Environmental relevance of adsorption of Doxycycline, Enrofloxacin, and Sulfamethoxypyridazine before and after the removal of organic matter from soils

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Abstract

In this work batch-type experiments were used to study the adsorption of the antibiotics doxycycline (DC), enrofloxacin (ENR), and sulfamethoxypyridazine (SMP) in cultivation soils, before and after the removal of soil organic matter. The results indicate a very different behavior depending on the type of antibiotic, showing the adsorption sequence DC>ENR>SMP. Specifically, DC adsorption was very high in untreated soil samples (with organic matter), and was still high (although decreased) after the removal of soil organic matter. Furthermore, the adsorption behavior of DC was clearly dependent on the pH of the medium. Regarding ENR, it also showed high adsorption, although to a lesser extent than DC. However, when soil organic matter was removed, ENR adsorption significantly decreased in all soil samples. As regards SMP, it was adsorbed to a much lesser extent, and the removal of soil organic matter caused an additional drastic decrease in adsorption, reaching negligible values in some samples. Desorption followed the reverse sequence of adsorption, specifically in the order DC<ENR<SMP. In the case of DC, desorption was negligible, both in samples with and without organic matter, while for ENR and SMP, desorption clearly increased for soil samples where organic matter was removed. These results may be of relevance as regards environmental quality and public health, especially to facilitate a correct use of soils and organic amendments in areas that receive the application of substances containing the investigated antibiotics.

Keywords: adsorption-desorption; doxycycline; enrofloxacin; soil organic matter; sulfamethoxypyridazine

1 Introduction

Environmental and public health issues due to the presence of antibiotics in soils and waters have been pointed out for years by different authors (Thiele-Bruhn, 2003; Kemper, 2008; Cycoń et al., 2019; Zhao et al., 2019; Duan et al., 2020; Lyu et al., 2020). Antibiotics reach agricultural soils mainly due to the spreading of animal manure or slurry as fertilizers (Kuppusamy et al., 2018) and to the addition of sewage sludge, in the case of antibiotics used in human medicine (Verlicchi and Zambello, 2015). Once in the soil, some of the most relevant processes determining the fate of antibiotics are physical, chemical and biological degradation, as well as adsorption-desorption processes, which deeply influence their retention and transport to water bodies. Adsorption and desorption depend on the characteristics of antibiotics, but also on soils properties (Wang and Wang, 2015). In fact, both the content and the quality of soil organic matter (SOM) are of special relevance as regards adsorption-desorption of different antibiotics. Specifically, SOM has been highlighted as main variable affecting the fate of tetracycline antibiotics (Laak et al., 2006; Zhao et al., 2011a; Zhao et al., 2011b; Zhao et al., 2012; Conde-Cid et al., 2019a; Conde-Cid et al., 2020; Álvarez-Esmorís et al., 2020a), sulfonamides (Lertpaitoonpan et al., 2009; Chu et al., 2013; Conde-Cid et al., 2019b; Conde-Cid et al., 2019c; Álvarez-Esmorís et al., 2020b), fluoroquinolones (Martínez-Mejia et al., 2017; Álvarez-Esmorís et al., 2020b), macrolides (Hu and Coats, 2009; Kodešová et al., 2015), and diaminopyrimidines (Kočárek et al., 2016). The influence of organic matter on the desorption of different antibiotics has also been evidenced in various publications (Conde-Cid et al. 2019b, Conde-Cid et al. 2019c; Álvarez-Esmorís et al., 2020a).

In addition, as further crucial aspect to be taken into account, an accelerated loss of SOM is taking place in different geographical areas of the planet, especially due to intensive agriculture (Haddaway et al., 2017; Purwanto and Alam, 2020), which supposes a direct effect of soil degradation, but could also have an additional negative effect due to the decrease in the retention capacity of pollutants, and among them the antibiotics that reach the edaphic environment (Pignatello, 1998; Antilen et al., 2016; Lasota et al., 2020).

With this in mind, the study of the influence of SOM on the adsorption/desorption of antibiotics can be carried out through three different approaches:

-A first approach consists of studying a high number of soil samples, relating adsorption and desorption parameters with different edaphic variables. Several recent works have used this methodology (Leal et al, 2013; Conde-Cid et al. 2019b).

-A second approach consists of adding organic matter to the soil or to soil components, then studying the changes that occur as regards the adsorption and desorption processes (Zhao et al., 2011a; Zhao et al., 2011b; Zhao et al., 2012).

-The third approach consists of the removal of organic matter from the soil, to assess to what extent it affects the adsorption/desorption process. These types of studies dealing with the elimination of organic matter are very scarce, which would make necessary to deepen and increase knowledge for this kind of experiments.

In view of that, the objective of this work is to evaluate the effect of SOM on the adsorption-desorption processes of three different antibiotics in cultivated soils. To do that, the removal of SOM is carried out in order to compare adsorption/desorption characteristics in its presence and absence. The hypothesis is that the elimination of SOM would decrease the adsorption of antibiotics, thus increasing their mobility in soils. The three different antibiotics investigated have been previously detected in soils, but their behavior as regards mobility could be clearly different for each one. These antibiotics are: a) doxycycline, a tetracycline antibiotic, which is one of the most modern antibiotics with respect to its use in veterinary medicine fundamentally; b) enrofloxacin, which is a second generation antibiotic belonging to the group of fluoroquinolones; and c) sulfamethoxypyridazine, which belongs to the group of sulfonamides and is also widely used in veterinary medicine. The results of this study could be of high relevance due to its environmental and public health implications, and could be used as a guide to make an appropriate management of soils and organic amendments when focusing on antibiotics pollution.

2 Material and Methods

2.1 Soil Samples

To carry out this study, surface samples (0-20 cm depth) were collected from 6 cultivation soils in Galicia (NW Spain), which constitute "Untreated samples": 4 soils from potato cultivation, and 2 soils from vineyards. Each of the 6 samples is the result of combining 10 subsamples taken randomly by means of an Edelman probe. Once in the laboratory, all soil samples were air-dried at room temperature, sieved through a 2 mm mesh, and then stored in polyethylene cans until analysis. Standard procedures (Tan, 1996) were applied to determine the main physicochemical characteristics of the studied soils, with results shown in Table 1.

These results indicate that soils 3, 5 and 6 have a sandy-loam texture, while the texture of soils 1, 2 and 4 is sandy-clay loam. Regarding the pH, it was measured potentiometrically by means of a pH-meter (model 2001, Crison, Barcelona, Spain), with soil:solution ratio 1:2.5, showing values in the range 4.2-7.0 for pH in water (pH_{H2O}), which were higher than those in 0.1 M KCl (pH_{KCl}), situated in the range 3.4-6.7, indicating the predominance of negative charges in these soils. The values of the effective cation exchange capacity (eCEC) were estimated by the sum of the exchangeable basic cations (Na_e, K_e, Ca_e, Mg_e) after displacement with 0.2 M NH_4Cl (Sumner and Miller, 1996), and exchangeable aluminum (Al_e) displaced with 1 M KCl (Bertsch and Bloom, 1996), and subsequent quantification of all cations by atomic spectrometry (AAnalyst200, PerkinElmer, USA), showing low scores (between 3.31 and 27.70 $cmol_c kg^{-1}$). The percentage of organic carbon was highly variable, with values between 0.3 and 10.9%, while the percentage of nitrogen varied between 0.04% and 0.84%, both parameters being measured by elemental analysis (Thermo Finnigan 1112 Series NC, USA). BET surface areas were determined by nitrogen adsorption-desorption isotherms, using ASAP 2020 equipment (MICROMERITICS, USA). The results of the untreated soils were between 1.4 and 4.0 $m^2 g^{-1}$ (Table 1). Replicate determinations were carried out (n = 3), and coefficients of variation were always lower than 5%. Standard calibration and measurement conditions were used to assure the quality/assurance of the results.

Aliquots of these 6 soil samples were subjected to calcination at 450 ºC for two hours in a muffle (calcined samples) to remove organic matter (Hoogsteen et al., 2015). The calcination of the samples resulted in carbon contents ≤ 0.1%, and in nitrogen contents ≤ 0.05% (Table 1). There was also an increase in exchangeable basic cations and surface area values (Table 1).

2.2 Antibiotics and chemical reagents

The antibiotics used were provided by Sigma-Aldrich (Buchs, Switzerland): sulfamethoxypyridazine (SMP), purity ≥98.0%, with chemical formula $C_{11}H_{12}N_4O_3S$; enrofloxacin (ENR), with purity ≥98.0%, formula $C_{19}H_{22}FN_3O_3$, and doxycycline (DC), with purity ≥95.0%, formula $C_{22}H_{24}N_2O_8 \bullet HCl \bullet 0.5C_2H_6O$. Table S1 (Supplementary material) shows the main characteristics of these three antibiotics.

For the quantification of the antibiotics by HPLC (see below), all the reagents used (acetonitrile, phosphoric acid, oxalic acid and triethanolamine) were of high purity analytical grade, supplied by Panreac (Barcelona, Spain) and Fisher Scientific (Madrid, Spain). All solutions were prepared with Milli-Q water (Millipore, Madrid, Spain). All determinations were made by triplicate.

2.3 Adsorption-desorption of the three antibiotics

Batch-type experiments were carried out, both for untreated (un-calcined) and calcined samples. To do that, 2 g of soil (in the case of SMP) and 1 g of soil (in the case of ENR and DC) were added with 5 mL of SMP solutions, and with 40 mL of ENR and DC solutions (respectively), containing 7 different concentrations of the antibiotics (2.5, 5.0, 10.0, 20.0, 30.0, 40.0 and 50.0 μM for SMP; and 10, 25, 50, 100, 200, 300 and 400 μM for ENR and DC), all of them including 0.005 M $CaCl_2$ as background electrolyte. The resulting suspensions were shaken at 50 rpm for 24 h (the time needed to assure that equilibrium was reached), and centrifuged at 4000 rpm for 15 minutes. The resulting supernatants were filtered (through 0.45 μm nylon syringe filters, Fisher scientific, Madrid, Spain) and then the antibiotic concentration was determined by HPLC (see section 2.4 below).

The amount of antibiotic adsorbed to the soil was calculated by the difference between the initial concentration and the concentration at equilibrium (measured in the supernatant). For the study of desorption, the soil samples previously subjected to the adsorption process were re-suspended in 5 mL of 0.005 M $CaCl_2$ and then shaken, centrifuged, filtered and analyzed under the same conditions as in the adsorption experiments. All experiments were carried out in triplicate.

The experimental data were adjusted to the Freundlich model (Eq. 1):

$$q_a = K_F.\ C_{eq}^{\ n} \qquad \text{(Eq. 1)}$$

where q_a (µmol kg^{-1}) and C_{eq} (µM) are the concentrations of SMP, ENR or DC retained by the soil, and the concentrations of the different antibiotics in the solution at equilibrium, respectively; K_F (L^n µmol^{1-n} kg^{-1}) and n (dimensionless) are the Freundlich coefficients.

2.4 Quantification of the three antibiotics

The quantification of the antibiotics was carried out by means of a HPLC equipment (Dionex Corporation, Sunnyvale, USA) connected to an ultraviolet-visible detector (UVD170U). For the chromatographic separation, a Luna C18 column from Phenomenex (Spain) was used. An injection volume of 50 µL and a flow of 1.5 mL min^{-1} were used. The mobile phase consisted of acetonitrile and 0.01 M phosphoric acid for SMP and ENR, and acetonitrile and 0.02 M oxalic acid/0.01M triethanolamine in the case of DC. The total analysis time was 15 min. For the detection of SMP and ENR, a wavelength of 270 nm was used, and it was 360 nm in the case of DC.

3 Results and discussion

3.1 Adsorption of the antibiotics

Fig. 1 shows adsorption data for DC. The results indicate that adsorption was very high, both for untreated and calcined samples. In general, calcination decreased DC adsorption, except for soil 6, where adsorption increased with calcination (Fig. 1). When data was modeled using the Freundlich equation (Eq. 1), K_F values were very high and followed the same trend commented above, with higher K_F in untreated samples (values between 3918-12882 L^n μmol^{1-n} kg^{-1}) (Table 2) than in calcined soil samples (values between 1080-4912 L^n μmol^{1-n} kg^{-1}), except for soil 6 (Table 2). The same pattern of behavior is observed for the n parameter (Table 2).

The percentages of decrease in the values of K_F in the calcined samples, compared to the untreated soil samples were quite high, oscillating between 50% and 72% (except for soil 6, with a negative percentage, since its adsorption increased with calcination). Therefore, in general, for these soils the SOM content is a very relevant factor in increasing the adsorption of DC, although other aspects would have more relevance in the case of soil 6. This effect of organic matter increasing adsorption has already been highlighted by other authors for other tetracycline antibiotics, such as tetracycline (Zhang et al, 2010; Zhao et al., 2012; Conde-Cid et al., 2019a), oxytetracycline and chlortetracycline (Kim et al., 2012; Conde-Cid et al., 2020), and also for DC itself (Álvarez-Esmorís et al., 2020a).

The fact that the calcined samples present such high adsorption may be due to the fact that DC adsorption also increases with clay contents, an aspect that has already been evidenced by Álvarez-Esmorís et al. (2020a), and that was also observed for other tetracycline antibiotics (Conde-Cid et al., 2019a; Conde-Cid et al., 2020). Furthermore, the surface area of the samples increased with calcination (Table 1), since new surfaces of the inorganic components are made accessible when organic matter is removed. This fact has been highlighted by some authors, indicating that it caused an increase in the adsorption of organic compounds such as quaternary ammonium herbicides (Pateiro-Moure et al., 2009), and also of other tetracycline antibiotics (Pils and Laird, 2007).

The high adsorption of DC found in the current study for soil samples even after calcination could be considered somehow surprising, as calcination caused the rise of soil pH (see Table 1), and of the pH of the solution at which adsorption takes place after 24 hours of contact (Table S2). In fact, this high adsorption after calcination could be not expected taking into account that previous studies have shown that the adsorption of tetracycline antibiotics decreased when pH increased (Sassman and Lee, 2005; Wang et al., 2010; Zhao et al., 2015; Chen et al., 2020).

In addition, the behavior of sample 6 is noteworthy, as DC adsorption increased when SOM was removed by calcination. This behavior could be related to the fact that new adsorbent surfaces become accessible in the inorganic components remaining, after destroying links between organic and inorganic components. In fact, surface area increased in this sample after calcination, going from 1.4 to 6.3 m^2 g^{-1}. However, the increase in surface area was greater in sample 4 (going from 2.1 to 11.0 m^2 g^{-1}), indicating that this would not be the fundamental reason for this behavior. It could be considered that the fundamental reason of that behavior is that the rise in pH linked to calcination causes a notable increase in the negative forms of DC, especially in the pH range of 8-10 (Table S3), as is the case of sample 6. This increase in negative forms of DC, together with the increase in the negative charge of soils that have clays with a variable charge, makes very relevant the formation of bonds between DC and soil clays through cationic bridges, which is favored by the increase of hydroxyl-aluminum groups, Mg^{2+} and Ca^{2+} in the calcined samples (Table 1). This does not occur with a comparable intensity in the other soils, because their pH values are lower than in sample 6, and at those lower pHs the presence of negatively charged forms of DC is also lower (Table S3). In this sense, Sithole and Guy (1987) indicate that the complexation of multivalent cations and tetracycline antibiotics is higher at high pH values, and also Figueroa et al. (2004) indicate that the complexation of another tetracycline (oxytetracycline) in clays increased with pH. But other processes that are yet to be elucidated, and that would need additional research, could also play a role. As an example, although it is not strictly related to soils, Elhalil et al. (2018) studied the removal of pharmaceuticals by means of layered double hydroxides phases intercalated by carbonates ions, that were calcined at 300, 400, 500 and 600 °C, and indicate that the optimum adsorption corresponded to a calcination temperature of 300 °C. Interestingly, these authors found that the adsorption kinetics follows a first rapid step, with adsorption on the external surface, and a second slow step, that the authors consider could be due to the reconstruction of layered double hydroxides structure (memory effect). Also, focusing on the retention of anions and cations, Lazaridis (2003) studied the sorbent capacity of hydrotalcite (a double-layered mixed-metal hydroxide included in the group of anionic clays), both for the uncalcined sorbent and after calcination at 500 °C, finding higher sorbent capacity for the calcined material. This author indicates that hydrotalcite containing carbonates as the interlayer anion would be decomposed into magnesium and aluminum oxide solid solution when calcined at 500 ºC, and then the calcined product could rehydrate and incorporate anions to rebuild the initial hydrotalcite structure.

Regarding modifications taking place in soils when subjected to calcination (in addition to removal of organic matter) Rodríguez et al. (2017) used an agricultural soil that was calcined at 500º C, which showed differences as compared to the non-calcined soil. In fact, these authors indicate that specific gravity increased around 10%, real density was reduced by 4%, BET area and pore volume decreased 50%, while SEM and XRD analyses confirmed the direct effect of soil calcination on the physicochemical characteristics and the structure of soil particles. The authors concluded that in their study (focused on the elucidation of effects due to morphological and physicochemical properties of soil affecting to phenanthrene degradation by ozonation) the main difference between soils was the removal efficiency. In addition, Ding et al. (2018) performed a study where the authors calcined soils at 200, 400, 600, and 800°C for 2 h, then characterizing them by means of XRD. These authors found that the intensities of the diffraction peaks changed correspondingly with the calcination temperature, even if in all cases a sharp diffraction peak indicated that the main component of calcined soils was SiO2. Also, SEM images indicated that the morphologies were closely related to the calcination temperature, with lumpy particles converted into plate-like particles when temperature increased from 200 to 800 °C.

Fig. 2 shows that, in the current study, ENR adsorption in untreated soils was also high in all cases, and calcination caused a decrease in adsorption in all samples. The K_F values obtained from modeling with Freundlich's equation oscillate in the range 1110-3019 L^n μmol^{1-n} kg^{-1} for untreated soils, and in the range 36-1181 L^n μmol^{1-n} kg^{-1} for calcined soils (Table 2). In this case, the percentages of decrease in the K_F parameter after calcining were in the range 19-98%, with soil sample 6 showing the highest value. In previous studies the adsorption of ENR has been fundamentally related to SOM, both in experiments in which the adsorption of ENR and other fluoroquinolones was evaluated based on the values of edaphic variables (Álvarez-Esmorís et al., 2020b), and in experiments where the adsorption of ENR was studied directly on humic acids (Martínez-Mejía et al., 2017; Afzal et al., 2019), or on soils amended with humic acids (Teixidó et al., 2014). In addition, the decrease in adsorption in calcined samples (where pH values increase, Table S2) is justified by the lower ENR retention that takes place as a function of the raise in pH, which has been described by several authors (Gu and Karthikeyan, 2005; Yan et al., 2012). As with DC, by increasing the pH in calcined samples, the formation of complexes between ENR and soil clays can take place, but in this case the complexes may be even more soluble than the ENR molecules themselves, and thus decrease their adsorption. In fact, this has been described for other fluoroquinolones, such as ciprofloxacin, which has a similar structure to that of ENR (Ross and Riley, 1993), and for other quinolones (Uivarosi, 2013), which justifies the different behavior as compared to DC, especially with regard to sample 6.

Fig. 3 shows that the adsorption of SMP was much lower than that of DC and ENR, and it was also lower in the calcined samples compared to the non-calcined ones, behaving in a similar way to ENR. In the case of SMP, the K_F values were much lower, in the range 1.2-33.5 L^n μmol^{1-n} kg^{-1} for untreated samples, and between 0.1-2.9 L^n μmol^{1-n} kg^{-1} for calcined soils (Table 2), which indicates that the adsorption was low in both kinds of samples. The percentages of decrease in K_F after calcination ranged between 25 and 99%, the smallest decrease being for sample 5, and the largest for sample 6. The low adsorption of sulfonamides in soils, as well as the positive influence of SOM on it has been highlighted by numerous authors (Lertpaitoonpan et al., 2009; Chu et al., 2013; Conde-Cid et al., 2019b; Conde-Cid et al., 2019c). In addition, it has been shown that its adsorption decreases as the pH increases, and this is one of the reasons for the decrease in its retention in calcined samples, in which the pH increases with respect to the untreated soils (Table S2). Likewise, sulfonamides have a low capacity to form chelates (Sukul and Spiteller, 2006), which justifies their even lower adsorption in calcined samples.

3.2 Desorption of the three antibiotics

Table 3 shows desorption results corresponding to the three antibiotics studied. Firstly, it should be noted that DC desorption is insignificant, generally achieving less than 1% for initial concentrations between 100-400 µM, both in untreated and calcined samples

(Table 3). Desorption pHs were similar to those of adsorption (Tables S2 and S4), and show an equivalent trend, since desorption pHs increases in calcined samples, which again indicates that calcination in general implies less retention of antibiotics. This behavior shows a high hysteresis, which is related to the multiple adsorption mechanisms of tetracycline antibiotics on soil surfaces (both organic and inorganic).

For ENR, desorption pHs follow the same trend and are similar to those of adsorption, as was the case for DC (Tables S2 and S4). ENR was desorbed to a greater extent, but also in low percentages (less than 10%) from untreated soils (Table 3). The removal of SOM by calcination causes an increase in desorption for all soils, highlighting the increase taking place in sample 6, where it exceeds 60% (Table 3). It should be noted that some of the bonds between antibiotics and inorganic surfaces of soils are reversible in nature, such as those that take place by cation exchange, which has been cited as one of the main retention mechanisms for ENR (Chen et al., 2016), and this reversibility would contribute to explain the increase in desorption in calcined samples, in which inorganic surfaces are those that can interact with the antibiotic. Previous experiments carried out on similar soils indicate that desorption appears to be correlated with the same variables as adsorption, but with the opposite sign (Álvarez-Esmorís et al., 2020b).

SMP desorbs in a greater proportion than the other two antibiotics, with desorption percentages ranging from 3 to 26% for untreated soils (Table 3), while removing SOM produces an increase in desorption, reaching values greater than 40% for sample 6 (Table S3). Desorption pHs follow the same trend as that indicated for DC and ENR (Tables S2 and S4). These results are in agreement with those reported by Álvarez-Esmorís et al. (2020b), which indicate that the soils with less organic matter and more sand fraction are those that suffer the greatest desorption.

The different behavior of the three antibiotics with respect to desorption is related to the retention mechanisms. DC molecules have multiple functional groups, whose combined action gives rise to a high affinity for the different components of the soil, with interactions taking place through mechanisms such as cation exchange, surface complexation with oxides or with octahedral layers of soil phyllosilicates, cationic bridges, or H bonds, all of which are of minor importance in the case of SMP, as well as for other sulfonamides (Hu et al., 2019). The mechanisms that intervene in the retention of ENR are also multiple, but weaker bonds predominate, which make their adsorption more reversible than that of DC.

4 Conclusions

The adsorption of the three different antibiotics evaluated in the present study taking place on the six cultivation soils investigated followed the order DC>ENR>SMP. The removal of SOM by calcination generally caused a decrease in adsorption for the three antibiotics. However, in the case of DC, a soil sample with higher pH showed an inverse behavior, which is supposed to be mainly due to the formation of cationic bridges in the calcined samples, since this calcination releases large concentrations of cations to the solution. Desorption behavior was the inverse to that of adsorption, following the sequence DC<ENR<SMP, which is related to the different adsorption mechanisms of the three antibiotics. In general, the conservation of organic matter in cultivation soils makes them maintain their ability to immobilize pollutants, such as antibiotics, which promotes the sustainability of soils over time, reducing the risks of contamination of surface, subsurface and ground waters, and the passage of antibiotics to the food chain, which has high relevance for public health and the environmental.

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Table 1. Physicochemical characteristics of the studied soils. Average values (n = 3), with coefficients of variation always <5%. Na_e, K_e, Ca_e, Mg_e: basic cations extracted with ammonium chloride; Al_e: aluminum extracted with potassium chloride; eCEC: effective cation exchange capacity. A: Untreated Soils; B: Calcined Soils

Parameter	Soil 1		Soil 2		Soil 3		Soil 4		Soil 5		Soil 6	
	A	B	A	B	A	B	A	B	A	B	A	B
pH_{H_2O}	4.2	4.9	5.7	5.8	4.8	4.8	4.5	6.9	4.6	5.0	7.0	9.7
pH_{KCl}	3.8	4.3	4.7	5.1	4.3	3.9	4.0	6.5	3.4	4.3	6.7	9.0
Sand (%)	60		51		70		49		70		58	
Silt (%)	19		21		12		19		17		27	
Clay (%)	21		29		18		32		14		15	
C (%)	2.0	<0.1	3.9	<0.1	1.1	<0.1	10.9	<0.1	0.3	<0.1	6.6	0.1
N (%)	0.19	<0.02	0.33	<0.02	0.09	<0.02	0.84	<0.02	0.04	<0.02	0.44	<0.02
Na_e ($cmol_{(c)}kg^{-1}$)	0.25	1.48	0.42	2.08	0.25	2.36	0.42	5.83	0.09	2.37	1.58	3.63
K_e ($cmol_{(c)}kg^{-1}$)	1.51	7.11	1.45	10.38	1.27	14.75	1.14	22.42	0.22	3.59	4.25	7.68
Ca_e ($cmol_{(c)}kg^{-1}$)	1.02	6.80	2.86	18.52	1.53	19.62	5.94	184.25	1.38	8.59	18.15	373.55
Mg_e ($cmol_{(c)}kg^{-1}$)	0.44	5.22	1.05	15.37	0.41	8.42	1.48	86.75	0.62	5.79	3.70	165.28
Al_e ($cmol_{(c)}kg^{-1}$)	1.51	3.59	0.81	1.00	0.61	5.57	2.66	3.30	1.00	3.55	<0.05	8.23
eCEC ($cmol_{(c)}kg^{-1}$)	4.74		6.59		4.08		11.64		3.31		27.70	
BET Surface area (m^2g^{-1})	3.8	6.7	4.0	8.2	1.7	1.6	2.1	11.0	2.1	2.4	1.4	6.2

Table 2. Freundlich parameters and R^2 values for the three antibiotics studied. K_F (L^n μmol^{1-n} kg^{-1}); n: dimensionless. DC: doxycycline; ENR: enrofloxacin; SMP: sulfamethoxypyridazine

	Untreated Soils			Calcined Soils		
			DC			
Sample	K_F	n	R^2	K_F	n	R^2
1	7735±1940	0.22±0.09	0.96	2368±742	0.58±0.10	0.97
2	12882±3552	0.13±0.22	0.81	4275±568	0.54±0.06	0.98
3	3918±551	0.37±0.05	0.98	1080±124	0.78±0.04	1.00
4	11676±705	0.50±0.09	0.98	4912±553	0.50±0.05	0.99
5	6786±737	0.24±0.04	0.98	3389±522	0.38±0.04	0.98
6	3164±437	0.62±0.06	0.98	14302±213	0.94±0.04	1.00
			ENR			
Sample	K_F	n	R^2	K_F	n	R^2
1	1527±150	0.51±0.03	0.99	1181±122	0.22±0.02	0.98
2	3019±135	0.54±0.02	1.00	2199±388	0.19±0.04	0.95
3	1110±125	0.48±0.03	0.99	895±164	0.19±0.04	0.94
4	2851±314	0.68±0.05	0.99	1377±144	0.42±0.03	0.99
5	1615±1124	1.14±0.34	0.69	1179±138	0.26±0.03	0.98
6	2339±296	0.60±0.05	0.99	36±18	0.71±0.10	0.97
			SMP			
Sample	K_F	n	R^2	K_F	n	R^2
1	4.9±0.2	0.85±0.01	1.00	2.9±0.4	0.78±0.04	0.99
2	7.1±0.5	0.81±0.03	1.00	2.6±0.4	0.49±0.04	0.98
3	2.4±0.6	0.85±0.08	0.98	0.9±0.2	0.71±0.05	0.99
4	33.5±1.5	0.84±0.04	1.00	1.5±0.6	0.54±0.12	0.89
5	1.2±0.5	0.78±0.11	0.95	0.9±0.2	0.91±0.06	0.99
6	13.3±1.6	0.81±0.06	0.99	0.1±0.0	1.09±0.12	0.98

Table 3. Percentages of desorption for each of the antibiotics from each of the untreated and calcined soil samples, for different initial concentrations added (C_o) of the three antibiotics. DC: doxycycline; ENR: enrofloxacin; SMP: sulfamethoxypyridazine

		C_o (μM)			
		100	200	300	400
Soil	Sample	DC			
1	Untreated Soil	<1	<1	<1	<1
	Calcined Soil	<1	<1	<1	<1
2	Untreated Soil	<1	<1	<1	1
	Calcined Soil	<1	<1	<1	<1
3	Untreated Soil	<1	<1	<1	<1
	Calcined Soil	<1	<1	<1	1
4	Untreated Soil	<1	<1	<1	<1
	Calcined Soil	<1	<1	<1	<1
5	Untreated Soil	<1	<1	<1	1
	Calcined Soil	<1	<1	<1	1
6	Untreated Soil	<1	<1	<1	2
	Calcined Soil	<1	<1	<1	<1
		ENR			
1	Untreated Soil	2	3	4	4
	Calcined Soil	11	17	19	24
2	Untreated Soil	1	1	2	2
	Calcined Soil	6	13	18	20
3	Untreated Soil	6	5	6	7
	Calcined Soil	19	26	30	34
4	Untreated Soil	1	2	2	2
	Calcined Soil	7	12	15	18
5	Untreated Soil	<1	1	1	1
	Calcined Soil	8	10	15	18
6	Untreated Soil	1	2	2	3
	Calcined Soil	20	27	45	63
		SMP			
1	Untreated Soil	14	15	17	18
	Calcined Soil	14	14	15	16
2	Untreated Soil	13	13	14	15
	Calcined Soil	21	20	20	19
3	Untreated Soil	18	19	19	22
	Calcined Soil	22	25	31	34
4	Untreated Soil	3	4	4	4
	Calcined Soil	18	16	18	22
5	Untreated Soil	22	25	26	26
	Calcined Soil	25	22	24	26
6	Untreated Soil	7	9	8	10
	Calcined Soil	44	41	37	39

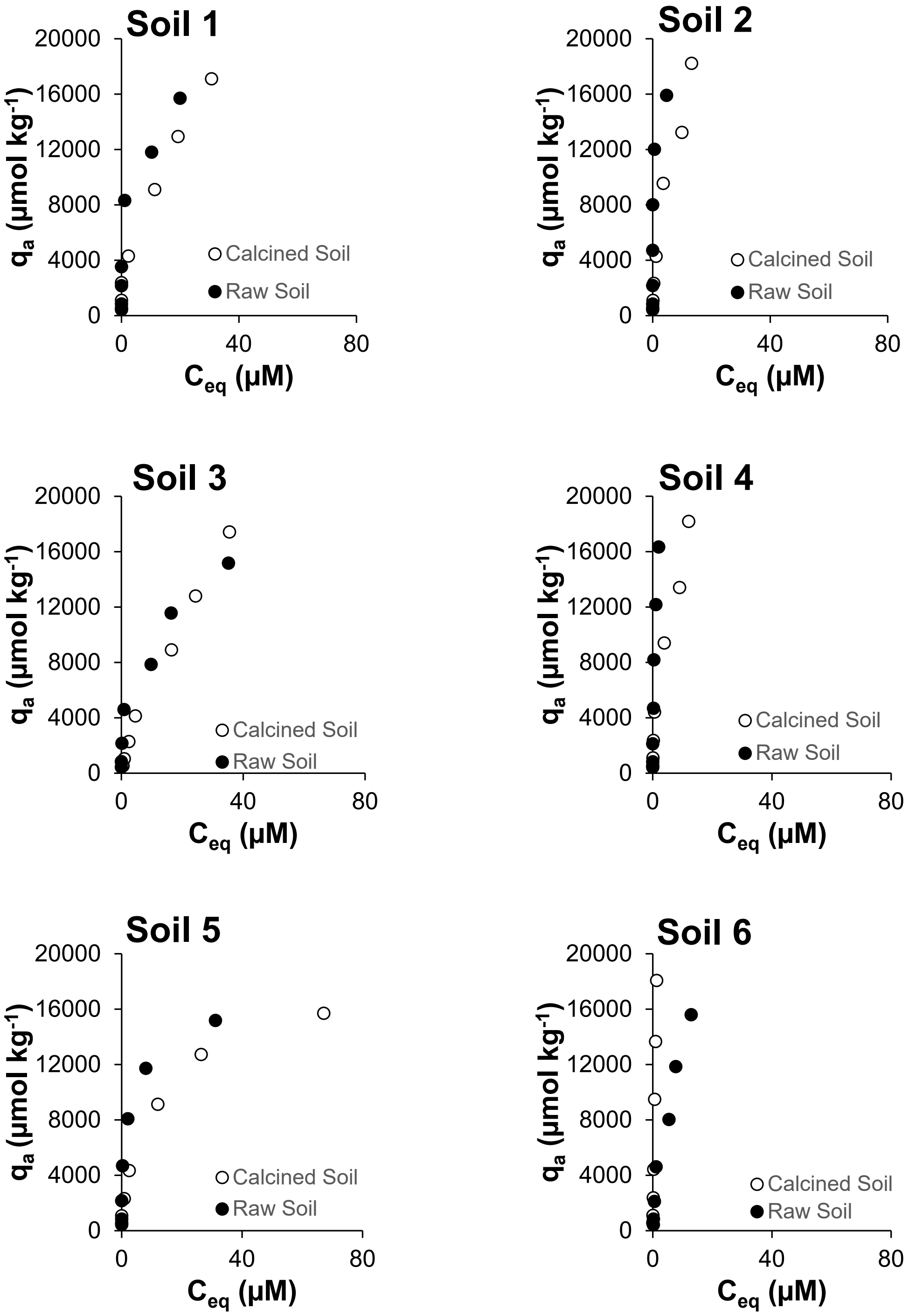

Fig. 1. Doxycycline (DC) adsorption in the six soil samples studied. Average values (n = 3), with coefficients of variation always <10%. q_a: adsorbed DC; C_{eq}: DC in the equilibrium

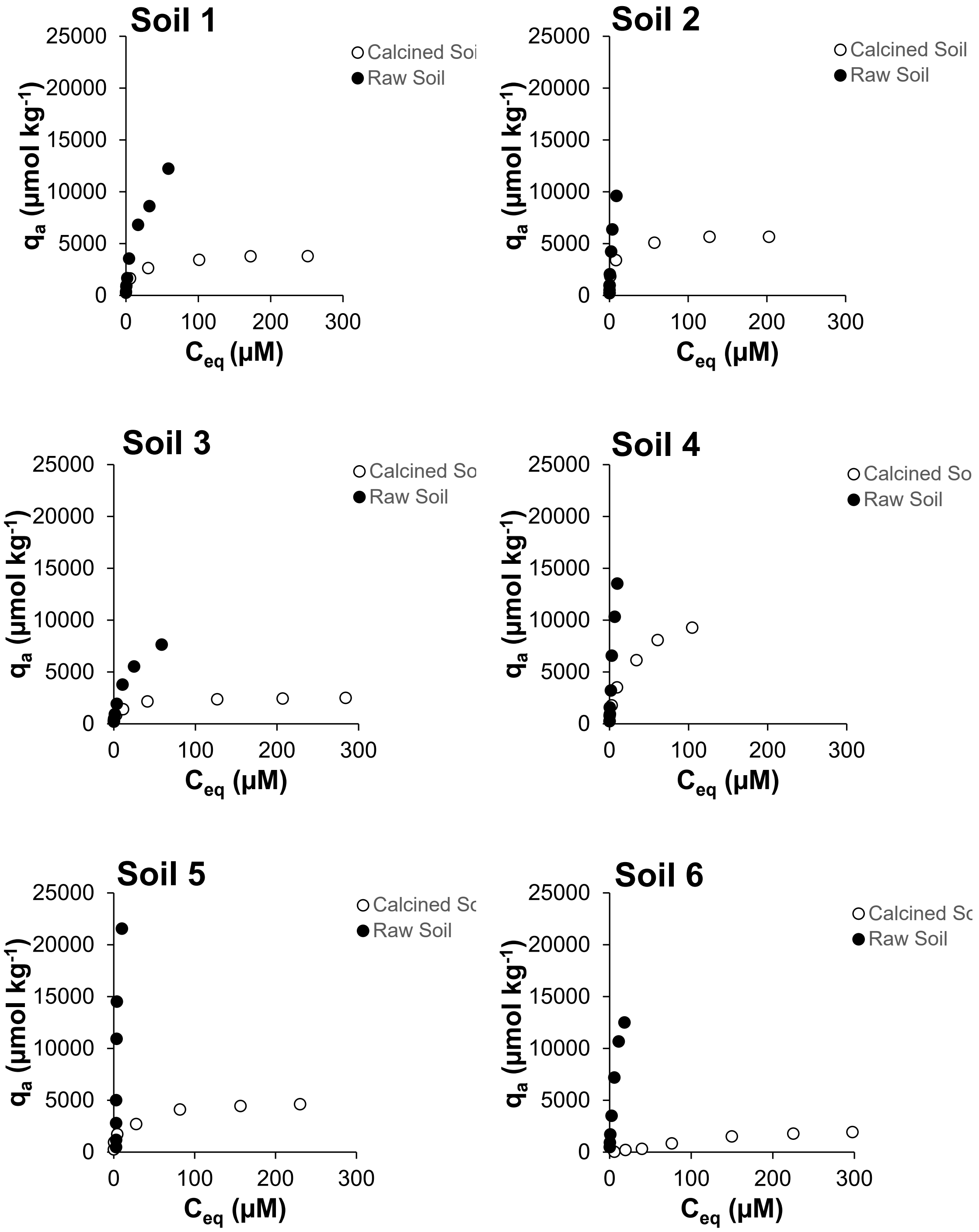

Fig. 2. Enrofloxacin (ENR) adsorption in the six soil samples studied. Average values (n = 3), with coefficients of variation always <10%. q_a: ENR adsorbed; C_{eq}: ENR in the equilibrium

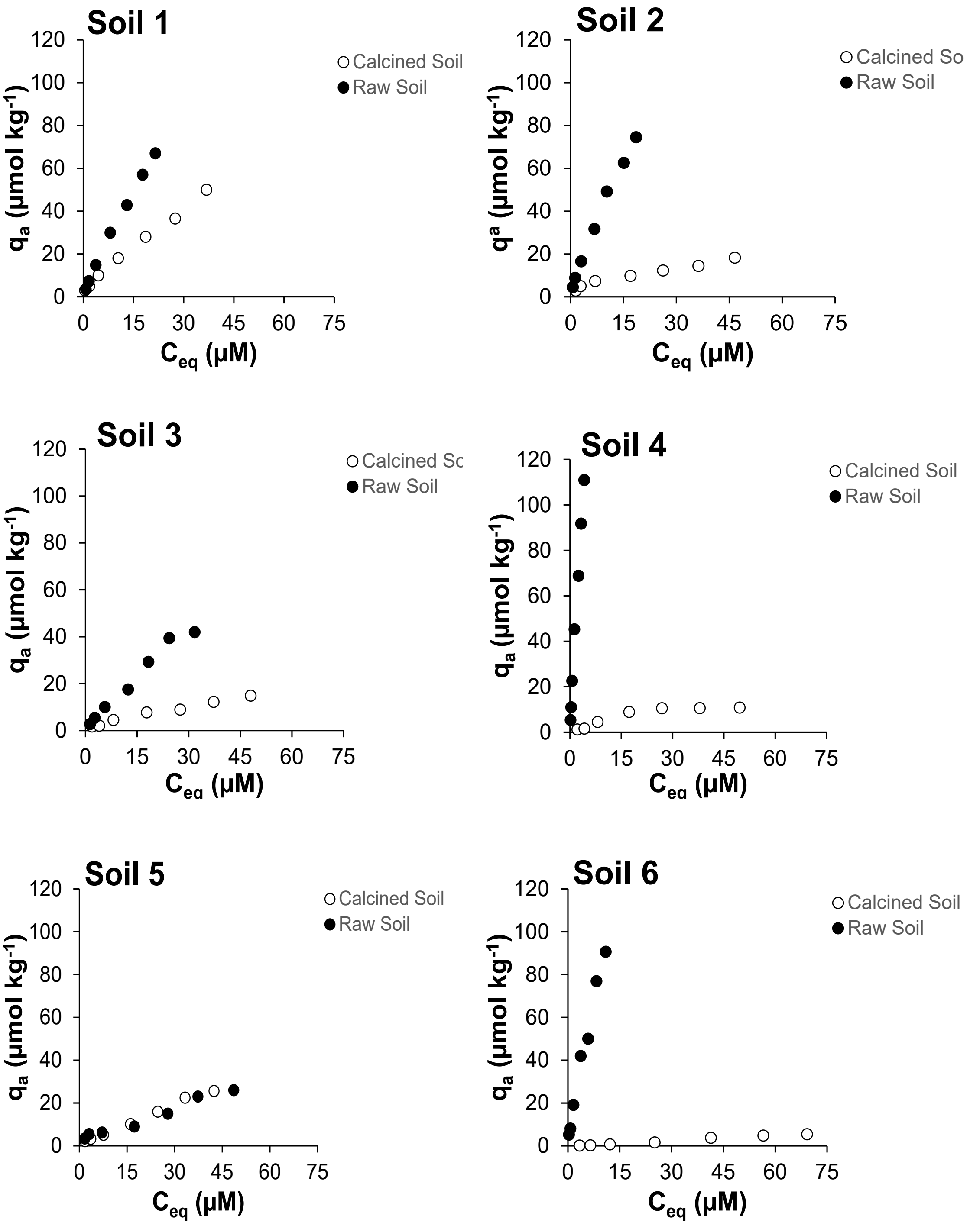

Fig. 3. Sulfamethoxypyridazine (SMP) adsorption in the six soil samples studied. Average values (n = 3), with coefficients of variation always <10%. q_a: SMP adsorbed; C_{eq}: SMP in equilibrium