

Sorption of Sulfonamides and Tetracyclines to Montmorillonite Clay

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Abstract The current study investigated the sorption of sulfadimethoxine (SMT), sulfamethoxazole (SMX), tetracycline (TET), and oxytetracycline (OTC) to Na-rich montmorillonite clay in synthetic effluent (SE) and field wastewater effluent (FE). Both SMT and SMX showed a low sorption capacity and are therefore likely to be highly mobile in the environment, while the sorption of TET to clay in environmental pH range (6.5–7.5) showed similarly high adsorption capacity. Differences in sorption capacities of TET and OTC to SE or FE were attributed to the various concentrations of divalent cations in the effluents. In addition, differences in sorption of OTC or TET to SE were attributed to their different molecular structure. Moreover, the adsorption of TET in SE and FE showed linear adsorption isotherms and fitted to Freundlich model.

Further experiments showed that addition of humic acid or SE to TET sorbed to clay did not enhance or suppress the sorption of TET to clay.

Keywords Sorption · Montmorillonite clay · Antibiotics · Pharmaceutical contaminants · Wastewater effluent

1 Introduction

Pharmaceuticals in the environment are becoming a subject of worldwide concern. Recent studies have shown that substances of antibiotic origin are only partially eliminated in wastewater treatment plants (WWTPs; Zwiener and Frimmel 2000; Karthikeyan and Meyer 2006; Heberer 2002). Some of the antibiotic residues will accumulate on activated sludge, while others will be found in the liquid phase (wastewater effluent) depending on the different characteristics of each compound.

According to the Israeli Ministry of Environment Protection, about 500 million cubic meters of wastewater effluent is produced in Israel per year. The wastewater effluent is used for different applications such as agricultural irrigation and stream rehabilitation and artificially recharged to groundwater. The different applications of wastewater effluent in the environment may result in the occurrence of antibiotic residues that will accumulate in soil or infiltrate to groundwater.

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Some of the major concerns from the presence of antibiotics in the environment involve possible elimination of trophic levels of bacteria in some ecosystems and the development of multiple drug-resistant bacteria that will flourish and make its way into the food chain. Other concerns involve for example the mobility, biodegradability, photolysis, stability, and sorption capacity of antibiotics in the environment and in soil matrix. In particular, the extent of sorption is affected by soil characteristics, ambient environmental conditions (pH, temperature, aqueous ionic strength, dissolved organic matter), and the physicochemical parameters of the antibiotic itself.

The exposures of antibiotic residues to the environment in different pathways result in detectable trace concentrations in various aquatic environments in soil and sludge. In a survey for pharmaceutical residues in US streams, at 27% of 139 river waters sampled, antibiotics were detected at concentrations of up to 0.7 $\mu\text{g/L}$ (Kolpin et al. 2002). Sacher et al. (2001) reported the occurrence of sulfamethoxazole (up to 410 ng/L) and dehydroerythromycin (up to 49 ng/L) in shallow groundwater samples nearby artificial recharge plant in Baden-Wurttemberg, Germany. Furthermore, sulfamethoxazole and sulfamethazine have been detected at low concentrations in a few shallow groundwater samples in the USA and Germany (Hirsch et al. 1999; Lindsey et al. 2001).

Several studies determined the occurrence of antibiotics in different water streams at the WWTPs. Hirsch et al. (1999) reported the highest concentration in wastewater effluent for erythromycin degradation product that reached a concentration of 6.0 $\mu\text{g/L}$. Golet et al. (2001) analyzed fluoroquinolone antibiotics in primary and tertiary wastewater effluent in Switzerland. In these samples, ciprofloxacin and norfloxacin occurred at concentrations between 249 and 405 ng/L and from 45 to 120 ng/L, respectively.

Some of the antibiotic tend to accumulate in the soil and will not reach groundwater. The persistence of antibiotic residues in sediment or soil mostly depends on a number of parameters as such as sorption capability, degradation rate, and mobility in water. In soils under conventional land farming fertilized with manure and monitored for 2 years, average concentrations of up to 199 $\mu\text{g/kg}$ tetracycline and 7 $\mu\text{g/kg}$ chlortetracycline were detected (Hamscher et al. 2002). Other survey carried out in northwestern Germany detected the occurrence of

various antibiotic residues in the top 0- to 30-cm surface of sandy soils fertilized with liquid manure (Hamscher et al. 2005). The maximum concentrations reported for the compounds screened in this study were 27 $\mu\text{g/kg}$ (oxytetracycline), 443 $\mu\text{g/kg}$ (tetracycline), and 4.5 $\mu\text{g/kg}$ (sulfamethazine).

Various studies concentrated on the sorption of antibiotic onto clays, as clay minerals imbedded within soils are well known for their ability to modulate organic molecules delivery in soils. Studies of the mobility and sorption of sulfonamides, tetracyclines, and fluoroquinolones have determined that sorption rates are highly dependent on soil particle size, pH conditions, and the presence of other ions in addition to the polarity and binding strength of the compounds (Thiele-Bruhn et al. 2004; Figueroa et al. 2004; Golet et al. 2003; Kulshrestha et al. 2004; Parolo et al. 2008). Sithole and Guy (1987) characterized the adsorption of tetracycline to clay by two processes of different kinetics that can be interpreted as a fast initial adsorption to outer surfaces, followed by a penetration into interlayer of clay minerals and micropores.

The main goal of the current study was to determine the sorption of selected antibiotics (sulfadimethoxine (SMT), sulfamethoxazole (SMX), tetracycline (TET), and oxytetracycline (OTC) to Na-rich montmorillonite clay in synthetic effluent and field wastewater effluent (originated from a WWTP). The montmorillonite mineral is widespread in Israel soils and is imbedded within the aquifer rocks; thus, it has a major impact upon the chemical fate and transport of organic contaminants through sediments of aquifer rocks. The study is focused on the sorption of antibiotics to clay since clay minerals imbedded within soils were found to take an important role in the transport of antibiotic residues in the environment.

2 Materials and Methods

2.1 Chemicals and Standards

Antibiotic analytical standards of SMT, SMX, TET, and OTC were obtained from Sigma-Aldrich (Israel). Humic acid (HA) was obtained from Sigma-Aldrich (Israel). All experiment solutions were prepared using deionized water (DI; Millipore Direct Q3 water purification system). All reagents used were analytical

reagent grade or higher purity, and solvents (ethanol, acetonitrile, and methanol) were high-performance chromatography (HPLC) and ultra liquid chromatography/mass spectrometry (ULC/MS) grade (water with purity higher than HPLC grade) obtained from Bio-Lab Ltd. (Jerusalem, Israel). Na-rich montmorillonite (SWy-2) was purchased from the Clay Mineral Society (USA).

2.2 Clay Suspension

Montmorillonite, the clay mineral used in the current study, is a member of the smectite family and characterized by a 2:1 layer structure. In addition to the unique structure, montmorillonite has a particularly large surface area (N_2 area; $32 \text{ m}^2/\text{g}$). Clay (1.5 g) was added slowly to 100 mL of ULC/MS water and properly stirred. The suspensions were left in 100-mL measuring cylinders for 72 h for size fractionation and sedimentation. After sedimentation, the upper layer consisting of the fine lamella layer was discharged, and the medium layer was collected into 50-mL centrifuge tubes and centrifuged at 3600 rpm for 35 min (IEC-HN-SII Damon centrifuge). The supernatant solution was removed and properly mixed with 10 mL of ULC/MS water then stored at 4°C .

2.3 Synthetic and Field Effluent

Synthetic effluent (SE) that represents biologically treated wastewater effluent used in this study was adopted from Smith et al. (2005). Representative constituents of the synthetic wastewater are as follows: COD 65 mg/L, TOC 13–30 mg/L (estimated ratio COD/TOC; Metcalf and Eddy 2004), and EC 90 $\mu\text{S}/\text{cm}$. Wastewater field effluent (FE) was collected from Kolchey Ha'Sharon wastewater treatment plant. The wastewater treatment included primary treatment (screening and sedimentation) and secondary treatment (activated sludge). The FE for the sorption experiment was collected from the secondary sedimentation area. In situ measurements included pH, conductivity, and temperature using IQ170-Scientific instruments. Afterwards, the sample was transferred under cooling to the hydrochemistry laboratory, Tel-Aviv University. Prior to refrigerator storage (at 4°C), the wastewater effluent samples were filtrated using a 0.22- μm stericup GP filter. Repre-

sentative constituents of the field wastewater effluent are as follows: COD 30 mg/L, TOC 6–15 mg/L (estimated ratio COD/TOC; Metcalf and Eddy 2004), and EC 1.27 mS/cm.

2.4 Sorption Experiments

Sorption of antibiotics by montmorillonite to both SE and FE was determined by a batch technique and in accordance with the OECD Test Guideline 106. A total of 50 mL solution containing different aqueous types and 1.8–180 mg/mL montmorillonite solution were added to 250 mL Erlenmeyer's and were shaken on an orbital shaker (Lab-line 3527-1 Environ shaker equipped chiller to control the temperatures) at 150 rpm for 12 h at constant temperature of 20°C to reach pre-equilibrium. After pre-equilibrium, the solution was spiked with antibiotic to reach a final concentration of 0.5 $\mu\text{g}/\text{mL}$ and shaken for additional 24 h until equilibrium achieved. All samples were kept in dark in order to avoid antibiotic photodegradation. After equilibrium, the solutions were filtered using 0.2- μm syringe filters and analyzed. Blank samples containing equivalent clay concentration and total aqueous solution volume of 50 mL (without antibiotic) was subjected to the same experiment procedure. This sample served as a background control during the analysis to detect interfering compounds or contamination originated from the clay and the aqueous solution.

2.5 Sorption to Clay and HS

The experimental procedure was as follows: samples with 18 mg/mL montmorillonite clay in water were spiked with TET to reach a final concentration of 0.5 $\mu\text{g}/\text{mL}$ and further shaken for 12 h to reach pre-equilibrium. After pre-equilibrium, concentrated SE solution was added to the sample to achieve final concentration (similar to previous experiment of adsorption of SA and TC to clay) and shaken for additional 24 h.

2.6 Analytical Analysis

Separation and detection of the antibiotics in the aqueous phases were achieved using (1) HPLC-UV/MS-MS (Agilent 1100 Series) equipped with a vacuum solvent degassing unit, a low-pressure gradient mixing, an automatic sample injector, a thermo-

static column compartment, and a UV diode array detector (DAD) and (2) a Finnigan LCQ ion trap mass spectrometer equipped with a heated capillary interface and electrospray ionization source. HPLC-UV-DAD analytical methods for detection of the selected antibiotic were developed. The sulfonamides were separated using an ACE C18 silica-based column, 250 mm, 2.1 mm, and 5 μm in combination with a guard column (2.1 and 4 mm) of the same type. The UV wavelength was monitored at a wavelength range of 260 and 280 nm. The mobile phase consisted of a multistep gradient combining (a) 0.1% formic acid (FA) in ULC/MS water (pH 3.4) and (b) 0.1% FA in acetonitrile (AcN). For the tetracyclines, the UV wavelength was monitored at a wavelength range of 268 and 360 nm. The selected LC column was a C8 column (ACE5, 250 and 2.1 mm) in combination with a guard column (2.1 and 4 mm) of the same type. The mobile phase consisted of a multi-step gradient combining (a) 0.1% FA in ULC/MS water (pH 2.3) and (b) 0.1% FA in AcN.

MS analytical methods were developed for the detection of the selected antibiotics. For the sulfonamide antibiotics, the instrument was operated in positive ion mode and the probe temperature set to 220°C. The flow from the HPLC was passed through a split connector with 60 $\mu\text{L}/\text{min}$ introduced into the MS interface. Ions in the range $m/z=85\text{--}400$ were registered in the conventional scanning mode. For the tetracycline antibiotics, the instrument was operated in positive ion mode and the probe temperature set to 220°C. Ions in full range of $m/z=85\text{--}500$ were registered in the conventional scanning mode. The product ion producing the highest intensity was used with SRM (Selected Reaction Monitoring) technique for quantification and to improve the signal–noise ratio in the MS chromatogram. Instrument control and data quantification were performed with ThermoQuest Xcalibur software version 3.1.

2.7 Data Presentation

The antibiotic sorption capacity, C_S (mmol/kg), was calculated by Eq. 1:

$$C_S = (C_{\text{in}} - C_{\text{eq}}) \times \frac{V}{m} \quad (1)$$

where C_{in} and C_{eq} (mmol/L) are the initial and equilibrium aqueous antibiotic concentrations, respec-

tively, V (L) is the volume of solution, and m (kg) is the mass of the clay.

The solid–water distribution coefficient (K_d , L/kg) is represented by Eq. 2. The coefficient is defined as the ratio between the concentration of the sorbate (C_S , mmol/kg) and the sorbent (C_{eq} , mmol/L).

$$K_d = \frac{C_S}{C_{\text{eq}}} \quad (2)$$

Most studies dealing with the adsorption of pharmaceuticals found the Freundlich model as the best fitting model (Eq. 3). The Freundlich model assumes that there are multiple types of adsorption sites that are acting in parallel.

$$C_S = K_F \times C_{\text{eq}}^n \quad (3)$$

where K_F is the Freundlich sorption coefficient which provides estimations of the absorbent capacity and n the linearity parameter which provides estimations of the adsorption intensity (Eq. 4). Those parameters can be obtained from the slope (n) and the intercept (K_F) of the logarithmic equation. When $n=1$, the isotherm is linear, the sorption is constant and indicates free energies at all sorbate concentrations; when $n<1$, the isotherm is concaved downward, indicating that added sorbates are bounded with weakening free energies (Schwarzenbach et al. 2002).

The percent of antibiotic sorbed to clay is calculated according to Eq. 4.

$$\% \text{ sorbed} = \frac{C_{\text{in}} - C_{\text{eq}}}{C_{\text{in}}} \times 100 = \frac{C_S}{C_{\text{in}}} \times 100. \quad (4)$$

2.8 Statistical Analysis

All main experiments were conducted in triplicates. The percentage of antibiotic adsorbed to the clay is presented by the averages, and standard deviation was performed on the repetitions. In addition, analysis of covariance was used to capture the interaction of the regression slope with a main effect such as antibiotic concentration with each sorption treatment. In this study, this method is accomplished by introducing the interaction between $\log q$ of antibiotic and $\log C_{\text{eq}}$ with each treatment (water type) which is the main effect.

2.9 Selected Antibiotics

Representative antibiotics from two major antibiotic classes have been selected in this research study:

Table 1 Representative physicochemical properties of TC and SA (Thiele-Bruhn 2003)

Compound class	Water solubility (mg/L)	$\log K_{ow}$	pK_a
Tetracyclines	230–52,000 Chlortetracycline, oxytetracycline, tetracycline	–1.3 up to 0.05	3.3/7.7/9.3
Sulfonamides	7.5–1,500 Sulfanilamide, sulfadiazine, sulfadimidine, sulfadimethoxine, sulfapyridine, sulfamethoxazole	–0.1 up to 2.5	2–3/4.5–10.6

sulfonamide (SA) and tetracycline (TC). For each antibiotic class, two specific compounds were chosen as representatives of the whole group: SMX and SMT from the SA and TET and OTC for the TC. The chemistry of sulfonamides consists of an aniline group and a sulfonamide group ($-\text{SO}_2\text{NH}_2$). The amine and sulfonamide groups must be para to one another. Only few sulfonamide derivatives are still in clinical use because resistance to these drugs has become widespread. The tetracycline molecular structure is derived from a system of four linearly annelated six-membered rings with a characteristic arrangement of double bonds. The TCs form chelate complexes with divalent metal ions and β -diketones and strongly bind to proteins and silanolic groups (Oka and Matsumoto 2000).

Antibiotics of different classes vary significantly in their molecular structure and physicochemical properties as presented in Table 1. Some substances as the TC are relatively hydrophobic, whereas other compounds as the SA are considerably hydrophilic and polar.

The $\log K_{ow}$ (octanol–water partition coefficient) is a measure of the equilibrium concentration of a compound between octanol and water that indicates the potential for partitioning into soil organic matter. Some investigators have expressed doubts over the usefulness of the method to predict the partitioning behavior of pharmaceuticals using the compounds $\log K_{ow}$ value (Tolls 2001; Loke et al. 2002). Loke et al. (2002) observed much higher sorption of oxytetra-

cycline to manure than anticipated only according to the negative $\log K_{ow}$ (–1.12) value of the compound.

The K_a value is a value used to describe the tendency of compounds or ions to dissociate. The pK_a is the dissociation constant and is derived from the K_a value. TCs are characterized by 3 pK_a values, whereas the SA is characterized by only 2 pK_a values.

Figure 1 presents the different species of SMX in varying pH levels. In acidic condition at pH below 1.39, it exists as a cation (1), between pH 1.39 and 5.8, the molecule is characterized by neutral charge (2), and above pH 5.8, SMX is negatively charged (3). Similarly, as can be seen in Fig. 2, tetracycline exists predominantly as a cation, +00, below pH 3.5 when the dimethylammonium group is protonated (1), as a zwitterion, +–0, between pH 3.5 and 7.5 resulting from the loss of proton from the phenolic diketone moiety (2), and as an anion, + – –(3) or 0– –, from the loss of protons from the tricarbonyl system and phenolic diketone moiety above pH 7.5.

3 Results and Discussion

3.1 pH and Ionic Strength Effect on Absorption of SA and TC

The adsorption behavior of two selected sulfonamide antibiotics to clay was primarily investigated by varying pH and ionic strength conditions. The effects of pH on the adsorption of SMX and SMT to clay

Fig. 1 Different forms of the sulfamethoxazole antibiotic in increasing pH

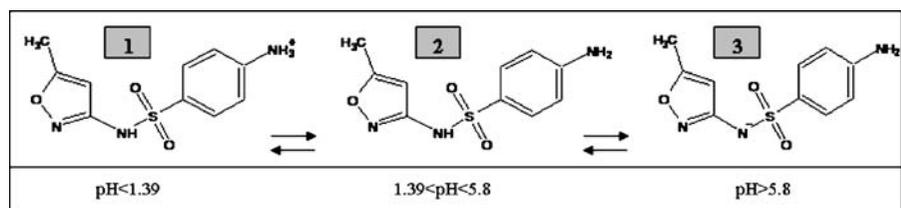
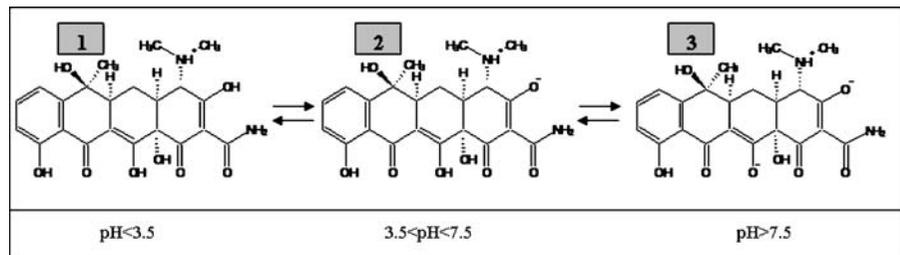


Fig. 2 Different forms of the tetracycline antibiotic in increasing pH



were determined in batch sorption experiments which were conducted using DI water at pH 2, 4, 6, and 7. The clay and antibiotic concentrations were 0.96 mg/mL (~0.1%) and 1 µg/mL, respectively.

The partitioning behavior of pharmaceuticals in the environment is likely to be dictated by a number of physicochemical parameters (Rabolle and Spliid 2000). The distribution coefficients K_d (Eq. 2) for SMT decreased as pH increased from a value of 0.91 to 0.07 L/kg, while for SMX, the distribution coefficients vary to a lesser extent at values of 0.31–0.085 L/kg. The isoelectric point (pI) of SMX and SMT is around pH 4.5, which means that at this specific pH, the molecules carry no net electrical charge. Below the pI , both molecules carry a net positive charge, and above, it the molecules carry a net negative charge. The montmorillonite surfaces are negatively charged, and therefore, the distribution coefficients of SA is expected to decrease with increasing pH, as both molecules and clay carry a net negative charge. These results are supported by studies that found SA as weakly absorbed to mineral soil colloids (Thiele-Bruhn et al. 2004) and that indicted pH as a crucial factor affecting the interaction of SA with soil (Thiele 2000; Boxall et al. 2002). The presented K_d values obtained for SMX and SMT in this study are in good agreement with partitioning coefficients reported for other sulfonamides. Boxall et al. (2002) reported for sulfachloropyridazine $K_d = 0.9$ –1.81 L/kg in soil and soil/slurry mixtures. Langhammer (1989) reported for sulfamethazine $K_d = 0.6$ –3.2 L/kg for different soils detected. The K_d values obtained were low and indicate that both antibiotics are anticipated to be mobile in soil and that after application are expected to enter aquatic environments. Similar effect of pH was observed by Boxall et al. (2002). The study detected the sorption and transport of a SA in two soil systems and indicated that sorption coefficients for sulfachloropyridazine in both soil systems increased as pH

decreased. It should be stated that in some papers, the authors refer to the K_d coefficient as normalized to the content carbon fraction. To summarize, the distribution coefficient was found to be pH-dependent and decreased with increasing pH.

The effect of solution ionic strength on sorption of the antibiotic to clay was evaluated at 0.1 and 0.01 M NaCl. The percent of SA sorbed to clay decreased with increasing ionic strength from 29% at 0.01 M to 14% at 0.1 M for SMX and from 14% at 0.01 M to 10% at 0.1 M for SMT. These results indicate a competition between SA species and Na⁺ cations for attractive surface sites. At higher electrolyte concentration, the Na⁺ cation concentration is high enough to compete with SA species and occupy surface sites decreasing the adsorbed amount of SA. On the contrary, when the NaCl concentration decreases, Na⁺ cations are no longer important competitors, and thus, the relatively higher adsorption of SA is reached. Ter Laak et al. (2006) described that the observed decreased sorption of tylosin and oxytetracycline with increasing ionic strength resulted from a competition of the electrolyte cations with the positively charged tylosin species and the positively charged oxytetracycline complexes.

To summarize, the ionic strength was also found to affect the adsorption, and higher adsorption capacity was observed in a lower ionic strength solution. While changes in soil pH and ionic strength affect the adsorption capacity of the SA, the observed effects were small, and under environmental conditions, the adsorption capacity of SMX and SMT to clay is expected to be low.

The TC antibiotic group is characterized by three different moieties of the molecule that can undergo protonation–deprotonation reactions and the possibility to adopt different ionic species and conformations depending on the pH of the aqueous solution in which TC is dissolved (Parolo et al. 2008). The influence of pH on the adsorption of TC antibiotic

was investigated. Several studies (Kulshrestha et al. 2004; Parolo et al. 2008) indicated a strong correlation between pH and the sorption capacity of TC. This study assessed the sorption capacity of TET to clay in varying environment pH values of 6.5, 7, and 7.5. The results show that at clay concentration of 1.8 mg/mL, the sorption was similar in all pH values and reached 93–94%.

Parolo et al. (2008) described a Patagonian montmorillonite that appeared as a good adsorbent of TC. Maximum adsorption detected was at pH 2–4; thus, the sorption capacity of this clay at pH 5–7 was still described as rather high. Kulshrestha et al. (2004) observed that the adsorption of OTC in two selected montmorillonite clays decreased with increasing pH in the order pH 1.5 > 5.0 > 8.7 > 11. This correlation was attributed to the cationic exchange interactions that are dominant at lower pH values when OTC is positively charge.

3.2 Adsorption of SA and TC to Clay

Figure 3 presents the sorption to clay in either SE or FE solution (pH 7.2) of SMX, SMT, TET, and OTC, respectively. It is important to note that SA and TC belong to different compound classes and the chemical composition of FE and SE also differ, which may impact the sorption of these molecules to clay.

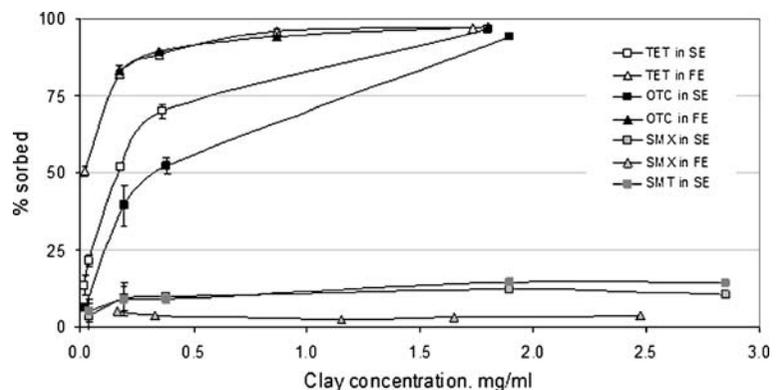
The percentage of antibiotic sorbed was calculated in accordance with Eq. 4. A difference was not observed between SMX and SMT in SE; however, the sorption of SMX in SE was higher than SMX in FE. The wastewater field effluent samples, which were collected from a wastewater treatment plant, contains a range of components such as cations, metals, salts, chlorides, dissolved organic matter, etc. Some of these

components may compete with the antibiotic molecules on available sorption sites on the negatively charged clay surfaces, which may result in lower sorption of SMX to FE compared to SE. The sorption of SA group is low at 0.038 mg/mL clay (5% sorption) and remains almost constant at a value of approximately 15% sorption above 0.2 mg/mL clay. Adsorption experiments in SE solution showed limited adsorption capacity at low clay concentration, which increased with increasing clay concentration. Adsorption experiments in FE present different results; first, the adsorption remained around 5% even with increasing clay concentrations. This may indicate that the presence of constituents of the FE suppressed the adsorption of SMX to clay.

At very small concentration of clay (0.01 mg/mL), the sorption of OTC and TET to FE was 47% and reached saturation at about 0.8 mg/mL of clay, while the sorption of OTC and TET to SE was 13% and 24% at low clay concentration (respectively) and increased steadily. For both water types at clay concentration higher than 1.8 mg/mL, the adsorption capacity was high and reached above 90%.

No difference between OTC and TET to FE was observed, suggesting that constituents of the FE solution did not affect the sorption of TC molecules to the montmorillonite clay as opposed to SE. Generally, ortho-substituted anionic and cationic groups on a zwitterionic molecule may result in undesirable repulsive interactions between the anionic moiety and the negatively charged surface of montmorillonite and disturbed sorption (Carrasquillo et al. 2008). However, both TET and OTC are zwitterions sorbed to a greater extent onto clay amended with FE compared to SE, possibly due to the greater concentration of divalent cations of the FE compared to SE.

Fig. 3 Sorption to clay in either SE or FE solution (pH 7.2) of SMX, SMT, TET, and OTC, respectively. Error bars represent the \pm standard deviation (STD)



For example, the concentration of Ca^{+2} and Mg^{+2} in FE is 88 and 24 mg/L, respectively, compared to less than 5 mg/L for both cations in SE. Both compounds function as chelating agents and form a bond with a single metal ion with the negative charge of the TC molecules. Metal bridging can only occur for pharmaceutical compounds that have functional groups that can complex with metal ions in solution. TCs are known to complex with divalent and trivalent cations. One positive charge of the divalent metal cation bonds with the carboxylic residue of the TC molecule while the other attracts to the negatively charged clay (cation bridging). It is likely that the structural suitability of TCs for surface complexation allowed for binding to montmorillonite and resulted in a higher extent of sorption.

The difference in sorption of OTC and TET to montmorillonite clay with SE solution can be explained by their molecular structure. The presence of adjacent hydroxyl group on the OTC can cause steric hindrance (Fig. 4) to the cationic amine residue,

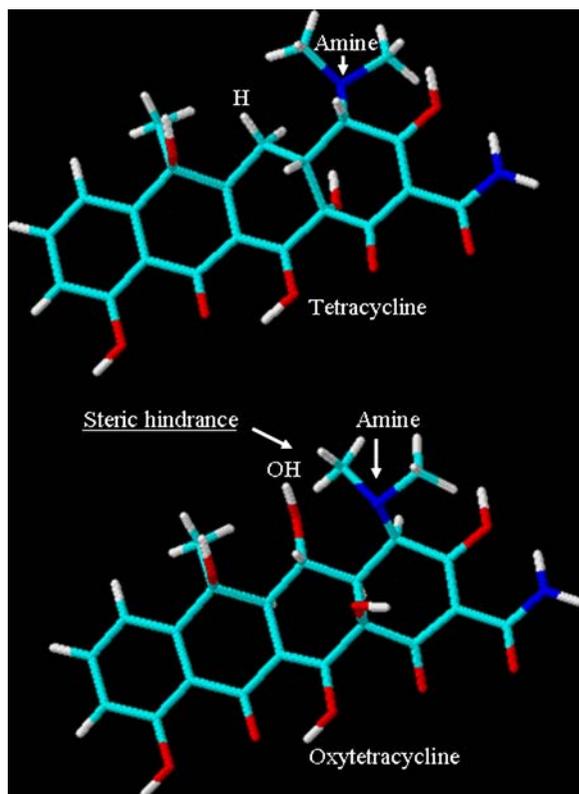


Fig. 4 TET and OTC molecules. The presence of adjacent hydroxyl molecule on the OTC can cause steric hindrance to the cationic amine residue

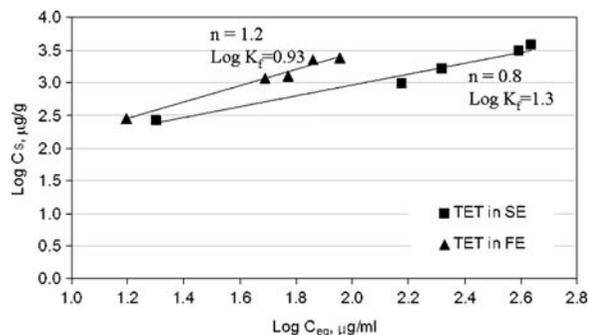


Fig. 5 Adsorption isotherms fitted to a Freundlich equation

which weakens its capability to interact with the clay and, hence, decrease sorption. In addition, the OH residue of the OTC is more electronegative than the hydrogen residue of the TET, which is expressed by lower pK_a in the OTC amine group than the pK_a value in the TET amine group. Thus, the OTC may decrease its sorption capability related to TET (Fig. 3).

3.3 Adsorption Isotherms

Generating Freundlich isotherms for SMX and SMT to clay was not relevant because of the low sorption of the sulfonamides to clay. In comparison to the sulfonamides, the TET showed linear sorption isotherms for both water types SE and FE when fitted to Freundlich model (Fig. 5). The differences between sorption of tetracycline to clay at both water types SE and FE were statistically significant, as the P value for the interaction is smaller than the significance level of 0.05.

In this study, it was assumed that the difference in sorption of TET to clay in different waters was due to the higher concentration of Ca^{+2} and Mg^{+2} in FE as detailed in the previous section. Differently, others observed that the mobility of tetracycline antibiotics may be increased or decreased by the presence of dissolved organic matter depending on the amount and pH of the medium which affects the sorption capacity (Kulshrestha et al. 2004). In addition, Thiele (2000) studied the adsorption of sulfapyridine antibiotic with various compositions of soil organic matter (SOM) and *p*-aminobenzoic acid in two loess chernozem soil samples. Freundlich coefficients indicated that differences in absorption were due to difference in quantity and composition of SOM, soil moisture, and the molecular structure of the antibiotics.

In this study, low K_F and n values are indicative of a relatively low adsorption capacity and weak

bonding (Schwarzenbach et al. 2002). Figure 5 presents values of Freundlich constants K_F and n which provide estimates of the adsorbent capacity and the adsorption intensity, respectively. Others reported $\log K_F$ values of 2.47–2.83 and n values ranging between 0.05 and 0.52 for the adsorption isotherms of OTC adsorbed in native montmorillonite at pH 1.5, 5.0, 8.7, and 11.0. Values of $n < 1$ indicate a situation in which at higher OTC concentrations, it becomes more difficult to adsorb additional molecules on the clay as binding sites become saturated (Kulshrestha et al. 2004). In the current study, the obtained n values for FE were slightly higher than unity, indicating that the isotherms convex upward, while n values for SE were slightly lower than unity ($n < 1$), indicating that the clay available sorption sites were saturated. Similarly, Boxall et al. (2002) presented isotherms fitting the Freundlich equation for sulfachloropyridazine in clay loam and in sandy loam. The n values obtained were 0.97 and 0.91 for clay loam and the sandy loam, respectively. Since the values of n were close to unity, sorption can be reasonably approximated with a linear sorption coefficient K_d (Eq. 2).

3.4 Sorption to Clay and HS

Decomposition of plants, animals, and microbial material in soil and water produces a variety of complex organic molecules, collectively called natural organic matter (NOM; Karthikeyan and Meyer 2006). Several studies (McCarthy and Zachara 1989; Murphy et al. 1994) found that the presence of NOM in soil, especially in high concentrations, can facilitate organic contaminant transport in soil and groundwater, thereby

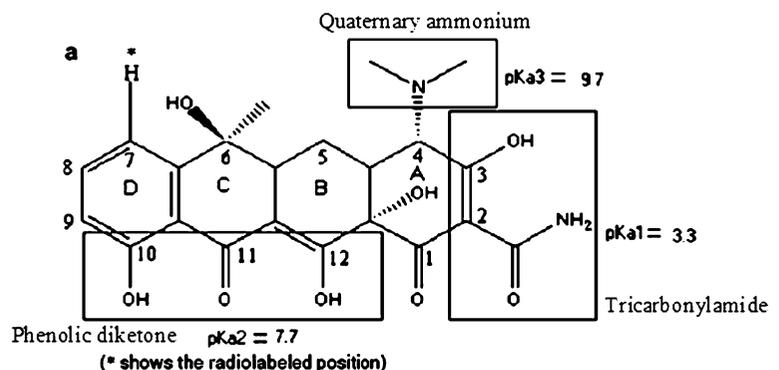
increasing their subsurface mobility. Humic substances (HS), derived from the decomposition of plant, animal, and microbial biomolecules (Sutton and Sposito 2005), is an element of NOM that can contribute as a sorbent phase for organic contaminants in soils (Schwarzenbach et al. 2002; Essington 2004). The HS are complex aromatic macromolecules with numerous acidic groups, polysaccharides, fatty acids, and long aliphatic side chains (Schulten and Schnitzer 1997).

The TCs are amphoteric molecules having multiple ionizable functional groups (Sassman and Lee 2005). TET acquire tricarbonylamide (C-1:C-2:C-3), phenolic diketone (C-10:C-11:C-12), and dimethylamine (C-4) moieties (Fig. 6). In addition, TET is characterized by three acid dissociation constants ($pK_a=3.3$, 7.6, and 9.6) and exists as a cationic, zwitterionic, and anionic species under acidic, neutral, and alkaline conditions (Gu et al. 2007).

Because of the pH-dependent speciation of the TC molecules, several potential mechanisms for interaction between TC, clay, and HS exist:

1. Positively charged quaternary ammonium functional group of TC may interact with negatively charged carboxylate groups sites on the HS ($pK_a \sim 4$) via cation exchange.
2. Basic amine moieties of TC will be strongly adsorbed by acidic moieties of soil organic matter (i.e., HS) and expanding lattice clay minerals; sulfonamide group acidic moiety of SA will be adsorbed in moderate amounts on organic matter and in relatively low amounts on clay minerals and hydrous metallic oxides.
3. Negatively charge phenolic diketone moiety of TC may interact with negatively charge sites via cation bridging.

Fig. 6 TET structure (Gu et al. 2007)



4. Hydrogen bonding between polar TC groups and acidic groups on clay and HS surfaces may also contribute to the sorption of TC.
5. Charged and polar functional groups on HS may interact directly with negatively charged sites on clay surfaces, thereby masking sites that would otherwise be available for interaction with functional groups of TC.
6. The negatively charged phenolic diketone moiety of TC may bind strongly to clay by interaction with divalent cations (e.g., Ca^{+2} , Mg^{+2}) which are bonded on the clay (creating chelates).
7. HS may block the access of TC molecule to the clay interlayer sorption sites.

The sorption of TET onto clay–NOM systems is complicated. An experiment was conducted to study the interaction of TET with clay and subsequent addition of SE or HA. Figure 7 presents the sequence setup of the experiment. It is important to emphasize that in environmental systems, it is not plausible that TET will first encounter clay and only afterwards the dissolved organic matter. Thus, the main purpose of this experiment was to isolate the impact of organics on the sorption capacity.

For all samples that contained clay (TET + clay + water, TET + clay + SE, TET + clay + HA), the sorption capacity reached 87–88%; thus, addition of SE or HA after pre-equilibrium did not enhance or suppress the sorption of TET to clay (Fig. 8). Additional comparable experiment which differed

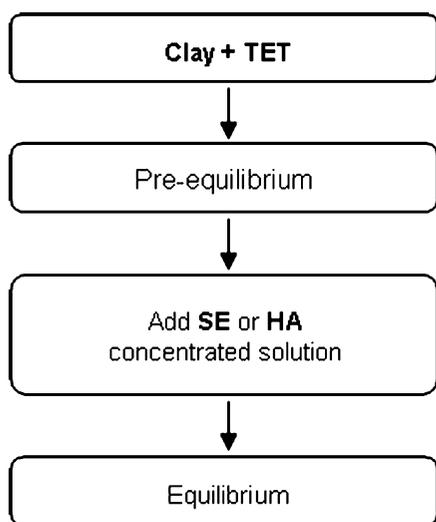


Fig. 7 Experiment sequence setup

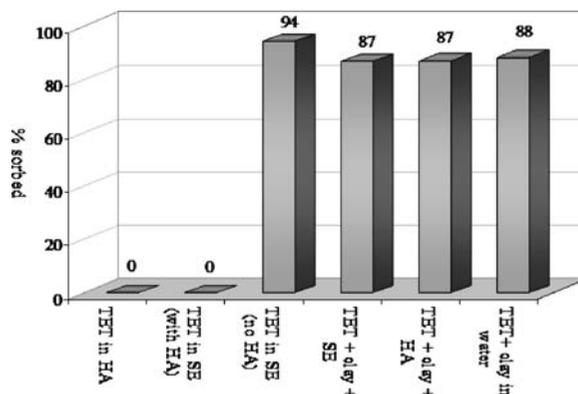


Fig. 8 Sorption of TET to clay in water, SE, and HA. Average values \pm STD for the TET clay samples in water with HA and with SE were 86.7 ± 3.5 , 87 ± 4 , and 88.2 ± 1.6 , respectively

only in the supplementation sequence of the different components resulted in similarly high sorption capacities of TET to clay. Hui et al. (2003), supporting these results, found that the presence of humic acid did not impact the sorption of pesticides by K^+ clays.

In samples void of clay that contained TET in HA or TET in SE (with or without HA), it was observed that when HA was in the system (either by itself or with SE), no sorption occurred (Fig. 8). The TET concentration in the aqueous phase with HA was identical to the concentration measured in the sample of TET in water. These results indicate that possibly, there was no interaction between the HA and the TET or, alternatively, that if interaction occurred, it was weak under HPLC constrained analysis conditions (pH 3). To confirm this hypothesis, an additional experiment (described subsequently) was investigated.

3.5 Interaction Between TET and HA

Our hypothesis was that TET and HA are expected to interact in water at pH 7 by ionic bonding. In order to confirm this hypothesis, an experiment was designed that included first reaching pre-equilibrium between TET and HA, followed by lyophilization (freeze and dry) of the samples, reconcentration of the precipitate with a limited volume of water, and then analysis of the aqueous phase by liquid chromatography–tandem mass spectrometry (LC-MS/MS). The main assumption was as follows: (a) if TET interacts with HA, then TET will remain in the precipitate with the HA or, alternatively, (b) if TET will not interact or interacts weakly with the HA, then TET should be

redissolved and should be detected by the LC-MS/MS. The results of the experiment showed that TET was fully detected in the aqueous phase, indicating that there was no interaction (or only weak interaction) between TET and HA. Moreover, Kulshrestha et al. (2004) found that the mobility of tetracycline antibiotics may be increased or decreased by the presence of dissolved organic matter depending on the amount and pH of the medium. Thus, an experiment at pH 11 showed that at lower concentration of HA (1 mg/L), increased sorption was observed, while a higher concentration of HA (10 mg/L) resulted in an opposite effect. This phenomenon was explained by hydrophobic partitioning of OTC between clay-associated HA and “free” HA in the solution phase.

4 Conclusions

Sulfonamide antibiotics showed increased sorption capacities at lower pH values (2–7). In environmental pH conditions, the adsorption capacity of SMX and SMT to clay is expected to be negligible. The obtained distribution coefficients were relatively low, indicating that both SMX and SMT will be highly mobile in soil and may reach aquatic environments. Indeed, these molecules were detected in groundwater aquifer located beneath an agricultural field (Avisar et al. 2009a). In comparison, the adsorption of OTC and TET to clay was high under environmental conditions, suggesting that this group of antibiotics will accumulate in soil and will be less mobile. In another study, these molecules were detected in a groundwater well located nearby a fishpond which was used as a point source for antibiotic (OTC) disposal. Due to the specific hydrological setting which occurred beneath the pond (saturated preferential flow paths), OTC rapidly leaked to the local groundwater and did not sorb to the sediment as was expected (Avisar et al. 2009b).

Tetracyclines are molecules that exist as cations, anions, and mainly zwitterions at environmentally relevant pH values, and as a result, their adsorption is demonstrated to be highly dependent on pH (Sassman and Lee 2005). In the current study, the sorption of tetracycline in environmental pH range (6.5, 7, and 7.5) was investigated and shows similarly high sorption capacities at all pH values. Sorption of TET and OTC to clay in FE was relatively high even at low clay concentrations. However, at SE, the sorption

of TET and OTC at similar low clay concentrations was lower than FE. This difference, observed in low clay concentrations (<0.17 mg/mL clay), was attributed to the higher concentration of divalent cations in FE. Moreover, the difference in sorption of OTC and TET to montmorillonite clay with SE solution can be explained by steric hindrance.

The sorption of TET in both SE and FE in the different experiments showed linear adsorption isotherms and fitted to Freundlich model. Additional set of experiments showed that addition of HA or SE to TET sorbed to clay did not enhance or suppress the sorption of TET to clay.

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