

More movement with manure: increased mobility of erythromycin through agricultural soil in the presence of manure

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ABSTRACT

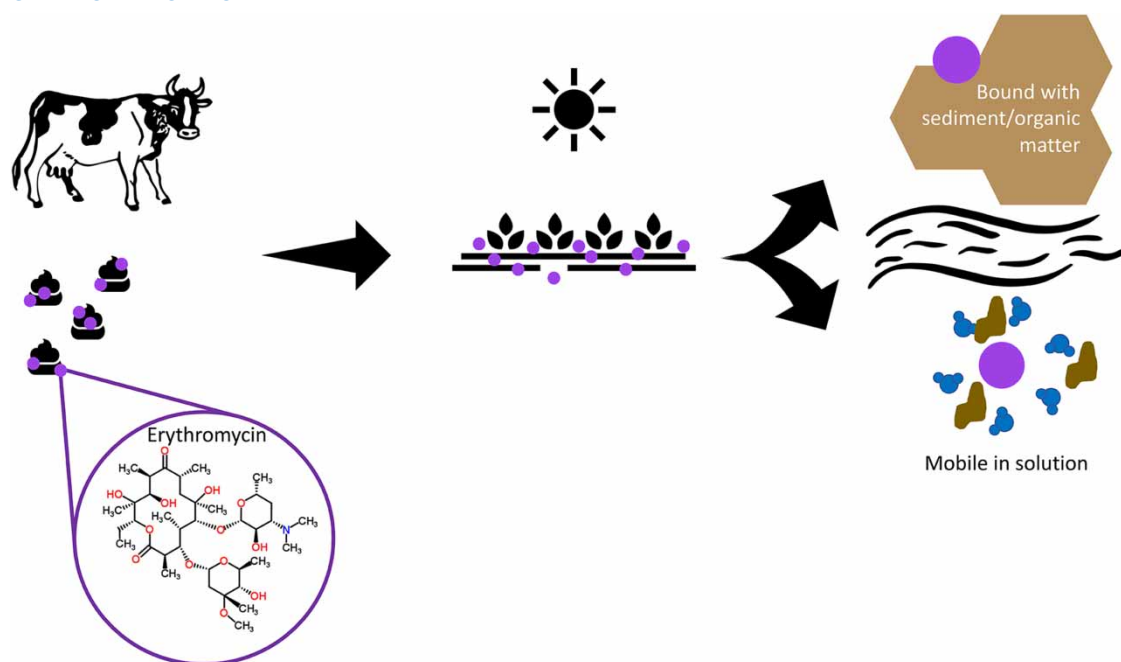
Antibiotic residues in the environment threaten soil and aquatic organisms and human and livestock health through the building of antimicrobial resistance. Manure spreading associated with animal agriculture is one source of environmental antibiotic residues. To better understand the risk of contamination, we studied the adsorption of erythromycin, a model macrolide antibiotic used across human and animal medicine. We conducted a series of equilibrium batch experiments to determine the kinetics and extent of adsorption and a continuous-flow column adsorption experiment to observe non-equilibrium adsorption patterns. We determined that the adsorption equilibration time to soil was approximately 72 h in our batch experiments. Erythromycin adsorbed to soil relatively strongly ($K = 8.01 \times 10^{-2}$ L/mg; $q_{\max} = 1.53 \times 10^{-3}$ mg/mg), adsorbed to the soil in the presence of manure with less affinity ($K = 1.99 \times 10^{-4}$ L/mg) at a soil: manure ratio of 10:1 by mass, and did not adsorb to manure across the solid ratios tested. We observed multi-phased adsorption of erythromycin to the soil during the non-equilibrium column experiment, which was largely absent from the treatments with both soil and manure present. These results suggest that erythromycin is more mobile in the environment when introduced with manure, which is likely the largest source of agriculturally sourced environmental antibiotics.

Key words: adsorption isotherm, antibiotic residue, antimicrobial resistance (AMR), erythromycin, one-health, pharmaceuticals

HIGHLIGHTS

- Erythromycin favored its aqueous phase when manure and manure–DOM were present.
- Erythromycin may display biphasic adsorption tendencies with multiple mechanisms.
- Manure management recommendation: reduce manure erythromycin content prior to field application when contaminant is aqueous.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Antibiotics and other emerging contaminants (e.g. pesticides, pharmaceuticals, and personal care products) are concerning environmental pollutants. Antibiotic residues have been detected in surface waters (e.g. Kolpin *et al.* 2002; Guo *et al.* 2016; Vatovec *et al.* 2016; Carpenter *et al.* 2018) and resistance genes found in the soil microbiome after manure applications (e.g. Fahrenfeld *et al.* 2014; Jacobs *et al.* 2019). These compounds pose threats through their interactions with aquatic and soil organisms (Väitalo *et al.* 2017), soil microbial activity inhibition, and microbial biodiversity reduction (Cycoń *et al.* 2019; Lucas *et al.* 2021). Though antibiotic resistance naturally occurs within the bacterial population, anthropogenically sourced antibiotic residues increase selection pressure for antibiotic resistance genes in the microbial community, allowing the buildup of resistance that subsequently reduces human and animal medicine efficacy (e.g. Martínez 2008). Agricultural antibiotic residue transport pathways are poorly understood, encumbering mitigation efforts of these emerging contaminants (Menz *et al.* 2018).

In livestock operations, antibiotics are used to treat and prevent diseases and to promote growth and productivity (McEwen & Fedorka-Cray 2002; Patel *et al.* 2020). Therapeutic, disease prevention, and growth promotion uses of antibiotics continue, though antibiotic usage for growth promotion and productivity has been reduced by the Veterinary Feed Directive in the United States in 2015 and amended in 2019 (U.S. FDA 2015). Europe continues to lead the way in reducing agricultural antibiotic use in comparison to the United States, though the spread of antimicrobial resistance and residence continues to be a global issue (Patel *et al.* 2020). Many animals absorb antibiotics poorly, with some animals excreting 50–90% of the active ingredient depending on antibiotic type (Feinman & Matheson 1978; Alcock *et al.* 1999; Kim *et al.* 2011). Excreted compounds can then move with water and sediments and interact with surrounding microbial communities. Understanding antibiotic transport will inform farm manure management systems to minimize the distribution of these potent compounds on farm-by-farm and industry-wide bases. Agricultural land has long been recognized as a major nonpoint nutrient source (e.g. Zaring 1996; Carpenter *et al.* 1998) but, more recently, has been associated with antibiotic pollution (Yi *et al.* 2019). Broadly, 6.2 million kg of medically important (i.e. antibiotics used in human medicine) and 5.2 million kg of non-medically important (i.e. not used in human medicine) antibiotics were sold and distributed for US agriculture use in 2019, with 41% of all medically important and 62% of all non-medically important antibiotics sold for use in cattle specifically (U.S. FDA 2020). However, farmer perceptions of these compounds as environmental contaminants vary widely across farms, management

styles, and farm sizes, with diverse views leading to numerous possible transport pathways and variable loading rates into environmental systems (Georgakakos *et al.* 2021).

Antibiotic partitioning between aqueous and adsorbed phases in soils likely controls the transport of these compounds. Due to the wide array of antibiotic chemical structures, generalizing the behavior of a class of these chemicals is difficult. Tolls (2001) reviewed existing literature on antibiotic adsorption to soils and found that the distribution coefficients (K_d) across-antibiotic classes varied from 0.62 to 1,030 L/kg for sandy loam. Tolls (2001) hypothesized that these compounds' functional groups may better indicate sorbate-sorbent interactions than the antibiotic classes themselves. Davis *et al.* (2006) also reinforced that solubility and adsorption vary widely among antibiotics. This within- and across-antibiotic class variability complicates antibiotic residue contamination mitigation. Some soil-bound antibiotics in equilibrium with a clay loam and loamy sand retained antimicrobial properties after desorption, suggesting a need to understand the sediment-organic matter-antibiotic relationship to predict not only transport but also impacts on soil and aquatic organisms (Chander *et al.* 2005). Post-excretion interactions of residues, metabolites, and bacterial resistance are not fully understood (Kemper 2008), with a major obstacle being the lack of soil adsorption coefficients in the literature and subsequent understanding of complex interactions with differing soils (Menz *et al.* 2018).

Erythromycin, a bacteriostatic macrolide antibiotic, inhibits the growth of both Gram-positive and Gram-negative bacteria, as well as some other parasitic pathogens (Amsden 1996). Though erythromycin is naturally produced as a waste byproduct by soil bacteria, the vast majority of environmentally present erythromycin originates from anthropogenic sources (Ashraf *et al.* 2021). Both animal and human medicine widely use erythromycin, making it an important contaminant to understand due to its broad medical applications. In cattle, erythromycin is used to treat bovine respiratory disease among other bacterial infections at a dosage rate of 8.8 mg/kg animal mass (e.g. Wittek & Constable 2005; Nouri *et al.* 2008). Macrolides are excreted unchanged and chemically active between 50 and 90% by mass of the ingested or injected active ingredient in most livestock, though excretion is lower among some animals, humans, for example, excrete about 12% (Van Epps & Blaney 2016). Erythromycin is particularly resistant to natural degradation, increasing its influence on soil and aquatic microbial communities (Islas-Espinoza *et al.* 2018). The pK_a of erythromycin is 8.8 (McFarland *et al.* 1997). Erythromycin contains a lone pair of electrons associated with the nitrogen atom, which is expected to attract a hydrogen ion in acidic environments, resulting in a positively charged molecule in the pH ranges expected of manure and subsequent soil environments ($pH = 4.6-7.4$) (Figure 1).

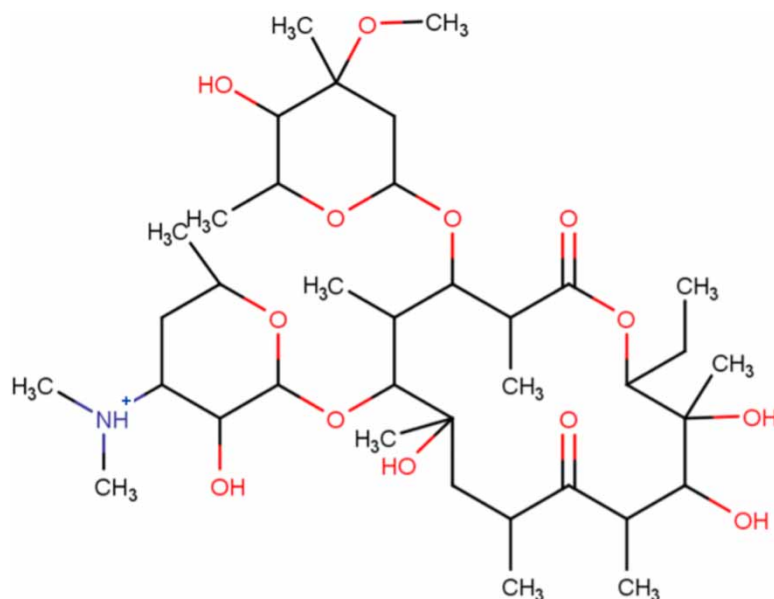


Figure 1 | Erythromycin's positive speciation with associated hydrogen ion as expected in manure and soil environments of pH ranges between 0 and 8.8. Visualization completed using the Marvin Chem Axon (version 21.17.0, ChemAxon, <https://www.chemaxon.com>).

Adsorption of erythromycin has been studied in relation to biochar (Ndoun *et al.* 2020) and activated carbon (Gholamiyan *et al.* 2020) with promising applications for removal from the water column with 35–75% removal efficiency depending on pyrolysis temperature of the biochar and low required interaction times with activated carbon. The half-life of erythromycin in manure storage lagoons was found to be 41 days, suggesting that the residue will be persistent in manure upon field application (Schlüsener *et al.* 2006). Erythromycin EPI Suite™ predictions suggest preferential partitioning into organic solvents over water ($\log K_{ow} = 3.06$) and organic carbon over water ($\log K_{oc} = 1.41$) (U.S. EPA 2019). In comparison to other macrolide antibiotics, erythromycin has comparable $\log K_{ow}$, $\log K_{oc}$, and biodegradation in wastewater but somewhat higher solubility and lower sorption to wastewater sludge (Table 1; U.S. EPA 2019). When comparing macrolides with β -lactam antibiotics, another class of medically important antibiotics, the two classes have comparable $\log K_{ow}$ and $\log K_{oc}$, while β -lactams have a higher solubility and lower sorption to wastewater sludge (Table 1; U.S. EPA 2019). These predictions suggest that erythromycin likely interacts with organic matter through adsorption to and partitioning into organic phases over aqueous phases. However, if the post-excretion or manure application environment is more alkaline, erythromycin's mobility may increase due to changes in pH-dependent charge, shifting erythromycin to a neutral or negatively charged molecule and increasing its solubility in water.

This work characterizes the adsorption and transport processes of erythromycin to help predict agricultural contamination risk to surrounding ecosystems. We characterized the adsorption isotherms of erythromycin to a characteristic agricultural soil with and without manure present through adsorption equilibrium batch reactions and column experiments to better understand mobility. We fitted Langmuir adsorption models to both treatments. This research fills existing gaps in the contaminant transport literature to assess and model antibiotic contamination from animal agriculture.

2. METHODS

2.1. Soil and manure collection

We collected soil from the top 10 cm of a fallow control plot (Cornell Recreation Connection, Freese Road, Ithaca, NY, USA) in the Caneseraga CaB soil series, an agricultural soil characteristic of New York's Finger Lakes region. We collected manure samples from four organic dairy farms. We collected manure from organic dairy farms to reduce the risk of antimicrobials present in manure (USDA organically marketed livestock cannot be treated with antibiotics) and confirmed with each farm that no animals had been treated with antibiotics or alternative organically approved therapies in the recent past. Dairy manure was chosen as the primary livestock manure land-applied regionally where soils were collected. On a per cow basis, farm 1 was 100% grass-fed with 0 kg grain/day, while farms 2, 3, and 4 fed 1.4 kg grain/day (3 lb/day), 4.5 kg grain/day (10 lb/day), and 17.2 kg grain/day (38 lb grain/day), respectively, at the time of manure collection. This diversity of feeding practices is representative of farms in the region. We combined the four manures in a 1:1:1:1 by dry mass ratio to achieve an average manure composition. All manures were individually analyzed for bulk parameters. Manure and soil solids were twice autoclaved at 135 °C for 25 min to sterilize and oven-dried before use to reduce microbial degradation or the alteration of erythromycin or organic molecules. Batches and column experiments were spiked with pure erythromycin, representative of an unchanged, biologically reactive antimicrobial upon excretion.

2.2. Bulk parameter characterization

We measured bulk solid parameters to understand the adsorption environment and allow comparison between treatments. We obtained bulk density, pH, particle size distribution, electrical conductivity, organic matter loss on ignition, surface area,

Table 1 | EPI Suite™ predictions for antibiotics in the macrolide and β -lactam classes

Class	Antibiotic	Solubility (mg/L)	Log K_{ow}	Log K_{oc}	Wastewater biodegradation (%)	Wastewater sorption to sludge (%)
Macrolide	Erythromycin	0.517	3.06	1.406	0.130	6.11
	Tildipirosin	0.0104	5.05	2.648	0.690	78.5
	Tilmicosin	0.007804	4.13	1.842	0.370	36.0
	Tulathromycin	0.0768	3.46	1.354	0.180	12.0
	Tylosin	1.59	1.05	-0.116	0.090	1.80
β -Lactam	Ampicillin	439.3	1.35	0.808	0.090	1.84
	Penicillin G	210.4	1.83	1.095	0.090	2.02

pore size, and zeta potential for both manure and soil substrates (Table 2). We also obtained percent sand, silt, and clay for the soil. To confirm the solid processing did not influence our results by altering the adsorption capacity of the solids, we compared oven-dried and autoclaved then oven-dried samples to assess the effect of substrate preparation on bulk parameters. Following this analysis, we chose to use autoclaved oven-dried solids to reduce the influence of biological activity without a significant change to solid parameters (all autoclaved versus oven-dried bulk parameters are provided in Supplementary Table S1).

We calculated soil bulk density by extracting a 270 cm³ cylindrical soil core (7.3 cm × 6.5 cm), obtained the total solid content of fresh manure by measuring 250 cm³ of fresh manure, and recorded the wet weights. Samples were oven-dried at 65 °C for 24 h before recording dry weights (U.S. Soil Quality Institute 1998). Using an Accumet Research AR50 Dual Channel pH/Ion Conductivity Meter (S/N AR 81202286), we measured pH and electrical conductivity. We prepared pH samples from 15 g of dry soil and manure, sieved through a 2 mm sieve, and rehydrated in 30 mL deionized (DI) water after a 30 min equilibration period (Robertson *et al.* 1999). We prepared electrical conductivity samples in a 1:5 ratio by mass of dry solids to water and left to equilibrate for 1 h prior to analysis (Rayment & Higginson 1992). Particle size distribution, specific surface area, and pore size were obtained using the Brunauer–Emmett–Teller (BET) method (Micrometrics ASAP 2640) from dry, sieved (2 mm) samples after subjecting the samples to a 100 °C vacuum for 24 h. We calculated percent loss on ignition by subjecting 5 g of oven-dried soil or manure to 500 °C for 2 h (Ferguson & Swenson 2017). Suspensions of 0.1 mg/mL were prepared for zeta potential analysis following Darrow *et al.* (2020) for soils. We obtained zeta potential from a Malvern Panalytical (zs90) Zetasizer set to a reflective index (RI) of 2 and absorbance index (*A*) of 1 for soil, and a RI of 1.4 and an *A* of 0 for manure (RI and *A* adjusted from estimates in Darrow *et al.* (2020) and Lafon *et al.* (2006)).

2.3. Batch equilibrium adsorption experiments

Autoclaved and then oven-dried solids were added to each batch and the volume was brought up to 150 mL with DI water after the addition of erythromycin (Cayman Chemical, item #: 16486, purity >98%). To determine solid ratios for experimental use, differing solid masses (0.01, 0.05, 0.5, 5, and 15 g) were tested to determine the detectable adsorption of erythromycin (at 667 ppb) (Supplementary Figure S1). Preliminary manure tests resulted in no adsorption across treatments, leading to the two-phased equilibrium experiment discussed below. A soil solid ratio of 5 g/150 mL was chosen as the first solid ratio tested with significant adsorption. For combined manure and soil experiments (SM treatment), 5 g of soil with 0.5 g of manure was used for a soil: dry manure ratio of 10:1 by mass. This ratio would be representative of the soil surface-manure contact site after field application using a manure spreader. Though this ratio would not represent soil: manure ratios across an entire field application, it is representative of the often non-homogenous manure distribution resulting in more manure concentrated in certain areas as is observed in practice. All batch experiments were in 250 mL amber glass vials. All amber glass vials were sonicated and autoclaved prior to usage and left on a dark, rotating shaker for the duration of the experiment. Control reactors with no solids were prepared in the same conditions. Because preliminary experiments with manure showed no erythromycin adsorption, no additional manure-only experiments were conducted.

Table 2 | Soil and manure bulk parameters

Characteristic	Soil	Manure ^a
Bulk density/total solids (g/cm ³)	0.872	0.149
Soil Water Content (SWC) (g water/g dry sample)	0.4752	5.9925
pH	4.37	6.68
Particle diameter (μm)	1.2	14.3
Electrical conductivity (μS/cm)	370	5.33
Organic matter (OM) loss on ignition (%)	9.81	87.55
Brunauer-Emmett-Teller (BET) surface area (m ² /g)	4.93	0.32
Pore diameter (nm)	14.93	33.62
Zeta potential (mV)	-13.11	-5.87

^aAverage values from four farms. Manure characteristic data from each farm are provided in Supplementary Table S2.

2.3.1. Soil adsorption equilibration time

For the determination of adsorption equilibration time, 10 mL of 10 ppm erythromycin solution was added to each batch reactor and the total volume was brought to 150 mL with a final concentration of 667 ppb. Each batch was sampled at 0, 3, 12, 24, 36, 48, 72, 96, 120, and 144 h. Samples were collected using a syringe and filtered through a 0.30 μm glass fiber filter immediately. Triplicate batch reactors were prepared for each equilibration time. We diluted samples to approximately 15 ppb erythromycin for analysis using an erythromycin enzyme-linked immunosorbent assay (ELISA) kit. All samples were analyzed within 24 h of filtration using a MyBioSource erythromycin ELISA kit (cat #: MBS282249) and a Molecular Devices M2 microplate reader.

We defined the adsorption equilibrium time to be the first sample with no statistical difference from both the previous ($t - 1$) and next ($t + 1$) samples using a Wilcoxon Rank Sum Test (with p -value of <0.1 indicating statistical difference). We calculated first-order reaction kinetics (Equation (1)):

$$q_t = q_e(1 - e^{-k_1 t}) \quad (1)$$

where q_t is the amount of erythromycin adsorbed at time t , q_e is the amount of erythromycin adsorbed at equilibrium, and k_1 is the kinetic rate constant.

2.3.2. Adsorption equilibrium isotherms

After establishing adsorption equilibrium time, erythromycin at 19 concentrations (10, 20, 40, 80, 100, 200, 400, 800, 1,000, 2,000, 5,000, 10,000, 25,000, 40,000, 50,000, 65,000, 75,000, 100,000, and 150,000 ppb) was added to 5 g of soil in 250 mL amber glass vials in triplicate (S treatment). Batch reactors were left to shake for 72 h, and the suspension was filtered through a 0.3 μm glass fiber syringe filter and analyzed within 24 h using an ELISA kit and a microplate reader. All samples were diluted (as needed) to the linear detection range of 0.2–25 ppb prior to analysis.

The SM treatment was also equilibrated for 72 h at initial erythromycin concentrations of 10, 100, 400, 1,000, 5,000, 10,000, 25,000, 50,000, 100,000, and 150,000 ppb. We combined 5 g of soil and 0.5 g of manure by dry mass in amber glass vials for a total volume of 150 mL.

We tested five nonlinear and linearized Freundlich and Langmuir models to S and SM adsorption isotherm data to determine the best model fit. Models that generated non-realistic (i.e. negative) adsorption parameters were excluded from further analysis. From the remaining models, the model with the greatest number of significant parameters and the smallest standard square error (SSE) was selected. Discussed here are the Langmuir nonlinear regression and one Langmuir linearization which fit the data best (Equations (2) and (3)); additional model discussion in the Supplementary Material).

$$q = q_{\max} \times K \times \frac{c}{1 + K * c} \quad (2)$$

$$\frac{c}{q} = a + b \times c \quad (3)$$

$$q_{\max} = \frac{1}{b}; K = \frac{1}{q_{\max} \times a} \quad (3.1)$$

In Equations (2) and (3), q is the equilibrium adsorption density (mg adsorbate)/(mg adsorbent), q_{\max} is the maximum adsorption density (mg adsorbate)/(mg adsorbent), c is aqueous concentration (mg/L), and K is the equilibrium coefficient for adsorption between adsorbate (erythromycin) and adsorbent (solids) (L/mg). In the modified linear model (Equation (3)), a and b are the intercept and slope of the linearized model, respectively. Linearized model parameters were then converted to parameters with physical meaning (Equation (3.1)).

2.3.3. Manure influence on adsorption

To assess manure's impact on erythromycin adsorption to the soil, we conducted a two-phased equilibration experiment. In the first phase, we equilibrated four manure concentrations (0.01, 0.05, 0.5, and 5 g) with erythromycin at 667 ppb for 72 h. In the second equilibration, we added the 0.3 μm filtrate from phase 1 to batch reactors with 5 g soil/150 mL. The second phase was equilibrated for an additional 72 h. Samples were analyzed after phase 2. Following this experiment, SM batch

experiments utilized a 10:1 soil:manure ratio (0.5 g of manure solids with 5 g of soil solids). We applied a semi-log transformation to the data after the second phase to generate a linear model:

$$c = a + b \times \log \text{SR} \quad (4)$$

where c is the aqueous concentration of erythromycin after the second equilibration, SR is the solid ratio of soil to manure by mass used in the first equilibration, and a and b are the empirically derived intercept and slope, respectively, of the resulting model.

2.4. Soil column experiments

We used 7 cm diameter columns, which are continuously infiltrated from the below using a pump. This design was chosen to reduce the effect of preferential flow paths and flow along the column walls as is sometimes confounding with smaller columns and columns infiltrated from above (e.g. [Show et al. 2022](#)). We infiltrated about 17 L of 500 ppb erythromycin solution through each column. A continuous, highly concentrated input of erythromycin was chosen to represent a worst-case scenario pollutant generating saturated adsorption site conditions. The column outflow was located 3 cm above the soil surface, which continuously drained the columns. Average outflow rates ranged from 28 to 31 mL/min. Total infiltration time was between 9.5 and 9.8 h. Treatments were run in triplicate. All columns contained 200.00 g of homogenized soil (S treatment). Columns in soil–manure (SM) treatment contained 20.00 g of manure in addition to 200.00 g of soil. We determined infiltration volume from initial column tests and high and low estimates of fitted adsorption isotherm models. Volumetric outflow was recorded continuously for each column independently. Samples were filtered, diluted, and analyzed within 12 h of experiment completion. Eight samples per column were analyzed for pH, and 30 samples per column were analyzed for aqueous antibiotic concentration. A non-parametric smooth local regression (loess) model was used to visualize column results.

2.5. Data analysis

The ELISA analysis method is a targeted approach, which is well equipped to assess the presence of a single compound in laboratory samples. This method required a standard microplate device, making it accessible to laboratories lacking high-performance liquid chromatography–mass spectrometry (HPLC-MS). Because ELISA methods are designed with a detection range of 0.2–25 ppb, this method is ideal when low concentrations are expected. We believe that some of the variability between our replicates may be attributed to the high dilution ratios required to reach the detection range of the ELISA.

All data were analyzed in R-studio (version 1.4.1106 for Mac) and Excel (version 16.57 for Mac). Model parameters were tested for statistical significance using a t -test.

3. RESULTS

3.1. Bulk solid parameter data

Bulk solid characteristics such as pH, zeta potential, organic matter content, particle surface area, and pore size can all influence the adsorption equilibrium between a solid and an adsorbate. A highly negative zeta potential drives the adsorption of positively charged erythromycin molecules at these pH conditions through complementary electrostatic interactions. The higher surface area allows for more adsorption sites and stronger van der Waals attractions. Hydrophobic organic matter tends to interact with organic contaminants in aqueous solutions, with compounds such as erythromycin favoring organic over aqueous phases. We, therefore, expect erythromycin to interact substantially with a component (dissolved or particulate) of the manure solids. The solution pH determines the charge of erythromycin from positive at low pH values to negative at high pH values, dictating that electrostatic interactions will be most influential. We obtained bulk characteristic data to understand the environment of these experiments ([Table 2](#)) and tested the effect of solid preparation methods (i.e. autoclaving versus oven-drying) ([Supplementary Table S1](#)).

3.2. Batch adsorption studies

3.2.1. Adsorption equilibration time

We applied a first-order reaction model and determined q_e and k_1 (Equation (1)) to be 1.47×10^{-5} mg/mg (p -value = 3.64×10^{-5}) and 6.399×10^{-2} h⁻¹ (p -value = 0.020), respectively, for the concentration tested (667 ppb), with both parameters as significant predictors (p -value < 0.05). We found erythromycin adsorption equilibration time to Caneserga soil to be

approximately 72 h (Figure 2). The 72-h sample was not statistically different from either the previous (48-h, p -value = 0.4) or the next sample (96-h, p -value = 0.4). Some additional adsorption was observed in the 144-h samples and may be indicative of an additional, slower adsorption mechanism. The control reactors experienced some reduction (~ 50 ppb) in erythromycin concentration over the test period, but this change was not statistically significant (p -value = 0.2) in a Wilcoxon rank sum test between the first and last control samples and therefore not considered in further analysis.

When testing the adsorption of erythromycin to pure manure substrate, we observed no change in aqueous concentration over the range of solid ratios tested (Supplementary Figure S1). Therefore, the adsorption equilibration time to manure was not determined. All subsequent tests contained a mixture of manure and soil solids.

3.2.2. Adsorption equilibrium isotherms

When comparing S and SM adsorption isotherms, it is apparent that the presence of manure increases the aqueous concentration of erythromycin and reduces the adsorbed mass (Figure 3). The two isotherms are distinct until the highest concentrations are tested (100,000 and 150,000 ppb). At these highest concentrations, adsorption of erythromycin begins to converge around the same values observed in the S treatments.

Both the S and SM isotherms were best fit by Langmuir models. In the S adsorption isotherm, the Langmuir nonlinear regression model best fits (Equation (2)) with parameters of maximum adsorption capacity (q_{\max}) and the equilibrium coefficient (K) that were 1.53×10^{-3} mg adsorbed erythromycin/mg soil and 8.01×10^{-2} L/mg erythromycin, respectively (Table 3). Parameters were statistically significant (p -value < 0.01) for both nonlinear and linear regression models when t -test statistics were computed. The 150,000 ppb samples did not show a large enough change to be detected. The 150,000 ppb sample has, therefore, been removed from the analysis and was not used to calculate the best fit.

Unlike the models run on the S isotherm, the SM isotherm models had fewer statistically significant parameters. The linear regression model (Equation (3)) best fits the data considering models with non-negative parameter values, at least one parameter that was a significant predictor of the data, and SSE values ($K = 1.99 \times 10^{-4}$ L/mg erythromycin, $q_{\max} = 4.63 \times 10^{-2}$ mg adsorbed erythromycin/mg soil + manure; only K was a significant predictor of the data) (Table 3).

We tested the influence of manure-derived dissolved organic matter (DOM) and other solutes on erythromycin adsorption by conducting a two-phased adsorption experiment: first between erythromycin and manure to generate an erythromycin–manure–DOM solution and then between this solution and the Caneseraga soil. These manure-derived solutions vary in

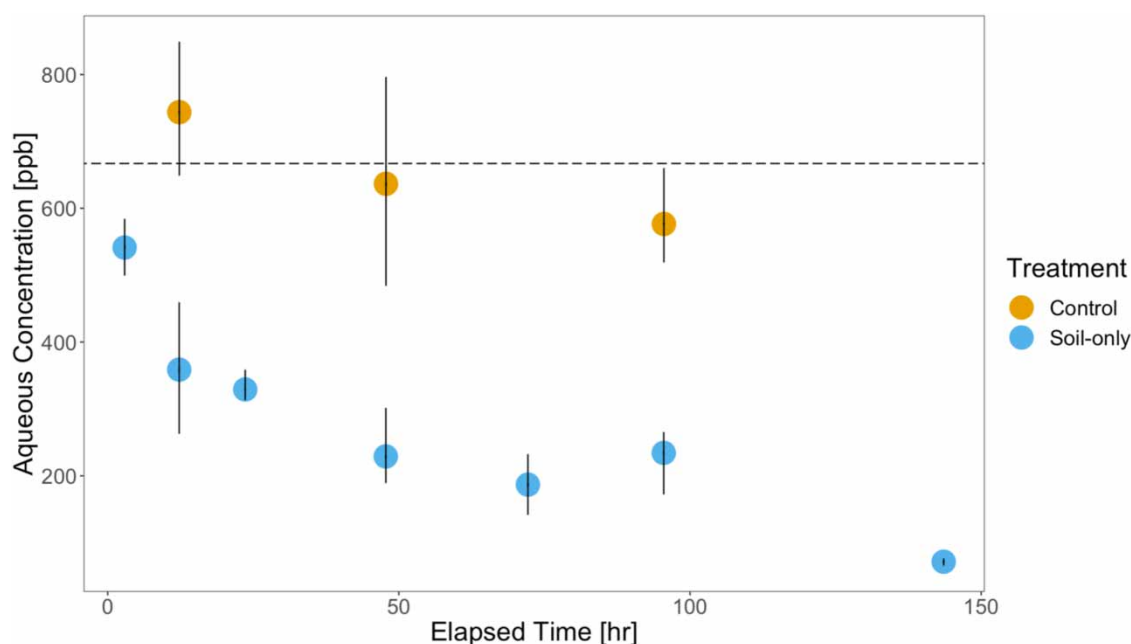


Figure 2 | Soil-only (S) equilibration time between erythromycin and Caneseraga soil (blue) compared to control reactors (yellow) with no soil. Error bars represent the range of the three replicates used to obtain the points plotted. Dashed line represented initial concentration of 667 ppb. Please refer to the online version of this paper to see this figure in colour: <http://dx.doi.org/10.2166/wh.2023.051>.

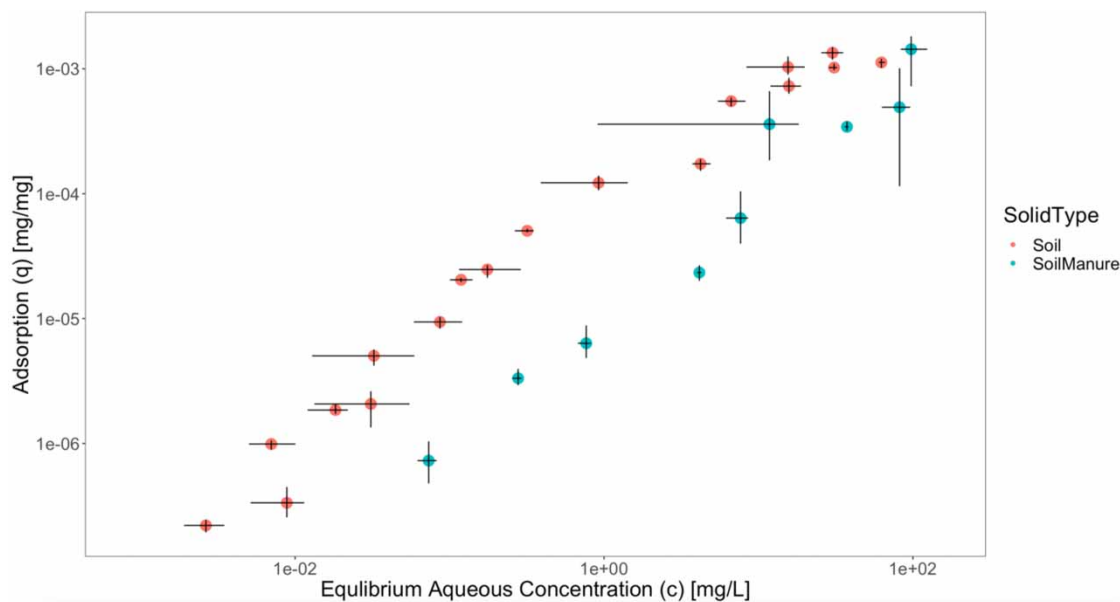


Figure 3 | Soil-only (S) and soil + manure (SM) isotherms. Error bars represent the range of the three replicates averaged for each point.

Table 3 | Converted model parameters from linearized and nonlinear regressions

Model	S parameters		SM parameters	
	K (L/mg)	q_{\max} (mg/mg)	K (L/mg)	q_{\max} (mg/mg)
Equation (2)	$8.01 \times 10^{-2*}$	$1.53 \times 10^{-3*}$	NA [†]	NA [†]
Equation (3)	$6.38 \times 10^{-2*}$	$1.52 \times 10^{-3*}$	$1.99 \times 10^{-4*}$	4.63×10^{-2}

Equation (2) is the nonlinear regression and Equation (3) is the linearization of Langmuir isotherms with K and q_{\max} parameters.

*Statistically significant model parameters (p -value < 0.05).

[†]Nonlinear regression modeling (Equation (2)) with the SM data did not generate a stable result.

the concentration of DOM and ionic strength. We found a semi-log relationship (Equation (4)) between the initial manure mass and the aqueous concentration of erythromycin (Figure 4). We found that manure hinders erythromycin adsorption to this soil. At the highest manure mass tested (5.00 g), samples resembled control batch experiments' aqueous concentrations at equilibrium. While at the lowest manure mass tested (0.01 g), samples resembled S treatments' aqueous concentrations. We found the adjusted R^2 for this relationship to be 0.9629. The semi-log model coefficients were 678.11 (p -value = 0.0039*) and -93.56 (p -value = 0.0124*) for a and b , respectively. This result suggests that at soil-to-manure ratios of 100:1 or less, erythromycin is likely to remain in a more mobile, aqueous phase. Ratios closer to 1:1 soil: manure are the exemplary of conditions at the soil–manure interface after manure application.

3.3. Soil column experiments

Initial breakthrough curves for both S and SM columns occurred between 0 and 5 L (infiltration depth between 0 and 130 cm) erythromycin solution that passed through the columns (Figure 5). The two column treatments overlap significantly infiltrated between 2.5 and 6 L (infiltration depth between 65 and 156 cm) after which they diverge for the remainder of the experiment (total of 16.6–17.6 L passed through the columns, infiltration depth of 431–457 cm). Although there is considerable overlap between the two treatments, the SM smooth loess model resulted in a higher aqueous concentration than the S treatment model for the entire experiment. Due to the slow adsorption kinetics determined in the adsorption equilibration time experiment, these results are expected. When the columns begin to diverge, the SM columns discharged a greater amount of erythromycin, consistent with lower adsorption extents.

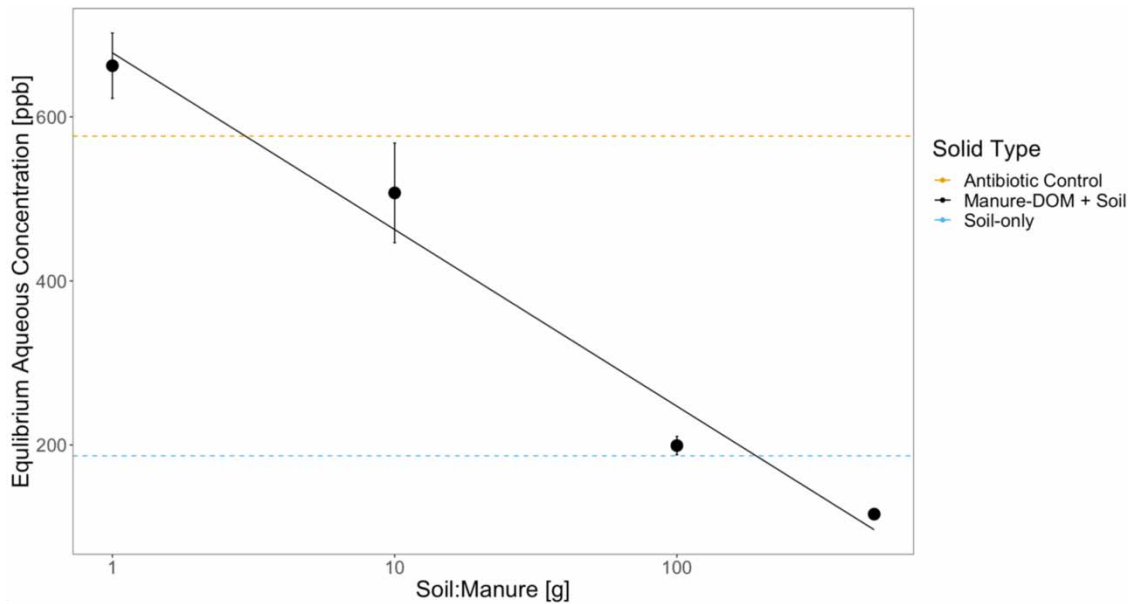


Figure 4 | Filtered manure influence on erythromycin aqueous concentration after 72 h reaction with 5 g of soil. Error bars represent the range of the data. The fitted model follows Equation (4) (black line). Antibiotic control (yellow line) and 72 h S sample (blue line) are provided for reference. Please refer to the online version of this paper to see this figure in colour: <http://dx.doi.org/10.2166/wh.2023.051>.

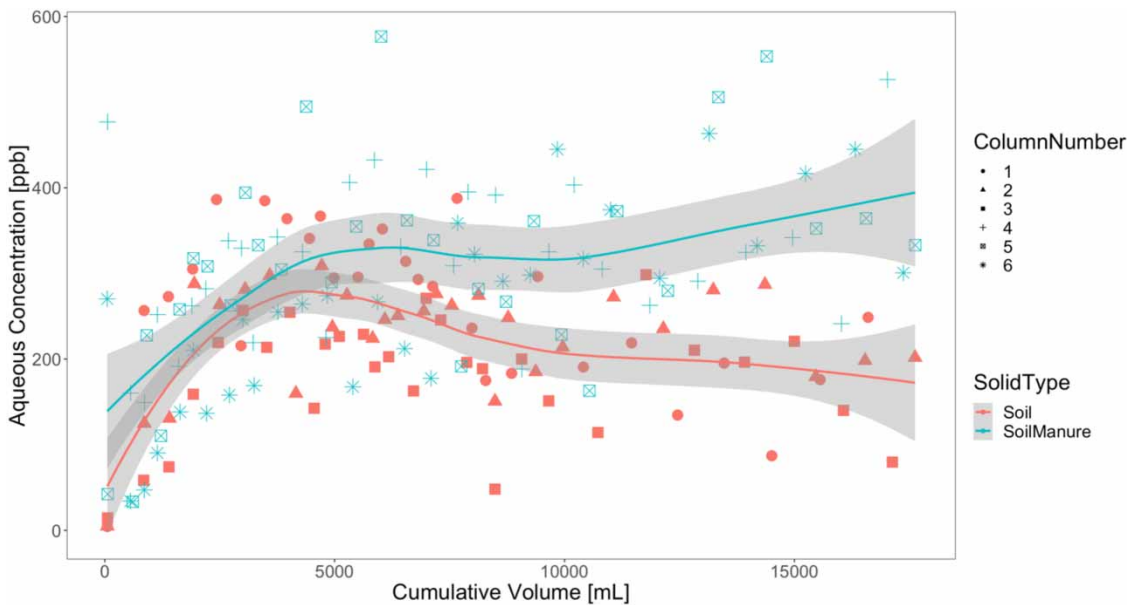


Figure 5 | Aqueous concentrations of erythromycin ($C_o = 500$ ppb) after passage through S (red) or SM (blue) columns. Volume represents exfiltrate from the column. Unique shapes indicate samples from the same columns. Columns 1–3 were S, and columns 4–6 were SM. Trend lines are fitted with a loess smoothing model. Please refer to the online version of this paper to see this figure in colour: <http://dx.doi.org/10.2166/wh.2023.051>.

The S column from 5 L through the end of the experiment displayed additional adsorption in comparison with SM columns. This may be caused by a secondary, slower adsorption mechanism with less affinity for erythromycin. This multi-phased adsorption may be a result of the complex, non-homogenous nature of the soil used and appears to be much less extensive in the SM treatment, which approaches the influent concentration of 500 ppb for some final samples. This

second mechanism of adsorption may have been captured by the 14-h samples in the adsorption equilibration time experiment, which displayed additional adsorption in comparison to the 72-h samples.

4. DISCUSSION

We found that erythromycin adsorbs to soil (S treatments), but in the presence of manure (SM treatments) erythromycin is characterized by an adsorption isotherm favoring its aqueous phase, leading to higher mobility and contamination risk. We found that primary adsorption equilibrium was established after approximately 72-h in soil batch reactors. Following the recommendation by *Menz et al. (2018)*, this work defines the soil–erythromycin adsorption interaction to better understand residue movement in the environment, especially under conditions of manure application, a likely erythromycin contamination source. We found that erythromycin is more mobile, i.e., it remains in the aqueous phase, when introduced with manure than it is when introduced alone. When considering the effect of our calculated partitioning coefficients on contaminant mobility, the difference between the retardation factors under the two conditions is illustrative. Considering the relationship

$$r_f = \frac{v_c}{v_w} = \frac{1}{1 + \frac{\rho_b}{\theta} K_d} \quad (5)$$

where r_f is the retardation factor, v_c is the velocity of the contaminant, v_w is the velocity of water, ρ_b is the bulk density of the substrate, θ is the porosity of the soil, and K_d is the distribution coefficient. We find r_f to be between 7.17×10^{-6} and 9.01×10^{-6} for the soil (S) conditions and 2.83×10^{-3} for the soil with manure (SM) conditions, assuming the bulk density equal to the bulk density of the soils tested (0.87 g/cm^3), a porosity of 0.5, and the distribution coefficients calculated during the batch isotherm experiments. Thus, erythromycin would move significantly slower than water; however, the estimate of that velocity depends on the manure or organic matter conditions present.

Differing from the organic carbon partitioning coefficient predicted by EPI Suite™ and previous soil adsorption experiments, which indicated increased erythromycin sorption to soil with organic matter presence (*Kruger 1961*), we found that erythromycin became more mobile when interacting with manure-derived dissolved organic matter. Though the soils we used did have 9.8% organic matter present, the addition of manure significantly increased the mobility of erythromycin. Our results suggest that adsorption sites previously taken by erythromycin are either instead occupied by a component from the manure, the manure alters the form of erythromycin to make it less likely to sorb to the soil, or that erythromycin–DOM–manure complexes formed, which were small enough to pass through the filter and unable to sorb to the soil. These DOM–erythromycin complexes could have effectively made the contaminant more mobile and would be expected given the propensity of this compound to partition into organic carbon. We did not explicitly test this mechanism and determination requires additional studies. DOM has been shown to influence contaminant adsorption to minerals by inhibiting mineral-catalyzed hydrolysis and oxidation reactions required for adsorption through competition for adsorption sites and through complexation reactions yielding compounds with higher or lower affinities for adsorption sites (*Polubesova & Chefetz 2014*), pushing the solid–aqueous-phase contaminant equilibrium toward the aqueous phase, as observed in our study. Clarithromycin and roxithromycin, two related macrolide antibiotics, have both shown similarly reduced adsorption to minerals in the presence of DOM (*Feitosa-Felizzola et al. 2009*). Humic acid–DOM was reported to reduce the adsorption of clarithromycin and roxithromycin onto manganese oxide and ferrihydrite minerals, likely by competing with the mineral surfaces for the macrolide antibiotics (*Feitosa-Felizzola et al. 2009*), a possible mechanism that may similarly influence erythromycin. Along with DOM, ions will dissolve into a solution from the manure that could potentially influence adsorption. In this study, we assessed the combination of all dissolved compounds, though the above-mentioned studies suggest that DOM interactions are a likely mechanism. To the author's knowledge, this is the first time manure has been shown to inhibit the adsorption of erythromycin to soils.

Other antibiotics have previously displayed biphasic adsorption, as seen in our column experiments. Tetracycline has shown biphasic adsorption to a highly porous human hair-based substrate, filling external adsorption sites prior to less accessible internal adsorption sites (*Ahmed et al. 2017*). Biphasic *mineralization* of erythromycin has been observed and associated with desorption of the compound from sediments (*Kim et al. 2004*), but no prior studies have noted biphasic adsorption of the erythromycin to soils to the author's knowledge.

4.1. Study implications and limitations

We found that erythromycin was more mobile in SM treatments in comparison with S treatments. This conclusion was held across adsorption isotherm experiments and column experiments under the conditions tested. The goals of these experiments were to determine distribution coefficients under worst-case, high-contaminant, and heavy manure application scenarios. We found that erythromycin contamination sourced from agricultural manure application may pose a higher risk of entering surface water and groundwater supplies than previously concluded from S experiments (Pan & Chu 2016). However, the concentrations tested in this experiment were the worst-case scenario, highly contaminated environments. These high concentrations are most applicable to soil–manure interfaces that may exist after the non-homogenous spreading of manure from treated animals on fields. This non-homogenous spreading effectively concentrates nutrients and contaminants over smaller surface areas. Though natural water body (i.e. streams and lakes) concentrations would not be expected to reach these levels (Ashraf *et al.* 2021), the above-discussed conclusions still provide valuable insights into the transport of erythromycin from highly contaminated sources. Lower contaminant concentrations should also be tested to ensure relationships hold across environmentally relevant levels.

To assess the risk of antibiotic contamination in other antibiotic classes and with other dominant functional groups, manure and SM interactions should be investigated similarly to and beyond those performed here to describe mechanisms of adsorption. Additionally, organic matter derived from differing sources may interact uniquely with each contaminant due to differing DOM compositions. The effect of differing DOM sources should also be investigated. We tested only dairy manure, but erythromycin is used across livestock, notably in swine production. Further investigation of the effect of differing manures on erythromycin mobility should also be studied. Reducing transport, and, more broadly antibiotic environmental impact, requires a deeper understanding of the controls of sorption of erythromycin to environmentally relevant particulate and dissolved organic compounds and soil surfaces.

Because erythromycin sorbs readily to soils, best management practices (BMPs) that reduce sediment transport may also reduce erythromycin transport. However, because of the continuous flushing of sediments in riparian zones and other frequently saturated areas (such as sediment control BMPs), conditions may favor the aqueous phase of the compound, increasing mobility and reducing the removal of erythromycin during high-flow conditions. The control of erythromycin residue transport may be more effective on the manure management level, e.g., breaking down this compound through heat treatment (Oliver *et al.* 2020) or biological degradation prior to the field application of manure. Kim *et al.* (2004) found that natural biological degradation of erythromycin was controlled by the desorption of erythromycin from stream sediments, suggesting that microbial degradation could be enhanced if a system was designed to allow degradation in manure storage prior to field application.

5. CONCLUSION

In high-concentration laboratory experiments, we found that erythromycin adsorbs more strongly to agricultural soil in the absence of manure, remaining more mobile in the presence of manure. These conditions may reflect the non-homogenous manure distribution that sometimes describes manure application, concentrating contaminants and nutrients over smaller than intended surface areas. We found that the characteristic adsorption isotherms that fit both environments were Langmuir isotherms, with the nonlinear regression fitting the soil-only (S) adsorption isotherm best and a linearization of the Langmuir fitting the SM condition best. In our column experiment, we observed more passage of erythromycin in the SM condition, as would be expected from the differences in their isotherms, and the possible biphasic adsorption breakthrough curve. To model the in-field transport of erythromycin and similar compounds, these experiments should be repeated under additional concentration and flow regimes, across soil types, and antibiotics. If we are to reduce the transport of compounds such as erythromycin, we must consider these physical and chemical processes alongside biological utilization and human decision-making pathways to predict the impact and risk of transport.

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AUTHOR CONTRIBUTIONS

C.B.G. conceptualized the study, performed data curation, completed data analysis & visualization, acquired funding, wrote the original draft, and reviewed and edited the manuscript. C.E.M. and D.E.H. assisted with method development, supervised the experiments, reviewed and edited the manuscript. M.T.W. acquired funding and reviewed and edited the manuscript.

DATA AVAILABILITY STATEMENT

Data is publicly available on Hydroshare at <https://www.hydroshare.org/resource/08084d979c5d4900a589f8232332a7c5/>.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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