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# Veterinary antimicrobials' sorption into a clay brazilian soil and peat

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**Abstract:** The fate of veterinary antimicrobials (VAs) in the environment is a concern due to the intensive systems for poultry and beef. Antimicrobials are widely applied in veterinary medicine, which may contaminate the environment through their release into soil, and undergo transformation, transport, and retention. Retention can be estimated by the partition coefficient between the soil and the soil solution (Kd), which is determined by sorption experiment. This study presents the validation of a method using high-performance liquid chromatography with fluorescence (Flu) and ultraviolet (UV) detection (HPLC-Flu-UV) of eight VAs from six classes, ciprofloxacin, enrofloxacin, and norfloxacin (fluoroquinolones), amoxicillin (β-lactam), tylosin (macrolide), lincomycin (lincosamide), sulfadiazine (sulfonamide), and trimethoprim (pyrimidine) to determine and compare their Kd values in a typical Brazilian clay soil and peat, rich in organic matter and high cation exchange capacity (CEC). Freundlich and Langmuir isotherms were tested and the best adjusted model was the Freundlich, with sorption coefficient values ranging from 0.008 to 3.921 µg1-1/n(cm3)1/ng-1 for soil (0.81% of organic carbon, 29.6 mmolc kg-1 CEC, pH 4.8) and from 1.046 to 9.708 µg1-1/n(cm3)1/ng-1 for peat (24.52% of organic carbon, 192.1 mmolc kg-1 CEC, pH 3.6). The high solubility of ENR and NOR in water could explain the low influence of organic matter or CEC in the retention of these substances in peat. The results highlight the importance of studies on the sorption of AVs in peat, a matrix that has been little studied and can influence the fate of VAs in the environment.

## Sorção de antimicrobianos veterinários em um solo argiloso brasileiro e em turfa

**Resumo:** O destino de antimicrobianos veterinários (AVs) no ambiente é um assunto preocupante devido aos sistemas intensivo de produção de aves e bovinos. Antimicrobianos são amplamente utilizados na medicina veterinária, podendo contaminar o meio ambiente por meio de lançamento no solo e sofrer transformações, transporte e retenção. A retenção pode ser estimada pelo coeficiente de partição entre o solo e a solução do solo (Kd), sendo determinada por ensaios de sorção. Este estudo apresenta a validação de método por cromatografia líquida de alta eficiência com detecção por fluorescência (Flu) e ultravioleta (UV) (HPLC-Flu-UV) de oito AVs, ciprofloxacina, enrofloxacina, norfloxacina (fluoroquinolonas), amoxicillina (β-lactâmico), tilosina (macrolídeo), lincomicina (lincosamida), sulfadiazina (sulfonamida) e trimetoprima (pirimidina) para determinar e comparar os valores de Kd em um típico solo brasileiro argiloso e em turfa, rica em matéria orgânica e alto valor de capacidade de troca catiônica (CTC). As isotermas de Freundlich e Langmuir foram testadas e o modelo melhor ajustado foi o de Freundlich, com valores de coeficiente de sorção variando de 0,008 a 3,921 µg1-1/n(cm3)1/ng-1 para o solo (0,81% carbono orgânico, 29,6 mmolc kg-1, pH 4,8) e de 1,046 a 9,708 µg1-1/n(cm3)1/ng-1 para a turfa (24,52% carbono orgânico, 192,1 mmolc kg-1 CTC, pH 3,6). A alta solubilidade em água pode explicar a baixa influência da matéria orgânica ou da CTC na retenção dos AVs ENR e NOR em turfa. Os resultados ressaltam a importância dos estudos de sorção dos AVs em turfa, uma matriz pouco estudada e que pode influenciar no destino dos AVs no ambiente.

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## Introdução

Veterinary antimicrobials (VAs) are widely employed in the poultry and beef industries for disease prevention and growth promotion (MAK *et al.*, 2022). Most VAs are poorly absorbed in the animal' gut and, consequently, are excreted through urine and feces (ZHOU *et al.*, 2022). These substances may enter in the environment through the release of urine and manure directly into the soil or indirectly through the addition of slurry or manure compost to the soil. Concern about soil microorganisms' resistance and possible impacts to animal and human life cause by VAs is discussed elsewhere (LARSSON; FLACH, 2022).

Similar to other organic contaminants, once in the environment, VAs can participate in different processes of retention, transformation and transport being able to reach different environmental matrices. The sorption process is important to understand antimicrobial bioavailability and transport in soil, leaching to groundwater and runoff to surface water. Soil organic matter and minerals, such as clay minerals and oxides are pointed out as important factors for the sorption of antimicrobials and other contaminants into soil (FRANKLIN *et al.*, 2022; ASSIS *et al.*, 2021; SILVA JÚNIOR *et al.*, 2019). Nowadays, there is an increase in the number of studies involving the occurrence and behavior of VAs in environmental matrices, such as in surface water, groundwater, soil, and manure (STANDO *et al.*, 2022; BARROS *et al.*, 2021). However, information regarding the behavior of these substances in tropical soil conditions is still scarce and no study has considerate the sorption of VAs in solid phase with high organic matter content such as peat, that is used in agriculture.

Recently, Rath *et al.* (2019) studied sorption behaviors of antimicrobial and antiparasitic veterinary drugs in Brazilian soils and found that the drugs with chemical structures in common and belonging to the same family presented similar sorption and desorption in different soils and results generally agreed with soils from temperate regions. Vaz Júnior (2016) assessed the sorption of the agricultural antimicrobial oxytetracycline in Brazilian soils and the influence of the organic matter on the sorption capability. Doretto and Rath (2013) found higher sorption of sulfadiazine in Brazilian soils with high organic carbon (ranging from 0.89 to 1.87%, w/w) and clay (ranging from 6.2 to 54.6 %, w/w) when investigating four types of Brazilian soils, including sandy and clay soil.

Despite the importance of livestock production for the Brazilian agribusiness, there is a lack of data about the amount and types of VAs commercialized for livestock production, the occurrence of VAs in the environment, their effects on the ecosystem and their dynamics in soil. In Brazil, official data on antimicrobials used in animal production are not publicly available (LENTZ, 2022).

Taking into account the range and complexity of tropical soil, and the possibility of applying manure or slurry from the poultry industry to soil and the risk of antimicrobial runoff from the treated soil, the present study evaluates the sorption of eight VAs from six different classes, ciprofloxacin, enrofloxacin, and norfloxacin (fluoroquinolones), amoxicillin (β-lactam), tylosin (macrolide), lincomycin (lincosamide), sulfadiazine (sulfonamide), and trimethoprim (pyrimidine) in a typical clay soil and peat from Brazil.

## Material and methods

### Chemicals

All organic solvents were HPLC grade (acetonitrile and methanol, Mallinckrodt, USA), and other reagents were analytical grade (phosphoric acid 85%, Chemis Brazil, and calcium chloride 75%, J. T. Baker Japan). Ultra-pure water was obtained from a Milli-Q purification system (Millipore, USA). Before HPLC analysis, samples and standard solutions were filtered with 0.25 µm syringe filters (PVDF).

Solid standards of the veterinary antimicrobials amoxicillin (AMO) (99.3 %), ciprofloxacin (CIP) (99.6 %), norfloxacin (NOR) (99.8 %), lincomycin (LIN) (100.3 %), sulfadiazine (SUL) (99.7 %), tylosin (TYL) (83.9 %), and trimethoprim (THR) (99.1 %) were obtained from Sigma-Aldrich GmbH Laborchemikalien (Germany). Enrofloxacin (ENR) (99.0%) was obtained from Dr. Ehrenstorfer GmbH (Germany). Individual stock solutions (1000 mg $L^{-1}$) of each VA standard were prepared by dissolving

10 mg of the ingredient in 10 mL of methanol for CIP, ENR, LIN, SUL, THR, and TYL, while AMO and NOR were dissolved in 10 mL of ultra-pure water. Working solutions of the VAs were prepared with the appropriate dilution of the standard stock solution with 0.01 mol $L^{-1}$ calcium chloride ($CaCl_2$). All solutions were stored at 5 ℃ and were renewed every 30 days.

### Soil and peat characteristics

Soil and peat samples were collected from two regions of São Paulo State (Brazil) in 2012 with no history of agricultural or animal cultivation. Peat was collected (50 cm depth) from the Taquaral city area (21° 3′ 37.70″ S, 48° 24′ 33.30″ W) and a clay soil (20 cm depth) from the Araraquara city area (21° 47′ 23.18″ S and 48° 11′ 51.58″ W). Both samples, properties showed in Table 1, were air-dried at room temperature (25 °C) and sieved using a 2 mm sieve prior to batch sorption experiments.

**Table 1 -** Physical and chemical properties of a typical Brazilian soil and peat employed in this study.

| Matrix | Sand (%) | Silt (%) | Clay (%) | Organic matter (g $dm^{-3}$) | Organic carbon (%) | pH | CEC (mmolc $kg^{-1}$) |
|---|---|---|---|---|---|---|---|
| Soil | 16.8 | 17.7 | 65.5 | 14 | 0.81 | 4.8 | 29.6 |
| Peat | 43.3 | 19.7 | 37.0 | 424 | 24.52 | 3.6 | 192.1 |

CEC: Cation Exchange Capacity. Source: the authors.

### Sorption experiment

Batch equilibrium method based on the Test Guideline 106 from the Organization for Economic Co-operation and Development (OECD) for soil (OECD, 2000) were employed to determine the sorption of VAs in a clay soil and peat. Briefly, the optimal soil/solution ratio and sorption equilibrium time were at 1:5 (weight:volume) and 9 up to 48 h, according to each VA. An amount of 5.0 g of dried soil and peat were separately added to 20 mL of 0.01 mol $L^{-1}$ $CaCl_2$ in amber glass flasks, and after an equilibration period of 12 h, 5 mL of each veterinary antimicrobial prepared in 0.01 mol $L^{-1}$ $CaCl_2$ was added to adjust the final volume to 25 mL. The flasks were spun in a horizontal shaker (150 rpm) during 48 h for LIN, SUL, THR, TYL, and AMO and 9 h for NOR, CIP, and ENR. The time was shorter for fluoroquinolones since they sorbed in a shorter period compared to other antimicrobials. After these periods, samples were kept at rest for 15 minutes for solid decantation. Aliquots of 250 µL of the supernatant were filtered through a 0.25 µm syringe filter (PVDF) and analyzed by HPLC-Flu-UV.

The amount of each veterinary antimicrobial sorbed onto the soil or peat particles ($C_s^{ads}$), in µg $g^{-1}$, was calculated from the difference between the initial concentration and the concentration after the respective contact times ($C_{aq}^{ads}$) in µg $mL^{-1}$, according to equation 1.

$$C_s^{ads} = \frac{V_0}{m_{soil}} \text{ x } (C_0 - C_{aq}^{ads}) \qquad (1)$$

Where $V_0$ is the initial volume of solution, $m_{soil}$ is the mass of soil or peat, and $C_0$ is the initial concentration of each veterinary antimicrobial.

Two sorption isotherms, Freundlich and Langmuir, were used to fit the sorption of all the VAs in soil and peat as follows (equations 2 and 3):

Langmuir sorption isotherm:

$$C_s^{ads} = \frac{K_L \text{ x } C_{aq}^{ads} \text{ x } b_L}{1 + K_L \text{ x } C_{aq}^{ads}} \qquad (2)$$

Where $K_L$ is the Langmuir sorption constant ($cm^3$ $g^{-1}$), and $b_L$ is the maximum sorption capacity (µg $g^{-1}$).

Freundlich sorption isotherm:

$$C_s^{ads} = K_F \text{ x } C_{aq}^{ads\ 1/n} \tag{3}$$

Where $K_F$ is the Freundlich sorption constant ($\mu g^{1-1/n}(cm^3)^{1/n}g^{-1}$), and 1/n is dimensionless and represent the amount of chemicals sorbed and the sorption intensity and the isotherm shape (GILES *et al.* 1974).

Since organic matter is highly variable between soils, an alternative is to normalize the results as a function of the soil organic carbon content (OC). The normalized sorption coefficient ($K_{OC}$) reduces the variability in the sorption data between soils for a given compound. $K_{OC}$ values were calculated according to Test Guideline 121 from the OECD (2001) and using equation 4 as follows:

$$K_{OC} = \frac{K_d}{\%\ OC} \text{ x } 100 \tag{4}$$

Where $K_d$ is the sorption coefficient obtained thoroughly linear isotherm (equation 5):

$$C_s^{ads} = K_d \times C_{aq}^{ads} \tag{5}$$

**Chromatographic analysis**

Chromatographic analyses were performed in a Varian 920 HPLC (Agilent, USA) equipped with fluorescence (Flu) and ultraviolet (UV) detectors (HPLC-Flu-UV), a Gemini reversed-phase column (C18, 150 mm, 4.6 mm, 5 µm, 110 Å, Phenomenex®, USA), and the data-acquisition software Varian Galaxie for data acquisition. Based on previously studies (ZHAO *et al.*, 2010; USLU *et al.*, 2008; CHRISTIAN *et al.*, 2003; CAMPAGNOLO *et al.*, 2002), chromatographic conditions consisted of isocratic mode with $H_3PO_4$ 0.02 mol $L^{-1}$ as solvent A and a proportion of solvent B (acetonitrile) for which VAs (Table 2), consisting of individual analysis. LIN, SUL, THR and TYL were determined by UV detection using the maximum wavelength for each VA. Fluorescent determination was employed for AMO, NOR, CIP and ENR according of each VA characteristic excitation and emission wavelengths.

**Table 2 -** Chromatographic conditions for VAs analysis using HPLC-Flu-UV.

| | | |
|---|---|---|
| **Column temperature** | 27 °C | |
| **Mobile phase components** | $H_3PO_4$ 0.02 mol $L^{-1}$ (A) and Acetonitrile (B) | |
| **Mobile phase composition by antimicrobial (isocratic mode)** | Antimicrobial | Acetonitrile (B) |
| | AMO | 12% |
| | LIN | 16% |
| | SUL | 16% |
| | THR | 16% |
| | NOR | 20% |
| | CIP | 20% |
| | ENR | 20% |
| | TYL | 31% |
| **Run time by antimicrobial** | 5 min | |
| **Flow rate** | 1 mL $min^{-1}$ | |
| **Injection volume** | 20 µL | |
| **Wavelengths (UV)** | LIN (205 nm); SUL and THR (264 nm); TYL (280 nm) | |
| **Wavelengths (Flu)** | AMO (Ex: 235 nm/ Em: 310 nm); NOR, CIP, and ENR (Ex: 280 nm / Em: 480 nm). | |

Source: The authors.

The reliability of the chromatographic method was following up the validation parameters linear range, matrix effect due the presence of calcium chloride, selectivity, precision, and limit of quantification (LOQ). The linear range was established through analytical curves, in triplicate analyses, of seven concentration levels for each antimicrobial: 2.9 to 89.7 mg $L^{-1}$ for LIN, SUL, THR, and TYL; 0.6 to 51.6 mg $L^{-1}$ for AMO; and 0.0009 to 0.131 mg $L^{-1}$ for NOR, CIP, and ENR. All solutions were prepared in 0.01 mol $L^{-1}$ $CaCl_2$ in water.

Method selectivity was evaluated comparing the chromatograms obtained from extracts of soil and peat without VAs addition and the chromatograms of a mixture solution containing the VAs prepared in $CaCl_2$ 0.01 mol $L^{-1}$. The LOQs for Flu and UV determination were based on the lowest standard solutions' concentration of individual VAs prepared in $CaCl_2$ 0.01 mol $L^{-1}$, within the linear range.

## Results and discussion

### Preliminary studies to ensure the reliability of the results

Degradation and possible VAs retention on the surface of test flasks were not observed previously to the sorption experiment. Chromatographic response of individual VAs prepared in 0.01 mol $L^{-1}$ $CaCl_2$ (20 mg $L^{-1}$ SUL; 30 mg $L^{-1}$ LIN, THR, and TYL; 1.0 mg $L^{-1}$ AMO; 0.05 mg $L^{-1}$ NOR, CIP, and ENR), measured using Flu or UV detection, showed stable during the required time for the sorption study and that no retention occurred onto the surface of the test flasks. The highest response decrease for an individual VAs was 3.7 % compared to the initial concentration. The chromatograms of the control samples (without VAs) showed that the extracts of soil and peat were free of interference, which indicates the selectivity of the chromatographic methods for all substances.

Since calcium chloride 0.01 mol $L^{-1}$, the saline solution employed in batch sorption experiments, can increase or decrease chromatographic response of analytes when in $H_3PO_4$ 0.02 mol $L^{-1}$ (a major component of the chromatographic mobile phase), the matrix effect was investigated for the studied VAs comparing the slopes of the analytical curves prepared in 0.01 mol $L^{-1}$ $CaCl_2$ solution and the ones obtained in 0.02 mol $L^{-1}$ $H_3PO_4$ (Table 3). As described elsewhere (TOLEDO NETTO *et al.*, 2012), when the difference for the angular coefficient is above 10%, matrix effect is significant, which may influence the quantitative analysis. Besides satisfactory determination coefficient values ($r^2 > 0.9$) were obtained for all VAs for both curves (0.02 mol $L^{-1}$ $H_3PO_4$ and 0.01 mol $L^{-1}$ $CaCl_2$), effect on the chromatographic response for VAs, calculated as the difference between the angular coefficients for both curves (Table 3), was pronounced for LIN, TYL, and ENR, higher than 10. These values indicate that the $CaCl_2$ solution is causing an increase in the chromatographic response and, therefore, to minimize this effect, the quantification of VAs was performed using the analytical curves prepared in $CaCl_2$ 0.01 mol $L^{-1}$.

**Table 3 -** Analytical curve parameters, matrix effect on the chromatographic response, and LOQs.

| VAs | Equation in $H_3PO_4$ 0.02 mol $L^{-1}$ | $r^2$ | Equation in $CaCl_2$ 0.01 mol $L^{-1}$ | $r^2$ | Matrix effect (%)* | LOQ (mg $L^{-1}$) |
|---|---|---|---|---|---|---|
| AMO | y = - 6.906 + 167.810x | 0.99 | y = 10.162 + 180.806x | 0.99 | + 7.7 | 0.6 |
| LIN | y = 2.175 + 7.438x | 0.99 | y = 0.058 + 8.671x | 0.99 | + 16.6 | 2.9 |
| SUL | y = - 46.802 + 58.522x | 0.99 | y = -47.529 + 56.256x | 0.99 | - 3.9 | 2.9 |
| THR | y = 10.187 + 20.267x | 0.99 | y = 10.993 + 19.794x | 0.99 | - 2.3 | 2.9 |
| TYL | y = - 45.873 + 19.048x | 0.99 | y = -0.175 + 16.402x | 0.99 | - 13.9 | 2.9 |
| CIP | y = - 7.311 + 35.129x | 0.99 | y = -22.497 + 31.886x | 0.99 | - 9.2 | 0.0048 |
| ENR | y = 499.960 + 89. 093x | 0.98 | y = 30.790 + 112.911x | 0.99 | + 26.7 | 0.0009 |
| NOR | y = 7.495 + 68.594x | 0.99 | y = -36.101 + 66.085x | 0.99 | - 3.7 | 0.0053 |

$r^2$: determination coefficient. *matrix effect due $CaCl_2$ 0.01 mol $L^{-1}$.

Source: The authors.

Relative standard deviations for the chromatographic area and retention time ranged from 0.8 to 4.9 % and from 0.12 to 0.48 %, respectively (data not shown), shows the suitability of the chromatographic methods with good inter-day precision under the same operating conditions over a short period of time.

### Sorption isotherms

Sorption experiments were fulfilled using five VAs concentration levels as follow 30, 40, 50, 70, and 90 mg L$^{-1}$ for LIN, SUL, THR, and TYL; 1.5, 5.0, 10.0, 15.0, and 20.0 mg L$^{-1}$ for AMO; and 0.08, 0.11, 0.14, 0.20, and 0.22 mg L$^{-1}$ for NOR, CIP, and ENR. AMO, NOR, CIP, and ENR were determined at lower concentrations than other VAs considering the better sensibility of fluorescence detector. The concentration chosen in the sorption studies were environmentally relevant based on works in the literature that report concentrations ranging from parts per trillion to parts per million in different types of environmental matrices. As reviewed and presented elsewhere (TOLEDO NETTO *et al.*, 2014) concentrations of different groups of antimicrobials were detected in soil from 0.72 μg kg$^{-1}$ to 378 mg kg$^{-1}$, poultry litter from 31 μg kg$^{-1}$ to 1420 mg kg$^{-1}$, biosolid from 8.7 μg kg$^{-1}$ to 562 mg kg$^{-1}$, surface water from 10 ng L$^{-1}$ to 1 mg L$^{-1}$, groundwater from 0.16 to 1.02 μg L$^{-1}$, and wastewater from 3.7 to 211 μg L$^{-1}$.

Sorption isotherms in soil play an important role regarding the fate and transport of substances in the environment, providing information that can contribute to estimate the potential risk of water contamination. Different sorption models have been reported in the literature, as described by Giles *et al.* (1974). The linear, Langmuir and Freundlich models were considered in this study, but the results of the last two are presented in Table 4 because they are the most frequently cited models in the literature to describe the sorption of VAs in soil or soil associated with animal manure (DORETTO and RATH, 2013; KIM *et al.*, 2012; LEAL *et al.*, 2012; KASTEEL *et al.*, 2010).

**Table 4 -** Sorption isotherm parameters obtained using the Freundlich and Langmuir models for VAs in a typical Brazilian soil and peat.

| VAs | Adsorbent | Freundlich | | | Langmuir | | |
|---|---|---|---|---|---|---|---|
| | | $K_F$ | 1/n | $r^2$ | $K_L$ | $b_L$ | $r^2$ |
| AMO | Peat | 4.84 (0.56) | 0.80 | 0.985 | 0.06 (0.01) | 85.636 | 0.998 |
| | Soil | 3.47 (0.35) | 0.79 | 0.728 | -0.027 (0.003) | -82.194 | 0.950 |
| LIN | Peat | 8.00 (0.71) | 0.55 | 0.892 | 0.023 (0.002) | 130.363 | 0.814 |
| | Soil | 2.25 (0.24) | 0.82 | 0.944 | $8.05\ 10^{-3}$ ($8.53\ 10^{-4}$) | 194.590 | 0.907 |
| SUL | Peat | 1.73 (0.04) | 0.72 | 0.686 | 0.017 (0.001) | 62.581 | 0.656 |
| | Soil | 0.08 (0.01) | 1.60 | 0.857 | $-6.48\ 10^{-3}$ ($9.27\ 10^{-5}$) | -81.090 | 0.945 |
| THR | Peat | 9.71 (1.54) | 0.67 | 0.894 | 0.014 (0.002) | 337.437 | 0.848 |
| | Soil | 0.20 (0.01) | 1.49 | 0.919 | $-1.06\ 10^{-2}$ ($6.01\ 10^{-4}$) | -62.311 | 0.886 |
| TYL | Peat | 1.05 (0.03) | 1.22 | 0.987 | -0.005 (0.001) | -361.036 | 0.988 |
| | Soil | 0.49 (0.01) | 1.16 | 0.844 | $-4.51\ 10^{-3}$ ($7.22\ 10^{-5}$) | -139.763 | 0.976 |
| CIP | Peat | 6.82 (0.52) | 1.28 | 0.982 | -8.32 (0.63) | -0.219 | 0.962 |
| | Soil | 0.60 (0.03) | 0.20 | 0.847 | 192.40(8.20) | 0.363 | 0.626 |
| ENR | Peat | 2.87 (0.15) | 0.82 | 0.915 | 5.58 (0.29) | 1.121 | 0.880 |
| | Soil | 3.15 (0.07) | 0.96 | 0.987 | 0.75 (0.02) | 4.872 | 0.990 |
| NOR | Peat | 1.18 (0.04) | 0.53 | 0.910 | 49.90(1.70) | 0.305 | 0.851 |
| | Soil | 3.92 (0.11) | 0.92 | 0.888 | -0.161 (0.004) | -30.359 | 0.910 |

$r^2$: determination coefficient; $K_F$: Freundlich sorption coefficient in $\mu g^{1-1/n}(cm^3)^{1/n}g^{-1}$; n: isotherm shape (dimensionless); $K_L$: Langmuir sorption coefficient in cm$^3$ g$^{-1}$; $b_L$ in μg g$^{-1}$; Values in parentheses refer to the standard deviation.

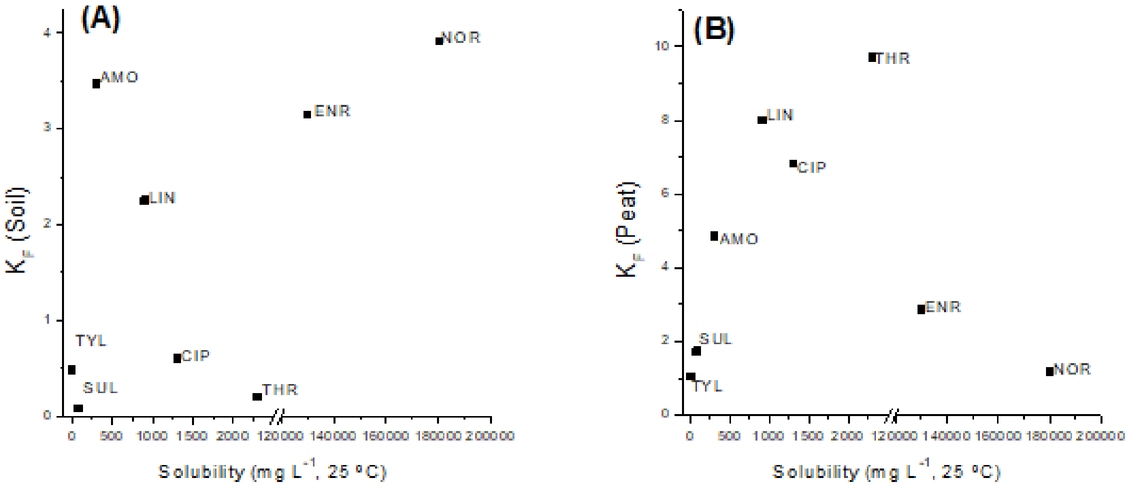
Source: The authors.

Among the isotherm models evaluated, Langmuir was not responded to sorption mechanisms because the sorption coefficients ($K_L$) were negative for many of the trials, which are not plausible. Negative values of these constants for organic and inorganic compounds were also reported elsewhere, indicating the inadequacy of the Langmuir model (KIM *et al.*, 2012; FUNGARO and BRUNO, 2009; FUNGARO *et al.*, 2009). Kim *et al.* (2012), for example, evaluated the Langmuir model in the sorption process of AMO in soil and obtained negative values for this constant. Therefore, the results obtained suggests that the sorption process behavior does not agree with the propositions of the Langmuir model.

Pereira *et al.* (2012) gathered sorption data for antimicrobials in soils, determining that Freundlich is the most representative model used in the different assessed studies. The Freundlich model suggests that the sorption energy decreases logarithmically when the surface of the adsorbent becomes covered by the analyte. This model considers the sorption as a heterogeneous process, differing from the Langmuir model. The Freundlich model was the most appropriate in the current study to represent the sorption processes, with determination coefficients higher than 0.844, except for AMO in soil ($r^2 = 0.728$) and SUL in peat ($r^2 = 0.686$). Therefore, the Freundlich model was chosen as most plausible to represent sorption of the VAs investigated in this study and employed to calculate $K_{OC}$ values.

When peat was considered as the sorbent for the studied VAs, values of $K_F$ were higher than $K_F$ values determined with soil as solid support, except for ENR and NOR, with $K_F$ 1.1 and 3.3 times higher in soil than in peat for ENR and NOR, respectively. For the other VAs, peat resulted in 48.6 (THR), 21.6 (SUL), 11.4 (CIP), 3.6 (LIN), 2.1 (TYL) and 1.4 (AMO) times higher for $K_F$ than in soil. Among the family fluoroquinolones, only CIP presented higher sorption in peat than soil. Regarding the characteristics of the studied sorbents, peat presents 24.52 % of organic carbon, 192.1 mmolc kg$^{-1}$ CEC and pH as 3.6, compared to 0.81 %, 29.6 mmolc kg$^{-1}$ and 4.8 from the studied soil, respectively. Soil has abundant clay content, 65.5 % compared to 37.0 % of peat. The high solubility of ENR and NOR in water could explain the low influence of organic matter or CEC in the retention of these substances in peat. Figure 1 presented the distribution of $K_F$ for the VAs in soil (A) and peat (B) according to their water solubility. For peat (B) it is possible to see an increase of the $K_F$ values according to the increase of solubility for TYL, SUL, AMO, Lin and THR.

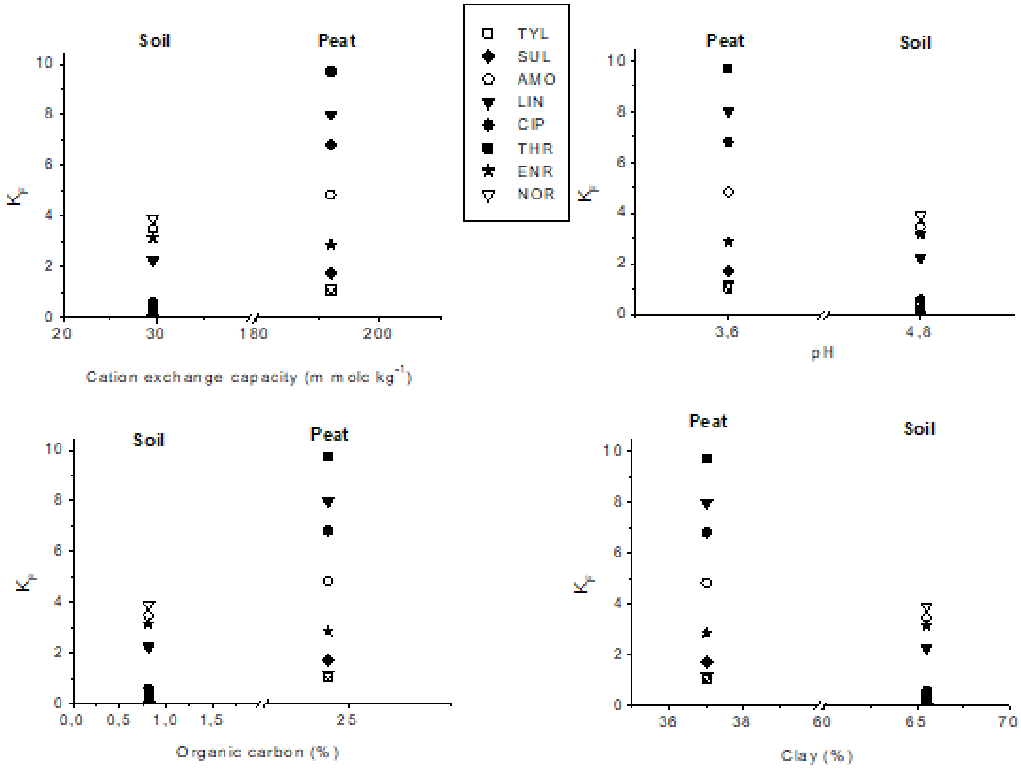
**Figure 1 -** $K_F$ distribution according to the water solubility for VAs, obtained in soil (A) and in peat (B).



Source: The authors.

Trends in VAs sorption $K_F$ coefficient with sorbent's properties presented less dispersive values for soil than for peat (Figure 2). Since peat (rich in organic matter and high CEC) has not been studied and sorbent for VAs, it was not possible to compare the distribution pattern of the values with other, so we maintained the comparison between a typical soil from São Paulo State, Brazil and peat. Values of $K_F$ in peat increased according to: TYL (1.05) < NOR (1.18) < SUL (1.73) < ENR (2.87) < AMO (4.84) < CIP (6.82) < LIN (8.00) < THR (9.71), and in soil according to: SUL (0.08) < THR (0.20) < TYL (0.49) < CIP (0.60) < LIN (2.25) < ENR (3.15) < AMO (3.47) < NOR (3.92).

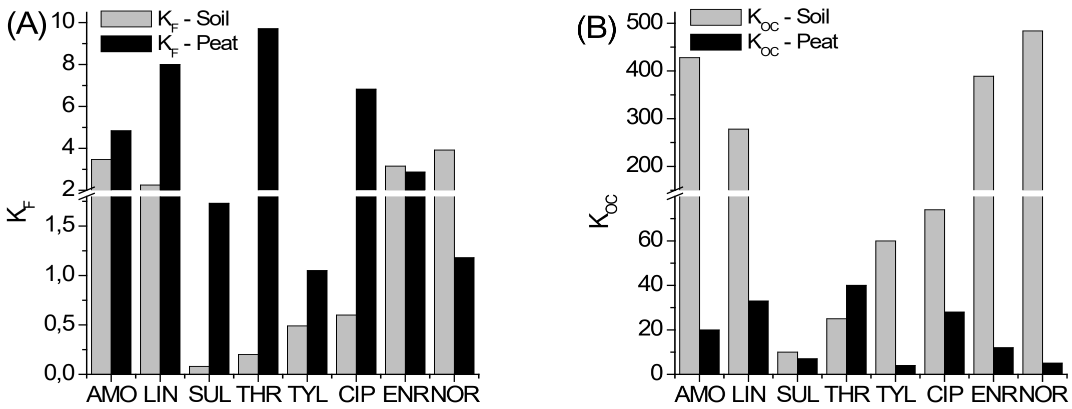
**Figure 2 -** $K_F$ distribution for soil and peat according to the sorbent's properties.



Source: The authors.

Freundlich sorption coefficients ($K_F$) were normalized to organic carbon content for data obtained from soil and peat according to equation 4. The comparison of $K_{OC}$ values is presented to be more appropriate than the $K_F$ values, since organic matter is highly variable between soils (OECD, 2001) (Figure 3 - B). $K_{OC}$ values were higher for all VAs in soil, except for THR.

**Figure 3 -** Comparison of $K_F$ and $K_{OC}$ distribution for VAs in soil and peat.



Source: The authors.

The high values of $K_{OC}$ for VAs in soil than in peat would suggest that the compounds are less mobile in soil than in peat, but in this case, for this situation the ionizable functional groups of the molecules need to be considered. Thus, these results alerts for the use of $K_{OC}$ in leaching estimation of VAs in soil. Except for the THR, the $K_{OC}$ obtained for soil when compared to values from peat was higher for all VAs studied: SUL (1.5 times), TYL (14 times), AMO (22 times), NOR (101 times), CIP (3 times), ENR (33 times), and LIN (9 times).

The objective of studying the VAs sorption' in peat was to verify the influence of its sorbent, rich in organic matter and high CEC in this process. Some references explain that the main interactions involved between the soil and antimicrobials are due to polyphenolic and ionizable carboxylic groups from the humic substances (present in large amounts in peat) and to metal hydroxides, which have negative or positive charges depending on pH. Several mechanisms may be involved, such as ion exchange, the formation of complexes with metal ions ($Ca^{2+}$, $Mg^{2+}$, $Fe^{3+}$ and $Al^{3+}$), hydrogen bonds between polar groups of the antimicrobial and soil humic acids, sorption to mineral constituent surfaces, and sorption to organic matter (PEREIRA *et al.*, 2012). However, it is not possible to identify which are the interactions involved between the antimicrobials studied and the soil and peat analyzed in this work, since it would be necessary to perform spectroscopic analysis to observe the possible physicochemical mechanisms at the molecular level of the interaction process, using different techniques such as Absorption and Fluorescence Spectroscopy in the UV-VIS region and Spectroscopy in the medium and near Infrared region. Spectroscopic analysis was not the focus of this work, which sought to estimate the sorption potential of VAs in soil, based on isotherms and sorption constants obtained experimentally. This study contributes with sorption data in typical Brazilian soil and peat, serving as a basis for further studies as for diagnosis of surface water contamination by veterinary antimicrobials that poultry litter can cause.

## Conclusion

The analytical method for VAs identification and quantification by HPCL-Flu-UV) employing calibration curves in $CaCl_2$ 0.01 mol $L^{-1}$ reduced the matrix effect for VAs determination and showed accurate to be employed in the sorption experiments. The best adjusted model for the sorption isotherms was the Freundlich model, which contributed to obtain the sorption behaviour of eight VAs in peat, a sorbent rich in organic carbon and high CEC, which is not common in the literature. The three VAs from a same family, fluoroquinolones, did not presented similar partner for sorption in peat and soil. ENR and NOR presented higher $K_F$ values for soil, unlikely CIP (also fluoroquinolones) and all the other studied VAs. Values of $K_F$ in peat resulted in 48.6 (THR), 21.6 (SUL), 11.4 (CIP), 3.6 (LIN), 2.1 (TYL) and 1.4 (AMO) times higher than $K_F$ in soil.

The results obtained highlight the sorption results of VAs in peat, a matrix that has been understudied and which can influence the fate of VAs in the environment if manure or poultry industry effluents are applied to this type of material.

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## References

ASSIS, F. X.; NETTO, A. M.; XAVIER, B. T. L.; LIMA, V. F. L.; SILVA, J. P. S. Imazapic interaction and mobility in soil cultivated with sugarcane in northeast Brazil. **Brazilian Journal of Water Resources**, v. 26, n. 16, p. 1-13, 2021. https://doi.org/10.1590/2318-0331.262120210007

BARROS, V. H. O.; COUTINHO, A. P.; ALVES, A. T. A.; SANTOS NETO, S. M.; ASSIS, F. X.; LIMA, V. F.; LIMA, J. R. S.; ANTONINO, A. C. D. Sorption studies of Cd and Cu on Brazilian alluvial soils. **Revista Ambiente & Agua**, v.

16, n. 4, p. 1-16, 2021. https://doi.org/10.4136/ambi-agua.2717

CAMPAGNOLO, E.R.; JOHNSON, K.R.; KARPATI, A.; RUBIN, C.S.; KOLPIN, D.W.; MEYER, M.T.; ESTEBAN, J.E.; CURRIER, R. W.; SMITH, K.; THUG, K. M.; MCGEEHIN, M. Antimicrobial residues in animal waste and water resources proximal to large-scale swine and poultry feeding operations. **Science of the Total Environment**, v. 299, p. 89-95, 2002. https://doi.org/10.1016/S0048-9697(02)00233-4

CHRISTIAN, T.; SCHNEIDER, R.J.; FARBER, H.A.; SKUTLAREK, D.; MEYER, M.T.; GOLDBACH, H.E. Determination of antibiotics residues in manure, soil and surface waters. **Acta Hydrochimica et Hydrobiologica**, v. 31, p. 36-44, p. 2003. https://doi.org/10.1002/aheh.200390014

DORETTO, K. M.; RATH, S. Sorption of sulfadiazine on Brazilian soils. **Chemosphere**, v. 90, p. 2027-2037, 2013. https://doi.org/10.1016/j.chemosphere.2012.10.084

FUNGARO, D.A.; BRUNO, M. Remoção de azul de metileno de solução aquosa usando zeólitas sintetizadas com amostras de cinzas de carvão diferentes. **Química Nova**, v. 32, p. 955-959, 2009. https://doi.org/10.1590/S0100-40422009000400024

FUNGARO, D.A.; IZIDORO, J.C.; BRUNO, M. Aplicação de material zeolítico sintetizado de cinzas de carvão como adsorvente de poluentes em água. **Eclética Química**, v. 34, p. 45-50, 2009. https://doi.org/10.1590/S0100-46702009000100007

FRANKLIN, A.M.; WILLIAMS, C.; ANDREWS, D.M.; WATSON, J.E. Sorption and desorption behavior of four antibiotics at concentrations simulating wastewater reuse in agricultural and forested soils. **Chemosphere**, v. 289, p. 133038, 2022. https://doi.org/10.1016/j.chemosphere.2021.133038.

GILES, C.H.; SMITH, D.; HUITSON, A. A general treatment and classification of the solute adsorption isotherm. **Journal of Colloid and Interface Science**, v. 47, p. 755-765, 1974. https://doi.org/10.1016/0021-9797(74)90252-5

KASTEEL, R.; M.B.O.H, C.M.; UNOLD, M.; GROENEWEG, J.; VANDERBORGHT, J.; VEREECKEN, H. Transformation and sorption of the veterinary antibiotic sulfadiazine in two soils: a short-term batch study. **Environmental Science & Technology**, v. 44, p. 4651-4657, 2010. https://doi.org/10.1021/es100141m

KIM, Y-K.; LIM, S-J.; HAN, M-H.; CHO, J-Y. Sorption characteristics of oxytetracycline, amoxicillin, and sulfathiazole in two different soil types. **Geoderma**, v. 185-186, p. 97-101, 2012. https://doi.org/10.1016/j.geoderma.2012.03.016

LARSSON, D.G.J.; FLACH, CF. Antibiotic resistance in the environment. **Nature Reviews Microbiology**, v. **20**, p. 257–269, 2022. https://doi.org/10.1038/s41579-021-00649-x

LEAL, R.M.P.; FIGUEIRA, R.F.; TORNISIELO, V.L.; REGITANO, J.B. Occurrence and sorption of fluoroquinolones in poultry litters and soils from São Paulo State, Brazil. **Science of the Total Environment**, v. 432, p. 344-349, 2012. https://doi.org/10.1016/j.scitotenv.2012.06.002

LENTZ, S.A.M. **Atualização sobre Uso Racional de Antimicrobianos e Boas Práticas de Produção**. 2022. Available in: https://www.gov.br/agricultura/pt-br/assuntos/insumos-agropecuarios/insumos-pecuarios/resistencia-aos-antimicrobianos/publicacoes/Apostila_AtualizaosobreUsoRacionaldeAntimicrobianoseBoasPrticasdeProduo.pdf. Access in December 2023.

MAK, P.H.W.; REHMAN, M.A.; KIARIE, E.G.; TOPP, E.; DIARRA, M.S. Production systems and important antimicrobial resistant-pathogenic bacteria in poultry: a review. **Journal of Animal Science and Biotechnology**, v. 13, p. 148, 2022. https://doi.org/10.1186/s40104-022-00786-0

OECD. **Adsorption–Desorption using a Batch Equilibrium Method: Technical Guideline 106**. 2000. Available in:http://www.oecd-ilibrary.org/environment/test-no-106-adsorption-desorption-using-a-batch-equilibrium-method_9789264069602-en. Access in March 2012.

OECD. **Estimation of the Adsorption Coefficient ($K_{oc}$) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC): Technical Guideline 121**. 2001. Available in: http://www.oecd-ilibrary.org/environment/test-no-121-estimation-of-the-adsorption-coefficient-koc-on-soil-and-on-sewage-sludge-using-high-performance-liquid-chromatography-hplc_9789264069909-e. Access in March 2012.

PEREIRA, L.A.; JARDIM, I.C.S.F.; FOSTIER, A.H.; RATH, S. Ocorrência, comportamento e impactos ambientais provocados pela presença de antimicrobianos veterinários em solos. **Química Nova**, v. 35, p. 159-169, 2012. https://doi.org/10.1590/S0100-40422012000100028

RATH, S.; FOSTIER, A.H.; PEREIRA, L.A.; DIONISO, A.C.; FERREIRA, F.O.; DORETTO, K.M.; PERUCHI, L.M.; VIERA, A.; OLIVEIRA NETO, O.F.; BOSCO S.M.; MARTÍNEZ-MEJÍA, M.J. Sorption behaviors of antimicrobial and antiparasitic veterinary drugs on subtropical soils. **Chemosphere**, v. 214, p. 111-122, 2019. https://doi.org/10.1016/j.chemosphere.2018.09.083

SILVA JÚNIOR, E.D.; COUTINHO, A.P.; SANTOS NETO, S.M.; RABELO, A.E.C.G.C.; ANTONINO, A.C.D.; LIMA, V.F.; GONDIM, M.V.S. Sorção da sulfadiazina em solo urbano: experimentação e modelagem. **Águas Subterrâneas**, v. 33, p. 368-377, 2019. https://doi.org/10.14295/ras.v33i4.29543

STANDO, K.; KORZENIEWSKA, E.; FELIS, E.; HARNISZ, M.; BUTA-HUBENY, M.; BAJKACZ, S. Determination of antimicrobial agents and their transformation products in an agricultural water-soil system modified with manure. **Scientific Reports**, v. 12, p. 17529, 2022. https://doi.org/10.1038/s41598-022-22440-5

TOLEDO NETTO, P; ARAÚJO, D. S.; LOURENCETTI, C.; TEIXEIRA JÚNIOR, O. J.; RODRIGUES DE MARCHI, M. R. Antimicrobianos veterinários: uso na avicultura e ocorrência em cama de frango. **Revista Uniara**, v.17, n. 2, p. 9-21, 2014. https://doi.org/10.25061/2527-2675/ReBraM/2014.v17i2.21

TOLEDO NETTO, P.; TEIXEIRA JÚNIOR, O.J.; DE CAMARGO, J.L.V.; RIBEIRO, M.L.; DE MARCHI, M.R.R. A rapid, environmentally friendly, and reliable method for pesticide analysis in high-fat samples. **Talanta**, v. 101, p. 322-329, 2012. https://doi.org/10.1016/j.talanta.2012.09.034

USLU, M.O.; YEDILER, A.; BALCIOGLU, I.A.; SCHULTE-HOSTEDE, S. Analysis and sorption behavior of fluoroquinolones in solid matrices. **Water, Air and Soil Pollution**, v. 190, p. 55-63, 2008. https://doi.org/10.1007/s11270-007-9580-0

VAZ JÚNIOR., S. Sorption behavior of the oxytetracycline antibiotic to two Brazilian soils. **Chemical and Biological Technologies in Agriculture**, p. 3-6, 2016. https://doi.org/10.1186/s40538-016-0056-6

ZHAO, L.; DONG, Y.H.; WANG, H. Residues of veterinary antibiotics in manures from feedlot livestock in eight provinces of China. **Science of the Total Environment**, v. 408, p. 1069-1075, 2010. https://doi.org/10.1016/j.scitotenv.2009.11.014

ZHOU, J.; YUN, X.; WANG, J.; L.I, Q.; WANG, Y. A review on the ecotoxicological effect of sulphonamides on aquatic organisms. **Toxicology Reports**, v. 9, p. 534-540, 2022. https://doi.org/10.1016/j.toxrep.2022.03.034.