–475.
- Lee, L.S., Carmosini, N., Sassman, S.A., Dion, H.M., Sepulveda, M.S., 2007. Agricultural contributions of antimicrobials and hormones on soil and water quality. *Adv. Agron.* 93, 1–68.
- Li, J., Werth, C.J., 2001. Evaluating competitive sorption mechanisms of volatile organic compounds in soils and sediments using polymers and zeolites. *Environ. Sci. Technol.* 35, 568–574.
- Malecki-Brown, L.M., White, J.R., 2009. Effect of aluminum-containing amendments on phosphorus sequestration of wastewater treatment wetland soil. *Soil Sci. Soc. Am. J.* 73, 852–861.
- Malecki-Brown, L.M., White, J.R., Reddy, K.R., 2007. Soil biogeochemical characteristics influenced by alum application in a municipal wastewater treatment wetland. *J. Environ. Qual.* 36, 1904–1913.
- Matamoras, V., Bayona, J.M., 2006. Elimination of pharmaceuticals and personal care products in subsurface flow constructed wetlands. *Environ. Sci. Technol.* 40, 5811–5816.
- Pico, Y., Andreu, V., 2007. Fluoroquinolones in soil – risks and challenges. *Anal. Bioanal. Chem.* 387, 1287–1299.
- Robinson, A.A., Belden, J.B., Lydy, M.J., 2005. Toxicity of fluoroquinolone antibiotics to aquatic organisms. *Environ. Toxicol. Chem.* 24, 423–430.
- Sander, M., Lu, Y.F., Pignatello, J.J., 2006. Conditioning-annealing studies of natural organic matter solids linking irreversible sorption to irreversible structural expansion. *Environ. Sci. Technol.* 40, 170–178.

- Sassman, S.A., Lee, L.S., 2005. Sorption of three tetracyclines by several soils: assessing the role of pH and cation exchange. *Environ. Sci. Technol.* 39, 7452–7459.
- Schwarzenbach, R.P., Gschwend, P.M., Imboden, D.M., 2003. *Environmental Organic Chemistry*, second ed. Wiley-Interscience, Hoboken, New Jersey.
- Tolls, J., 2001. Sorption of veterinary pharmaceuticals in soils: a review. *Environ. Sci. Technol.* 35, 3397–3406.
- Trahan, L., Bradley, J.J., Nolde, R., 1990. *Soil Survey of St. Tammany Parish, Louisiana*. USDA, Soil Conservation Service.
- Vasudevan, D., Bruland, G.L., Torrance, B.S., Upchurch, V.G., MacKay, A.A., 2009. pH-dependent ciprofloxacin sorption to soils: interactions mechanisms and soil factors influencing sorption. *Geoderma* 151, 68–76.
- Verhoeven, J.T.A., Meuleman, A.F.M., 1999. Wetlands for wastewater treatment: opportunities and limitations. *Ecol. Eng.* 12, 5–12.
- Vieno, N.M., Harkki, H., Tuhkanen, T., Kronberg, L., 2007. Occurrence of pharmaceuticals in river water and their elimination a pilot-scale drinking water treatment plant. *Environ. Sci. Technol.* 41, 5077–5084.
- Visser, J.M., Sasser, C.E., 2009. The effect of environmental factors on floating fresh marsh end-of-season biomass. *Aquat. Bot.* 91, 205–212.
- Wang, H., Jawitz, J.W., White, J.R., Martinez, C.J., Sees, M.D., 2006. Rejuvenating the largest municipal treatment wetland in Florida. *Ecol. Eng.* 26, 132–146.
- White, J.R., Reddy, K.R., 2000. Influence of phosphorus loading on organic nitrogen mineralization of everglades soils. *Soil Sci. Soc. Am. J.* 64, 1525–1534.
- White, J.R., Belmont, M., Metcalfe, C., 2006. Pharmaceutical compounds in wastewater: wetland treatment as a potential solution. *Sci. World J.* 6, 1731–1736.
- Xia, G.S., Pignatello, J.J., 2001. Detailed sorption isotherms of polar and apolar compounds in a high-organic soil. *Environ. Sci. Technol.* 35, 84–94.
- Zhang, J.Q., Dong, Y.H., 2008. Effect of low-molecular-weight organic acids on the adsorption of norfloxacin in typical variable charge soils of China. *J. Hazard. Mater.* 151, 833–839.
- Zhang, H., Huang, C.-H., 2007. Adsorption and oxidation of fluoroquinolone antibacterial agents and structurally related amines with goethite. *Chemosphere* 66, 1502–1512.
- Zhu, D.Q., Pignatello, J.J., 2005. A concentration-dependent multi-term linear free energy relationship for sorption of organic compounds to soils based on the hexadecane dilute-solution reference state. *Environ. Sci. Technol.* 39, 8817–8828.