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VETERINARY ANTIBIOTIC SORPTION TO AGROFORESTRY BUFFER, GRASS BUFFER AND CROPLAND SOILS

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Abstract: Veterinary antibiotics are used to treat infectious animal diseases and enhance animal growth. In Missouri, the increased growth of confined animal feeding operations (CAFOs) and the need to dispose of manure generated by CAFOS may be problematic due to co-application of antibiotics during land application of manure. Surface runoff events from claypan or claypan-like soils are relatively frequent; thus, there is a need to develop and evaluate the use of vegetative buffer strips (VBS) as management tools to reduce antibiotic transport to surface water resources. The objectives of this study were to (1) investigate oxytetracycline (OTC) and sulfadimethoxine (SDT) sorption to agroforestry (tree/grass) buffer, grass buffer, and cropland soils, (2) evaluate differences in antibiotic sorption between soils collected from different vegetative species, and (3) elucidate relationships between soil properties and antibiotic sorption. Sorption/desorption isotherms generated using batch techniques were well-fitted by the Freundlich isotherm model ($r^2 > 0.80$). Oxytetracycline was strongly adsorbed by all soils, and the antibiotic was not readily desorbed; hysteresis was observed between all adsorption and desorption isotherms. Solid-solution distribution coefficients (K_d) values of OTC are an order of magnitude greater than those of SDT. Statistical analyses indicate that OTC K_d values are significantly greater for VBS soils relative to cropland soil, and STD K_d values are significantly greater for agroforestry soils as compared to other soils studied. Regression analyses correlating antibiotic sorption to soil properties are in progress. Results indicate that agroforestry and grass buffers may effectively mitigate antibiotic loss from agroecosystems due to enhanced antibiotic sorption properties.

Key Words: Oxytetracycline (OTC); Sulfadimethoxine (SDT); Sorption/Desorption Isotherms; Vegetative Buffer Strips; Solid-Solution Distribution Coefficients (K_d).

INTRODUCTION

Antibiotics are used to treat infectious diseases and as animal feed supplements to promote growth of food-producing animals. However, significant proportion (up to 80%) of the antibiotics added to animal feed is excreted in urine or feces as the parent compound (Thiele-Bruhn 2003). Land application of manure as fertilizer is a common agricultural practice in many parts of the U.S. However, manure-amended lands may serve as a non-point source for antibiotics that enter surface and ground waters via runoff and leaching (Meyer et al. 2000; Koplín et al. 2002). The concentrations of antibiotics in manure range from trace levels to concentrations as high as 200 mg L⁻¹ (Kumar et al. 2004; 2005). In most soils, antibiotic

concentrations do not reach therapeutic levels that can inhibit bacterial growth and population, but they may still have an influence on the selection of antibiotic resistant bacteria in the environment (Nygaard et al. 1992; Kümmerer, 2003). However, in particular terrestrial settings, it is possible that antibiotics can affect the quantity and quality of the native microbial communities (Nygaard et al. 1992). The development of antibiotic-resistant microorganisms, which are difficult to treat with existing antibiotics, is also a risk if infectious bacteria are frequently exposed to antibiotics. Land application of manure containing veterinary antibiotics is one of the main reasons attributed to the development of antibiotic resistant bacteria in the environment (Thiele-Bruhn 2003; Sarmah et al. 2006). Therefore, there is a need to evaluate land management practices that may reduce the fate and transport of antibiotics in the environment.

It has been demonstrated that a well-designed vegetative buffer can be a cost-effective method to mitigate non-point sources of agricultural pollutants from crop land (Schultz et al. 1995; Lowrance et al. 1997). Vegetative buffers may be planted to trees, shrubs, grasses, or a combination of species grown within or on the edges of fields and along stream banks in riparian zones. Mechanisms of pollutant removal within vegetative buffers include physical, chemical and biological processes that can increase solute-soil interaction, improve solute uptake by the soil, and enhance degradation of organic agrochemicals (Mandelbaum et al. 1993). However, no previous studies have evaluated vegetative buffer impacts on antibiotic adsorption and transport.

The objective of this study was to investigate antibiotic sorption to cropland, grass buffer and agroforestry (tree/grass) buffer soils, evaluate differences in antibiotic sorption between soils collected from different vegetative species and elucidate relationships between soil properties and antibiotic sorption.

MATERIALS AND METHODS

Sampling Site Information

Soil samples were collected from grass and tree/grass buffers located at the University of Missouri Horticultural and Agroforestry Research Center (HARC; 39°01'N, 92°45'W), University of Missouri Greenley Memorial Research Center (GMRC; 40°01'N, 92°11'W), and University of Missouri Agricultural Experiment Station Southwest Center (SWC; 37°05'N, 93°52'W). The soil series sampled at HARC, GMRC, and SWC were Menfro silt loam (Typic Hapludalfs), Armstrong silt loam (Aquertic Hapludalfs), and Huntington silt loam (Fluventic Hapludolls), respectively. Soil (3 - 5 kg) was randomly sampled at multiple points in the vegetated areas from a 0 - 10 cm depth. In the case of sampling the tree/grass buffer soils, samples were collected from multiple trees (3 or 4) at a distance of 30 - 50 cm from the base of the tree. Cropland soil samples were collected in farmlands adjacent to these vegetative buffers. At each site, samples were bulked by vegetation type, thoroughly mixed, air-dried, sieved through a 2 mm mesh sieve, and stored in plastic bags. Soil samples < 2 mm were analyzed using standard methods of analysis (Ross 1995; Loeppert and Inskeep 1996; Burt 2004). Samples were analyzed for particle size analysis, cation exchange capacity (NH₄Cl exchangeable), base saturation, organic C content, and pH in salt and water. The soil properties are listed in table 1.

Table 1 Selected properties of the studied soils.

Soil Series	Vegetation	Clay g kg ⁻¹	Organic Carbon g kg ⁻¹	pH	CEC [†] cmol kg ⁻¹	Base Saturation %	Al _{CBD} [‡] g kg ⁻¹	Fe _{CBD} g kg ⁻¹
Armstrong	Grass	245	30	7.0	23.4	71.4	1.08	16.0
	Tree/Grass	253	23	6.7	26.8	65.1	0.97	13.8
	Corn/Soybean	223	22	7.1	25.5	63.4	1.03	15.2
Huntington	Grass	158	12	5.2	13.8	74.5	0.62	5.50
	Tree/Grass	190	23	4.7	9.00	57.0	0.85	5.60
	Corn/Soybean	199	13	6.6	15.1	42.3	0.74	6.84
Menfro	Grass	247	20	6.2	18.0	49.3	0.98	8.73
	Tree/Grass	247	22	5.8	22.6	62.5	0.96	9.28
	Corn/Soybean	211	19	6.3	23.5	57.4	0.68	7.22

[†]CEC, cation exchange capacity.

[‡]CBD, citrate-bicarbonate-dithionite extracted element.

Experimental Design

Oxytetracycline and sulfadimethoxine (Figure 1) were two antibiotics chosen for study because they are widely used in veterinary medicine (Halling-Sorensen et al. 1998). The antibiotic OTC has three functional groups that undergo proton dissociation ($pK_{a1} = 3.33$, $pK_{a2} = 7.68$, $pK_{a3} = 9.69$ (Tolls 2001); SDT has two pK_a s which are 2.44 and 6.0 (Thiele and Aust 2004). Subsequently, these antibiotics may be cationic, zwitterionic, or anionic depending on pH, thus influencing their interaction with soil minerals and organic matter.

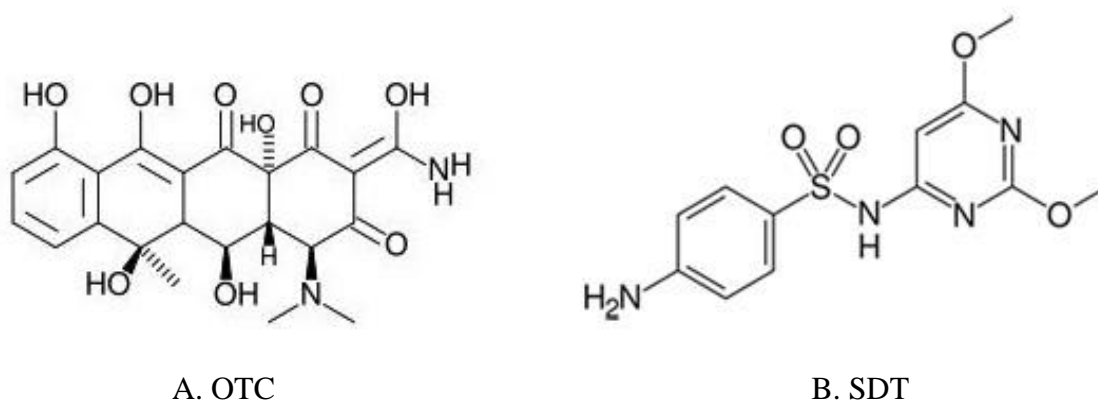


Figure 1. Chemical structures of (a) oxytetracycline (OTC) and (b) sulfadimethoxine (SDT).

Oxytetracycline sorption experiments were conducted over a range of initial aqueous phase concentrations (0.0, 0.005, 0.01, 0.025, 0.05, 0.1, 0.175, 0.25, 0.5 mmol L⁻¹), and SDT was reacted with soil at a single concentration (0.10 mmol L⁻¹). Air-dried soils (0.350 g) were added to 50 mL polypropylene co-polymer (PPCO) centrifuge tubes and suspended in CaCl₂ background electrolyte solution ($I = 0.005$ M CaCl₂). To inhibit microbial growth, samples were

spiked with concentrated NaN_3 to achieve a final concentration of 0.0015 M NaN_3 (Wolf et al., 1989); total solution volume in each reaction vessel was 35 mL. After solution addition, tubes were wrapped in aluminum foil to prevent photo-degradation and agitated for 24 h on an end-over-end shaker (7 rpm) in a constant temperature room (25°C). Reactions at each individual concentration consist of samples reacted in triplicate and blanks (no soil) reacted in duplicate. After the reaction, tubes were centrifuged at 15,000 rpm for 15 min followed by removal of the supernatant solution and filtration through 0.45 μm nominal pore size PTFE membrane filters. Samples were then analyzed to determine antibiotic concentration remaining in solution and solution pH.

Soil samples were demineralized using a modified hydrofluoric acid (HF) treatment as a pre-treatment before Fourier transformed infrared (FTIR) spectroscopy analysis to investigate differences in structural composition of soil organic matter (SOM). Using modification of the method described in Skjemstad et al (1994), 20 g of soil sample was reacted three times in 250 mL of 5% HF for 1.5 h using a rotary shaker, followed by an 18 h extraction period. Soil organic matter residue was washed five times with ultrapure water and freeze-dried. Freeze-dried SOM samples were mixed with ground KBr powder to create a powder with sample concentration of 5%. Samples were analyzed by diffuse reflectance infrared Fourier transform infrared (DRIFT) spectroscopy using a Thermo-Nicolet 4700 spectrometer with Smart DRIFT accessory. All DRIFT spectra were obtained by averaging 400 scans at 2 cm^{-1} resolution.

Aqueous Phase Analyses

Antibiotic concentrations in solution were analyzed using high performance liquid chromatography (HPLC) with an ultraviolet (UV) detector (Beckman, San Ramon, CA). Analysis of OTC was performed at a column temperature of 40°C using a reverse-phase Phenomenex Luna C₈(2) column (250 × 4.6 mm, 5 μm particle size; 100 Å pore size; Torrance, CA). The mobile phase consisted of 0.1% phosphoric acid (H_3PO_4) buffer in water (pH 2.2) and 100% acetonitrile (solvents A and B, respectively). The initial solvent mixture consists of 95% A : 5% B from 0 - 10 min. The solvent ratio changed to 75% A : 25% B between 10 and 25 min, and this ratio was kept constant from 25 to 30 min. For SDT analysis, an Agilent ZORBAX SB-CB, (150×4.6 mm, 5 μm particle size, 80 Å pore size; Santa Clara, CA) column was employed. The mobile phase for SDT analysis consisted of 20 mM KH_2PO_4 buffer and 100% acetonitrile (solvents A and B, respectively). The initial solvent mixture consists of 75% A : 25% B and this ratio is kept constant from 0 to 5 min. The solvent ratio was gradually changed to 45% A : 55% B from 5 to 15 min. At the 15 min mark, the ratio is reduced to 75% A : 25% B and held constant for final 10 min.

Mathematical Description of Sorption Data

The amount of antibiotics adsorbed to soil after reaction was calculated from,

$$q_{\text{ads}} = \frac{(C_{\text{ads,B}})(V_{\text{B}}) - (C_{\text{ads,S}})(V_{\text{S}})}{m_{\text{s}}}$$

where q_{ads} is the surface excess of antibiotic (i.e., amount adsorbed) after the reaction period (mmol kg^{-1}), $C_{\text{ads,B}}$ and $C_{\text{ads,S}}$ are the equilibrium antibiotic concentrations (mmol L^{-1}) in blank

(B) and samples (S) after reaction, V_B and V_S are the volume of solution (L) added to samples and blanks, and m_S is mass of soil (kg). The partition coefficient, K_d , was determined using the following equation,

$$K_d = \frac{q_{ads}}{C_{ads,S}}$$

Desorption experiments were initiated immediately after the adsorption step by adding a volume of methanol equivalent to the volume of supernatant removed as a means to determine total desorbable antibiotic (Thiele-Bruhn et al. 2004). Adsorbate retention was calculated from,

$$q_{des} = q_{ads} - \left\{ \frac{(C_{des,S})(V_{des}) - (C_{ads,S})(V_{ent})}{m_S} \right\}$$

where q_{des} is the surface excess remaining on the surface after the desorption period (mmol kg^{-1}), $C_{des,S}$ is the equilibrium antibiotic concentration in solution after the desorption reaction period, V_{des} is the volume of solution in the reaction vessel during desorption phase, and V_{ent} is the volume of entrained solution remaining in the adsorption pellet after removal of adsorption phase supernatant. Adsorption and desorption data were fitted by the Freundlich isotherm model to provide a concise set of parameters for comparison between the soils,

$$q_{ads\ or\ des} = K_f C_{ads\ or\ des}^N$$

where the parameters K_f and N are positive valued adjustable parameters. The parameters are obtained from a log-log plot of the adsorption isotherm data (K_f is the intercept and N the slope of the resulting line).

RESULTS AND DISCUSSION

Adsorption/desorption experiments were conducted to investigate OTC sorption and retention in different soils (Fig. 1). The isotherm data demonstrate that OTC was strongly adsorbed by all soils investigated and OTC had a relatively high affinity for the soil surface at low surface coverage. Desorption isotherms show that the antibiotic was not readily desorbed from soil in the presence of methanol as hysteresis is evident between all adsorption and desorption isotherms.

Sorption of OTC was best fitted by the Freundlich equation with correlation coefficients (R^2) ranging from 0.80 to 0.98 (Table 2). The Freundlich parameters, $\log K_f$ and N , indicate the affinity of the antibiotic for the solid and the degree of isotherm curvature, respectively. Adsorption $\log K_f$ values ranged from 3.20 to 3.51 and from 1.35 to 3.98 for desorption isotherms. The values of N varied from 0.58 to 0.98 for adsorption and from 0.12 to 0.57 for desorption. These values indicate that adsorption isotherms range from nearly linear ($N = 1$) to nonlinear, L-shaped isotherms ($N < 1$). Although mechanisms of adsorption and more detailed processes are not revealed by adsorption isotherms, a higher degree of isotherm nonlinearity suggests a greater degree of heterogeneous adsorption sites may be present in the soil and specific mechanisms of adsorption may be occurring (Essington 2004; Thiele-Bruhn et al. 2004). At higher initial aqueous antibiotic concentrations, the specific binding sites become saturated via antibiotic adsorption and reduce the affinity of OTC for the soil particle surfaces.

Figure 3 is a comparison of K_d values of OTC and SDT at an initial concentration of 0.1 mM antibiotic. The mean K_d values for OTC were 1203, 586 and 1781 L kg⁻¹ for Armstrong, Huntington and Menfro soils, respectively. The mean K_d values for SDT were 27, 130 and 157 L kg⁻¹ for Armstrong, Huntington and Menfro soils, respectively. Distribution coefficient values of OTC are an order of magnitude greater than K_d values of SDT. The K_d values for OTC are in agreement with previous studies; however, K_d values for SDT are greater than values reported by others (Tolls 2004). We attribute this difference to differences in experimental conditions used in this study relative to others.

ANOVA results demonstrate that K_d values for OTC were significantly greater ($p < 0.05$) for VBS soils relative to cropped soils. For SDT, K_d values were significantly greater ($p < 0.05$) for tree/grass buffers relative to the other vegetation types studied. Irrespective of the antibiotic studied, K_d values were always significantly different ($p < 0.05$) between the soil types. The

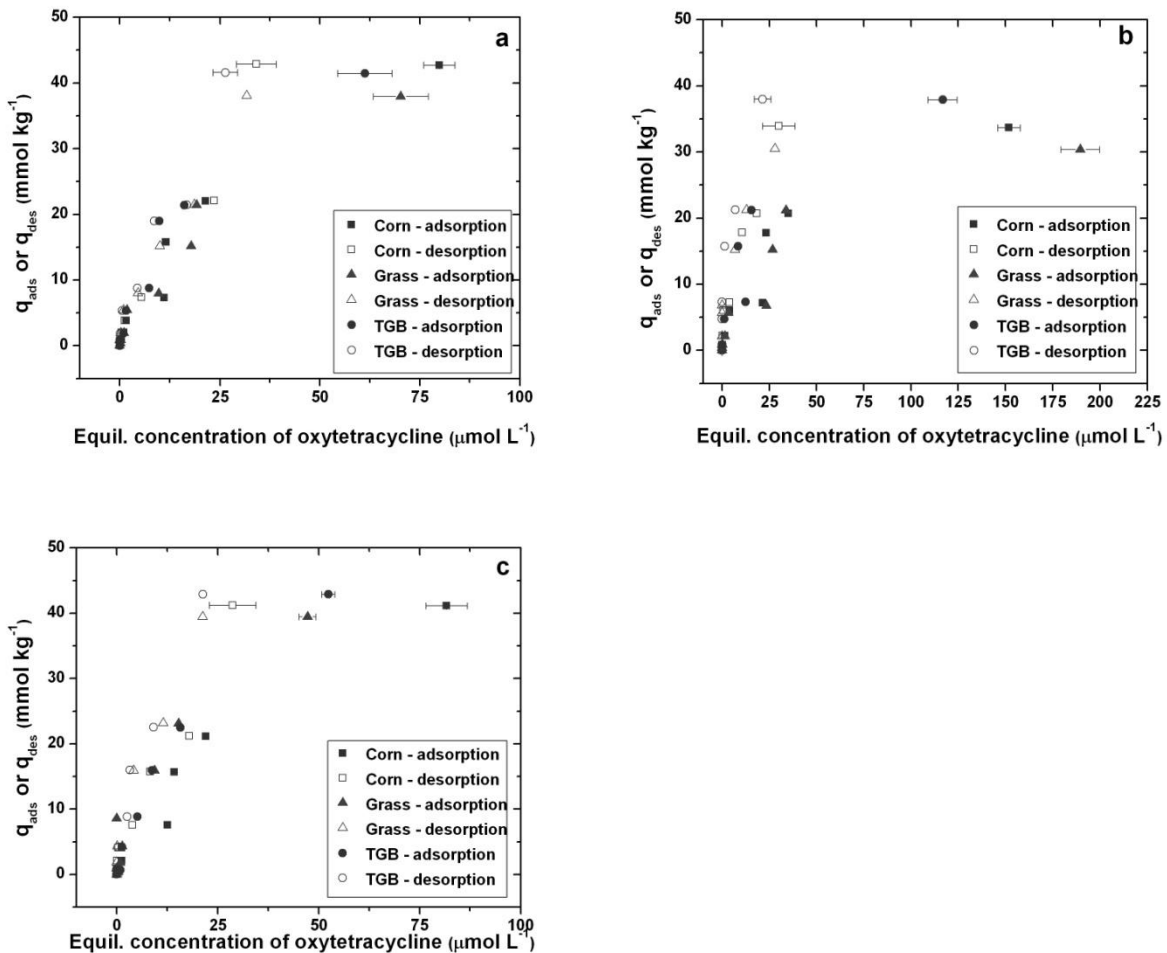


Figure 2. Oxytetracycline adsorption/desorption to the (a) Armstrong, (b) Huntington, and (c) Menfro soils. Error bars, where observed, represent the 95% confidence interval.

Table 2. Freundlich model parameters for OTC adsorption/desorption isotherms.

Soil	Phase	$\log K_f \pm 95\% \text{C.I.}$	$N \pm 95\% \text{C.I.}$	$R^2 (n)$
Menfro Corn/Soybean	Adsorption	3.51 ± 0.11	0.80 ± 0.13	0.90 (20)
	Desorption	3.81 ± 0.06	0.43 ± 0.06	0.94 (18)
Menfro Grass	Adsorption	3.20 ± 0.25	0.98 ± 0.22	0.82 (20)
	Desorption	3.98 ± 0.04	0.41 ± 0.04	0.98 (12)
Menfro Tree/Grass	Adsorption	3.36 ± 0.10	0.78 ± 0.10	0.92 (24)
	Desorption	1.35 ± 0.28	0.12 ± 0.01	0.92 (27)
Armstrong Corn/Soybean	Adsorption	3.33 ± 0.06	0.70 ± 0.05	0.97 (24)
	Desorption	3.60 ± 0.06	0.55 ± 0.06	0.95 (21)
Armstrong Grass	Adsorption	3.33 ± 0.06	0.70 ± 0.05	0.97 (24)
	Desorption	3.64 ± 0.06	0.57 ± 0.06	0.96 (18)
Armstrong Tree/Grass	Adsorption	3.42 ± 0.05	0.72 ± 0.05	0.97 (24)
	Desorption	3.74 ± 0.06	0.54 ± 0.06	0.96 (18)
Huntington Corn/Soybean	Adsorption	3.25 ± 0.07	0.63 ± 0.05	0.96 (24)
	Desorption	3.92 ± 0.10	0.35 ± 0.10	0.80 (17)
Huntington Grass	Adsorption	3.26 ± 0.07	0.58 ± 0.05	0.96 (24)
	Desorption	1.38 ± 0.36	0.12 ± 0.02	0.86 (27)
Huntington Tree/Grass	Adsorption	3.45 ± 0.08	0.61 ± 0.07	0.93 (24)
	Desorption	1.46 ± 0.37	0.12 ± 0.02	0.85 (27)

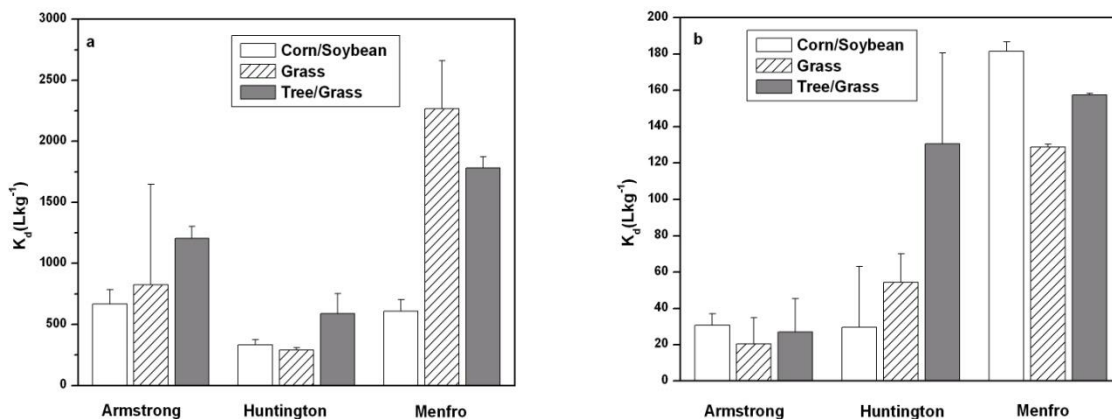


Figure 3. Solid-solution distribution coefficients (K_d) for (a) oxytetracycline and (b) sulfadimethoxine at 0.10 mM initial antibiotic concentration.

differences of the K_d between vegetation types may due to soil pH differences. Both OTC and SDT have multiple pK_a values; when pH is less than pK_{a1} , the cationic species are dominant and, at pH greater than pK_{a2} , the anionic species are dominant. Zwitterionic species are predominate within the pH range between these pK_a values. Within each site studied, soil pH is lowest in the tree/grass buffers and highest in the cropland soils, and this coincides with tree/grass buffers and grass buffers soils having greater K_d values than cropland soils. Thus, it appears that reduced pH within the buffer soils enhances antibiotic sorption via effects on antibiotic speciation. More acidic pH results in higher concentrations of cationic and zwitterionic antibiotic species that may interact to a greater extend with negatively charged sorption sites on clay minerals and soil organic matter.

Diffuse reflectance Fourier transform (DRIFT) spectroscopic analysis of HF-treated soils suggests that organic matter chemical composition is similar under the vegetative species planted on the three soil types (Fig. 4). Therefore, it does not seem likely that differences in soil organic matter (SOM) chemical composition play an important role in sorption differences observed between the vegetative buffer and cropland soils. Although, the quantity of SOM and subsequent effects on soil CEC may be important factors influencing antibiotic sorption capacity of the soils studied. Regression analyses further exploring correlations between soil properties and antibiotic sorption are in progress and will further elucidate interpretation of the data.

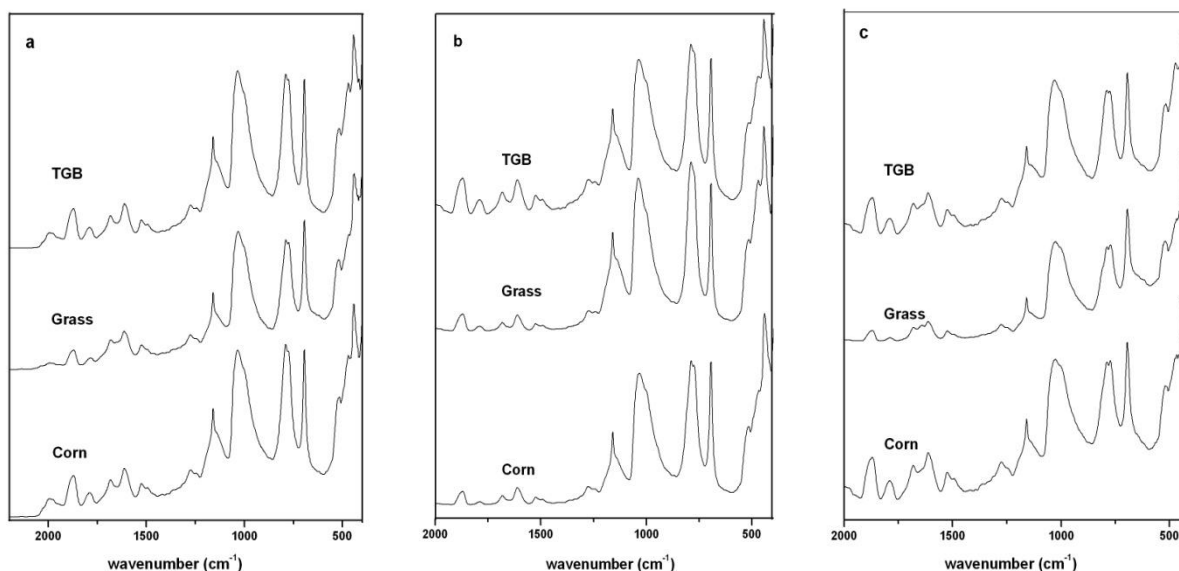


Figure 4. Diffuse reflectance Fourier transform (DRIFT) spectra of hydrofluoric acid-treated soils: (a) Armstrong soil, (b) Huntington soil, and (c) Menfro soil.

CONCLUSIONS

The OTC has high affinity to all soils studied and OTC was not readily desorbed by methanol; hysteresis was observed between all OTC adsorption and desorption isotherms. Distribution coefficient values of OTC are an order of magnitude greater than those of SDT, and K_d values were significantly different between soil types. More importantly, K_d values for OTC were significantly greater for VBS soils relative to cropped soils, and K_d values of SDT were

significantly greater for tree/grass buffers relative to the other vegetation types studied. Spectroscopic analysis of HF-treated soils suggests that organic matter chemical composition is similar under the vegetative species planted on the same soil type. Soil pH appears to have a significant influence on antibiotic adsorption due to effects on antibiotic species present in solution. Overall, the results indicate that VBS may help mitigate antibiotic loss from agroecosystems.

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