Adsorption/desorption of three tetracycline antibiotics on different soils in binary competitive systems

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Abstract

Taking into account environmental and public health issues due to emerging pollutants, and specifically to antibiotics spread into environmental compartments, this work focused on the competition among three tetracycline antibiotics (tetracycline, CT; oxytetracycline, OTC; and chlortetracycline, CTC) for adsorption sites in six different soils. Batch-type desorption/desorption tests were carried out, with 24 h as contact time. The six soils were from two different farming areas, and were selected according to pH value and organic matter content. Binary systems (pairs of antibiotics present simultaneously) were used to study competition, setting the dose of one antibiotic at 200 µmol L^{-1}, and varying the concentration of another from 50 to 600 µmol L^{-1}. In the case of the concentration of 200 µmol L^{-1}, the results of the binary systems were also compared with those obtained in simple and ternary systems. The results showed that those soils with the highest organic matter content (soils 50AL and 71S) adsorbed 100% of the three antibiotics, with desorption being <10% in all cases. The other four soils showed some degree of competition for adsorption sites in binary systems, with adsorption decreasing between 25 and 47% compared to simple systems, and with desorption increasing, especially in soils with higher pH and less organic matter. This competition was even more pronounced in ternary systems, affecting to these same soils, while the effects were very scarce in soils with greater organic matter content. The results indicate that most of the studied soils have high adsorption capacity for tetracycline antibiotics, retaining them with high energy even in multiple systems. It was also shown that hysteresis affected adsorption/desorption processes. These results have environmental and social relevance, given the growing concern about antibiotics pollution, and the need of promoting their retention and inactivation when spread in the environment.

Keywords: competitive adsorption; chlortetracycline; desorption; oxytetracycline; tetracycline

1 Introduction

Tetracycline antibiotics include molecules such as chlortetracycline (CTC), oxytetracycline (OTC), and tetracycline (TC), active against a wide range of microorganisms. These antibiotics are widely used in livestock, as therapeutic, as prophylactic, and (in certain countries) even as feed additives for intensive production systems (Charuaud et al., 2019). These molecules contain a central octahydronaphthacene core, and a wide variety of functional groups that bind to the four rings of the structure. Between 80 and 90% of the administered dose of these antibiotics are excreted via feces, causing that their presence is common in manures, slurries, and soils (Cycoń et al., 2019). In fact, concentrations of 0.9, 4.0 and 35.0 mg kg^{-1} were found for CT, CTC and OTC, respectively, in slurry, and up to 0.6 and 0.2 mg kg^{-1} for CT and CTC, respectively, in agricultural soils in Galicia (NW Spain) (Conde-Cid et al., 2018).

When antibiotics reach soils, they can remain in it for a time, enter surface waters or accumulate in plant tissues, and consequently enter the food chain. In addition, different studies have been warning about the emergence of bacterial resistance to antibiotics, with adverse and chronic effects on human and animal health (Sun et al., 2010; Huijbers et al., 2019). It has been indicated that the number of victims attributable to antimicrobial resistance has exceeded 700,000 since 2014, and that by 2050 it would reach 10 million (Scarafile, 2016). Antibiotics spread into environmental compartments are considered emerging pollutants, which can reach waters, wastewaters, soils, plants and animals, causing deleterious effects. In view of that, their behavior, eventual retention and mobility in soils, adsorbents and water should be investigated.

Antibiotics adsorption/desorption onto soils are some of the main processes conditioning the dynamics and evolution of these molecules in the environment, affecting its mobility, passage to waters, plant uptake, and even its biotransformation or microbial degradation. Adsorption mechanisms are conditioned by soil characteristics (pH, organic matter, non-crystalline compounds, etc.) and by the physico-chemical properties and structure of the antibiotics (Kemper, 2008). Thus, even within the same group of antibiotics, adsorption processes may vary for different antibiotics. Authors such as Graouer-Bacart et al. (2015) and Zhou et al. (2019) pointed out electrostatic forces, cation-π interactions, cationic bridges and surface complexation as main mechanisms for adsorption of antibiotics to soils. Previous studies reported on the existence of a primary step in adsorption based on a reversible equilibrium process, for which any subsequent release would be slow, which coincides with the frequent presence of hysteresis in the adsorption of antibiotics, including those into the group of tetracyclines (Gu et al., 2007; Fernández-Calviño et al., 2015a).

Up to now, most of the studies on adsorption/desorption of antibiotics were carried out in simple systems, while results corresponding to multiple systems (more than one antibiotic present simultaneously) are scarce. Within the latter, Fernández-Calviño et al. (2015b) conducted a study on competitive adsorption of tetracycline antibiotics in two soils from Galicia (NW Spain), using a binary system (two antibiotics present simultaneously). These authors detected the existence of strong competition between antibiotics, and pointed out differences in the adsorption trends for each antibiotic, depending on whether these molecules were in a competitive or individual system. These authors attributed it to eventual changes in characteristics of the antibiotics when compete for adsorption sites, which would affect retention processes. Recently, Conde-Cid et al. (2019) went a step ahead studying competitive adsorption/desorption for CT, OTC and CTC in six agricultural soils in Galicia (NW Spain), in ternary systems (with the three antibiotics present simultaneously), thus providing further information on the retention of these antibiotics in these specific experimental situation. However, much more work is needed, specially taking into account that the simultaneous presence of different antibiotics in environment compartments is frequent, making necessary to expand the variety of experiments in this regard. It would allow to increase the degree of knowledge on mechanisms of interaction, synergies and competition among antibiotics in these complex media, in different circumstances. In this way, experiments using binary systems would complement those previously performed in simple and ternary systems.

In view of that background, the objective of this work is to carry out binary experiments on competitive adsorption/desorption of the tetracycline antibiotics TC, OTC and CTC onto six different soils, also comparing with simple and ternary systems, aiding to elucidate characteristics of the processes and mechanisms involved. The results of this study could be relevant and useful in view of the environmental implications of these antibiotics when reaching soils, and their subsequent evolution in different compartments, as well as an aid to program correct management practices for soils exposed to this kind of pollution.

2 Materials and methods

2.1 Study area, soil sampling and characterization

The soil samples used in this work were previously characterized by Conde-Cid et al. (2018). Details on soil sampling, soil parameters determined, methods and values are included in Supplementary Material. Briefly, six soil samples were taken in two agricultural areas with intensive farming activities: three from A Limia (Ourense province, Galicia, Spain) (AL samples), and three from Sarria (Lugo province, Galicia, Spain) (S samples). These six soils were selected from a set of 65 previously described by Conde-Cid et al. (2018). AL soils had pH values from 4.5 to 4.8, while S soils had pH from 6.2 to 7.0. Three of these soils had organic carbon content <2%, while it was between 3.4-10.9% for the other three. In addition, these six soils had differences regarding other relevant characteristics, such as non-crystalline Fe and Al contents (Table S1, Supplementary Material).

2.2 Adsorption and desorption experiments

In a previous work, Conde-Cid et al. (2019) studied the competitive adsorption/desorption of the tetracycline antibiotics TC, CTC and OTC in ternary systems (with the three antibiotics present simultaneously), working with six soils also used in the present study. To complement that previous work and go a step ahead, in the present study binary tests were carried out (with pairs of these three antibiotics added simultaneously), using the same 6 soils. The binary experiments were performed adding in each case a constant concentration of one of the antibiotics (200 µmol L^{-1}), and then adding a second antibiotic in increasing concentrations (0, 50, 100, 200, 400, and 600 µmol L^{-1}), resulting in a total of 6 different binary combinations for each soil.

Adsorption tests were carried out by means of batch-type experiments, stirring for 24 h 1 g of soil with 40 mL of a 0.005 M $CaCl_2$ solution containing the corresponding concentration of antibiotic in each case. Previous kinetic studies guaranteed that the 24-hour contact time was sufficient to achieve the equilibrium (Fernández-Calviño, 2015a, b; Conde-Cid, 2019). Subsequently, the samples were centrifuged at 4000 rpm (6167 *x g*) for 15 minutes. Next, the concentration of each of the antibiotics remaining in the equilibrium solution was determined (see details below). The amount of antibiotic adsorbed was calculated by the difference between the concentration added and that remaining in the equilibrium solution.

Desorption of each antibiotic in each soil was determined by adding 40 mL of 0.005 M $CaCl_2$ to each of the samples resulting from the previous adsorption test, then following a procedure analogous to that indicated above, stirring samples for 24 hours and centrifuging at 4000 rpm (6167 *x g*) for 15 minutes, and finally quantifying antibiotics present in the solution.

2.3 Quantification of tetracycline antibiotics

The procedure described by López-Peñalver et al. (2010) and by Fernández-Calviño et al. (2015a, b) was used to quantify tetracyclines (TCs), with just slight modification. Details on the whole procedure are included in Supplementary Material.

2.4 Adsorption models and statistical treatment

The Freundlich model can be adapted for binary competitive systems, as previously shown for metals by Arias et al. (2006). In the current research, a first step could be to calculate the total amount adsorbed for each pair of antibiotics in each binary system (Eq. 1):

$$Q_{ad(1)} + Q_{ad(2)} = K_F(Q_{eq(1)} + Q_{eq(2)})^{1/n} \qquad \text{(Eq. 1)}$$

where Q_{ad} is the amount adsorbed for a specific antibiotic (antibiotic 1 or antibiotic 2, among TC, CTC or OTC) (µmol kg^{-1}), C_{eq} is the concentration of antibiotic 1 or 2 in the equilibrium solution (µmol L^{-1}); K_F and n are parameters of the Freundlich equation (L^n µmol^{1-n} kg^{-1}, and dimensionless, respectively).

The model of Murali and Aylmore (1983) can be used to study competition between tetracycline antibiotics in binary systems (Eq. 2):

$$Q_{ad(1)} = \frac{K_{F(1)} \times C_{eq(1)}^{n_{(1)}+1}}{C_{eq(1)} + a_{(1)} \times (C_{eq(1)} + C_{eq(2)})} \qquad \text{(Eq. 2)}$$

where a is a parameter related to competition between tetracycline antibiotics (by pairs, in binary systems). It must be noted that, a being situated in the denominator of the equation, higher a values result in lower Q_{ad} scores, and thus lower adsorption of the antibiotics.

The amounts of antibiotics desorbed were expressed as concentration (µmol kg^{-1}), and as the percentage released referred to the amount previously adsorbed.

The tools used to perform the fitting of experimental data to adsorption models were the statistical software R version 3.1.3 and the *nlstools* package for R (Baty et al., 2015). In addition, the SPSS 21 software was used to carry out bivariate Pearson correlations between adsorption and desorption data and soil variables, as well as multiple linear regression analysis, and analysis of variance (ANOVA), with Tukey test.

3 Results and discussion

3.1 Tetracycline antibiotics adsorption in binary systems

Adsorption of the three tetracycline antibiotics was studied for each of the six soils in any of the possible binary combinations (TC + (OTC or CTC); OTC + (TC or CTC); and CTC + (TC or OTC)). Fig. 1 shows adsorption percentages for each of the three antibiotics and for each soil. Binary combinations resulted from adding a constant concentration of 200 µmol L^{-1} of one antibiotic, and increasing concentrations (from 0 to 600 µmol L^{-1}) of one of the other two antibiotics, separately. It is shown that the effects of presence of OTC or CTC on the adsorption of CT are very similar. In both cases, the soils with the highest contents in organic matter (50AL and 71S) are those showing the highest adsorption capacity for CT (close to 100%). In general, CT adsorption shows a downward trend as a function of increasing concentrations of OTC or CTC added, with more pronounced decrease in the four soils having lower organic matter content (19AL, 3AL, 6S, 51S).

The decrease in CT adsorption was slightly higher in the presence of CTC than when OTC is present, although the differences depend on the type of soil. In fact, when organic matter is abundant (soils 50AL and 71S), the effects of the presence of CTC or OTC are quite similar. However, when the organic matter content is scarce (mainly in soil 19AL), the difference in the adsorption of CT is clear, reaching 47% comparing adsorption in the absence of CTC with that in the presence of 600 µmol L^{-1} of CTC is, while the difference is clearly lower when OTC is present (specifically, 15%).

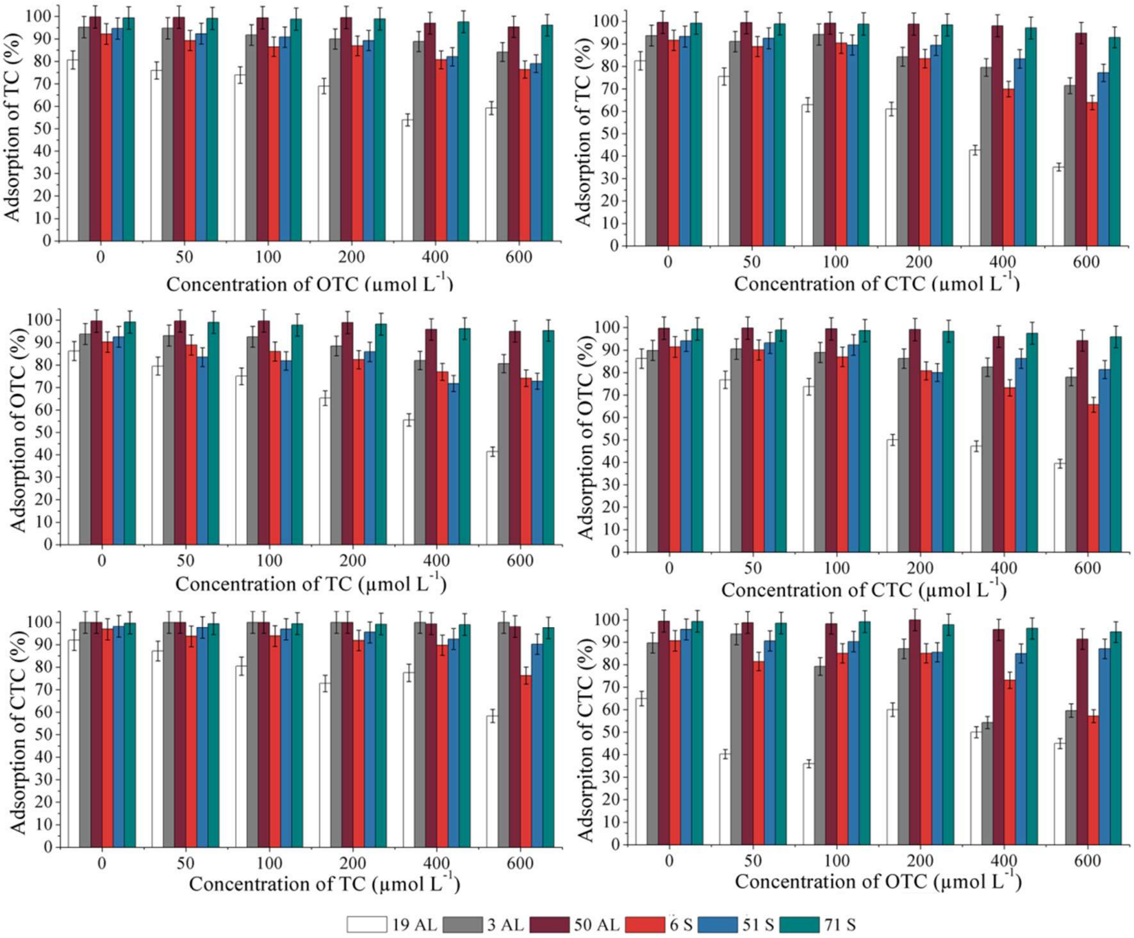

Fig. 1. Percentage of adsorption for the three tetracycline antibiotics (TC, OTC, and CTC) in binary systems, on soils of A Limia (19AL, 3AL and 50AL) and Sarria (6S, 51S and 71S), when the concentration added is kept constant (200 µmol L^{-1}) for the antibiotic indicated on the Y axis of the graphs, and is increased from 0 to 600 µmol L^{-1} for the second antibiotic (as indicated on the X axis). Average values (n =3), with error bars showing that coeficientes of variation were always <5%

Regarding the adsorption of OTC in the presence of the other two antibiotics (Fig. 1), a similar trend was found. Specifically, soils with higher organic matter content (50AL and 71S) adsorbed OTC as much as 90-100%, regardless of the dose of CT or CTC added. In the other four soils, OTC adsorption decreased as the concentration of CT or CTC added increased, with very similar effects due to both antibiotics in each soil. As a result, soil 3AL always showed the smallest decrease in adsorption (12-13%), while the highest decrease (46-47%) corresponded to soil 19AL. The soil with the lowest organic matter content (19AL) was the one showing more marked decrease in OTC adsorption as a function of increasing doses of CT or CTC added. In the binary systems CTC + (TC or OTC), those soils with the highest organic matter content (50AL and 71S) adsorbed close to 100% of CTC in all cases. However, for the other four soils the behavior varied. Specifically, regarding the effects due to the presence of CT, soil 3AL adsorbed close to 100% of CTC in all cases, while for the other soils CTC adsorption decreased as a function of the dose of CT added. The highest decrease (30%) corresponded to the soil with less organic matter (19AL), while adsorption decreased 20% and 8% in soils 6S and 51S, respectively. In the presence of OTC, the decrease in CTC adsorption was generally higher. The adsorption results in these binary systems indicate that soils with high organic matter content have sufficient adsorption sites to adsorb the antibiotics added, both in simple and binary systems, and regardless of the concentration of antibiotic. In this situation the competition among antibiotics would be minimal. However, in soils with lower organic matter content, the decrease in the adsorption of antibiotics would reveal the appearance of certain effects due to the competition for adsorbent surfaces, especially when high doses are added.

Bansal et al. (2013), studying CT, OTC and CTC adsorption in simple systems, found a higher affinity of CTC for adsorption sites. Similarly, in the present study, the highest effect on CT adsorption was due to CTC. In addition, a pronounced competitive effect was detected for CTC adsorption in the presence of OTC, with decreased adsorption even in soils with high organic matter content (50AL and 71S).

As noted by Kemper (2008), antibiotics adsorption/desorption on/from soils and are regulated by complex mechanisms, dependent on both physico-chemical properties of the antibiotics (structure, solubility, hydrophobicity, etc.) and soil characteristics (pH, organic matter and Al and Fe oxides contents, texture, etc.). Gu et al. (2007) have considered pH as a key factor in the adsorption of antibiotics, due to its influence on antibiotic-soil interactions. Specifically, pH has marked effects on chemical speciation of antibiotics and on the electric charge of soil components (Figueroa-Diva et al., 2010). In this regard, it is of relevance that tetracycline antibiotics have several ionizable functional groups (such as hydroxyl, ketone and amino) that undergo protonation-deprotonation reactions, giving different ionic species depending on the pH of the solution, which can result in positive, neutral or negative charges (Sun et al., 2010). However, CT, OTC and CTC have very similar values for pK_{a1}, pK_{a2} and pK_{a3} (Hamscher et al., 2005; López-Peñalver et al., 2010), suggesting that the adsorption of the three antibiotics could be similar, thus promoting competition for the same adsorption sites. This fact would facilitate the saturation of adsorbent surfaces at high doses of antibiotics added, especially in soils with low organic matter content. Other authors have found that the higher affinity of CTC for reactive soil surfaces might be related to the ionization of some functional groups (specifically, dimethylamine and phenolic b-diketone), structural aspects that have been considered of importance regarding adsorption of tetracycline antibiotics (Pils and Laird, 2007; Chang et al., 2009a, b).

3.2 Adjustment of experimental results to adsorption models

Table 1 shows the adjustment of adsorption results to the Freundlich model modified for binary systems according to Eq. 1, considering the six possible combinations of the three antibiotics in binary systems. In some cases, error values were too high to allow fitting, but in most cases the adjustment was appropriate, and R^2 was higher than 0.95. Regarding the K_F parameter (related to the adsorption capacity of a certain adsorbent -Bhaumik et al., 2012), the highest values corresponded to those soils with higher organic matter content (50AL and 71S), coinciding with what was observed in laboratory tests (Table 1). The CTC-fixed + TC-variable combination showed the highest values for this parameter in 4 of the 6 soils, coinciding with that observed in Fig. 1, while low K_F values were obtained in binary systems in which CTC concentration was variable (Table 1).

Table 1. Values of the Freundlich parameters corresponding to the competitive binary experiments

Soil	Antibiotics	------------------- Freundlich parameter --------------------				
		K_F	Error	n	Error	R^2
3AL	TC (Fix) + OTC	2277.04	694.24	0.50	0.07	0.967
	TC (Fix) + CTC	3035.57	635.40	0.42	0.04	0.981
	OTC (Fix) + TC	3163.97	380.50	0.40	0.03	0.993
	OTC (Fix) + CTC	1293.69	287.44	0.63	0.05	0.989
	CTC (Fix) + TC	-	-	-	-	-
	CTC (Fix) + OTC	2906.30	776.27	0.23	0.06	0.892
19AL	TC (Fix) + OTC	-	-	-	-	-
	TC (Fix) + CTC	1661.19	506.22	0.36	0.05	0.961
	OTC (Fix) + TC	3436.76	513.63	0.22	0.03	0.971
	OTC (Fix) + CTC	2102.69	175.56	0.34	0.02	0.996
	CTC (Fix) + TC	2255.74	327.73	0.31	0.03	0.987
	CTC (Fix) + OTC	-	-	-	-	-
50AL	TC (Fix) + OTC	11321.05	330.76	0.28	0.01	0.998
	TC (Fix) + CTC	10640.16	872.55	0.36	0.03	0.988
	OTC (Fix) + TC	10525.49	692.93	0.28	0.02	0.991
	OTC (Fix) + CTC	10954.45	656.52	0.31	0.02	0.993
	CTC (Fix) + TC	12213.92	1754.88	0.25	0.05	0.951
	CTC (Fix) + OTC	5361.35	2602.18	0.33	0.15	0.597
6S	TC (Fix) + OTC	2238.15	344.06	0.44	0.03	0.990
	TC (Fix) + CTC	2687.16	595.67	0.42	0.05	0.980
	OTC (Fix) + TC	1782.20	150.00	0.47	0.02	0.998
	OTC (Fix) + CTC	2062.12	190.99	0.47	0.02	0.997
	CTC (Fix) + TC	3350.36	544.76	0.34	0.03	0.983
	CTC (Fix) + OTC	4127.85	1001.03	0.10	0.05	0.698
51S	TC (Fix) + OTC	2359.08	408.06	0.47	0.04	0.989
	TC (Fix) + CTC	992.16	123.92	0.76	0.03	0.998
	OTC (Fix) + TC	1899.09	499.38	0.45	0.05	0.976
	OTC (Fix) + CTC	1512.03	226.68	0.67	0.04	0.995
	CTC (Fix) + TC	4018.21	174.52	0.33	0.01	0.999
	CTC (Fix) + OTC	2076.50	598.92	0.36	0.06	0.946
71S	TC (Fix) + OTC	7366.16	440.90	0.44	0.02	0.996
	TC (Fix) + CTC	7480.65	546.79	0.45	0.02	0.995
	OTC (Fix) + TC	6574.01	574.32	0.41	0.03	0.993
	OTC (Fix) + CTC	7209.66	565.71	0.50	0.03	0.994
	CTC (Fix) + TC	7959.06	710.94	0.39	0.03	0.988
	CTC (Fix) + OTC	5356.61	568.34	0.34	0.04	0.981

-: Error values too high for fitting

An analysis of variance showed that average K_F values were higher in binary systems in which CTC concentration was fixed, while the lowest K_F values corresponded to binary systems where CTC concentration was variable (Table 2), indicative of the higher adsorption of CTC onto the studied soils.

Table 2. Comparison of average values of Freundlich parameters obtained in binary systems for the soils under study. Different letters indicate significant differences ($p<0.05$) in the Tukey test

Antibiotics	K_F	n
TC (Fix) + OTC (Var)	4649.25^{a}	0.410^{a}
TC (Fix) + CTC (Var)	4249.48^{a}	0.460^{a}
OTC (Fix) + TC (Var)	4563.58^{a}	0.370^{ab}
OTC (Fix) + CTC (Var)	4189.10^{a}	0.480^{a}
CTC (Fix) + TC (Var)	5959.45^{a}	0.320^{ab}
CTC (Fix) + OTC (Var)	9391.98^{a}	0.130^{b}
Minimum significant difference	10732.93	0.263
Error	2461.98	0.060

The Freundlich's n parameter indicates the reactivity and heterogeneity of the active sites of the adsorbents. When $n = 1$ the adsorption is linear, while when $n > 1$ the adsorption process is chemical in nature, and when $n < 1$ there are heterogeneous sites of high adsorption energy, with adsorption being predominantly physical, and the high-energy sites being the first to be occupied (Behnajady and Bimeghdar, 2014; Foo and Hameed, 2010; Khezami and Capart, 2005).

As shown in Table 1, t n values are in all cases <1, so that adsorption is mainly physical, and adsorption sites are of high energy. Table 2 also shows n values <1 in all cases, and indicates that the binary systems that have the lowest n values (and therefore access to the highest energy sites) are those that have a fixed concentration for CTC (with TC or OTC variable), while the highest n values corresponded to combinations in which CTC is added in increasing concentrations. Vijayaraghavan et al. (2006) indicated that the highest adsorption capacity and affinity between adsorbent and sorbate are associated to the highest K_F values and to the lowest n values. Therefore, our results would indicate a greater adsorption capacity for those soils having high organic matter contents, and also would indicate higher affinity of adsorbent surfaces for CTC.

Table 3 shows the adjustment of adsorption results to the Murali and Aylmore model (Eq. 2), in which the *a21* index indicates the competition of the antibiotic added in fixed concentration (Fix) over the antibiotic added in variable concentration (Var). The values of the *a21* index showed good fitting for binary systems, with R^2 between 0.859 and 0.999, unless in the case of some combinations for soils 19AL and 3AL. In most soils, the *a21* index has negative values, indicating low competition between antibiotics for adsorption sites.

Table 3. Fitting of experimental results to the Murali and Aylmore model (Eq. 2), including values for the *a21* index, indicative of the degree of competition between the antibiotic added in fixed concentration (Fix) over the antibiotic added in variable concentration (Var)

Soil	Antibiotics	*a21*	R^2	Soil	Antibiotics	*a21*	R^2
3AL	TC (Fix) OTC (Var)	-1.78	0.971	3AL	TC (Fix) OTC (Var)	-4.06	0.984
	TC (Fix) CTC (Var)	-0.72	0.972		TC (Fix) CTC (Var)	-0.08	0.975
	OTC (Fix) CTC (Var)	-4.98	0.980		OTC (Fix) CTC (Var)	-0.71	0.989
	OTC (Fix) TC (Var)	-0.30	0.995		OTC (Fix) TC (Var)	0.31	0.999
	CTC (Fix) TC (Var)	-	-		CTC (Fix) TC (Var)	-7.93	0.968
	CTC (Fix) OTC (Var)	-1.61	0.971		CTC (Fix) OTC (Var)	-2.09	0.859
19AL	TC (Fix) OTC (Var)	-1.84	0.491	19AL	TC (Fix) OTC (Var)	-1.19	0.998
	TC (Fix) CTC (Var)	-0.19	0.976		TC (Fix) CTC (Var)	1.77	0.998
	OTC (Fix) CTC (Var)	-0.02	0.989		OTC (Fix) CTC (Var)	-3.80	0.996
	OTC (Fix) TC (Var)	-1298.39	-		OTC (Fix) TC (Var)	-1.35	0.981
	CTC (Fix) TC (Var)	-0.02	0.989		CTC (Fix) TC (Var)	-0.88	0.992
	CTC (Fix) OTC (Var)	-0.20	0.113		CTC (Fix) OTC (Var)	1.87	0.993

50AL	TC (Fix) OTC (Var)	-1.97	0.993	50AL	TC (Fix) OTC (Var)	-3.90	0.999
	TC (Fix) CTC (Var)	-312.64	0.997		TC (Fix) CTC (Var)	-9.49	0.996
	OTC (Fix) CTC (Var)	-1.15	0.991		OTC (Fix) CTC (Var)	-17.96	0.984
	OTC (Fix) TC (Var)	-0.72	0.993		OTC (Fix) TC (Var)	-0.97	0.982
	CTC (Fix) TC (Var)	-54.53	0.981		CTC (Fix) TC (Var)	-71.23	0.970
	CTC (Fix) OTC (Var)	-7.39	0.993		CTC (Fix) OTC (Var)	-10.37	0.992

The average values of the *a21* index for each pair of combinations (antibiotic-1 fixed + antibiotic-2 variable; antibiotic-2 fixed + antibiotic-1 variable) are presented in Table 4. In the binary system TC + OTC, the highest values for *a21* were obtained when the OTC concentration was fixed. In the TC + CTC binary system, *a21* was higher when the CTC concentration was fixed. Similarly, in the OTC + CTC combination, *a21* was higher when the CTC concentration was fixed. According to Arias et al. (2006), this would indicate that CTC and OTC reduce the adsorption of CT, and in addition CTC would also reduce the adsorption of OTC. This coincides with what was observed in laboratory experiments.

Table 4. Average values for the *a21* index (Eq. 2) corresponding to the 6 binary systems

Binary system	Index *a21*	Binary system	Index *a21*
TC (Fix) OTC (Var)	-2.580	CTC (Fix) TC (Var)	-26.918
OTC (Fix) TC (Var)	-0.606	OTC (Fix) CTC (Var)	-4.770
TC (Fix) CTC (Var)	-77.785	CTC (Fix) OTC (Var)	-3.918

3.3 Desorption of the three tetracycline antibiotics in the six binary systems

Desorption of the three antibiotics was studied for the six binary systems (TC + (OTC or CTC); OTC + (TC or CTC); and CTC + (TC or OTC)). Fig. 2 shows CT desorption in the presence of increasing doses of OTC or CTC. Desorption rates were generally higher in the presence of OTC, although not exceeding 15% in any case. The lowest desorption corresponded to soils 50AL and 70S, with values below 2.8% and 2.45% (in the presence of OTC and CTC, respectively). These soils had the highest organic matter contents and the highest adsorption of antibiotics. Those soils having low organic matter content, particularly 51S and

6S, showed a slight increase in CT desorption as a function of increasing doses of the other antibiotics, especially OTC. Specifically, CT desorption went from 3.5% (in absence of OTC) to 11.5% (when OTC was added at a dose of 600 µmol L^{-1}).

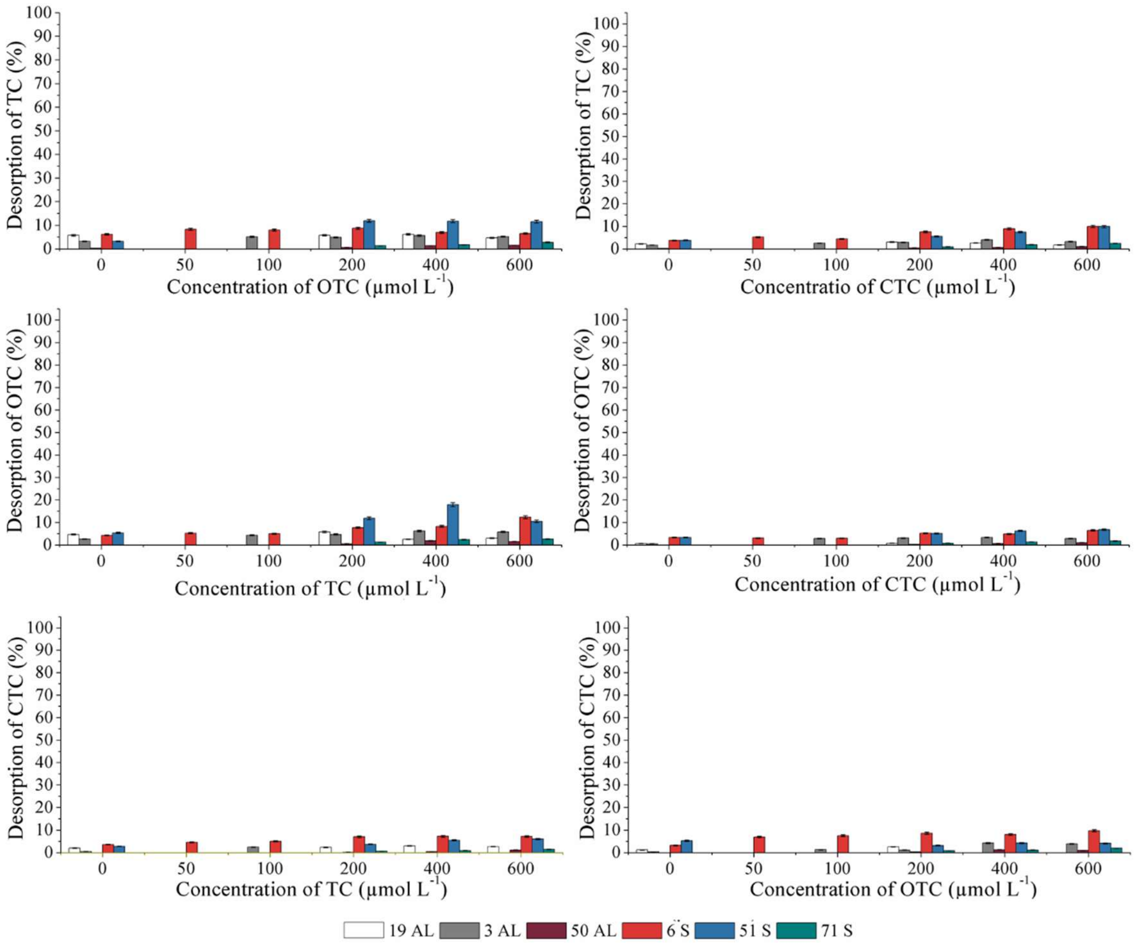

Fig. 2. Desorption percentage for each of the three tetracycline antibiotics (TC, OTC, and CTC) in binary systems, in soils of A Limia (19AL, 3AL and 50AL) and Sarria (6S, 51S and 71S), when the concentration added is kept constant (200 µmol L^{-1}) for the antibiotic indicated on the Y axis of the graphs, and is increased from 0 to 600 µmol L^{-1} for the second antibiotic (as indicated on the X axis). Average values (n = 3), with error bars showing that coefficients of variation were always <5%

With regard to OTC desorption (Fig. 2), it was generally higher in the presence of CT (18% or lower) than of CTC (7% or lower). Again, the soils with the highest organic matter contents (50AL and 71S) showed the lowest desorption (<2.7% and <l.8% of the amounts adsorbed in the presence of CT and CTC, respectively). However, in soils 6S and 51S (with higher pH and lower organic matter content), desorption increased by 10%, approaching 20% for soil 51S in the presence of high doses of CT. Taking into account

that these two soils had a relatively low organic C content (<2%), and pH >6.3, this would indicate that organic matter, as well as pH, would play an important role in desorption processes, in addition to influence adsorption. At the pHs of these soils, both the tetracycline antibiotics and the variable charge components of the soil can be negatively charged, and cationic bridges would be of main importance in adsorption process. However, in the case of the AL soils, with average pH values between 4.5 and 4.8, the tetracycline antibiotics could continue with a negative charge, and would be directly bound to the positive charges present on the adsorbent surfaces of these acidic soils. In this regard, Gu et al. (2007) indicated that the maximum adsorption between tetracyclines and humic substances takes place at pH <4.3, while at higher pH the cationic and zwitterionic fractions of these antibiotics decrease, and the organic substances are deprotonated. This causes that, at pH> 6.5, the complexation or the H bonds between these antibiotics and humic substances is not favored. All these facts can influence the degree of stability of the adsorption process.

Regarding CTC desorption (Fig. 2), no large differences were observed depending on the presence of one of the other two antibiotics. When the dose of CT or OTC are increased, there is a tendency to also increase CTC desorption, more pronounced in soil 6S. In any case, desorption values were always <10%. In simple systems, Fernández-Calviño et al. (2015a) also found desorption <15% for these antibiotics in agricultural soils from the same geographical area. Despite the slight increase in CT desorption taking place in the binary compared to the simple system, especially in soils with low organic matter content and higher pH, desorption percentages remain always low, even for high concentrations of antibiotics added.

This would indicate that hysteresis is affecting these adsorption/desorption processes, coinciding with what was previously indicated by Gu et al. (2007), and it also shows that most of the soils studied have a high adsorption capacity for tetracycline antibiotics, retain them with high energy in binary systems.

3.3 Comparison of adsorption/desorption results for TC, OTC and CTC in simple, binary and ternary systems

Figs. 3, 4 and 5 show information to compare CT, OTC and CTC adsorption/desorption results obtained in this work with those from previously published experiments. In these previous experiments, Conde-Cid et al. (2019) used ternary systems to study the competitive adsorption of the same three antibiotics in the same six soils of the current work. The results of simple, binary and ternary systems are compared in Fig. 3, with 200 µmol L^{-1} being the dose of each of the antibiotics added in all cases. In addition, the differences between simple systems and binary and ternary systems are shown in Fig. 4 (for adsorption) and Fig. 5 (for desorption), to visualize the variations in adsorption and desorption for the three antibiotics when they are at the same concentration in multiple systems.

Trends regarding adsorption are similar for all soils, regardless of the antibiotic in question (Fig. 3). In those soils with the highest organic matter contents (50AL and 71S), almost any difference can be found between simple and binary systems for all three antibiotics, while the ternary system is characterized by a certain decrease in adsorption compared to both simple and binary systems. This decrease is slightly more pronounced in soil 50AL, reaching between 5% and 14% in relation to the simple system (Fig. 4). In the rest of the soils, adsorption of each antibiotic decreases from the simple to the binary, and finally to the ternary system. In soil 19AL (with low content in organic matter), this effect is very evident for all three antibiotics, with a decrease that exceeds 40% comparing the simple with the ternary system (Fig. 4).

An analysis of bivariate correlations was performed for the differences in adsorption between a simple and ternary system and soil organic matter contents. Positive and significant correlations were obtained ($p < 0.10$), with very similar coefficients for the three antibiotics ($r = 0.781$ for CT; $r = 0.703$ for OTC; and $r = 0.726$ for CTC), showing the relevance of soil organic matter in adsorption, as well as the strong competition in ternary systems for adsorption sites present in organic fractions. As indicated above, similar pK_a values in the three antibiotics would favor competition. In addition, the increase in concentration and ionic strength from simple to binary, and later to ternary systems, can cause some saturation and increased competition for adsorption sites in these soils (Sun et al., 2010). In this regard, at the pH of these soils, tetracyclines are present mainly as cationic and zwitterionic species, with the former having higher affinity for adsorption sites negatively charged; however, the increase in concentration and ionic strength affects more to the decrease of cationic than zwitterionic forms (Figueroa et al., 2004), which would result in a decrease in the adsorption of the antibiotics in the ternary system.

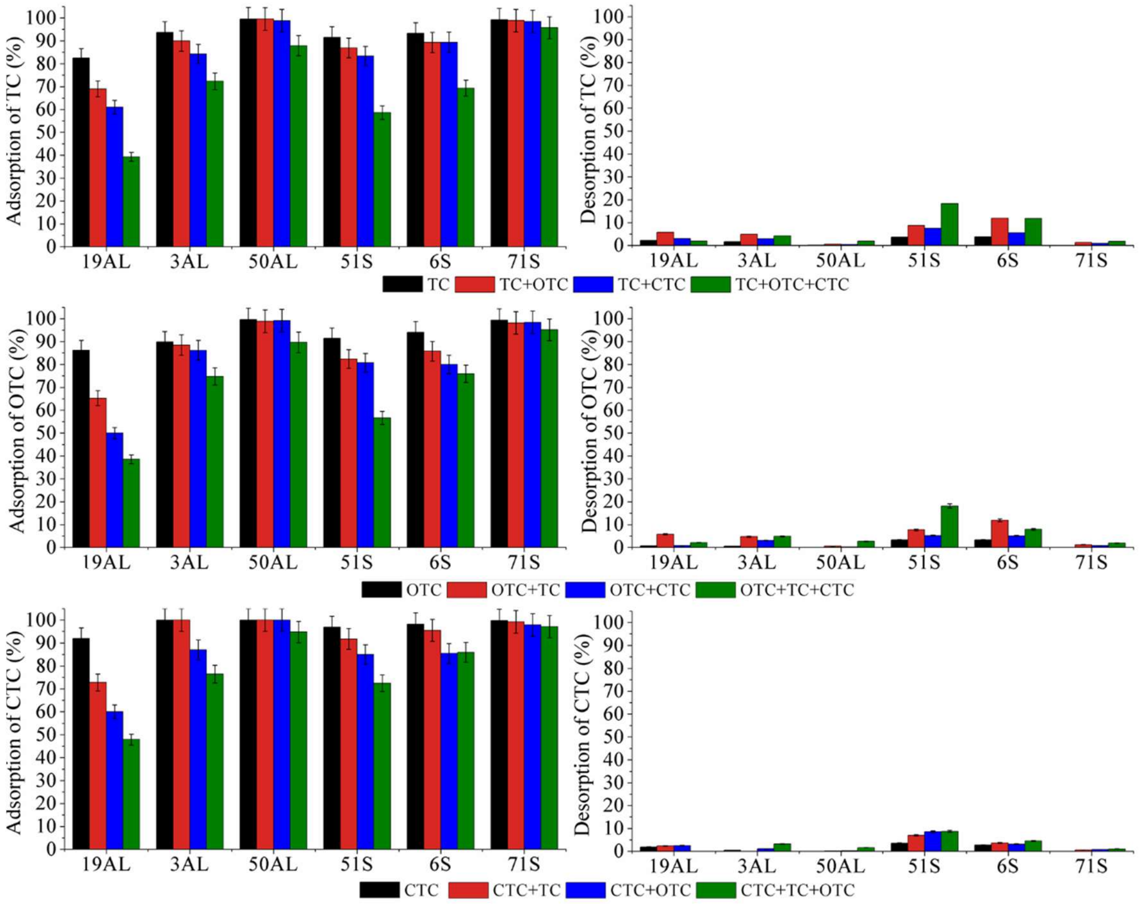

Fig. 3. Comparison of the adsorption and desorption for each of the three tetracycline antibiotics (CTC, TC and OTC) in simple, binary, and ternary systems, for soils of A Limia (19AL, 3AL and 50AL) and Sarria (51S, 6S and 71S), with 200 µmol L^{-1} being the dose of each antibiotic added in all cases. Average values (n = 3), with error bars showing that coefficients of variation were always <5%

Figures 3 and 4 allow to visualize the competition between antibiotics in the binary and ternary systems, highlighting competition taking place in soils with low organic matter content, especially soil 19AL. The adsorption of CT is lower when this antibiotic is in the presence of CTC (61%), than when OTC is present (69%), and the value is even lower when CT is in the presence of both antibiotics (39%). Regarding OTC, its adsorption also has lower values when it is presence of CTC (50%) than in the presence of CT (65%), while adsorption decreases to 39% when both antibiotics are present. Finally, CTC adsorption is more reduced in the presence of OTC (60%) than of CT (72%), decreasing to 45% in the presence of both antibiotics.

When the three graphs in Fig. 3 are compared, it is shown that the adsorption of CTC is generally higher than that of the other two antibiotics, especially in ternary systems. This is also appreciated comparing the differences in the adsorption of each antibiotic among the various systems. Thus, Fig. 4 shows that the smallest differences regarding adsorption percentages among simple, binary, and ternary systems, generally correspond to CTC, indicating a lesser influence of the other antibiotics on its adsorption, which would confirm that CTC competes more effectively for adsorption sites.

In view of the similarity of the pK_a values of the three antibiotics, the differences in adsorption/desorption to soils should be attributed to the respective molecular structures. In this regard, CTC has a Cl atom in its structure, which implies a lower electronic density of the ring, as well as a lower pK_{a2} (7.4) of the phenolic diketone moiety, compared to CT (7.8) and to OTC (7.5), increasing its polarity and water solubility (water solubility at 28 °C = 8.6, 1.7 and 1.4 mg mL^{-1} for CTC, TC and OTC, respectively) (Pils and Laird, 2007). These differences would aid to explain the higher affinity of CTC for adsorption sites.

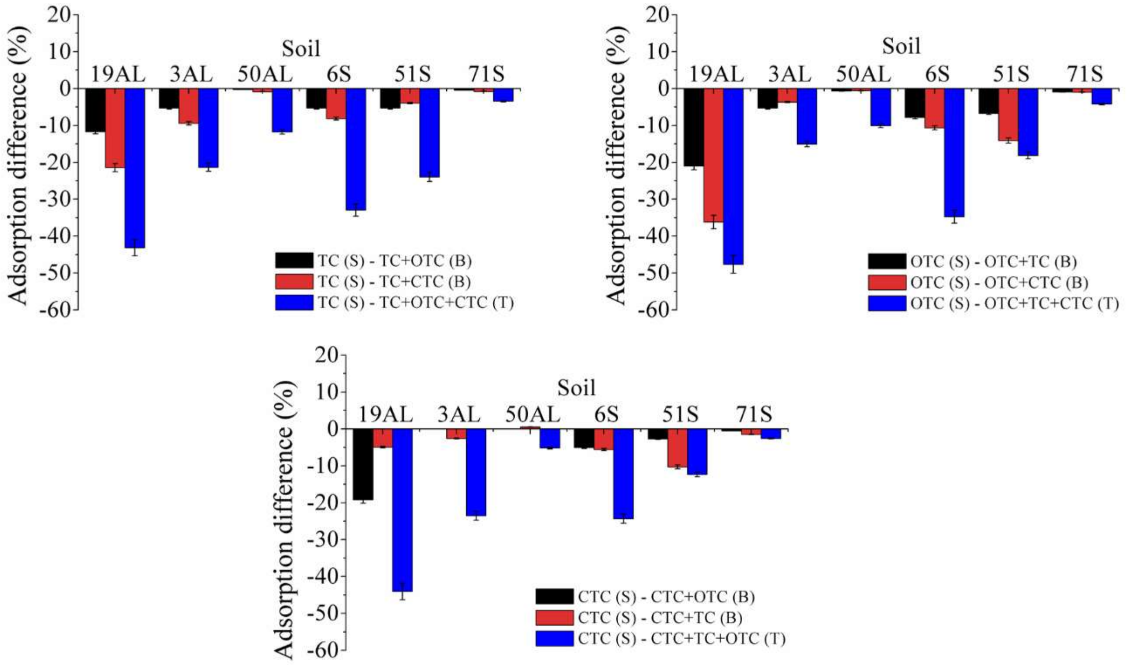

Fig. 4. Difference in adsorption among simple (S), binary (B), and ternary (T) systems, in soils of A Limia (19AL, 3AL and 50AL) and Sarria (51S, 6S and 71S) for each of the three tetracycline antibiotics (CTC, TC and OTC), 200 µmol L^{-1} being the dose of each antibiotic added in all cases. Average values (n = 3), with error bars showing that coefficients of variation were always <5%

Regarding desorption, Fig. 3 shows that the maximum values corresponded to soils with low organic matter content and high pH (soils 6S and 51S), being more pronounced in ternary systems, reaching scores close to 11% and 18%, respectively. For the rest of the soils, desorption percentages are low, especially for CTC, always remaining at <9%.

As shown in Fig. 5, differences regarding desorption were generally small between simple and multiple systems. This means that, under the experimental conditions of the study, the adsorption of these antibiotics is in many cases practically irreversible, even in binary and ternary system. This hysteresis has been reported for simple systems, and related to the existence of strong bonds between antibiotic molecules and adsorbent surfaces (Gu et al., 2007; Fernández-Calviño et al., 2015a). Braida et al. (2003) and Xiang-Rong and Xiao-Yan (2010) indicate that hysteresis processes taking place in soils rich in organic matter may be due to deformations of pores caused by solutes, which makes the adsorption route different from that of desorption, with partial capture of solutes due to collapse of polyaromatic structures during desorption. This additional retention would aid to reduce the toxicity of antibiotics in soils, also increasing their residence time in the edaphic environment.

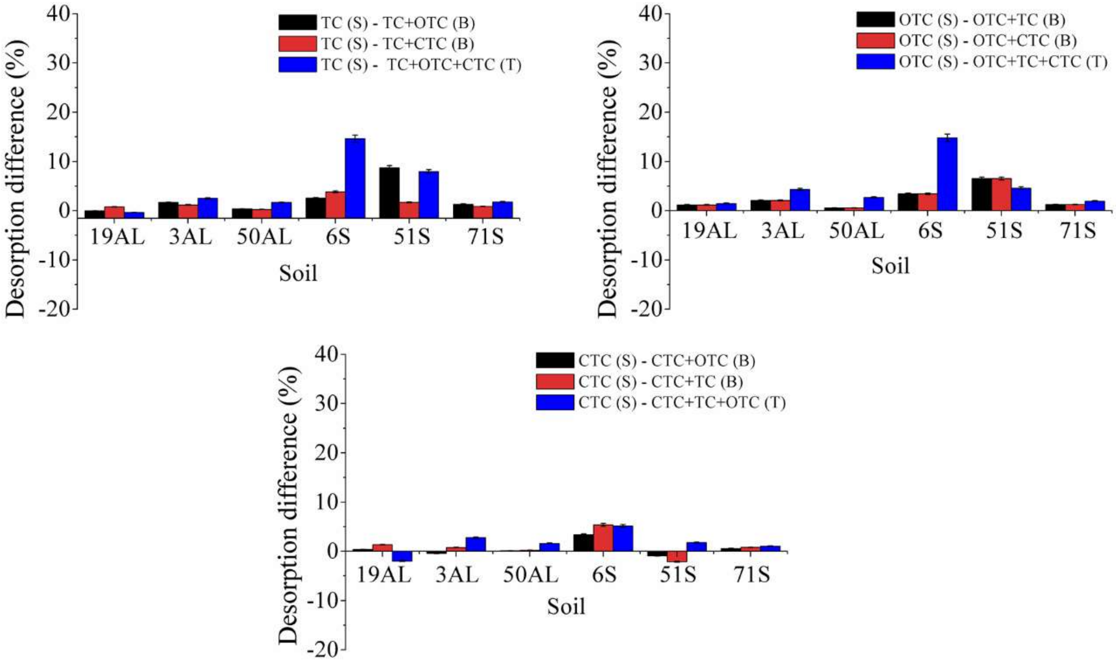

Fig. 5. Difference in desorption between simple (S), binary (B) and ternary (T) systems, in soils of A Limia (19AL, 3AL and 50AL) and Sarria (51S, 6S and 71S), for each of the three tetracycline antibiotics (CTC,

TC and OTC), with 200 µmol L^{-1} being the dose of each antibiotic added in all cases. Average values (n = 3), with error bars showing that coefficients of variation were always <5%

4 Conclusions

Soil organic matter was the main factor conditioning adsorption/desorption of the three tetracycline antibiotics studied (TC, OTC and CTC) in the soils used, whether they were in simple, binary or ternary systems. In soils where this component was abundant, adsorption was close to 100%, while desorption was very low, even in binary and ternary systems. In soils with lower organic matter content, competition for adsorbent surfaces was more pronounced, and adsorption decreased as the dose of added antibiotic increased. An adsorption model used for the binary systems here studied pointed out higher adsorption capacity for soils with more organic matter, and greater affinity for CTC. Desorption depended mainly on pH, increasing for higher pH values, where the cationic and zwitterionic fractions of the antibiotics decrease, and the organic compounds are deprotonated, making difficult complexation and H bonds between antibiotics and humic substances. In multiple systems, competition was intensified, with CTC generally presenting more affinity for adsorbent surfaces due to its structural characteristics. The results of the study indicate that those soils with higher organic matter content are associated to low risk of mobilization to waters (and subsequent entry into the food chain) for the studied antibiotics, even when they are in high doses and present simultaneously. In soils with less organic matter, competition among antibiotics reduced their adsorption and increased desorption (especially when pH was high). However, desorption was generally low, with most of the soils retaining with high energy the three antibiotics, even in multiple systems. These results can be considered of relevance as regards the development of means to protect the environment and public health, given the increasing appearance of multiple antibiotics in different environmental compartments, where they can cause direct and indirect harmful effects. In view of that, facilitating their immobilization and inactivation should be considered essential objectives.

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