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Sorption behavior of the oxytetracycline antibiotic to two Brazilian soils

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Abstract

Background: This study comprises the assessment of sorption of the agricultural antibiotic oxytetracycline to Brazilian soils, to prove the sorption to soil and the influence of the organic matter on the sorption capability, noting that tetracyclines are one of the major classes of antibiotics used in the global livestock industry, either for the treatment of diseases or as growth promoter, besides to be applied in agriculture to control bacteria and fungi. The high-performance liquid chromatography with UV detector was used to obtain quantitative data allowing the construction of soil-oxytetracycline sorption isotherms at pH 4.8, in order to confirm the sorption and to identify the influence of the organic matter on the sorption capacity.

Results: The Freundlich isotherm presented himself as a mathematical model suitable for the verification of the sorption by means of the application of chromatographic method, proving the sorption and a homogeneous molecular process of interaction, as well as the effect of organic matter content on the sorption capacity (K_d values up to 9290 kg L⁻¹).

Conclusions: The sorption capacity (K_d) was quantified as high, denoting an achievement of the groundwater by the antibiotic OTC.

Keywords: Environmental chemistry, Soil chemistry, Emerging environmental pollutants

Background

Considering the many sources of pollution to which the environment is exposed due to human activities related to production and usage of several chemicals, studies for fate assessment have become a fundamental tool for determination of the polluting sources, exposure routes, and the potential risk to the environment and human health [1].

As a global trend, the Brazilian environmental legislation is advancing in order to monitor and restrict the presence of several organic and inorganic polluting species in the environment [2–4]. However, there is still a wide variety of chemicals that are not included in such legislation, being able to highlight care products, pharmaceuticals, and veterinary, a broad family of emerging pollutants, including antibiotics [5, 6]. Antibiotics are

compounds that effectively inhibit the bacteria growth, and are commonly produced by microorganisms present in the environment, such as fungi [7]. The estimated global production of antibiotics is on the order of 200,000 tons per year, and half that amount used for use in animals, and the tetracyclines (Fig. 1) ranks among the most used classes of antibiotic [8]. Furthermore, the global consumption of antibiotics in food animal production was estimated at 63,151 tons in 2010 and is projected to rise by 67 %, to 105,596 tons, by 2030 [9].

The main representatives of the tetracyclines class are classified in the following descending order of veterinary use: oxytetracycline (OTC) > tetracycline > chlortetracycline [10]. The use of tetracycline antibiotics as growth promoter (GP) and to treat bacteria from the genus *Salmonella* in poultry is widespread in Brazil [11], and it is one of the major classes of antibiotics marketed and applied to the Brazilian livestock [12].

One concern in the use of veterinary tetracyclic antibiotics is that a large fraction of these, between 20 and 90 %, is not absorbed by the animal organism and is

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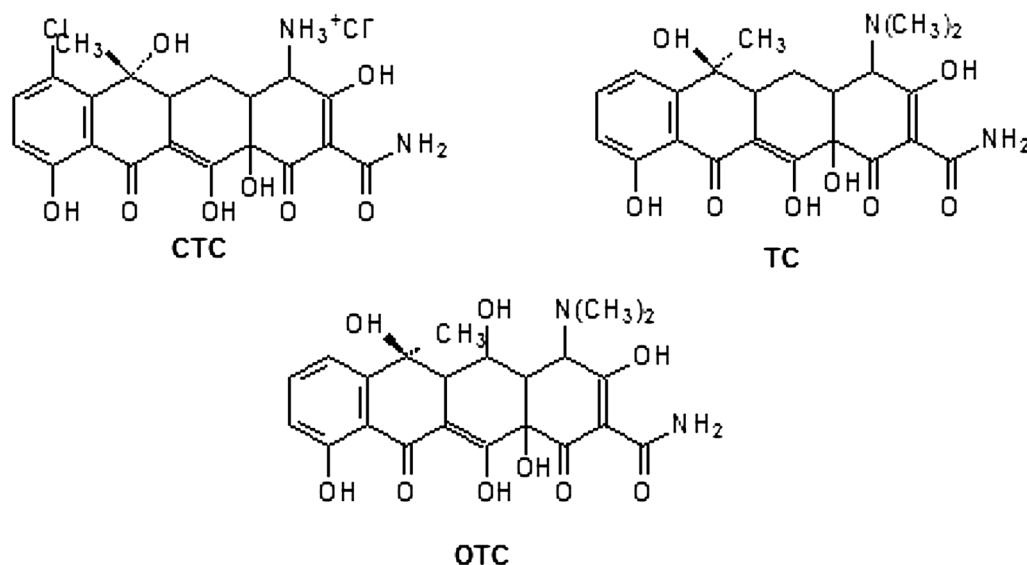


Fig. 1 Molecular structures of the tetracycline antibiotics. *CTC* chlortetracycline, *TC* tetracycline, *OTC* oxytetracycline

excreted unchanged in the urine and feces [13]. It can promote resistance to these antibiotics and, depending on its transport in the soil, reached the groundwater and confer resistance to the microorganisms in this water. If humans and animals will consume this water, microorganisms present inside them may transmit genes of resistance to the antibiotic to humans and animals [14, 15]. The application of animal manure in farmland stands out as a source of soil pollution by antibiotics [16], with the evidence of the presence of residues in soil resulting from this type of agricultural application [17, 18]. Besides this issue of environmental and public health concerning, the fact that the new generation of GP is not absorbed from the intestinal tract to avoid residues in meat [19] may increase the presence of these compounds in environmental matrices, as soil and water.

The soil organic matter (SOM) originates from decomposition of vegetable and animal biomass by means of chemical, biological and physical processes, with structural modifications giving rise to a number of organic compounds whose main representatives are humic substances (HS)—humic and fulvic acids and humin [20]. HS interact with various organic pollutants such as pesticides and oil, and inorganic such as heavy metals [21]. A recent study on the interaction between humic acid extracted from Brazilian soil and the OTC pointed to the existence of different physicochemical mechanisms of interaction that depends on pH values [22], which most probably should be repeated for organic matter (OM) present in the soil.

The study reported here aimed to verify, through the application of HPLC-UV analytical technique, the occurrence of sorption between the OTC and SOM molecules, followed by the construction of sorption isotherms—Freundlich isotherm. It was also of particular interest to evaluate the effect of OM content on the sorption. It is important to highlight the inexistence of studies on this theme for Brazil and tropical soils.

Materials

M1 soil, a red-yellow dystrophic latosol, was collected in a tropical forest in the São Paulo State (21040'4" S, 47050'33" W); T1 soil, an organosol (peat), was collected in a farm field, also in the São Paulo State (21033'20" S, 47055'08" W). Both samples were collected at depth from 0 to 15 cm and used in soil-OTC sorption experiment. Soil samples were dried at room temperature for 7 days. After this period, samples were homogenized in 2 mm sieve and sent to the laboratory for analyses and sorption experiments. Such soils were analyzed according to Embrapa routine procedures for soil analysis [23].

Methods

The soil-OTC sorption experiment was carried out in duplicate using the M1 and T1 soils as adaptation of the procedure developed by Jones et al. [24]. As the charge and speciation of OTC and soil are dependent on pH values, it chose a medium with a pH value of 4.8, close to the maximum mass fraction of the OTC zwitterion [25] and to minimize possible interference of H⁺ and other electronic charges on the sorption process.

A solution of OTC (oxytetracycline hydrochloride, HPLC grade ≥ 95 % purity, Sigma-Aldrich) was previously prepared at 240 mg L^{-1} (stock-solution) in a buffer solution of sodium acetate (Synth)/acetic acid (Synth) at 0.1 mol L^{-1} in ultrapure water (Milli-Q). The prepared working concentrations from the stock-solution were 120, 60, 30, 20, 10, and 5 mg L^{-1} , with a control solution of 0 mg L^{-1} of OTC (buffer solution). A 10 mL aliquot of each solution was transferred to amber glass flask and, immediately, added to the soil mass to a final concentration of 5 g L^{-1} of soil at OTC solution. The mixtures were shaken for 48 h, protected from light and at room temperature ($25 \text{ }^\circ\text{C}$), and after this period followed for chromatographic analysis, the results were used in the construction of the sorption isotherms.

High-performance liquid chromatography analyses were carried out in a chromatograph Prostar Varian with a UV detector, with a column for reverse mode of polystyrene-divinylbenzene with 15 cm length, 4.6 mm internal diameter, and $5 \text{ }\mu\text{m}$ particle size (Phenomenex), according to the conditions adapted from Loke et al. [26]: isocratic mobile phase (v/v) of 26 % of acetonitrile (JT Backer)/74 % of aqueous solution of trifluoroacetic acid (Mallinckrodt) at 0.05 % (v/v), with previous ultrasound treatment by 15 min; column temperature of $30 \text{ }^\circ\text{C}$; flow of mobile phase of 1 mL min^{-1} ; and wavelength of UV detector at 355 nm. Acetonitrile of HPLC grade (JT Baker) and trifluoroacetic acid (Mallinckrodt) were used for the mobile phase, with a minimum purity grade of 99 %. An external calibration curve was constructed for OTC whose points were 5, 10, 20, 30, 60, 120, and 240 mg L^{-1} . From this curve was determined the limit of quantification (LOQ) of the method, according to the IUPAC recommendations [27].

The determination of the partition coefficient (K_d) was done by means of Eq. 1 [28], followed by Eqs. 1 and 3, which enables an estimative of the sorption intensity of xenobiotics on soil:

$$K_d = C_s/C_e \left(\text{L g}^{-1} \right), \quad (1)$$

where C_s is the OTC concentration adsorbed to the SOM (mmol g^{-1}) and C_e is the OTC concentration in the aqueous phase at equilibrium condition (mmol L^{-1}). K_d was chosen expressed as L kg^{-1} , instead of L g^{-1} .

The Freundlich isotherm applied to heterogeneous surfaces is defined by

$$S = K_f C_e^N, \quad (2)$$

where S is the concentration of solute sorbed (mg g^{-1}), K_f is the sorption coefficient ($\text{mg}^{1-N} \text{ L g}^N$), C is the concentration of solute in equilibrium in aqueous solution (mg L^{-1}), and N is the linearity parameter. In a

simple case, $N = 1$ and K_f is equivalent to K_d for linear isotherms. The logarithmic form of the equation is used for the linear fit of the Freundlich isotherm:

$$\text{Log } S = \text{log } K_f + N \text{ log } C_e. \quad (3)$$

Results and discussion

Table 1 shows the results of the compositional analysis and physicochemical characterization of the soil samples. The data presented demonstrated that the T1 sample had a larger content of OM and metal, which resulted in higher cationic exchange capacity (CEC) at high pH values, respectively, when compared to the sample M1.

Isotherms were built to verify the influence of soil type on the sorption of OTC. However, it is worth mentioning that the external calibration curve of the analytical method showed a satisfactory value for linear correlation coefficient ($R = 0.999$) [29]. Then, was obtained a LOQ of 4.0 mg L^{-1} . Nevertheless, were obtained negative values for C_e for the initial concentrations of OTC of 10.0 and 5.0 mg L^{-1} , which demonstrated that the LOQ was inefficient for two concentrations, most likely due to the decreasing in the method sensitivity [29].

Freundlich, Langmuir, and partition isotherms were tested (not shown), with the Freundlich isotherm being the more suitable mathematical model, as shown in Fig. 2.

It should be noted that the N value of the Freundlich isotherm allows infer the shape of the isotherm and the sorption mechanism, where $N = 1$ represents an linear isotherm C, or partition, $N < 1$ the isotherm L, and $N > 1$

Table 1 Compositional analysis and physicochemical characterization of the soil samples T1 and M1

| Parameter | Unity | Soil sample | |
|-------------------------------|-----------------------|-------------|------|
| | | M1 | T1 |
| pH in CaCl_2 | – | 4.0 | 6.3 |
| Al^{3+} | mmol L^{-1} | 8 | 0 |
| $\text{H}^+ + \text{Al}^{3+}$ | mmol L^{-1} | 72 | 15 |
| K^+ | mmol L^{-1} | 0.5 | 6.3 |
| Ca^{2+} | mmol L^{-1} | 4 | 80 |
| Mg^{2+} | mmol L^{-1} | 2 | 42 |
| CEC (total) | mmol kg^{-1} | 79 | 143 |
| OM | g kg^{-1} | 26 | 119 |
| Sum of bases | mmol L^{-1} | 7 | 128 |
| Saturation of bases (%) | – | 8 | 90 |
| Granulometry | – | | |
| Sand (%) | – | 4 | 19.2 |
| Silt (%) | – | 78 | 19.2 |
| Clay (%) | – | 18 | 61.6 |

CEC cationic exchange capacity, OM organic matter

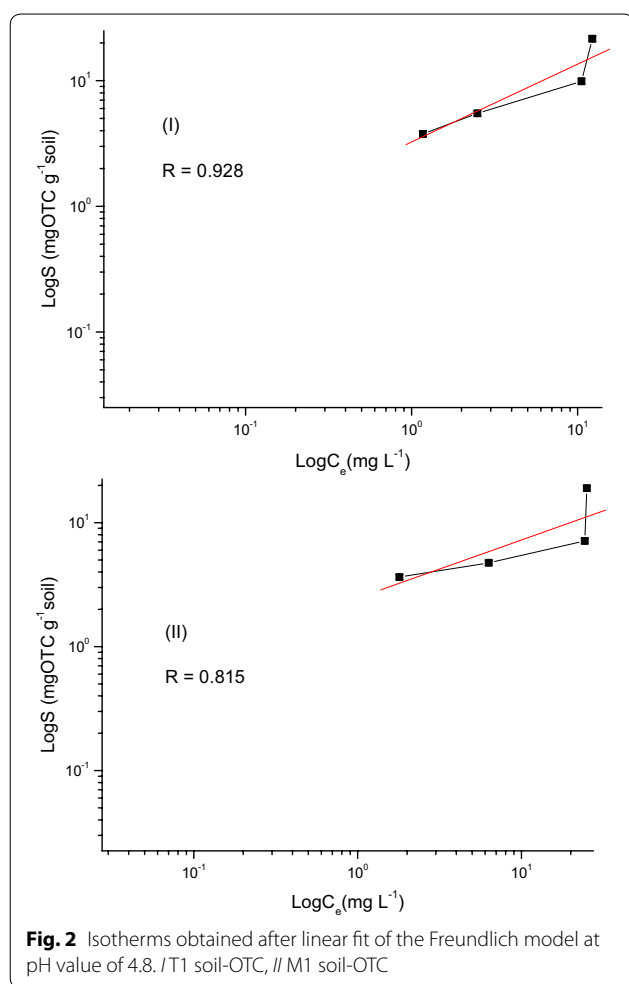


Fig. 2 Isotherms obtained after linear fit of the Freundlich model at pH value of 4.8. // T1 soil-OTC, // M1 soil-OTC

the isotherm S [30]. The higher the value of N , more heterogeneous is the sorption process [31].

From the linear fit—Eq. 3—of the curves obtained, it was found that the Freundlich isotherm, with the logarithmic linear fit done by the use of the software Origin[®], was the best model. This isotherm is commonly used for the experimental representation of the sorption of organic compounds, as veterinary drugs, on heterogeneous surfaces (e.g., soil and OM) [31, 32]. Table 2 lists the fit parameters.

The values obtained for the K_f from Eq. 2 showed that soil with higher content of OM (T1) showed the highest

sorption capacity—a L-type isotherm—which increases with increasing concentration of OTC in the aqueous medium [33]. K_f values obtained were in accordance with those from literature for OTC sorption to temperate soils [24], demonstrating that this sorption was weak, probably due to a lower OM content. However, Kong et al. [34] also observed the need to calculate the K_d partition coefficient for sorption and desorption of OTC to soils. Thus, K_d was calculated for both soils according to Eq. 1 described above, with values shown in Table 3.

Figure 3 shows the graphic profile with the K_d for soil sample.

The behavior observed in Fig. 3 can be interpreted from the physicochemical characteristics of each soil. Soil texture (particle size), CEC, and OM content are the main factors influencing the OTC sorption to soil [24, 34]. Regarding the OM, probably its influence is limited to its content allied to the physicochemical particularities from its molecular composition, as considered by Schwarzenbach et al. [35].

To correlate the sorption profiles presented in Fig. 2 with the compositional analysis and physicochemical characterization in Table 1, it can be observed that the soil with higher content of OM and higher CEC (T1) showed the higher K_d value (9290 kg L⁻¹) in the presence of the higher initial concentration of OTC (120 mg L⁻¹), probably due to the fact that the higher OTC concentration had a larger amount of sites available for intermolecular interactions. Another factor also considered for the largest value of K_d for T1 is the greater presence of metallic ions, that leads to an increasing in the interaction between OTC and OM, since OTC is a strong chelating agent for metallic cations at pH about 5 [26, 36].

However, according to the values established by the Brazilian environmental legislation to evaluate the mobility capacity of chemicals in soils [37], K_d values ranging from 0 to 24 kg L⁻¹ feature a low sorption capacity to transport the OTC molecule from soil to groundwater by means of leaching process. The K_d values in Table 3 were different from those observed in the literature for soils in other regions of the world [38], especially for the soil T1. However, these values expressed the behavior of OTC sorption to Brazilian soils.

Obeying the Freundlich isotherm, the sorption of OTC to soils occurred by means of the formation of multilayers

Table 2 Values calculated for the Freundlich isotherm following linear equation fit in its logarithmic form

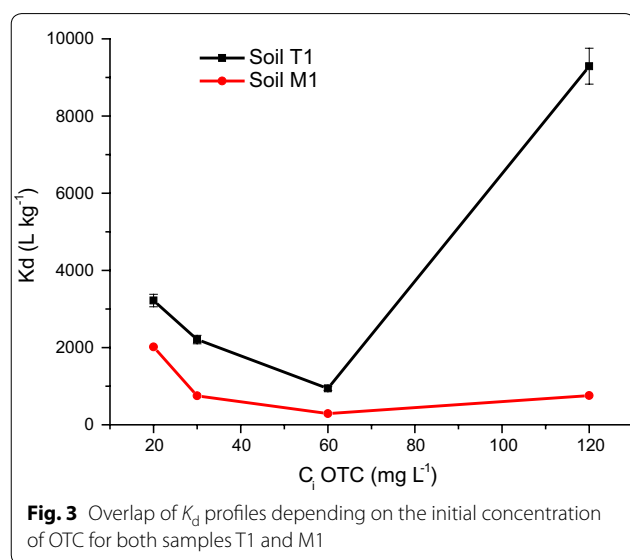
| Freundlich isotherm equation | K_f (mg ¹⁻ⁿ L g ⁿ) (mean ± standard error) | N (mean ± standard error) | Isotherm type | R | Soil sample |
|---|---|-----------------------------|---------------|-------|-------------|
| $\text{Log}S = 0.51 + 0.62\text{log} C_e$ | 3.24 ± 0.09 | 0.62 ± 0.03 | L | 0.928 | T1 |
| $\text{Log}S = 0.39 + 0.47\text{log} C_e$ | 2.45 ± 0.06 | 0.47 ± 0.01 | L | 0.815 | M1 |

K_f , sorption coefficient, N linearity parameter, $\text{Log}S$ logarithmic form of the concentration of solute sorbed, $\text{Log}C_e$ logarithmic form of the concentration of solute in equilibrium in aqueous solution

Table 3 Determined K_d values for the OTC sorption to the T1 and M1 soils

| C_i OTC (mg L^{-1}) | K_d (L kg^{-1}) (mean \pm standard error) | Soil sample |
|----------------------------------|--|-------------|
| 120.0 | 9290 \pm 108 | T1 |
| | 760 \pm 45 | M1 |
| 60.0 | 940 \pm 17 | T1 |
| | 290 \pm 32 | M1 |
| 30.0 | 2210 \pm 74 | T1 |
| | 750 \pm 28 | M1 |
| 20.0 | 3220 \pm 86 | T1 |
| | 2020 \pm 39 | M1 |

C_i initial concentration, OTC oxytetracycline, K_d partition coefficient



[39], from the sorbed species (OTC) on the sorbent surface (soils).

Conclusions

There was a weak sorption of OTC to Brazilian soils, where it was dependent on the OM content, presenting a potential risk to the environment arising from their weak sorption capacity to such soils, making it available to transport the antibiotic to the groundwater. OTC adsorption to soil obeyed Freundlich isotherm (L-type), for a multilayer OTC deposition on the soil surface. From such findings, it is reasonable to assume that environmental agencies and legislative bodies should provide more attention to the presence of antibiotics in environmental matrices, which leads to the need for further studies related to this subject to produce data enabling stipulate the maximum values allowed for the presence in soil.

Abbreviations

CEC: cationic exchange capacity; GP: growth promoter; HPLC: high-performance liquid chromatography; HS: humic substances; IUPAC: International Union of Pure and Applied Chemistry; LOQ: limit of quantification; OM: organic matter; OTC: oxytetracycline; K_d : partition coefficient; K_f : sorption coefficient; SOM: soil organic matter; UV: ultraviolet.

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Competing interests

The author declares that he has no competing interests.

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