THE EFFECTS OF SOIL PROPERTIES ON THE SORPTION OF SELECTED CEPHALOSPORIN ANTIBIOTICS

By

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To the faculty of Washington State University:

The members of the Committee appointed to examine the thesis of REUVEN MIROPOLSKIY find it satisfactory and recommend that it be accepted.

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Abstract

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Cephalosporin antibiotics are widely used and could be a reason for the development of antibiotic resistance in certain strains of pathogenic bacteria. This threat of cephalosporin antibiotics to the environment depends on their transport and bioavailability in soil and water environments, for which their sorption to soil is a key factor. Laboratory sorption experiments have not yet been conducted for the wide range of cephalosporins, including cephalothin, cefoxitin, and ceftriaxone. This study examined the sorption of these three antibiotics to soils exhibiting distinct physicochemical properties, such as organic carbon content, clay content, cation exchange capacity and pH. Batch equilibrium experiments were conducted to determine sorption properties for the three antibiotics each interacting with the three types of soil.

Linear sorption coefficients (K_d) were obtained for each cephalosporin and soil combination. Cefoxitin was weakly sorbed to all soils, as its estimated K_d values ranged from 0.495 to 1.530 L/kg and were not highly affected by the soil properties examined. In contrast, ceftriaxone sorption to soil was affected by the soil properties; the estimated K_d values for

ceftriaxone varied from 1.57 to 1,103.4 L/kg. This large range of K_d values between the three soils may result from the highly ionizable nature of ceftriaxone which would lead to an increased number of sorption mechanisms. Of all the antibiotics tested, cephalothin had the highest sorption propensity, with a K_d value of 7.442 L/kg for the soil with the lowest organic and clay content; complete sorption was observed for the other soils.

The high sorption of cephalothin and ceftriaxone to soil suggests that these antibiotics are unlikely to leach to groundwater, but could undergo overland transport to surface waters as soil-bound contaminants during erosion processes. On the other hand, cefoxitin would more readily leach due to poor sorption characteristics. Hence this study underlines the importance of collecting sorption data for individual antibiotics in order to better predict their fate and bioavailability in aqueous and terrestrial environments.

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Chapter 1: Introduction

Surface and subsurface water contamination by cephalosporin antibiotics is an emerging environmental concern that has stimulated recent research on the transport and fate of antibiotics released into the environment, including sorption processes. This is a significant question since the total worldwide production of antibiotics per year is estimated to be 100,000 to 200,000 tons (Wise and Soulsby, 2002). Cephalosporins are a β-lactam class of antibiotics, accounting for approximately 37% of the total worldwide antibiotic production (Nwosu, 2001). Most of the cephalosporins consumed are commonly excreted in urine, feces, and manure as active substances or as metabolites (Qiang et al., 2006). Thus unmetabolized antibiotics may reach surface water through agricultural or municipal pathways (Kanda et al., 2003; Meyer et al., 2000). Evidence exists that cephalosporin usage has increased the development and spread of multi-drug resistant pathogenic bacteria (Dancer, 2001) that may enter the food chain. Due to the associated decreased effectiveness of antibiotics, there is public concern that we are returning to a pre-antibiotic age. Therefore, it is important to understand the fate of cephalosporins and their bioavailability once they are released into soil and water environments in the hope that the spread of multi-drug resistance can be controlled.

Sorption plays a major role in determining the environmental fate and bioavailability of organic chemicals such as cephalosporins. Several reviews of sorption studies have compiled linear sorption coefficients (K_d) for a variety of antibiotics and soils (Boxall et al., 2004; Thiele-Bruhn, 2003; Tolls, 2001), which range from 0.2 to 6,000 L/kg. High sorption coefficients are associated with low antibiotic mobility in aqueous environments and high sorption to soil. Antibiotics are considered to be highly mobile when $K_d \leq 5$ L/kg and slightly mobile when 5 L/kg $< K_d <$ 50 L/kg (Tolls, 2001). K_d values for cephalosporins, such as cephapirin, were found

to be in the range of 0.94 to 3.45 L/kg in soils with low organic carbon content (Peterson and O'Mears, 2008). These K_d values were comparable with other antibiotics studied in sandy soils, such as oxytetracycline, sulfamethazine, olaquindox, metronidazole, and chloramphenicol. The low K_d values indicate a low affinity of these antibiotics to soil and therefore make them more bioavailable (Lawrence et al., 2000).

There is a need to understand the interactions between soil and cephalosporins in order to predict their bioavailability, fate and transport. The majority of sorption literature has focused on non-cephalosporin groups of antibiotics such as tetracycline and sulfonamides. Current literature is lacking information in reference to cephalosporin sorption to soil particles except for cephapirin (Peterson et al., 2009; Peterson and O'Mears, 2008). No sorption studies were found for three cephalosporins: cephalothin, cefoxitin, and ceftriaxone. Thus, the goal of this study was to obtain sorption coefficients for these three cephalosporins by the batch equilibrium method while focusing on the effects of soil properties on sorption. This information can then be used to predict the mobility and bioavilability of cephalosporins in the soil and water environment. Finally, this study aimed to provide parameters that can be used to model the fate and transport of these antibiotics in natural systems.

Chapter 2: Literature Review

Batch equilibrium experiments have been used to determine sorption coefficients and examine the sorption kinetics for many chemicals (OECD, 2000). This method involves agitating a small amount of soil with a solution containing the compound of interest, then separating the liquid and solid phases and measuring the compound concentration in the aqueous phase. The sorbed concentration can be found by subtracting the equilibrium aqueous concentration from the initial concentration of the solution and is then used to deduce the ratio of the amount of chemical sorbed to the amount of sorbent used. Sorption isotherms are obtained by plotting the equilibrium aqueous concentration versus the sorbed concentration.

There are four general types of isotherms: S, H, C, and L-type (Figure 1). S-type isotherms indicate a low surface affinity for the sorbate at low concentrations. As the sorbate concentration increases more sorption occurs. This phenomenon could be a result from solute-solute interaction at the surface (Sparks, 1995). As concentration increase the affinity for sorbent decreases and less additional sorption is observed. An H-type isotherm indicates strong sorbate-soil interactions such as inner-sphere complexes. Compared to outer surface interactions, sorption of a chemical by inner complexes occurs much slower because it is not driven by the surface charge of the sorbent: however, the resulting interaction is much stronger. C-type isotherms or linear isotherms indicate a partitioning of the sorbed chemical between the liquid and solid phase with no specific sorption mechanism. L-type (Langmuir) isotherms can be found when sorbate affinity to sorbent is strong at low sorbate concentrations. This interaction decreases as the concentration of sorbate increases because the number of vacant sites decreases. Ultimately, isotherms do not answer the question of which sorption mechanism is involved in the

sorption phenomenon they reflect. However, they do show the macroscopic measurement of sorption (Sparks, 1995).

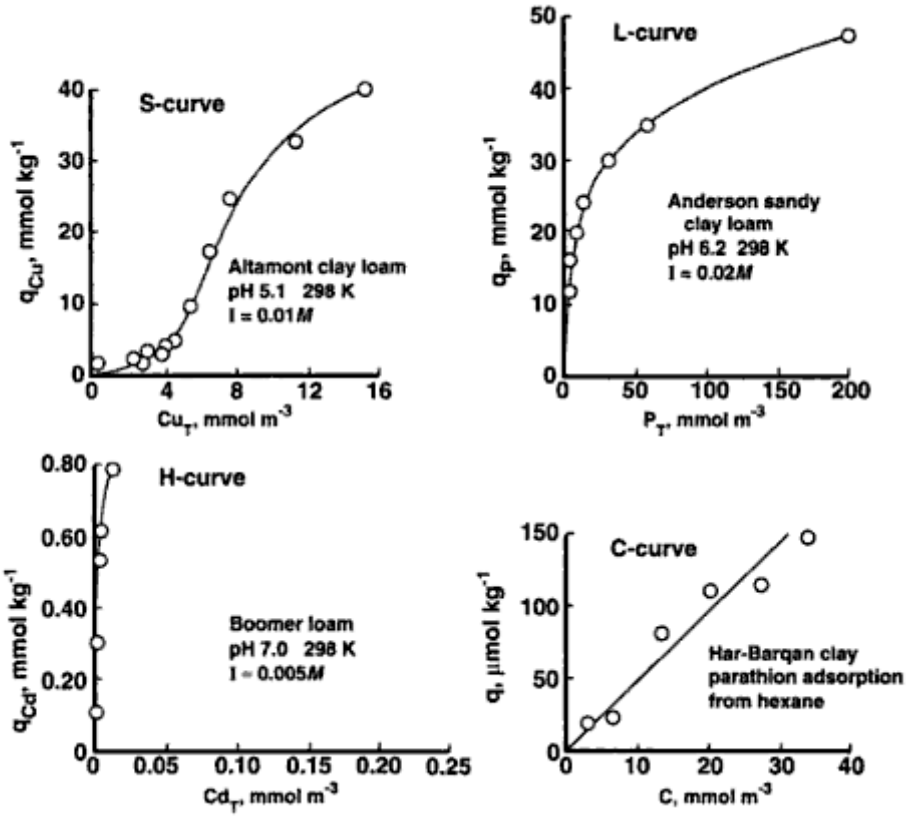


Figure 1. The four general types of adsorption isotherms (Sparks, 1995).

Sorption coefficients describe the extent to which the partitioning of a compound occurs between soil and aqueous solution at equilibrium. The linearity of sorption isotherms depends on the aqueous sorbate concentration, properties of the compound, and the soil being investigated. Linear isotherms are found most often at low concentration of contaminant. Linear partition coefficients, K_d, can be obtained from the slope of a linear isotherm. A nonlinear isotherm is better represented by nonlinear equations such as that of Freundlich or Langmuir (Sparks, 1995). Freundlich and Langmuir equations are sorption models widely used in environmental soil

chemistry to mathematically describe sorption. The Freundlich equation is an empirical model that can be used to fit any sorption data for organic and inorganic contaminants. Langmuir isotherms are often used when sorption increases until it reaches a sorption maximum or all the vacant sites on the surface become occupied. These two and other equations can be used to mathematically describe isotherms (Hinz, 2001). According to Hinz (2001), S-shape isotherms are most often described by Freundlich (when $1/n > 1$), modified Langmuir, or Fowler-Guggenheim equations. Although isotherms do not indicate the mechanism by which soil interacts with the antibiotic, they are often coupled with such equations to describe and predict the sorption behavior on macroscopic scale for organic chemicals.

It is difficult experimentally to probe the minutiae of microscopic mechanisms underpinning macroscopic sorption behavior. The mechanisms by which soil may interact with organic chemicals include hydrophobic bonding, van der Waals interactions, electrostatic interactions, hydrogen bonding, charge transfer, ligand exchange, direct and induced dipole-dipole interactions, and chemi-sorption (Boethling and Mackay, 2000). According to Boethling and Mackay, because of the complex nature of soils, it is difficult to determine which sorption mechanism is involved. Usually, for many soil and pollutant interactions, one or two mechanisms dominate the sorption process and generalizations regarding sorption behavior can be made. For example, the sorption of neutral, hydrophobic, organic chemicals correlates well with the organic carbon content of the soil. In the case of ionizable and polar organic compounds, such as many antibiotics, the organic carbon may not be a major factor affecting sorption. Instead, sorption of these chemicals more readily correlates with clay content, cation exchange capacity (CEC), pH, iron and aluminum oxides (Tolls, 2001). Hence if such

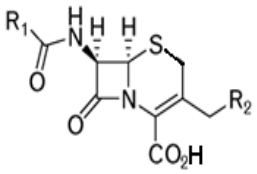
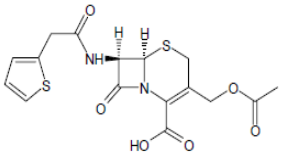
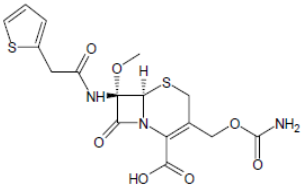
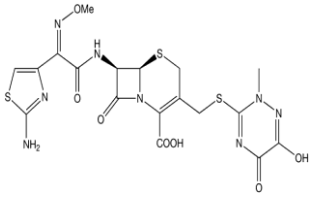
generalization about sorption behavior can be demonstrated, the sorption behavior of a compound could be better understood.

2.1 Cephalosporins

Three cephalosporins were investigated in this study. Cephalosporins belong to a larger class of β-lactam antibiotics which have a unique nonpolar core structure consisting of a four-membered ring (or β-lactam ring) which is fused with a six-member dihydrothiazine. All cephalosporins have the same core structure, but differ in their side chain substituents R_1 and R_2 as shown in Table 1.

Cephalosporin antibiotics are generally polar, hydrophilic, non-volatile, and thermolabile (Pehourcq and Jarry, 1998). Table 1 lists the pK_a values for the three cephalosporins used in this study as well as the octanol-water partition coefficients (K_{ow}) which can be used as a measure of the hydrophobicity. The polarity and low hydrophobicity is due to a carboxylic acid group (Hornish and Kotarski, 2002). More than one pK_a value may exist due to multiple functional groups that may be present in the R_1 and R_2 side chains of the cephalosporin. These functional groups may be protonated based on the solution pH and pK_a of the functional groups. For these specific cephalosporins, at a natural pH of 4 to 8 these functional groups are mostly deprotonated, resulting in negatively charged anions. At low soil pH, soil may gain a positive charge due to the presence of aluminum and iron oxides on the surface of clay particles. These charges increase the soil's capacity to attract anions. As the polarity, number of functional groups, and ionic nature of a soil increases, so does the number of possible mechanisms involved in sorption (Boethling and Mackay, 2000).

Table 1. Chemical structures of selected cephalosporins and their properties

Name	Chemical structures	Molecular weight (g/mol)	Log K_{ow}	pK_a
Cephalosporins*		334.4 – 470.3	0.9-2.9	2.7(COOH)
Cephalothin	$C_{16}H_{16}N_2O_6S_2$ 	396.44	2.11	2.2 (COOH)
Cefoxitin	$C_{16}H_{17}N_3O_7S_2$ 	427.454	2.11	3.5 (COOH)
Ceftriaxone	$C_{18}H_{18}N_8O_7S_3$ 	554.58	0.78	3.0 (COOH) 3.2 (NH^{3+}) 4.1 (OH)

Note: pK_a values were obtained from El-Shaboury et al. (2007), Log K_{ow} from Ferreira and Kiralj, (2004), and cephalosporin properties were obtained from the β-lactam general properties (Thiele-Bruhn, 2003).

2.2 Sources and Transport of Antibiotics in the Environment

Antibiotics reach aquatic and terrestrial environments primarily through urban and agricultural routes (Figure 2). Households, hospitals and manufacturing centers in urban systems release antibiotics into the environment through the effluent of sewage treatment plants. Veterinary antibiotic use presents an agricultural pathway for these compounds to enter the

environment. Infections, such as mastitis and respiratory diseases in cattle and swine, are treated by cephalosporins such as ceftiofur (Hornish and Kotarski, 2002). A large portion of these cephalosporins are excreted in urine; about 65% is excreted as chemically unchanged or as metabolites (BeconiBarker et al., 1996). Cephalosporins can be excreted directly to the soil by grazing animals or enter the soil following land application of livestock manure to fields. Continuous exposure of soil organisms to antibiotic residues from the application of manure can be a significant source of resistance development in microbial organisms (Nwosu, 2001). Residual cephalosporins can further migrate through groundwater flow within the soil column or be transported by runoff to surface waters.

In the receiving waters, cephalosporin antibiotics can remain as a parent compound or be hydrolyzed, conjugated, or decomposed into secondary byproducts. Unmetabolized cephalosporins and their derivatives can be further transported by stream flow, sorbed to sediments, biodegraded and/or slowly released back into the water column. The transport and fate of these antibiotics is largely affected by their binding affinity to organic and inorganic matter in soils. Trace levels of antibiotics in the water may assist the development of antibiotic resistant microbial organisms (Witte, 1998).

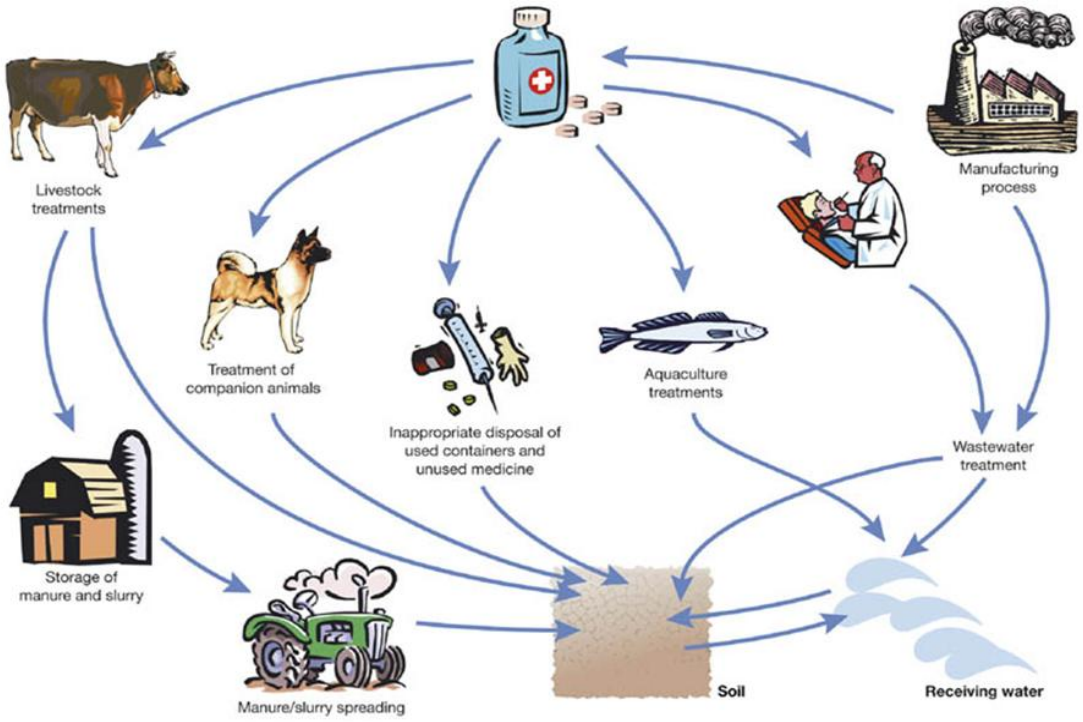


Figure 2. Routes by which antibiotic enter the aquatic and terrestrial environments (Tung and Christensen, 2007).

Slow biodegradation of various antibiotics has been observed (Al-Ahmad et al., 1999; Gilbertson et al., 1990). Al-Ahmad et al. (1999) found limited biodegradation of 7% after 28 days and 10% after 40 days for cephalosporins such as cefotiam. Gilbertson et al. (1990) reported half-lives of 22.2, 49.0 and 41.4 days for ceftiofur in three soils collected in California, Florida, and Wisconsin. No biodegradation rates were found for the antibiotics selected in this study: however, based on studies with other cephalosporins, a low percentage of biodegradation is expected during the 24-hour sorption experiments used here (< 5%). Gilbertson et al. (1990) also found that the hydrolysis half-lives of ceftiofur at 22 °C increased with increasing pH and were 100.3, 8.0, and 4.2 days at pH 5, 7, and 9, respectively. Similar results were obtained for cefoxitin which exhibited maximum stability in water at pH 5-7, where only 10% was hydrolyzed in 2 days at 25 °C (Oberholtzer and Brenner, 1979). However, this study involved

experiments with no soil addition, which could have significantly change hydrolysis rates. Depending on soil type, the degradation of cefoxitin in solution would greatly depend on soil constituents and the potential presence of enzymes that may enhance hydrolysis. Cefoxitin hydrolysis was also examined in the study presented in this thesis. Although cephalosporins are not the most persistent antibiotics due to their unstable core structure, the trace levels present in the environment can also have an effect on inducing resistance in the microbial community in soil and water ecosystems (Halford, 2008).

2.3 Cephalosporin occurrence and concentrations

Cephalosporin concentrations and occurrence have not been extensively studied. Based on currently available studies, β-lactams have not been found in some U.S. wastewater effluents or in the aquatic environment (Cha et al., 2006; Hirsch et al., 1999). The conclusion was that β-lactams were hydrolyzed or degraded in the environment. But another reason for the limited detection of these antibiotics in environmental waters may be a lack of appropriate and sensitive testing technology. However, β-lactam antibiotics from hospital effluents were found in Australian watershed in significant concentration (Watkinson et al., 2009). The total concentration of antibiotics ranged from 0.01 to 14.5 μg L^{-1} and was dominated β-lactam, quinolone and sulphonamide groups. Watkinson et al. (2009) also observed the highest concentration in WWTP influent for cephalexin, a cephalosporin antibiotic, up to 64 μg/L. The Watkinson's study investigated 28 antibiotics in five WWTPs, three hospital effluents, six rivers, and a drinking water source. The removal efficiency of the WWTPs, however, was approximately 80% and effluent contained β-lactam antibiotics in the low- to mid-parts per trillion (ppt) range. Watkinson concluded that although WWTP removal of antibiotics is relatively high, antibiotic input from WWTPs into surface water streams is still significant

compared to stream samples with no WWTP input. No antibiotics were found in drinking water, indicating that antibiotics in surface waters were not reaching drinking water sources at significant concentrations.

2.4 Antibiotic Determination

Currently, liquid chromatography (LC) is the most commonly used technique for the detection of cephalosporin antibiotics in environmental samples. Numerous methods using ultraviolet (UV) and fluorescence array detector (FAD) are available (Rao et al., 2008). Improved sensitivity was achieved using liquid chromatography/triple quadrupole tandem mass spectrometry (LC-MS/MS), and detailed in two reviews of analytical methods used to determine pharmaceutical compounds in surface water (Grujic et al., 2009; Hao et al., 2007). LC-MS/MS analytical equipment allows for pharmaceutical determination at parts per quadrillion (pg/L) concentrations in environmental samples (Kolpin et al., 2002). Newer MS/MS electrospray technologies allow the determination of unique fragment ions to be monitored, which in turn increases the capability to more reliably detect pharmaceuticals at trace levels compared to high performance liquid chromatography (HPLC) or simple LC/MS analysis (Rao et al., 2008).

2.5 Objectives

The objective of this study was to determine sorption coefficients for cephalothin, cefoxitin, and ceftriaxone by use of the batch equilibrium method. Sorption coefficients were also related to the properties of soil to determine the effects of soil properties on the sorption behavior of selected cephalosporins.

Chapter 3: Methods

3.1 Chemicals and Solutions

The cephalosporins considered were added to a 0.01M $CaCl_2$ solution to mimic the typical ionic strength of the aqueous and soil solutions found in the environment. The 0.01M $CaCl_2$ solution was prepared by dissolving 1.11 g of anhydrous calcium chloride from Fisher Scientific (Fairlawn, NJ) in a 1 L volumetric flask with milli-Q water. The 0.01M $CaCl_2$ solution was used as a background solution for controls (with no soil) and for samples with soil. The solution pH of experimental samples was controlled by the natural pH of each specific soil tested. The final solutions of antibiotics in the experimental samples contained less than 0.1% methanol (v/v), so the co-solvent effect was minimized.

Cephalothin, cefoxitin, and ceftriaxone were purchased as sodium salts from Sigma-Aldrich (St. Louis, MO). A stock solution of each antibiotic at 1 g/L was prepared in HPLC–grade methanol from JT Baker (Phillipsburg, NJ). Antibiotic stock solutions were prepared by measuring 50 mg of the selected cephalosporin in powder form and dissolving it in methanol using a 50 mL volumetric flask. This solution was transferred to two 35 mL amber glass vials and stored at 5 °C as the primary standard stock solution. A secondary standard solution was obtained by adding 0.05 mL of the primary stock solution to a 50 mL volumetric flask and diluting it with 0.01M $CaCl_2$. Secondary stock solutions were used in the experiment for preparing calibration standards and to spike samples in order to reach a desired concentration of antibiotic. All stock solutions were stored in amber glass volatile organic analysis (VOA) vials to minimize photodegradation.

3.2 Soils

Three soils representative of various locations throughout Washington State were collected and analyzed for physicochemical properties by a previous investigator in the Dr. Ullman's lab (Paternostre, 2008). The Pullman, Wenatchee, and Quincy soils had different texture and color as shown in Figure 3. All soils were collected from the top 10 cm of the soil profile, and were homogenized, sieved through a 2 mm sieve, and air-dried.

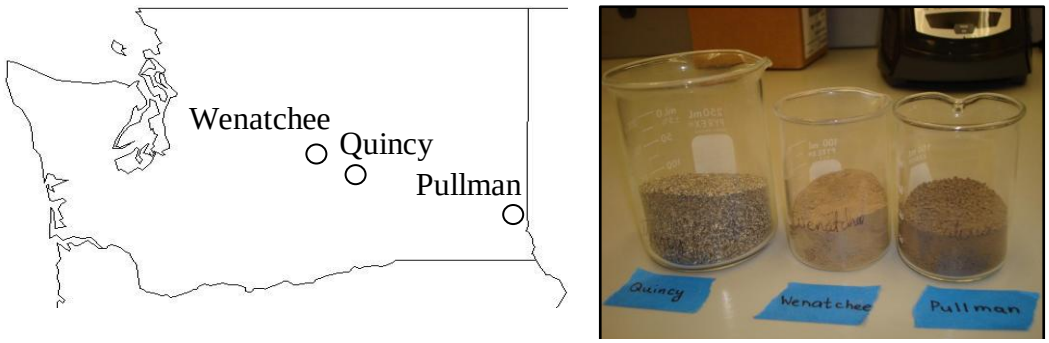


Figure 3. Sampling locations and soils from Washington State

Paternostre (2008) measured the following soil properties: pH, organic carbon (OC), cation exchange capacity (CEC), sand, silt, and clay content (Table 2). These soils covered a wide range of physicochemical properties. Pullman soil had the lowest pH and the highest organic matter, clay content, and CEC. In contrast, Quincy soil had lowest organic carbon, clay content, and CEC, and the highest pH. The Wenatchee soil properties fell in the middle of those of the two previous soils. The pH of the analyzed soils ranged from 4.7 to 7.8. The organic carbon content ranged from 0.24% to 2.27%, and the clay content ranged from 0% to 17%.The general trend for the selected soils was that as pH decreased the OC, CEC, and clay content increased.

Table 2. Physicochemical properties of soils (modified from Paternostre, 2008)

Soils	pH	OC %	CEC cmol(+)/kg	Sand %	Silt %	Clay %	Textural class
Pullman	**4.71**	**2.27**	**22**	**4**	**79**	**17**	**Silt Loam**
Wenatchee	**6.39**	**0.96**	**16**	**50**	**44**	**6**	**Sandy Loam**
Quincy	**7.75**	**0.24**	**7.3**	**98**	**2**	**0**	**SandSandy Loam**

3.3 Hydrolysis

Cefoxitin loss due to hydrolysis was monitored over 48-hours in a 0.01M $CaCl_2$ aqueous solution. The hydrolysis experiments were performed at an initial concentration of 1 mg/L and analyzed at 12, 24, 36, and 48 hours using four replicates. The experiments were conducted in 50 mL glass centrifuge tubes with Teflon caps, where 4.5 mL of water was spiked with 0.5 mL of cefoxitin at an initial concentration of 10 mg/L. Samples were placed on a reciprocating shaker in a dark room at 20°C. A staggering technique was implemented by which samples were spiked and placed on the shaker at specific times to account for the 15 minutes per sample runtime of the HPLC. At the set time intervals described earlier, the samples were removed and analyzed using the HPLC. The lowest quantification limit for cefoxitin in 0.01M $CaCl_2$ was 0.1 mg/L. A full calibration curve covering concentrations from 0.1 to 10 mg/L was prepared at 12 and 48 hours and quality controls were included at 24 and 36 hours.

3.4 Equilibrium Time Experiment

An equilibrium time experiment was performed with the Pullman soil, which exhibited the highest organic carbon and clay content of the soils used in this study. The purpose of this experiment was to find the time when the cefoxitin concentration reached a steady-state (dC/dt = 0). This experiment was performed using a 1:5 soil to solution ratio and at the natural pH of 4.7

for the Pullman soil. Two grams of Pullman soil were placed in a centrifuge tube and 9 mL of 0.01 M $CaCl_2$ were added. Twelve samples were examined and three replicates were included at four different sampling times. One control with no soil and one blank sample with soil and no spike were also included. Samples were vortexed for 30 seconds and placed on a reciprocating shaker and equilibrated in a dark walk-in environmental chamber for one hour at 150 rpm and 20 °C. The samples and control were then spiked with 1mL of cefoxitin at 1000 µg/L to bring the initial concentration of cefoxitin to C_0= 100 µg/L. Then samples were vortexed for 30 seconds and placed back onto the reciprocating shaker. Three samples were removed at 6, 24, 48, and 72 hours, and the supernatant was analyzed.

Sample preparation procedures were as follows: after centrifugation at 3000 x g for 30 minutes, supernatants were removed with a 3 mL syringe and needle. Two mL of supernatants were filtered through a 0.45 µm nylon syringe filter. The first few drops that saturated the filter were discarded and 1.5 mL was then placed into a 2 mL amber glass GC vial. These samples were analyzed on LC-MS/MS. Aqueous concentration of cefoxitin was normalized to the initial concentration and plotted versus time.

3.5 Sorption Experiments

A batch equilibrium method was used to determine the sorption characteristics of cephalothin, cefoxitin, and ceftriaxone to various soils. Experiments were conducted by following standard procedures (OECD, 2000). These procedures involved agitation of a small amount of soil with a solution containing the chemical of interest, followed by separation of the phases, and measurement of the concentration of the compound of interest in the aqueous phase. The experiments were conducted using glass centrifuge tubes with Teflon caps, continuously shaken on reciprocating shakers at 150 rpm.

Batch equilibrium sorption experiments were performed to determine sorption isotherms for cephalothin, cefoxitin, and ceftriaxone for the three different soils. Cefoxitin sorption experiments were conducted at two different levels of initial aqueous concentration: low level initial concentrations were 0.1, 1, 50, and 100 µg/L, and high levels were 10, 100, 500, and 1,000 µg/L. Sorption experiments with cephalothin and ceftriaxone were performed at initial aqueous concentrations of 1, 50, 100 and 200 µg/L. These concentrations include and exceed the range currently present in hospital effluent (0.01 to 14.5 µg L^{-1} as described by Watkinson et al., 2009), allowing for observation of changes in isotherm shape at higher initial concentrations than are present in the environment to determine whether the soil binding sites may become saturated. Triplicates were prepared for each concentration. In addition, a control of 100 µg/L with no soil and a blank with no antibiotic were included.

The experiment was performed using a 1:5 soil to solution ratio, as recommended by standard procedures (OECD, 2000). Accordingly, two grams of soil were placed into a 50-ml glass centrifuge tube and 9 mL of 0.01M $CaCl_2$ was added (leaving room for a 1 ml spike of antibiotic). The samples were vortexed for 30 seconds and equilibrated in a dark walk-in environmental chamber at 20 °C on a reciprocating shaker at 150 rpm for one hour. Following soil/solution equilibration, samples were spiked with the antibiotic, vortexed for 30 seconds, and placed back onto the shaker. Triplicates of each sample were removed after an assumed pseudo-equilibrium time of 24 hours. The samples preparation procedures were similar to that of equilibrium time experiment.

Detailed standard operating procedures are included in Appendix A. Raw data for all sorption experiments, as well as hydrolysis and equilibrium time experiments for cefoxitin, can be found in Appendix B. The pH was measured at the beginning and end of each experiment in

order to make sure that the pH did not significantly change throughout the experiment: the experimental pH can be found in Appendix B.7.

3.6 Chemical Analysis

The hydrolysis experiment was analyzed using an Agilent HP 1100 HPLC with diode array detection (DAD), as shown in Table 3. Conditions were similar to those discussed in Sigma-Aldrich Report 107 (Hugh Cramer, 2004). The injection volume was 50 µL and the mobile phase flow rate was set at 1 mL/min. The compounds were separated on a C-18 reversed phase column. The mobile phase A and B were 25% methanol and 75% 0.01 M KH_2PO_4, respectively. The detection wavelength was set at 254 nm.

Analysis of the equilibrium time and sorption experiments was performed on an Agilent 6460 triple quad liquid chromatographer (LC-MS/MS), as shown in Table 3. Compounds were separated on a Agilent XDB-C18 rapid resolution column and carried by a 60:40 mobile phase solution, consisting of 20 mM formic acid:acetonitrile. The LC-MS/MS produced a response, in terms of areas under peaks, which in turn related to the concentration of chemicals of interest by use of calibration curves. Calibration was performed before and after sample analysis. All calibrations were based on standards at 0.05, 0.1, 1, 10, 50, 100 and 200 µg/L of cephalothin and ceftriaxone. Cefoxitin calibration standards captured the range of concentrations from 0.05 to 1,000 µg/L.

Table 3. HPLC and LC-MS/MS types and operating parameters.

Conditions	HPLC (Agilent HP 1100)	LC-MS/MS (Agilent 6460 triple quad)
Column	C-18 reversed phase	Agilent XDB-C18 rapid resolution column
Mobile phase	25% methanol and 75% 0.01 M KH_2PO_4	60:40 (20 mM formic acid:acetonitrile).
Detection	DAD (254nm)	Precursor Ion: 389 (cefoxitin) Product Ion: 345.1 (cefoxitin)
Injection	50 µL	40 µL
Runtime	10 minutes	5 minutes
Gas Temperature		300 °C
Gas Flow		7 mL/min
Nebulizer		20 psi
Sheath gas temp		250 °C
Sheath gas flow		9 L/min
Capillary		4000 V

3.7 Data Analysis

After building the isotherms, a relationship between the equilibrium aqueous concentration of the chemical and the sorbed concentration of the chemical was described by the following equations: the linear isotherm (eq. 1) and the nonlinear Freundlich, modified Langmuir, and Fowler-Guggenheim isotherms (eq. 2, 3, and 4, respectively):

$$C_s = K_d C_e \qquad \text{eq. (1)},$$

$$C_s = K_f C_e^{\,1/n} \qquad \text{eq. (2)},$$

$$C_s = \frac{bK_{1L} \cdot C_e}{(1+K_{1L} \cdot C_e) \cdot (1+K_{2L} \cdot C_e)} \qquad \text{eq. (3)},$$

$$C_e = \frac{\frac{C_s}{c}}{\left(1-\frac{C_s}{c}\right) \cdot K_1 * \exp\left(K_2 \cdot \frac{C_s}{c}\right)} \qquad \text{eq. (4)},$$

where C_s and C_e are sorbed and aqueous concentrations of the antibiotic at equilibrium in units of (µg/kg) and (µg/L), respectively. K_d, K_f, K_{1L}, and K_{2L} are linear, Freundlich, and Langmuir partition coefficients in units of (L kg^{-1}), (µg $^{1-1/n}$ L $^{1/n}$ kg^{-1}), and (L kg^{-1}), respectively. K_1 and K_2

are Fowler-Guggenheim coefficients. Constants n, b, and c are the Freundlich, the Langmuir, and the Fowler-Guggenheim constants.

Using C_e and C_s, the sorbed equilibrium concentration of a cephalosporin in the soil is found by equation 5, and the percentage of initial mass sorbed to soil is given by equation 6,

$$C_S = \frac{(C_0 - C_e) * V_{solution}}{m_{soil}} \qquad \text{eq. (5)},$$

$$A(\%) = \frac{(C_0 - C_e)}{C_0} * 100\% \qquad \text{eq. (6)},$$

where C_o is the initial aqueous concentration of the contaminant (µg/L), $V_{solution}$ is the initial volume (L), and m_{soil} is the mass of soil (g).

To build the isotherms the equilibrium concentration in aqueous solution was plotted versus the equilibrium concentration in the soil. Then the Linear, Freundlich and Langmuir equations were fit to the resulting isotherms using a nonlinear fitting algorithm embedded in Igor Pro software (WaveMetrics Inc.).

3.8 Batch Equilibrium Method Assumptions

In order to use the batch equilibrium method, several assumptions must be made. First, the assumption that equilibrium has been reached must be correct in order to accurately predict sorption coefficients. Secondly, no chemical loss due to microbial, photo or chemical degradations should occur during the experiment. Photolysis can be prevented by performing the experiment in the dark. In the literature, biodegradation was not found to be a significant factor over short time periods, such as the 24 hour duration used in these experiments. Degradation by hydrolysis should not play a significant role in altering the aqueous concentration of cefoxitin in 0.01M $CaCl_2$ after 48 hours based on the literature (Oberholtzer & Brenner, 1979). Use of controls and blanks ensures that there is no significant loss of a compound or contamination

during the experiment. The batch equilibrium technique also assumes good mixing occurs to ensure homogeneity and a constant temperature. Mixing was accomplished by placing centrifuge tubes at a 45° angle and placing them on reciprocating shaker at 150 rpm. Experimental temperature was kept constant in a walk-in environmental chamber at 20 °C. Keeping samples refrigerated at 5 °C, and preparing fresh standards for each experiment is advised for further prevention of hydrolysis.

Chapter 4: Results and Discussion

4.1 Hydrolysis

The cefoxitin hydrolysis experiment conducted at an initial concentration of 1 mg/L showed no apparent decrease in cefoxitin aqueous concentration after 48 hours (Figure 4). The average aqueous concentration was maintained at 1 mg/L, with a standard deviation of 0.02 mg/L. Based on these results, cefoxitin in a 0.01M $CaCl_2$ aqueous solution did not undergo rapid hydrolysis. This observation is in accord with other hydrolysis studies which indicated no significant cefoxitin hydrolysis in 48 hours (Oberholtzer and Brenner, 1979).

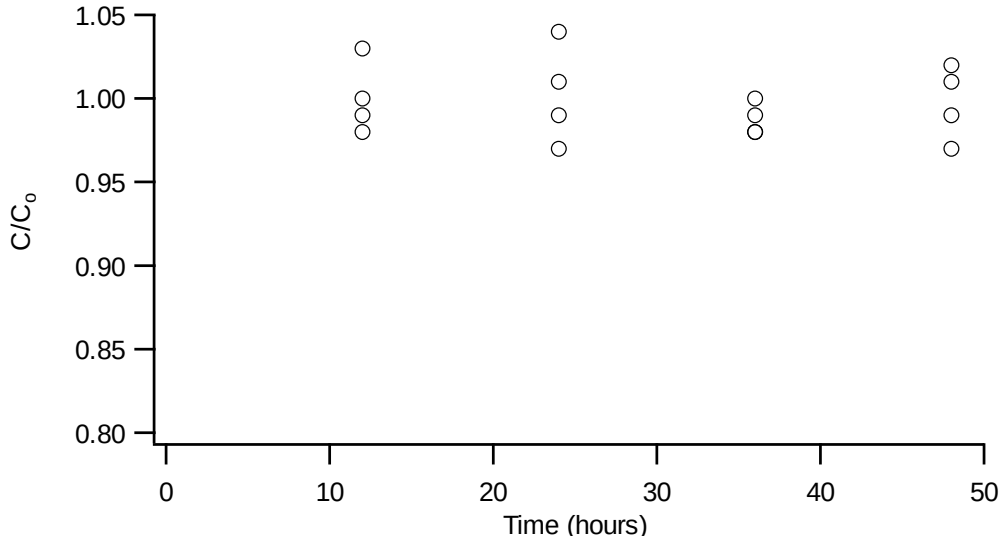


Figure 4. Hydrolysis experiment for cefoxitin at C_o = 1 mg/L did not show significant decrease in concentration after 48 hours.

4.2 Equilibrium Time Experiment

Results for the preliminary equilibrium time experiment failed to confirm the expected 24 hour cefoxitin equilibrium time. After 72 hours, the concentration of cefoxitin in the aqueous

solution was still steadily decreasing, as shown in Figure 5. The Environmental Protection Agency (EPA) defines equilibrium time as 5% or less change in solution concentration over a 24 hour period (Roy et al., 1992). However, the aqueous concentration decreased by 6% over each 24 hour period. Less rigorous definitions for equilibrium times (compared to the EPA definition) have been used, such as a 10 % or less change in solution concentration during a 6 hour period (Jones et al., 1977).

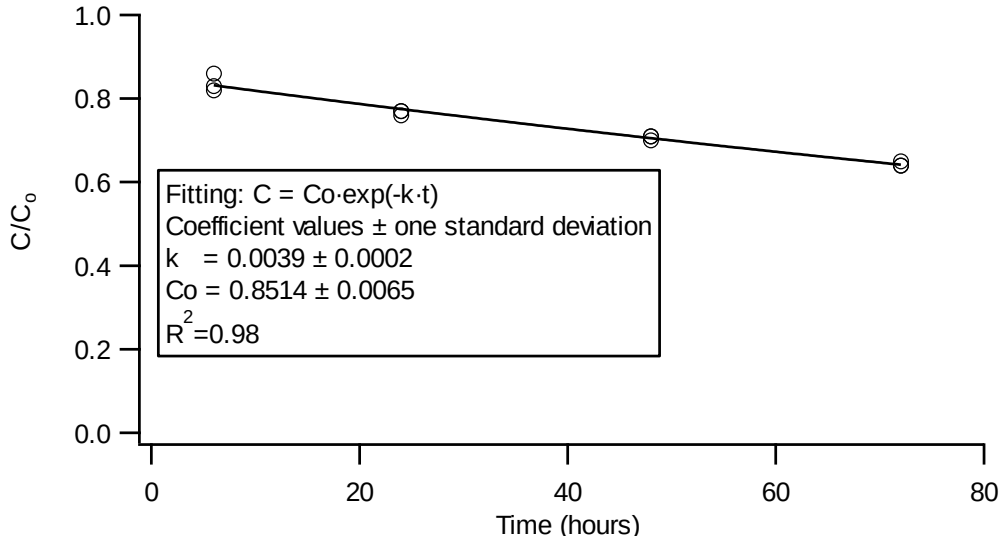


Figure 5. Equilibrium time experiment for cefoxitin at C_o = 100 µg/L and 1:5 soil/solution ratio

The EPA equilibrium definition was satisfied after six days into the experiment using the pseudo-first order sorption kinetics equation from Figure 5. This equation was used to predict percent changes in concentration at time periods beyond the conclusion of the experiment (Table 4). The control value of 86 µg/L matched the initial concentration predicted by the fitted line. However, hydrolysis could have occurred in 72 hours of experiment, because the control was lower than the initial concentration of 100 µg/L.

Table 4. Predicted percent change in aqueous cefoxitin concentration over time

Days	C/Co	% Change
0	0.85	
1	0.78	7.61
2	0.71	6.93
3	0.64	6.31
4	0.59	5.75
5	0.53	5.23
6	0.49	4.76

The equilibrium times for cefoxitin and other cephalosporins used in this study were not available in the literature. However, sorption experiments with other cephalosporins, such as cephapirin, used a 24 hour equilibrium time (Peterson and O'Mears, 2008). Thus, a 24-hour equilibrium time was selected for all sorption experiments in this study.

4.3 Sorption Isotherms

Sorption isotherms were obtained using an assumed 24 hour equilibrium time for cephalothin, cefoxitin, and ceftriaxone using the three different soils (Pullman, Wenatchee, and Quincy). Data were fit to linear, Freundlich, modified Langmuir, and Fowler-Guggenheim equations, using an algorithm embedded in the Igor Pro ® software. This algorithm allowed for the determination of the unknown parameters in each of the applied equations.

4.3.1 Cefoxitin

Separate cefoxitin isotherms for low and high initial concentrations were combined (Figure 6). The two experiments were combined because t-test showed no significant difference at a 0.01 significance level between the two trials with the initial concentration of 100 µg/L (Appendix C). A p-value of 0.045 was obtained for the Pullman soil. This value was slightly lower than 0.05. Hence, the null hypothesis of no difference in the mean values could be rejected

for the Pullman soil alone at a 0.05 significance. However, the null hypothesis could not be rejected at the 0.01 significance level for all the soils.

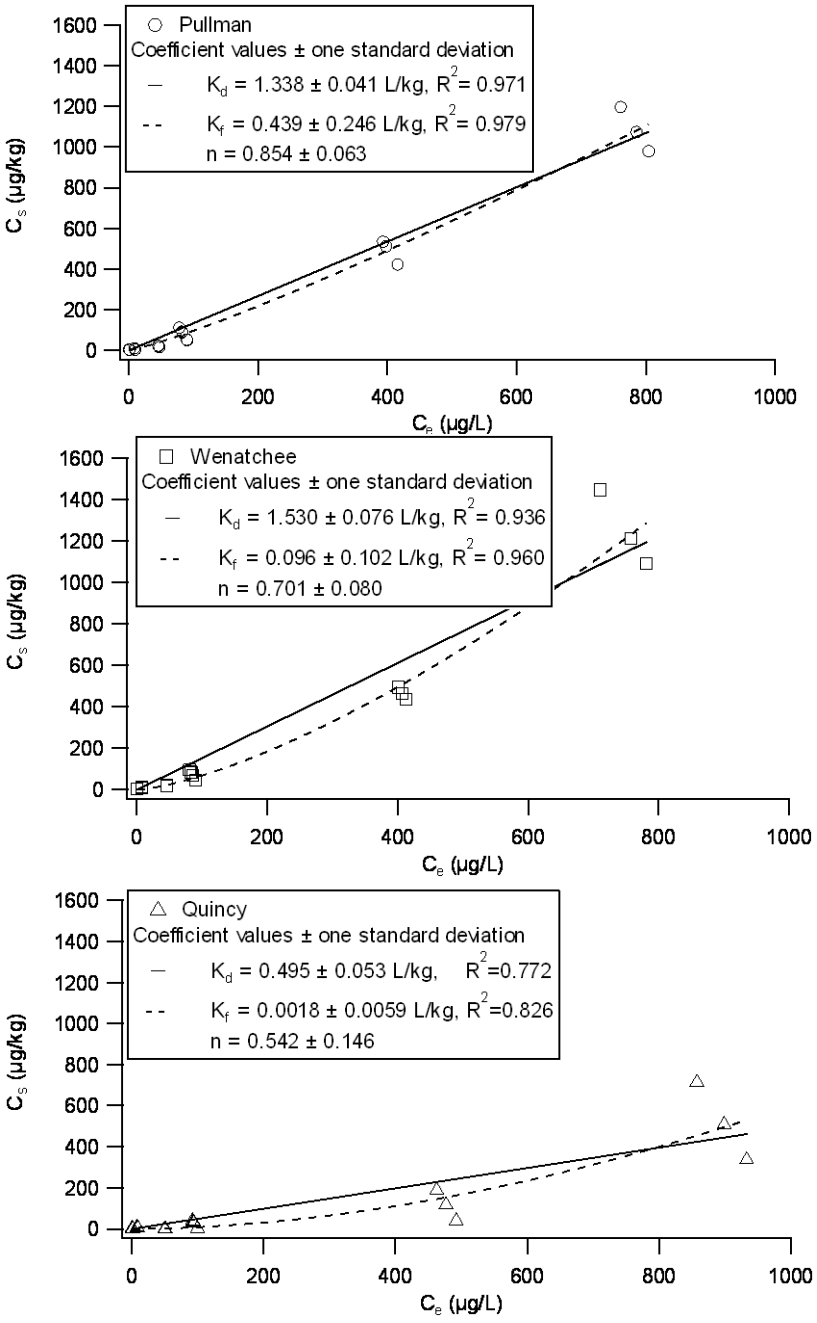


Figure 6. Combined cefoxitin isotherms in Pullman, Wenatchee and Quincy soil were fitted with linear and Freundlich equations.

The linear sorption coefficients (K_d) for the combined experiments ranged from 0.495 to 1.530 L/kg. Cefoxitin K_d values were close to the range of K_d values obtained for another cephalosporin, namely cephapirin, which exhibited a K_d range of 0.94 to 3.45 L/kg in a dune sand (Peterson and O'Mears, 2008). Samples with the lowest initial concentration 0.1 µg/L were below the lowest calibration standard of 50 ng/L, and therefore not quantifiable. The controls (initially at 100 µg/L) were 94 and 105 µg/L for the low and high level experiments, respectively. These controls indicate no loss of chemical during the 24-hour experiments.

S-type isotherms were obtained for this concentration range for Wenatchee and Quincy soils. Nonlinearity was indicated by the Freundlich nonlinearity constant $n < 1$ in Wenatchee and Quincy soils. Based on the R^2 value, nonlinear S-type data fit better with Freundlich and Fowler–Guggenheim isotherm equations compared to modified Langmuir and Linear (Table 5). However, due to the large standard deviation on all the nonlinear models, these models should be used with caution and the linear model may still be the most appropriate form to use to characterize sorption.. The large standard deviation might be due to the insufficient number of data points and a larger spread of replicates at higher concentrations. This S-type isotherm was also obtained for cephapirin in quartz filter soil (Peterson and O'Mears, 2008).

Table 5. Combined cefoxitin sorption data and goodness of fit for the Linear, Freundlich, modified Langmuir and Fowler-Guggenheim isotherms for Wenatchee and Quincy soils.

Sorbent	Wenatchee	Quincy
Linear	K_d = 1.530 ± 0.076 R^2=0.936	K_d = 0.495 ± 0.053 R^2=0.772
Freundlich	K_f = 0.096 ± 0.102 n = 0.701 ± 0.079 R^2=0.960	K_f = 0.0018 ± 0.0059 n = 0.542 ± 0.146 R^2=0.826
Modified Langmuir	K_{1L} = 0.00024 ± 0.00267 K_{2L} = -0.000603 ± 0.000876 b = 4339.1 ± 4.6e+004 R^2=0.893	K_{1L} = 3.36e-005 ± 0.00495 K_{2L} = -0.000595 ± 0.00157 b = 7647.7 ± 1.12e+06 R^2=0.716
Fowler–Guggenheim	K_1 = 5.64e-005 ± 0.00182 K_2 = 9.30 ± 265 c = 15076 ± 4.88e+005 R^2=0.988	K_1 = 3.06e-005 ± 0.00167 K_2 = 14.2 ± 713 c = 6918.4 ± 3.79e+005 R^2=0.912

The highest K_d value was observed in the Wenatchee soil, followed by the Pullman and the Quincy soils. The Pullman soil, with its low pH value, was expected to have the highest K_d value relative to the other soil types, because it exhibits the highest organic carbon and clay content. Furthermore, the Pullman soil has the lowest pH and anion binding is expected to increase with decreasing pH. This inconsistency suggests an unidentified soil property may influence the sorption propensity of cefoxitin to different soils. As an alternative, the nonlinear nature of the Wenatchee and Quincy isotherms could have artificially inflated their K_d results. Nevertheless, only a small difference in K_d values was observed between the Pullman and Wenatchee soils, despite their large differences in soil properties. However, larger differences were found between the Quincy soil K_d values and those for the Wenatchee and Pullman soils, but these differences are still relatively small compared when considering the large difference among the properties exhibited by the three soils. Thus, the results indicate that soil organic carbon and clay content, pH and CEC do not exert an overwhelming dominance in governing cefoxitin sorption and other unidentified influencing factors may play a significant role.

Experimental partition coefficients were correlated using Pearson's correlation coefficients (Table 6). Pearson's correlation coefficients (r) measure the strength of linear dependence between two variables and range from -1 to 1. Results indicated a low correlation between cefoxitin K_d values and the soil properties. These results underscore the weak dependency of cefoxitin sorption on soil properties. Better correlation was found for cefoxitin K_f values and the soil properties. Hence, it suggests that sorption of cefoxitin was better explained by Freundlich equation.

Table 6. Pearson correlation coefficients for soil properties correlated with the linear and Freundlich sorption coefficient for cefoxitin

	Pearson's r	
Soil Properties	Kd (L/kg)	Kf (L/kg)
pH	-0.725	-0.967
OC (%)	0.649	0.989
Clay (%)	0.647	0.989
CEC (cmol(+)/kg)	0.829	0.912

Pearson correlation coefficients for organic carbon and clay content were almost identical, because of the coincidentally high linear correlation between these two properties (r=1). The correlation between CEC and K_d was lower than that found for organic and clay content. Therefore, organic carbon and clay probably play a more important role in ceftriaxone sorption. The main driving force for sorption of anions to soil would be positively charged surface soil chemistry. Therefore, it would be more relevant to correlate anion exchange capacity (AEC) with K_d rather than CEC, and it would be beneficial to find AEC and correlate it with K_d or K_f. Unfortunately, the AEC was not obtained for this experiment, so the role AEC plays in these experiments remains unknown.

4.3.2 Ceftriaxone

The ceftriaxone isotherms exhibited mostly linear behavior at the concentration ranges used ($R^2 > 0.9$), taking either C or H shapes (Figure 7). In other words, the concentration of the antibiotic sorbed to soil increased proportionally with an increase in the aqueous concentration. This linear behavior is often found in natural systems where contaminant concentrations are low or the soils exhibit a high binding affinity for organic compounds (Sparks, 1995). The control measurement of 104 µg/L (for C_o = 100 µg/L) indicated that no hydrolysis had occurred, and the instrument performed within acceptable error limits.

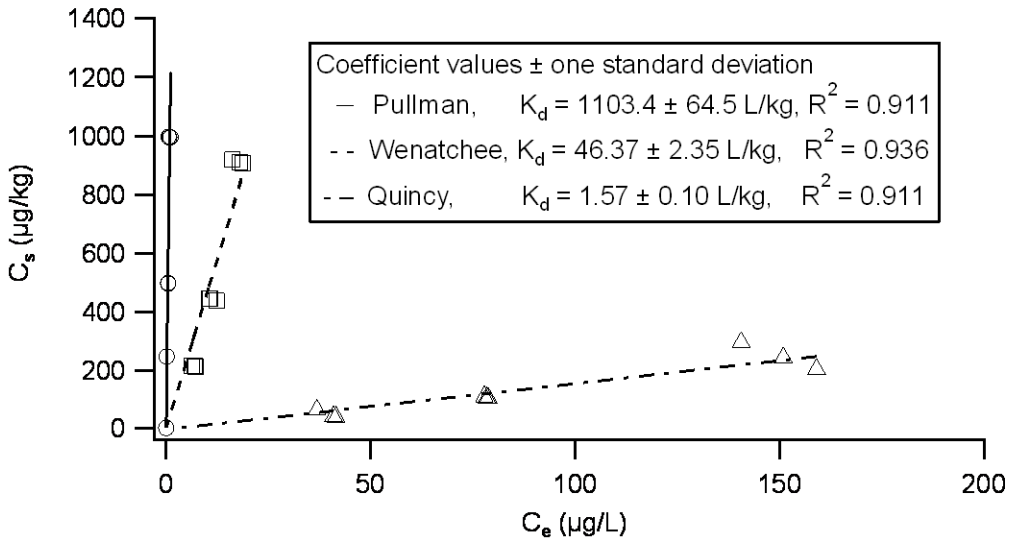


Figure 7. Ceftriaxone isotherms in Pullman, Wenatchee and Quincy soil. Isotherms were fitted with linear equation.

The highest sorption coefficient was observed with the Pullman soil (K_d = 1103.4 ± 64.5 L/kg), followed by the Wenatchee soil (K_d = 46.37 ± 2.35 L/kg), and the Quincy soil (K_d = 1.57 ± 0.10 L/kg). The ceftriaxone K_d value for the Pullman soil was almost three orders of magnitude higher than that of the Quincy soil and about 20 times higher than for the Wenatchee soil. This pattern found for the K_d values between soil types relates to the corresponding organic carbon and clay content, CEC and pH parameters for each soil, indicating that some combination of these parameters play a governing role in ceftriaxone sorption to soil. With three functional groups, ceftriaxone is highly ionizable, meaning that a higher number of sorption mechanisms may participate and it is possible that ceftriaxone sorption has a degree of pH dependence. At the experimental pH values between 4 and 8, most ceftriaxone species are present as anions in the solution. Ceftriaxone anions therefore are capable of interaction with the positively charge aluminum and iron oxides that can form at low pH. Experimental partition coefficients were correlated using Pearson's correlation coefficients (Table 7). Ceftriaxone K_d and K_f correlated

well with all soil properties, suggesting that ceftriaxone sorption is affected by the soil properties described here. However, other properties that were not measured, such as aluminum and iron oxides, could be also involved. These results also suggest that ceftriaxone isotherms can be adequately described with linear and Freundlich equations because 1/n is close to 1. Other ionizable antibiotics such as tetracyclines showed the same behavior of increasing sorption with decreasing pH (Figueroa et al., 2004).

Table 7. Pearson correlation coefficients for soil properties correlated with the linear and Freundlich sorption coefficient for ceftriaxone

	Pearson's r	
Soil Properties	Kd (L/kg)	Kf (L/kg)
pH	-0.910	-0.899
OC (%)	0.949	0.941
Clay (%)	0.949	0.941
CEC (cmol(+)/kg)	0.829	0.815

4.3.3 Cephalothin Isotherms

Of all the antibiotics tested, cephalothin had the highest sorptive capacity, with K_d value exceeding 7.442 ± 0.228 L/kg for the Quincy soil (Figure 8). The Quincy isotherm was linear with R^2=0.98. Sorption isotherms for Pullman and Wenatchee soil were not obtained, since after 24 hours most of the cephalothin in the aqueous phase had sorbed to the soil and the amount remaining in solution was below the 50 ng/L analytical detection limit. Based on these results, cephalothin was found to sorb to soils more strongly than cefoxitin and ceftriaxone. This can be explained by the structural differences in cefoxitin and cephalothin. There is no difference in hydrophobicity between the two chemicals based on the log K_{ow} from Table 1. However, cephalothin's nonpolar substitutional group in the R_2 position makes cephalothin less polar

compared to cefoxitin. The nonpolar part of cephalothin could participate in nonpolar interactions with organic carbon by such mechanism as van der Waals; while the carboxylate group can bind to iron and aluminum oxides or quartz (SiO_2) when pH is low. For example, this sorption behavior was studied for cephapirin carboxylate group sorption to positively charged quartz surface (SiO_2) using Raman spectroscopy (Peterson et al., 2009). Peterson et al. observed higher sorption of cephapirin onto quartz with decreasing pH because of the higher positive charge on the surface.

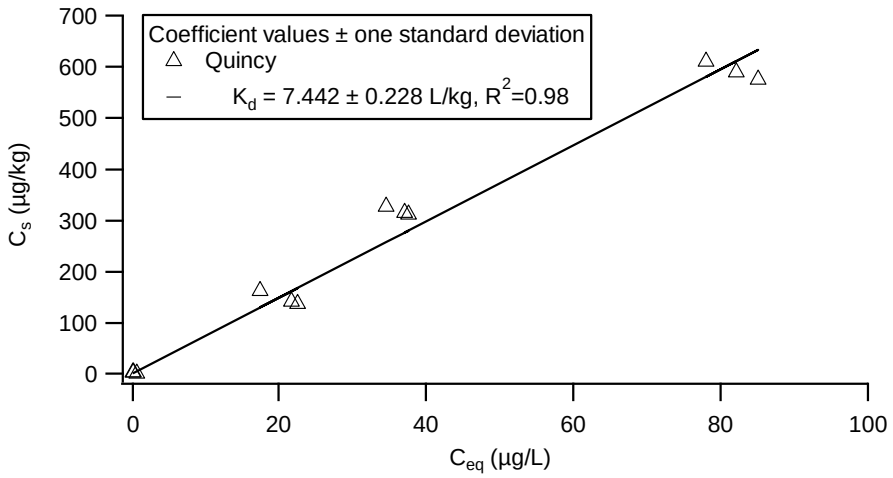


Figure 8. Cephalothin isotherm in Quincy soil was fitted with linear isotherm.

4.4 Sorption Comparison

The highest sorption propensity was observed for cephalothin followed by ceftriaxone and cefoxitin (Figure 9). The percentages of cephalosporin sorbed to the Quincy soil (initially at 100 µg/L) were 5.6, 21.7, and 63.6% for cefoxitin, ceftriaxone, and cephalothin, respectively. The amount of cephalothin sorbed to the Quincy soil was approximately 3 times higher than ceftriaxone and 10 times higher than that for cefoxitin. Cephalothin and ceftriaxone almost completely sorbed to the Wenatchee and Pullman soils. The higher organic carbon and clay

content, and lower pH of these soils seems to be responsible for greater sorption of cephalothin and ceftriaxone compared to sandy soil (Quincy).

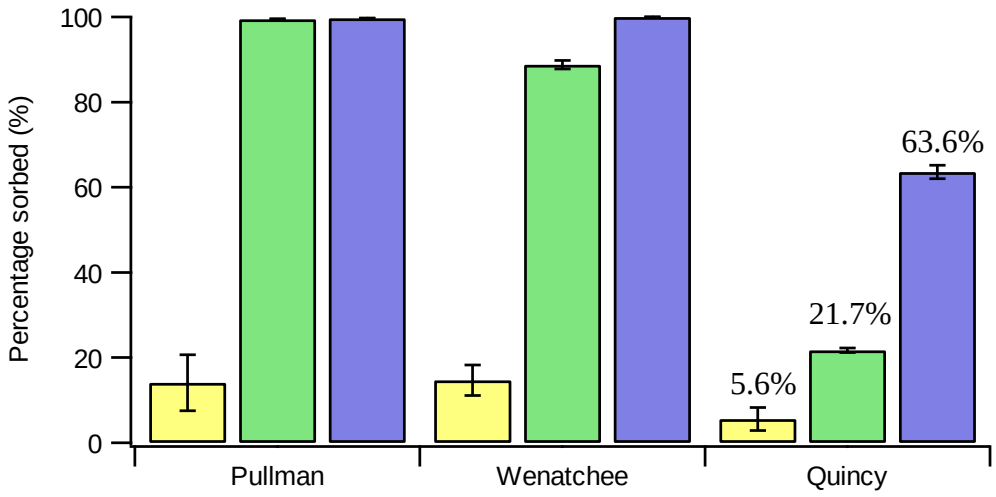


Figure 9. Percentage of cephalosporins sorbed to three soils at initial concentration of 100 µg/L. Cefoxitin (yellow), Ceftriaxone (green), and Cephalothin (blue) are placed in the same order from left to right for each soil.

The wide range of sorption coefficients obtained in this study is comparable to the trend exhibited by various antibiotic classes compiled by the Thiele-Bruhn (2003) (Figure 10). The low sorption affinity of cefoxitin is similar to such antibiotic classes as sulfonamides and imidazoles. Ceftriaxone's highest K_d value exceeded that of the macrolides, but was lower than tetracycline K_d values. Cephalothin appears to be in the range of the tetracyclines K_d values. However, we did not obtain values for the highest sorption capability of cephalothin to soils; therefore, it is difficult to predict the highest K_d for cephalothin. Fluorquinolones were not placed in the figure; however, their K_d values (7.7 to 5,612 L/kg) might also be in the range of cephalothin K_d values.

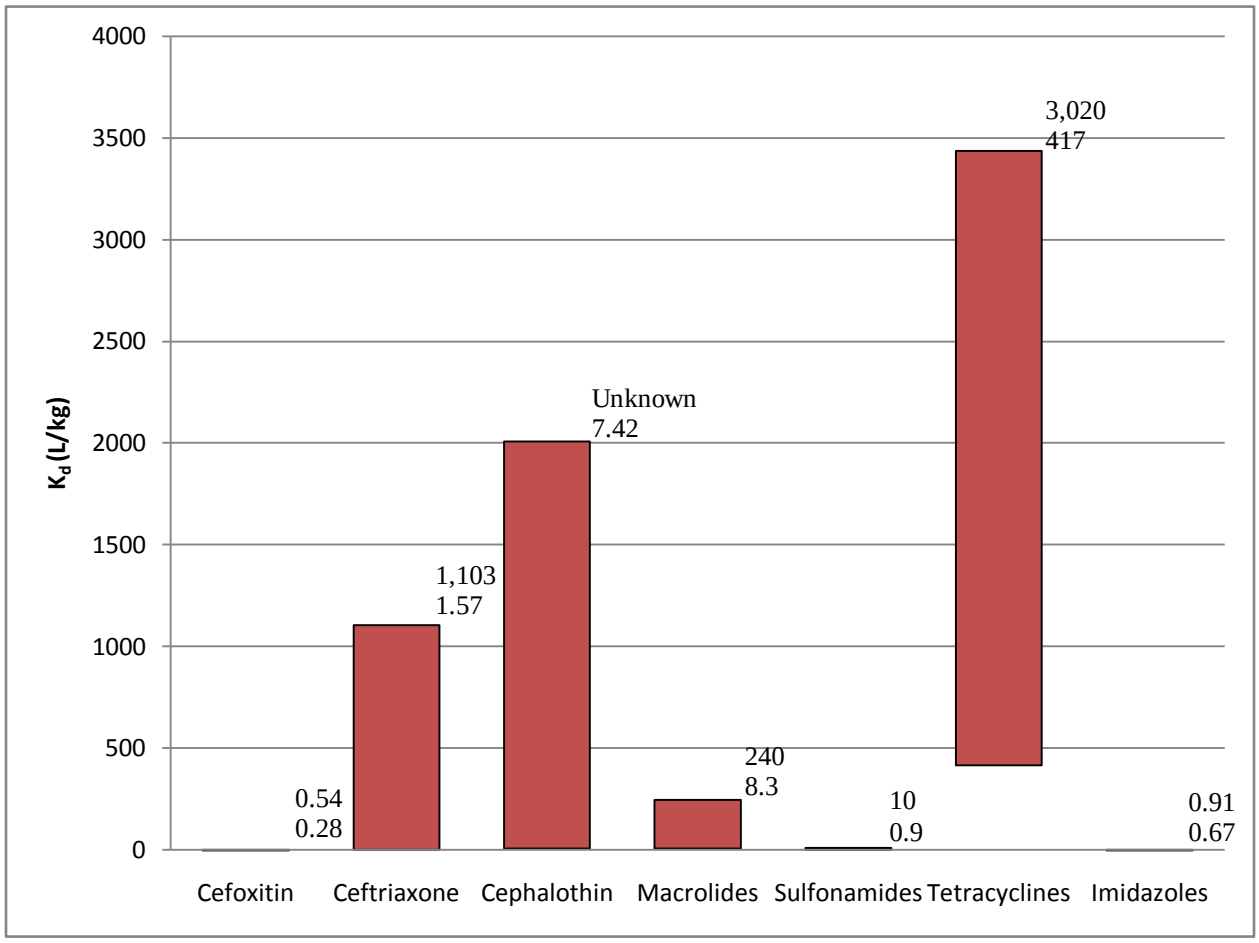


Figure 10. Experimental K_d ranges compared to that of major classes of antibiotics (obtained from Thiele-Bruhn, 2003)

Chapter 5: Conclusions

- The hydrolysis experiment showed no antibiotic loss due to hydrolysis degradation during the 48 hour duration of the experiment at 1 mg/L and 20 °C.

- Based on 24 hour batch equilibrium sorption experiments:

    - Cefoxitin sorption was found to be small and not strongly governed by the soil properties measured. Nonlinear isotherms were best described by the Freundlich equation.

    - Ceftriaxone sorption was highly dependent on the soil properties measured. Ceftriaxone isotherms were adequately described by both linear and Freundlich equations.

    - Cephalothin sorption was the highest out of three cephalosporins.

- Cefoxitin exhibited the highest bioavailability and mobility potentials out of the three cephalosporins.

    - Cefoxitin is more likely to leach through the soil and contaminate ground water. Cefoxitin can also be transported by runoff into surface water in the aqueous phase.

    - Cephalothin and ceftriaxone may reside longer in soil and have a longer effect on microbial organisms in the soil if it becomes bioavailable. They can also be transported as soil-bound contaminants during erosion processes.

Chapter 6: Suggestions for Future Research

- Perform a longer equilibrium time experiment with cefoxitin.
- Optimize the soil/solution ratio for cephalothin to find K_d values for the Pullman and Wenatchee soils.

OECD guidelines suggest using a lower soil to solution ratio for highly sorptive chemicals (OECD, 2000). The soil to solution ratio can be optimized before the equilibrium time experiment, followed by the determination of the sorption isotherms. These steps should be followed if the time frame of the project, funding, and equipment are available. OECD advises starting with a 1:5 ratio and changing the ratio until an optimal ratio is found, which should yield 20% sorption at the least, and preferably be higher than 50% (OECD, 2000). A ratio that gives more than 90% sorption will leave only 10% in the aqueous phase, which may be lower than the detection limit of the method. Therefore, the optimal ratio would be the one that yields a detectable aqueous concentration that still exhibits a significant change in concentration.

- Increase the number of soil samples to better predict dependency on simultaneously-acting soil properties.

For ceftriaxone, the antibiotic exhibiting the highest soil property dependence along with strong linearity, the partition coefficient can be found with more soils using multiple regression analysis in the form:

$$K_d = a + b \cdot (pH) + c \cdot (OC) + d \cdot (Clay) + e \cdot (CEC) \quad \text{eq. (5)},$$

where a, b, c, d, and e are empirical constants found by regression for the respective soil properties. This information is useful for predicting sorption parameters for a particular pollutant.

- Perform column studies with the lowest soil-sorbing antibiotic, cefoxitin, in order to determine its mobility in soil.
- Determine the effect of pH on ceftriaxone sorption to soil.
- Determine the effect of temperature to reflect hot and cold climates.

Appendix A: Standard Operating Procedures

Appendix A1: Hydrolysis Experiment

Introduction and Background:

Hydrolysis of cefoxitin or breakdown of this chemical in water was tested in a 48 hour long experiment. It is important to find the potential loss of cefoxitin due to reaction with water. All the conditions were the same as in other sorption experiments.

Equipment and Supplies:

To perform the experiment, the following equipment and supplies were used:

- 3 ml syringes
- Needles
- 0.45 um nylon syringe filters
- 50 mL centrifuge tubes with Teflon caps
- 2 ml amber glass GC vials with crimp caps
- Pasteur pipettes
- 0.2, 1 and 5 mL pipettes
- Volumetric flasks: 5, 10, 25 ml
- Digital scale
- Centrifuge
- Reciprocating shaker
- HPLC

Procedures:

1. Prepare 0.01 M $CaCl_2$ in milliQ-H_2O
2. Prepare 1 mg/L secondary standard in 0.01 M $CaCl_2$ water
3. Prepare 3 samples at 12, 24 , 36, and 48 hours at concentration of 100 ug/L cefoxitin, and 1:5 (g/mL) soil to solution ratio
4. Prepare 5 standards for calibration curve of cefoxitin at 0.1, 0.2, 0.5, 1, and 10 mg/L
5. This gives a total of 17 samples to be run on the LC-QQQ (12 samples + 5 standards)

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1. Prepare 0.01M $CaCl_2$ water:
    a. Add 1.11 g $CaCl_2$ to 1 L volumetric flask
    b. Place a stirrer on the bottom
    c. Add milliQ-H_2O to line
    d. Mix thoroughly for 30 min
2. Prepare 50 mL of 10 mg/L cefoxitin in 0.01 M $CaCl_2$ water:
    a. Add 0.5 mL of 1 g/L cefoxitin stock solution in methanol at room temperature to a 50 mL volumetric flask
    b. Add 0.01 M $CaCl_2$ water to line, stopping to mix thoroughly and finally inverting to mix at the correct level
    c. Transfer to 2, 35 mL amber vial
3. Sample preparation (12 samples)
    a. Add 4.5 mL of 0.01 M $CaCl_2$ to centrifuge using 5 mL pipette
    b. Add 0.5 mL of Cefoxitin at an initial concentration of 10 mg/L
    c. Vortex for 30 sec

d. Place samples on shaker

e. Shake for 12, 24, 36 and 48 hours

f. Stop shaker

g. Remove centrifuge tubes

h. Place an un-used hypodermic needle on a 3 mL syringe

i. Place needle in supernatant and extract 2 mL supernatant into 3 mL syringe

j. Take needle off and place in sharps container

k. Place a 0.45 um nylon membrane filter on the opening of the syringe

l. Depress supernatant into waste (about 10 drops)

m. Depress about 1.5 mL supernatant into labeled, amber GC vial

n. Secure GC vial cap

o. Label (samples A1-12)

p. Discard syringe and filter into waste container

4. Prepare standards from the same secondary stock solution as above at concentration of 0, .05, 0.1, 1, 10, 50, and 100 ug/L, on the day it is analyzed from 1 mg/L solution prepared in step 1

    a. Prepare each concentration using appropriate dilution

    b. Transfer solutions to GC vials and label accordingly

5. Samples are to be analyzed immediately on HPLC

Appendix A2: Equilibrium Time Experiment

Introduction and Background:

The purpose of equilibrium time experiment is to find the time where the concentration of antibiotics in aqueous phase does not change. The initial concentration cefoxitin in aqueous solution was 100 ug/L. Cefoxitin solution of 10 ml was applied to 2g of Pullman soil which gave the highest sorption based on previous experimentation. This samples were removed after 6, 24, 48, and 72 hours, samples were centrifuged, filtered and analyzed on the LC-QQQ to determine the filtrate concentration. One experimental blank and one control were tested at 72 hour. The concentration of cefoxitin in the soil was determined based on initial and final concentrations of cefoxitin in aqueous solution measured on the LC-QQQ.

Equipment:

To perform the experiment, the following equipment was used:

- 3 ml syringes
- Needles
- 0.45 um nylon syringe filters
- 50 mL centrifuge tubes with Teflon caps
- 2 ml amber glass GC vials with crimp caps
- Pasteur pipettes
- 0.2, 1 and 5 mL pipettes
- Volumetric flasks: 5, 10, 25 ml
- 10 mL beakers
- Digital scale
- Centrifuge

- Reciprocating shaker
- Agilent 6460 Triple Quad LC/MS

Procedures:

1. Prepare 1 mg/L secondary standard in 0.01 M $CaCl_2$ water
2. Prepare 3 soils: Pullman, Wenatchee, and Quincy
3. Prepare 12 samples, 1 blank, 1 control: Use one solution concentration 100 ug/L with cefoxitin at 6, 24, 48, and 72 hours shaking time.
4. Determine pH
5. Prepare 7 standards for calibration curve of cefoxitin (0, 0.05, 0.1, 1, 10, 50, and 100 ug/L)
6. This gives a total of 21 samples to be run on the LC-QQQ (12 samples + 1 blank + 1 controls + 7 standards).

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1. Prepare 50 mL of 1 mg/L cefoxitin in 0.01 M $CaCl_2$ water:
    a. Add 0.05 mL of 1 g/L cefoxitin stock solution in methanol at room temperature to a 50 mL volumetric flask
    b. Add 0.01 M $CaCl_2$ water to line, stopping to mix thoroughly and finally inverting to mix at the correct level
    c. Transfer to 2, 35 mL amber vial
2. Prepare soil:
    a. Obtain about 25 g of each soil
    b. Sieve through 2 mm sieve
3. Sample preparation (3 sample reps for 3 soils at 6, 24, 48, and 72 hours shaking time):

a. Weigh 2 g of soil in a clean 50 mL centrifuge tube (+/- 0.001 g). (Note: Skip this step for controls)
b. Add 9 mL of 0.01 M $CaCl_2$ to centrifuge using 5 mL pipette twice (once at 5 mL and once at 4 mL)
c. Vortex soil and water solution for 30 sec
d. Place on reciprocating shaker and shake for 1 hour at 150 rpm, 20°C, and in the dark at a 45° angle
e. Stop the shaker
f. Add 1 mL of 1 mg/L cefoxitin in 0.01 M $CaCl_2$ water; this gives a final initial cefoxitin concentration of 100 ug/L. (Note: skip this step for blank)
g. Vortex for 30 sec
h. Place samples back on shaker as described in step 4d
i. Shake for 24 hours
j. Stop shaker
k. Remove centrifuge tubes
l. Centrifuge at 3000g for 30 min (Setting between 4 and 5 on the constant angle centrifuge)
m. Place an un-used hypodermic needle on a 3 mL syringe
n. Place needle in supernatant and extract 2 mL supernatant into 3 mL syringe
o. Take needle off and place in sharps container
p. Place a 0.45 um nylon membrane filter on the opening of the syringe
q. Depress supernatant into waste (about 10 drops)
r. Depress about 1.5 mL supernatant into labeled, amber GC vial

s. Secure GC vial cap

t. Label (samples A1-15, blanks B1-3, controls C1-9)

u. Discard syringe and filter into waste container

4. Determine sample pH for soil-water slurries (at 0 and 72 hours)

    a. Calibrate the pH meter

    b. Weigh 2 g of soil in a clean 50 mL centrifuge tube (+/- 0.001 g)

    c. Add 10 mL of 0.01 M $CaCl_2$ to centrifuge using 5 mL pipette twice (once at 5 mL and once at 4 mL)

    d. Vortex for 30 sec

    e. Place stir bar in a test tube and insert pH probe; make sure that pH probe is thoroughly cleaned each time using milliQ-H_2O water

    f. Determine sample pH at the beginning of experiment

    g. Place sample on the shaker

    h. Determine sample pH at the end of experiment

5. Prepare standards from the same secondary stock solution as above at concentration of 0, .05, 0.1, 1, 10, 50, and 100 ug/L, on the day it is analyzed from 1 mg/L solution prepared in step 1

    a. Prepare each concentration using appropriate dilution

    b. Transfer solutions to GC vials with glass pipettes and label accordingly

6. Place all samples in refrigerator until being analyzed. Samples are to be analyzed the same morning

Notes:

1. Keep antibiotic stock solutions in the refrigerator, covered in aluminum foil

2. Blanks are soil, water and no spike

3. Controls are water and spike

Appendix A3: Sorption Equilibrium Experiment

Introduction and Background:

Batch equilibrium sorption experiments were performed to build sorption isotherms for cephalothin, cefoxitin, and ceftriaxone for three different soils. The simplified procedures are shown in Figure 11.Triplicates of each concentration was used to build isotherms at 1, 10, 100 and 200 µg/L. The control at 100 µg/L and blank with no spike were included. The experiment was performed by using a1:5 grams of soil to milliliters of solution ratio. Two grams of soil were placed into a glass centrifuge tube and 9 mL of 0.01M $CaCl_2$ were added. The samples were vortexed for 30 seconds and equilibrated in a dark walk-in environmental chamber at 20 °C on a reciprocating shaker at 150 rpm and for one hour. Following the one hour equilibration of soil and 0.01M $CaCl_2$, samples were spiked with the antibiotic at a desired initial concentration. Vortexed for 30 seconds and placed back onto the shaker. Triplicates of each sample were removed after equilibrium time of 24 hours. Following centrifugation at 3000 x g for 30 minutes, supernatants were removed with a 3 mL syringe and needle. The supernatants were then filtered with a 0.45 µm nylon syringe filter. The first few drops saturating the filter were wasted. Then about 1.5 mL filtrate was placed into a 2 mL amber glass GC vial. Samples were analyzed on a triple quad liquid chromatographer (LC-QQQ) to determine the aqueous concentration. The concentration of cefoxitin in the soil was determined based on the initial and final concentrations of cefoxitin in aqueous solution. The equilibrium concentration in aqueous solution was then plotted versus equilibrium concentration in the soil and various equations were applied to fit the data with a curve using Igor Pro software (WaveMetrics Inc.).

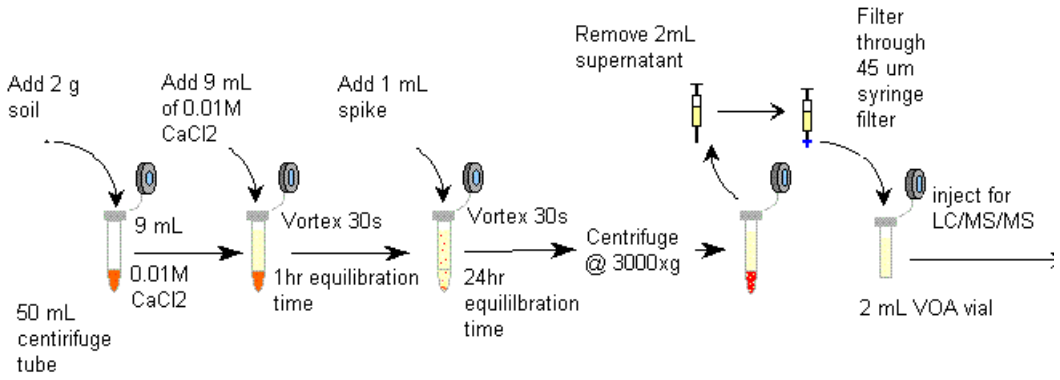

Figure 11. Schematic diagram of sample preparation for batch equilibrium experiment

Summary:

1. Prepare 1 mg/L secondary standard in 0.01 M $CaCl_2$ water
2. Prepare 3 soils: Pullman, Wenatchee, Quincy
3. Prepare 36 samples, 1 blank, and 1 control: Use five concentration solution at: 1, 50, 100, 200 µg/L; use 24 hours equilibrium time
4. Determine pH
5. Prepare 7 calibration standards for cefoxitin (0.05, 0.1, 1, 50, 100, and 200 ug/L)
6. This gives a total of 45 samples to be run on the LC-QQQ (36 samples + 1 blank + 1 control + 7 standards)

---

1. Prepare 50 mL of 1 mg/L cefoxitin in 0.01 M $CaCl_2$ water:
    a. Add 0.05 mL of 1 g/L cefoxitin stock solution in methanol at room temperature to a 50 mL volumetric flask
    b. Add 0.01 M $CaCl_2$ water to line, stopping to mix thoroughly and finally inverting to mix at the correct level
    c. Transfer to 2, 35 mL amber vial

2. Prepare soil:
    a. Obtain about 25 g of each soil
    b. Sieve through 2 mm sieve
3. Sample preparation (3 sample reps for 2 antibiotics and 3 ratios each soil):
    a. Weigh 2 g of each soil in a clean 50 mL centrifuge tube (+/- 0.001 g). (Note: Skip this step for controls, and A-Pullman, B-Wenatchee, C-Quincy)
    b. Add 9 mL of 0.01 M $CaCl_2$ to all centrifuge tubes except for A4-A6 which will be filled with 9.5 mL. Use 5 mL pipette twice (once at 5 mL and once at 4 mL).
    c. Vortex soil and water solution for 30 sec
    d. Place on reciprocating shaker and shake for 1 hour at 150 rpm, 20°C, and in the dark at a 45° angle
    e. Stop the shaker
    f. Add 1 ml of 1 mg/L cefoxitin to A1-A3; 0.5 mL of 1 mg/L cefoxitin to A4-A6; 1mL of 10 µg/L to A7-A9; 1mL of 1 µg/L to A10-A12; this gives a final initial cefoxitin concentration of 100, 50,1, and 0.1 ug/L. (Note: skip this step for blank, use 100 µg/L of cefoxitin for control)
    g. Repeat the same steps for Wenatchee and Quincy.
    h. Vortex for 30 sec
    i. Place samples back on shaker as described in step 4d
    j. Shake for 24 hours
    k. Stop shaker
    l. Remove centrifuge tubes

m. Centrifuge at 3000g for 30 min (Setting between 4 and 5 on the constant angle centrifuge)
n. Place an un-used hypodermic needle on a 3 mL syringe
o. Place needle in supernatant and extract 2 mL supernatant into 3 mL syringe
p. Take needle off and place in sharps container
q. Place a 0.45 um nylon membrane filter on the opening of the syringe
r. Depress supernatant into waste (about 10 drops)
s. Depress about 1.5 mL supernatant into labeled, amber GC vial
t. Secure GC vial cap
u. Label (samples A1-12, blanks B1-B12, controls C1-12)
v. Discard syringe and filter into waste container

4. Determine sample pH for soil-water slurries (after 24 hours)
    i. Calibrate the pH meter
    ii. Weigh 2 g of soil in a clean 50 mL centrifuge tube (+/- 0.001 g)
    iii. Add 10 mL of 0.01 M $CaCl_2$ to centrifuge using 5 mL pipette twice (once at 5 mL and once at 4 mL)
    iv. Vortex for 30 sec
    v. Insert pH probe; make sure that pH probe is thoroughly cleaned each time using milliQ-H_2O water
    vi. Determine sample pH at the beginning of experiment
    vii. Place sample on the shaker
    viii. Determine sample pH at the end of experiment

5. Prepare standards from the same secondary stock solution as above at concentration of 0, 0.05, 1, 50, 100, and 200 ug/L, on the day it is analyzed from 1 mg/L solution prepared in step 1
    a. Prepare each concentration using appropriate dilution
    b. Transfer solutions to GC vials with glass pipettes and label accordingly
6. Place all samples in refrigerator until being analyzed. Samples are to be analyzed the same morning

Appendix B: Raw Data

Appendix B1: Hydrolysis

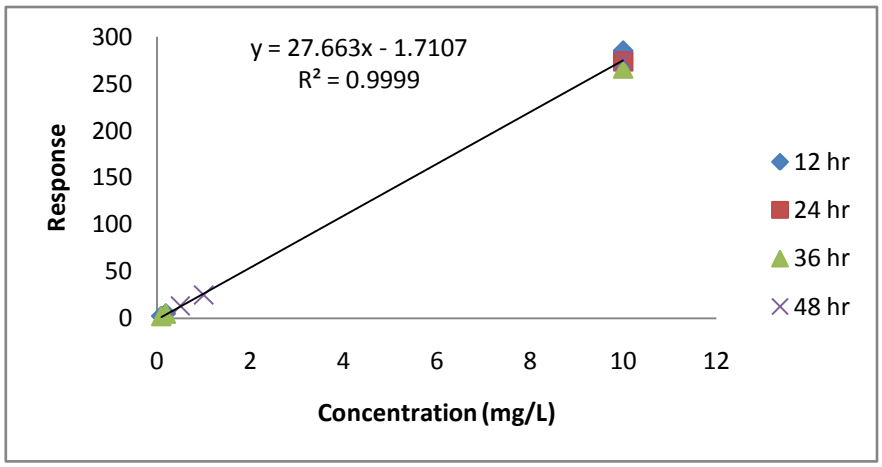

Figure B1. HPLC calibration curve for cefoxitin (hydrolysis experiment)

12hr

Slope	28.53288
Intercept	-0.329542

36 hr

slope	26.59394
intercept	-0.039194

average

slope	27.56167
intercept	-1.710729

Table B1. Calibration curves for hydrolysis experiment on HPLC at 12, 24, 36, and 48 hours

Date	Standard concentration (µg/L)	Response				Average Response
		12hr	**24hr**	**36hr**	**48hr**	
	0.1	2.6		2.6		2.6
	0.2	5.3		5.3		5.3
	0.5				13.25	13.25
	1				24.76	24.76
	10	285	273.65	265.9	274.98	274.8825

Table B2. Hydrolysis raw data (done on HPLC)

Sample	Day	Time(hrs)	Inoculation Time	Area	C_e (mg/L)	Average C_e (mg/L)	Std dev
W1	0	12	8:00 AM	28.13	1.00	1.00	0.02
W2		12	8:15 AM	27.9	0.99		
W3		12	8:30 AM	27.7	0.98		
W4		12	8:45 AM	29.1	1.03		
W5	1	24	8:00 PM	29.45	1.04	1.00	0.03
W6		24	8:15 PM	28.42	1.01		
W7		24	8:30 PM	27.88	0.99		
W8		24	8:45 PM	27.22	0.97		
W9		36	8:00 AM	26.045	0.98	0.99	0.01
W10		36	8:15 AM	25.9	0.98		
W11		36	8:30 AM	26.39	0.99		
W12		36	8:45 AM	26.59	1.00		
W13	2	48	8:00 PM	26.2	1.01	1.00	0.02
W14		48	8:15 PM	26.34	1.02		
W15		48	8:30 PM	25.69	0.99		
W16		48	8:45 PM	24.94	0.97		

Results for 12 and 24 hr are based on 12 hr calibration

Results for 36 hr are based on 36 hr calibration data
Results from 48 hr are done by use of 48 hr calibration curve

Table B3. Average concentration of Cefoxitin in aqueous solution. The error described as +/- is one standard deviation

Time (hrs)	12	24	36	48
Concentration (mg/L)	1±0.02	1±0.03	.99±0.01	1.00±0.02

Appendix B2: Equilibrium Time Experiment

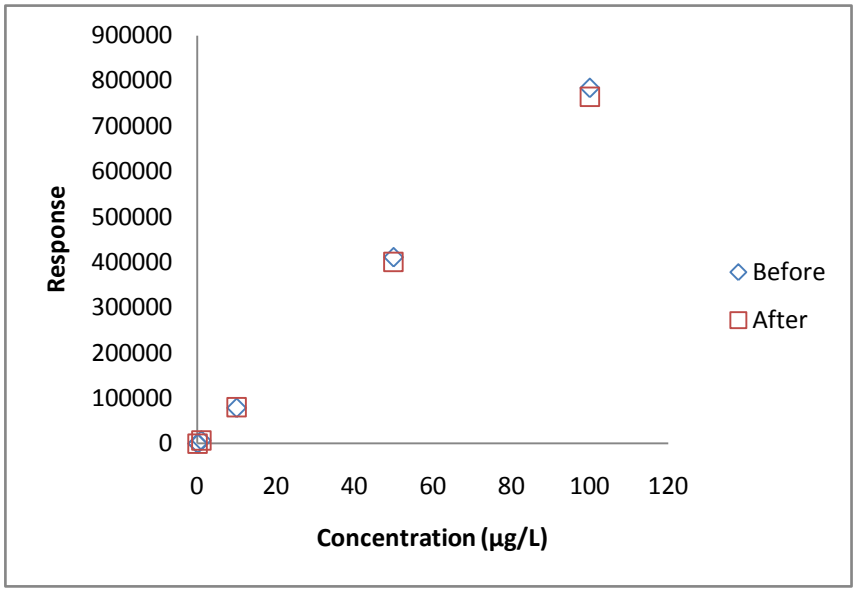

Figure B2. LC-MS/MS calibration curve for cefoxitin (equilibrium time experiment)

Slope	7794.189
Intercept	2100.812

Table B4. Calibration curve for equilibrium time experiment

Date	Standard Concentration (ug/L)	Response	Response	Average Response
9/30/2009	0.05	399	333	366
9/30/2009	0.1	748	756	752
9/30/2009	1	7259	7013	7136
9/30/2009	10	79129	80455	79792
9/30/2009	50	410899	401548	406223.5
9/30/2009	100	783814	764924	774369

Table B5. Equilibrium time experiment data with cefoxitin sorption on Pullman soil (P)

Sample	Time (hr)	C_0 (µg/L)	V (mL)	Soil Mass (g)	LC/MS/MS date	LC/MS/MS Response	C_e (µg/L)	Avg. C_e (µg/L)	C/Co
A1	6	100	10	2	9/30/2009	668609	86	84	0.86
A2	6	100	10	2	9/30/2009	650132	83		0.83
A3	6	100	10	2	9/30/2009	642890	82		0.82
A4	24	100	10	2	9/30/2009	599729	77	77	0.77
A5	24	100	10	2	9/30/2009	605566	77		0.77
A6	24	100	10	2	9/30/2009	594685	76		0.76
A7	48	100	10	2	9/30/2009	551079	70	71	0.70
A8	48	100	10	2	9/30/2009	558555	71		0.71
A9	48	100	10	2	9/30/2009	556467	71		0.71
A10	72	100	10	2	9/30/2009	506974	65	65	0.65
A11	72	100	10	2	9/30/2009	504725	64		0.64
A12	72	100	10	2	9/30/2009	504633	64		0.64
B1	72	0	10	2	9/30/2009	40	0		@
C1	72	100	10	2	9/30/2009	670974	86		0.86

Table B6. Cefoxitin aqueous sorbed percentage at specified

Time(hrs)	12	24	36	48
% sorbed	16%	23%	29%	35%

Appendix B3: Sorption Equilibrium Experiment (Cefoxitin - low concentrations)

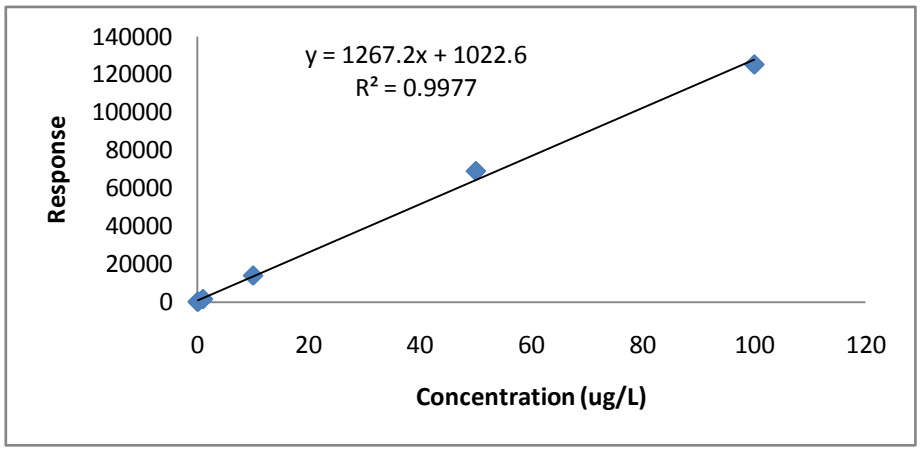

Figure B3. LC-MS/MS calibration sorption experiment (Cefoxitin – low concentrations)

Slope	1267.16
Intercept	1022.65

Table B7. Calibration curve for sorption experiment (Cefoxitin – low concentrations)

Date	Standard Concentration (µg/L)	GC/MS Response
10/14/2009	0.05	117
10/14/2009	0.1	210
10/14/2009	1	1566
10/14/2009	10	13985
10/14/2009	50	69104
10/14/2009	100	125356

Table B8. Cefoxitin sorption data (low concentrations) with Pullman (P), Quincy (Q), and Wenatchee (W) soil

Sample	Soil	C_0 (µg/L)	V (mL)	Soil Mass (g)	LC/MS/MS date	LC/MS/MS Response	C_e (µg/L)	C_s (µg/kg)
A1	P	100	10	2	10/8/2009	121672	95.2	24.1
A2	P	100	10	2	10/8/2009	115076	90.0	50.1
A3	P	100	10	2	10/8/2009	114099	89.2	53.9
A4	P	50	10	2	10/8/2009	59991	46.6	17.0
A5	P	50	10	2	10/8/2009	58591	45.5	22.5
A6	P	50	10	2	10/8/2009	58187	45.2	24.1
A7	P	1	10	2	10/8/2009	1303	0.4	3.1
A8	P	1	10	2	10/8/2009	1287	0.4	3.1
A9	P	1	10	2	10/8/2009	890	0.1	4.7
A10	P	1	10	2	10/8/2009	110	@	
A11	P	1	10	2	10/8/2009	42	@	
A12	P	1	10	2	10/8/2009	64	@	
B1	W	100	10	2	10/8/2009	112718	88.1	59.4
B2	W	100	10	2	10/8/2009	110075	86.0	69.8
B3	W	100	10	2	10/8/2009	115789	90.5	47.3
B4	W	50	10	2	10/8/2009	59732	46.4	18.0
B5	W	50	10	2	10/8/2009	59550	46.3	18.7
B6	W	50	10	2	10/8/2009	59168	46.0	20.2
B7	W	1	10	2	10/8/2009	1100	0.2	3.9
B8	W	1	10	2	10/8/2009	1142	0.3	3.7
B9	W	1	10	2	10/8/2009	1122	0.2	3.8
B10	W	0.1	10	2	10/8/2009	20	@	
B11	W	0.1	10	2	10/8/2009	39	@	
B12	W	0.1	10	2	10/8/2009	40	@	
C1	Q	100	10	2	10/8/2009	120087	93.9	30.4
C2	Q	100	10	2	10/8/2009	120505	94.3	28.7
C3	Q	100	10	2	10/8/2009	117653	92.0	39.9
C4	Q	50	10	2	10/8/2009	65256	50.7	0.0
C5	Q	50	10	2	10/8/2009	64280	50.0	0.0
C6	Q	50	10	2	10/8/2009	65302	50.8	0.0
C7	Q	1	10	2	10/8/2009	1407	0.5	2.7
C8	Q	1	10	2	10/8/2009	2447	1.3	0.0
C9	Q	1	10	2	10/8/2009	1442	0.5	2.5
C10	Q	0.1	10	2	10/8/2009	208	@	
C11	Q	0.1	10	2	10/8/2009	93	@	
C12	Q	0.1	10	2	10/8/2009	161	@	
Control		100	10	2	10/8/2009	119593	93.5	
Blank	P		10	2	10/8/2009	6	0	

Appendix B4: Sorption Equilibrium Experiment (Cefoxitin - high concentrations)

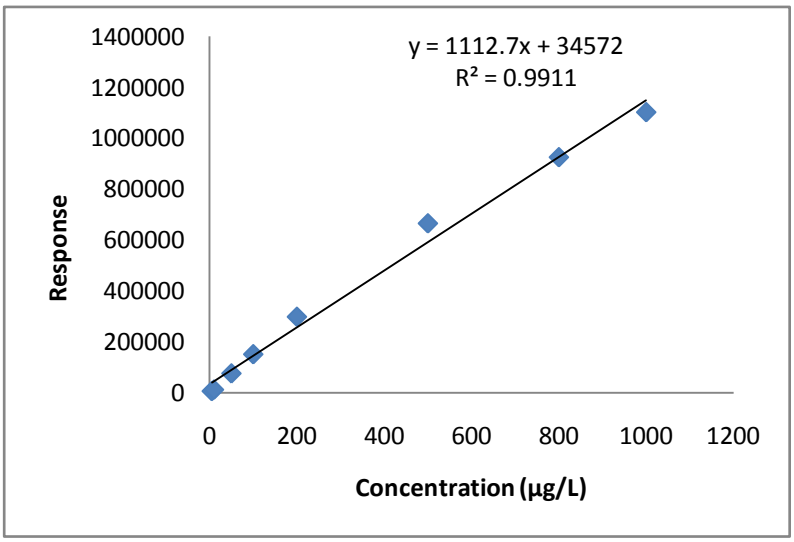

Figure B4. LC-MS/MS calibration sorption experiment (Cefoxitin – high concentrations)

Slope	1112.7
Intercept	34572.1

Table B9. Calibration curve for sorption experiment (Cefoxitin - high concentrations)

Concentration (µg/L)	Response	Response
5	6580	7615
10	12137	13323
50	73327	77083
100	137931	152325
200	276988	299162
500	588747	665626
800	805940	925143
1000	922077	1101757

Table B10. Cefoxitin data (high level) with Pullman (P), Quincy (Q), and Wenatchee (W) soil

Sample	Soil	C_0 (µg/L)	V (mL)	Soil Mass (g)	LC/MS/MS date	LC/MS/MS Response	C_e (µg/L)	C_s (µg/kg)
A1	P	1000	10	2	10/20/2009	930734	785.2	1074.0
A2	P	1000	10	2	10/20/2009	909510	760.5	1197.5
A3	P	1000	10	2	10/20/2009	946540	804.0	980.0
A4	P	500	10	2	10/20/2009	558213	415.5	422.8
A5	P	500	10	2	10/20/2009	537156	397.5	512.4
A6	P	500	10	2	10/20/2009	531685	392.9	535.5
A7	P	100	10	2	10/20/2009	121651	82.1	89.7
A8	P	100	10	2	10/20/2009	120888	81.5	92.3
A9	P	100	10	2	10/20/2009	115241	77.7	111.7
A10	P	10	10	2	10/20/2009	11517	7.9	10.6
A11	P	10	10	2	10/20/2009	13484	9.2	4.1
A12	P	10	10	2	10/20/2009	11504	7.9	10.7
B1	W	1000	10	2	10/20/2009	864525	710.1	1449.7
B2	W	1000	10	2	10/20/2009	927366	781.2	1093.8
B3	W	1000	10	2	10/20/2009	906469	757.0	1214.9
B4	W	500	10	2	10/20/2009	555047	412.7	436.3
B5	W	500	10	2	10/20/2009	548123	406.8	465.9
B6	W	500	10	2	10/20/2009	541021	400.8	496.1
B7	W	100	10	2	10/20/2009	123795	83.5	82.3
B8	W	100	10	2	10/20/2009	122984	83.0	85.1
B9	W	100	10	2	10/20/2009	119790	80.8	96.1
B10	W	10	10	2	10/20/2009	10718	7.3	13.3
B11	W	10	10	2	10/20/2009	11370	7.8	11.1
B12	W	10	10	2	10/20/2009	10715	7.3	13.3
C1	Q	1000	10	2	10/20/2009	120087	898.3	508.6
C2	Q	1000	10	2	10/20/2009	120505	857.2	714.2
C3	Q	1000	10	2	10/20/2009	117653	932.7	336.6
C4	Q	500	10	2	10/20/2009	65256	492.1	39.6
C5	Q	500	10	2	10/20/2009	612179	462.5	187.3
C6	Q	500	10	2	10/20/2009	138225	476.5	117.4
C7	Q	100	10	2	10/20/2009	627842	93.5	32.5
C8	Q	100	10	2	10/20/2009	147025	99.6	1.9
C9	Q	100	10	2	10/20/2009	137696	93.1	34.3
C10	Q	10	10	2	10/20/2009	12019	8.2034	9.0
C11	Q	10	10	2	10/20/2009	13517	9.1921	4.0
C12	Q	10	10	2	10/20/2009	12963	8.826	5.9
Control		100	10	2	10/20/2009	119593	104.6	
Blank	P		10	2	10/20/2009	6	0	

Appendix B5: Sorption Equilibrium Experiment (Ceftriaxone)

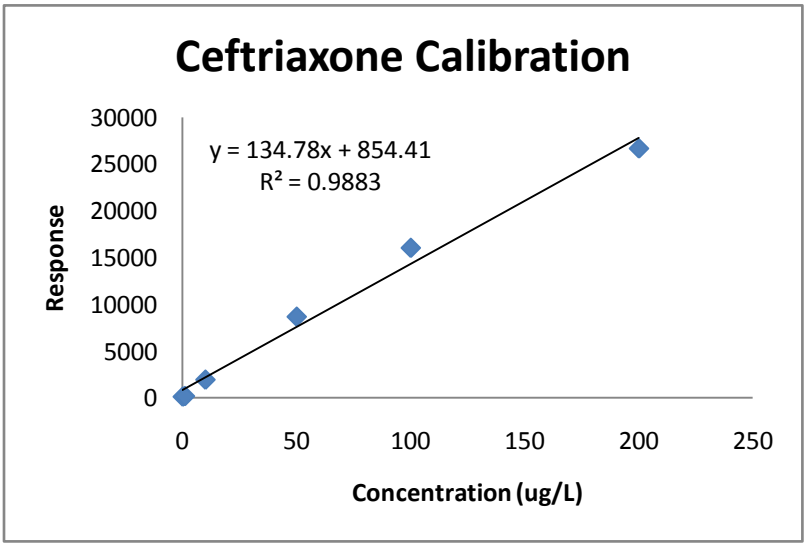

Figure B5. LC-MS/MS calibration sorption experiment (Ceftriaxone)

Slope	134.8
Intercept	854.4

Table B11. Calibration curve for sorption experiment (Ceftriaxone)

Standard Concentration (µg/L)	GC/MS Response
0.05	155
1	217
10	1971
50	8700
100	16060
200	26685

Table B12. Ceftriaxone sorption data with Pullman (P), Quincy (Q), and Wenatchee (W) soil

Sample	Soil	C_0 (µg/L)	V (mL)	Soil Mass (g)	LC/MS/MS Date	LC/MS/MS Response	C_e (µg/L)	C_s (µg/kg)
A1	P	200	10	2	10/14/2009	349	0.7	996.6
A2	P	200	10	2	10/14/2009	345	0.8	995.8
A3	P	200	10	2	10/14/2009	293	1.1	994.5
A4	P	100	10	2	10/14/2009	191	0.5	497.5
A5	P	100	10	2	10/14/2009	162	0.4	498.0
A6	P	100	10	2	10/14/2009	148	0.5	497.7
A7	P	50	10	2	10/14/2009	109	0.2	249.1
A8	P	50	10	2	10/14/2009	93	0.2	249.2
A9	P	50	10	2	10/14/2009	92	0.1	249.4
A10	P	1	10	2	10/14/2009	50	0.0	5.0
A11	P	1	10	2	10/14/2009	54	0.0	5.0
A12	P	1	10	2	10/14/2009	42	0.0	5.0
B1	W	200	10	2	10/14/2009	3043	16.3	918.5
B2	W	200	10	2	10/14/2009	3483	18.8	906.0
B3	W	200	10	2	10/14/2009	3327	17.9	910.4
B4	W	100	10	2	10/14/2009	2327	12.3	438.5
B5	W	100	10	2	10/14/2009	1995	10.5	447.7
B6	W	100	10	2	10/14/2009	2047	10.8	446.2
B7	W	50	10	2	10/14/2009	910	6.3	218.5
B8	W	50	10	2	10/14/2009	994	7.2	213.9
B9	W	50	10	2	10/14/2009	918	7.0	215.2
B10	W	1	10	2	10/14/2009	70	0.7	1.5
B11	W	1	10	2	10/14/2009	74	0.2	4.2
B12	W	1	10	2	10/14/2009	60	0.1	4.6
C1	Q	200	10	2	10/14/2009	22980	159.1	204.5
C2	Q	200	10	2	10/14/2009	22141	151.0	245.0
C3	Q	200	10	2	10/14/2009	21011	140.6	297.0
C4	Q	100	10	2	10/14/2009	13087	78.7	106.7
C5	Q	100	10	2	10/14/2009	12964	77.8	111.0
C6	Q	100	10	2	10/14/2009	13067	78.5	107.4
C7	Q	50	10	2	10/14/2009	7368	41.6	42.0
C8	Q	50	10	2	10/14/2009	7268	41.0	45.0
C9	Q	50	10	2	10/14/2009	6570	36.8	66.1
C10	Q	1	10	2	10/14/2009	353	0.8	0.8
C11	Q	1	10	2	10/14/2009	256	0.8	1.1
C12	Q	1	10	2	10/14/2009	241	0.9	0.5
Control		100	10	2	10/14/2009	16670	104.7	
Blank	P		10	2	10/14/2009	246	0.93	

Appendix B6: Sorption Equilibrium Experiment (Cephalothin)

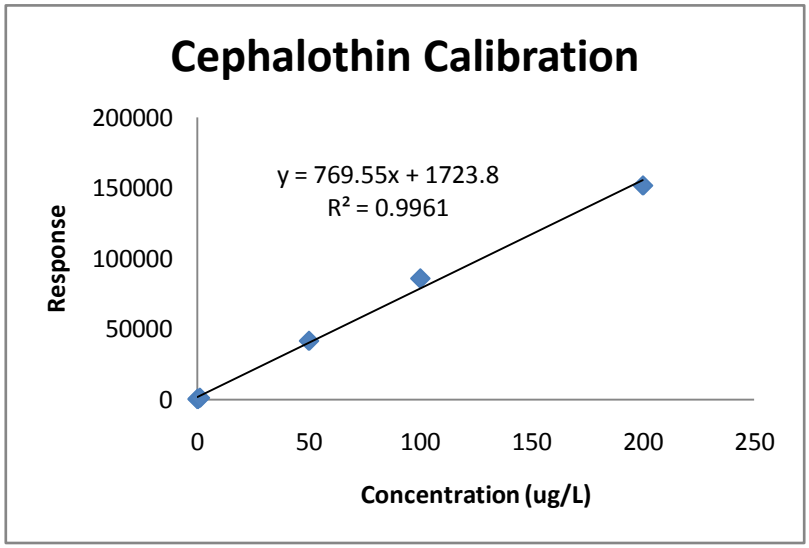

Figure B6. LC-MS/MS calibration sorption experiment (Cephalothin)

Slope	769.6
Intercept	1723.8

Table B13. Calibration curve for sorption experiment (Cephalothin)

Standard Concentration (µg/L)	GC/MS Response
0	14
0.05	435
1	1018
10	
50	41462
100	85798
200	151768

Table B14. Cephalothin sorption data to Pullman (P), Quincy (Q), and Wenatchee (W) soil.

Sample	Soil	C_0 (µg/L)	V (mL)	Soil Mass (g)	LC/MS/MS date	LC/MS/MS Response	C_e (µg/L)	C_s (µg/kg)
A1	P	200	10	2	10/21/2009	311	0.4	998.2
A2	P	200	10	2	10/21/2009	330	0.4	998.1
A3	P	200	10	2	10/21/2009	1014	1.1	994.3
A4	P	100	10	2	10/21/2009	267	0.3	498.4
A5	P	100	10	2	10/21/2009	202	0.2	498.8
A6	P	100	10	2	10/21/2009	229	0.3	498.6
A7	P	50	10	2	10/21/2009	75	0.1	249.5
A8	P	50	10	2	10/21/2009	181	0.2	248.9
A9	P	50	10	2	10/21/2009	1442	1.6	241.9
A10	P	1	10	2	10/21/2009	65	0.1	4.5
A11	P	1	10	2	10/21/2009	20	0.0	4.8
A12	P	1	10	2	10/21/2009	55	0.1	4.6
B1	W	200	10	2	10/21/2009	0	0.0	1000.0
B2	W	200	10	2	10/21/2009	0	0.0	1000.0
B3	W	200	10	2	10/21/2009	0	0.0	1000.0
B4	W	100	10	2	10/21/2009	8	0.0	499.9
B5	W	100	10	2	10/21/2009	0	0.0	500.0
B6	W	100	10	2	10/21/2009	0	0.0	500.0
B7	W	50	10	2	10/21/2009	0	0.0	250.0
B8	W	50	10	2	10/21/2009	0	0.0	250.0
B9	W	50	10	2	10/21/2009	114	0.1	249.3
B10	W	1	10	2	10/21/2009	11	0.0	4.8
B11	W	1	10	2	10/21/2009	8	0.0	4.9
B12	W	1	10	2	10/21/2009	7	0.0	4.9
C1	Q	200	10	2	10/21/2009	69646	82.1	589.5
C2	Q	200	10	2	10/21/2009	66399	78.0	610.0
C3	Q	200	10	2	10/21/2009	72012	85.1	574.5
C4	Q	100	10	2	10/21/2009	32616	37.1	314.7
C5	Q	100	10	2	10/21/2009	33119	37.6	311.8
C6	Q	100	10	2	10/21/2009	30547	34.6	326.8
C7	Q	50	10	2	10/21/2009	15599	17.5	162.7
C8	Q	50	10	2	10/21/2009	19316	21.7	141.6
C9	Q	50	10	2	10/21/2009	20117	22.6	137.0
C10	Q	1	10	2	10/21/2009	660	0.7	1.3
C11	Q	1	10	2	10/21/2009	253	0.3	3.5
C12	Q	1	10	2	10/21/2009	208	0.2	3.8
Control		100	10	2	10/21/2009	79433	94.6	
Blank	P		10	2	10/21/2009	14	0.036	

Appendix B7: pH data for all experiments

Table B15. Average pH data for all experiments

		pH	
Experiment	Date	Pullman	Blank
Eq.time	9/26/2009	6.2	4.43
Cefoxitin1	10/3/2009	6.05	4.48
Ceftriaxone	10/7/2009	6.1	4.41
Cephalothin	10/10/2009	6	4.37
Cefoxitin2	10/17/2009	6.1	4.3

Appendix C: Statistical Comparison

Table B16. t-test for cefoxitin equilibrium concentration of two experiments low (1) and high(2) at initial concentration Co=100 µg/L.

Caq (ug/L)	Equilibrium concentrations (µg/L) 1	2	Average 1	2	Std dev 1	2	p-value t-test
Pullman	95.2	82.1	91.5	80.4	3.3	2.4	0.02
	90.0	81.5					
	89.2	77.7					
Wenatchee	88.1	83.5	88.3	82.4	2.3	1.5	0.10
	86.1	83.0					
	90.6	80.8					
Quincy	94.0	93.5	93.4	95.4	1.2	3.6	0.37
	94.3	99.6					
	92.0	93.1					

Ho was rejected at 0.05 significant level for
Pullman soil since 0.02<0.05
Ho was not rejected at 0.01 sig. level for all soils

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