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Joint antibacterial activity of soil-adsorbed antibiotics trimethoprim and sulfamethazine

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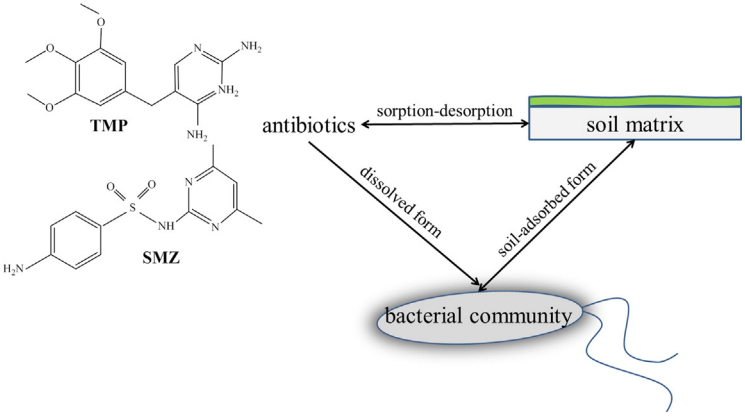
HIGHLIGHTS

- Sorption and antibacterial activity of TMP and SMZ in three soils were investigated.
- Co-solute sorption of TMP and SMZ was not different from the single solute sorption.
- The soil pH, CEC and OM are important factors affecting sorption of TMP and SMZ.
- Soil-adsorbed TMP still retained antibacterial activity.
- Co-presence of SMZ could enhance antibacterial activity of the soil-adsorbed TMP.

GRAPHICAL ABSTRACT



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ABSTRACT

Trimethoprim (TMP) and sulfamethazine (SMZ) are two antibiotics that are often administered in combination. We investigated the sorption and desorption behaviors of TMP and SMZ individually as single solute and in combination as co-solute in three representative soils, and evaluated joint antibacterial activity of the soil-adsorbed antibiotics to a reference strain *Escherichia coli* ATCC 25922. Comparative sorption tests showed that co-solute sorption of TMP and SMZ was not considerably different from their single sorption. Soil-adsorbed TMP was found to effectively inhibit the growth of *E. coli* at environmentally relevant concentrations in all three soils, and moreover co-presence of SMZ enhanced the antibacterial effects on bacteria both in its dissolved form and soil-adsorbed form. Overall, the results from this study suggest that various soil-adsorbed antibiotic residues could play a joint influencing role in soil bacterial community activity.

1. Introduction

Sulfamethazine (SMZ) is a widely used antibiotic of sulfonamides class in human and veterinary medicines to treat bacterial infections. Trimethoprim (TMP) is an antibiotic of diaminopyrimidines class and

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often used in combination with sulfonamides to inhibit folic acid synthesis due to their synergistic effects (Huovinen et al., 1995; Giguère et al., 2013). After administration, some of their residues would be excreted and finally end up in the receiving environment (Miao et al., 2004; Yang et al., 2011; Zhou et al., 2011; Zhou et al., 2013a, b, c). Previous studies showed detection of SMZ and TMP in wastewaters, manures and sludge of animal farms and municipal wastewater treatment plants (Hoa et al., 2011; Sim et al., 2011; Zhou et al., 2013a, b, c). Due to application of wastewaters and sludge on agricultural soils, antibiotics such as SMZ and TMP have been reported in soil environments (Li et al., 2011; Zhou et al., 2013a). The highest concentrations for SMZ and TMP in manure of swine farms in South China were up to 0.250 and 0.246 mg/kg, respectively (Zhou et al., 2013a). SMZ and TMP have been detected in manures and soils at concentrations up to 12.4 mg/kg in German farms (Haller et al., 2002; Miao et al., 2004). SMZ was detected in soils of South China at concentrations ranging from ND to 0.074 mg/kg (Li et al., 2011), while TMP was found in Austrian soils up to 0.1 mg/kg (Martínez-Carballo et al., 2007). Those antibiotic residues even at sub-inhibitory low concentrations in the environment could exert selective pressure on bacterial populations, leading to development and spread of antibiotic resistance among bacteria (Heuer et al., 2011; Kim et al., 2010; Kümmerer, 2009a, b). Therefore, it is important to investigate the bioavailability of these antibiotics in soils in order to understand their effects on soil microbial ecosystems.

Various environmental processes are involved in the fate of antibiotics in the soil environment. Abiotic reduction reactions have been reported to be an important removal pathway for chemicals in subsurface environments due to the presence of reduced sulfur compounds (e.g., bisulfide and polysulfides) in soils and associated pore waters (Zeng et al., 2011; Zeng et al., 2012). Sorption–desorption processes play an important role in understanding the bioavailability of chemicals in soil (Tolls, 2001; Huang et al., 2003; Kümmerer, 2009a). Thus, knowledge on sorption and desorption behaviors of TMP and SMZ in soils is necessary to evaluate their bioavailability in soils and to assess their bioactivity for microorganisms. Recent studies have reported that sulfonamides and diaminopyrimidines showed low sorption to soils (Leal et al., 2013; Sanders et al., 2008; Srinivasan et al., 2013; Thiele-Bruhn et al., 2004). H-bonding, cation bridging, ion-exchange, surface complexes and hydrophobic partition have been shown to be the main sorption mechanisms for sulfonamides (Gao and Pedersen, 2005; Kahle and Stamm, 2007; Leal et al., 2013). However, very few studies have investigated co-sorption of sulfonamides and diaminopyrimidines which are often used together. Srivastava et al. (2009) observed no considerable difference for sorption of sulfadimethoxine and ormetoprim between individual sorption and co-sorption, while Sanders et al. (2008) found that ormetoprim sorption was enhanced at high concentrations when in combination with sulfadimethoxine. Therefore, more research is needed to clarify the co-sorption characteristic of antibiotics.

As is known, the bioavailability and bioactivity of antibiotics are influenced by soil sorption. However, knowledge about the relationship between antibiotic sorption behavior and antibacterial activity in soil environments is still scarce (Chander et al., 2005; Goetsch et al., 2012; Halling-Sørensen et al., 2003; Subbiah et al., 2011). Furthermore, although soil adsorbed antibiotics are expected to have the potential to exert biological effects against soil microorganisms, this aspect has not been well-elucidated. Accinelli et al. (2007) found that concentrations much higher than 100 mg/kg would be necessary for sulfamethazine and sulfachloropyridine to affect sulfonamide degradation rates and soil microbial community structure and function. Thiele-Bruhn and Beck (2005) also demonstrated that concentrations of sulfapyridine up to 1000 mg/kg in soil had no effect on microbial respiration. While other studies revealed that sulfonamides and trimethoprim at much lower concentrations could reduce microbial activity and microbial respiration after application (Kotzerke et al., 2008; Liu et al., 2009). Moreover, to date, no studies have so far been attempted to elucidate the bioactivity of soil-adsorbed co-contaminants.

The aim of this study was to firstly investigate sorption and desorption behaviors of trimethoprim (TMP) and sulfamethazine (SMZ) as single solute or co-solutes at a mass ratio of 1:5 normally used for conjunction of these two agents (Huovinen et al., 1995; Giguère et al., 2013), in three representative soils, and further evaluate antibacterial activity of soil-adsorbed compounds to a reference strain. *Escherichia coli* ATCC 25922 (*E. coli* ATCC25922), a quality control strain for antimicrobial susceptibility testing, was applied in the antibacterial activity analysis.

2. Materials and methods

2.1. Soils

Experiments were conducted with three representative soils (A, B and C) with different pH, organic carbon (OC) and cation exchange capacity (CEC) (Table 1). Soil A, Soil B and Soil C were surface soil (0–15 cm) from Dezhou in Shandong, Guangzhou in Guangdong and Chenzhou in Hunan Province, respectively. Soils were air-dried, crushed, sieved through a 2 mm sieve, and stored in closed containers at room temperature prior to use. Soils were sterilized by autoclaving three times to ensure sterility and then dried in an oven at 60 °C. Soils were characterized in our previous study (Peng et al., 2014).

2.2. Standards and reagents

Sulfamethazine (purity >98%) and trimethoprim (purity >98%) were purchased from Dr. Ehrenstorfer GmbH (Germany) and stored at −20 °C. Both compounds are amphoteric molecules with two pK_a values, i.e. 3.23 and 6.76 for TMP, and 2.07 and 7.47 for SMZ (Qiang and Adams, 2004). As solution pH is below pK_{a1}, the antibiotics are in their cationic form due to the protonation. When solution pH is between pK_{a1} and pK_{a2}, the zwitterionic form antibiotics are the dominant species resulted from the charge balance of deprotonation and protonation. As the solution pH is above pK_{a2}, deprotonation results in the formation of anionic form antibiotics (Fig. 1).

All chemicals were analytical-reagent grade or higher purity and solvents were HPLC grade. Water obtained from a Milli-Q water purification system (Millipore, Darmstadt, Germany) was used for the preparation of all reagent solutions.

Aseptic operations were observed during the experiment. The stock solutions of SMZ and TMP for aqueous phase antibiotic activity experiment were prepared at 20 mg/L and 4 mg/L in pH 7.3 10 mM MOPS in amber vials. All stock solutions were stored at 4 °C and remade every time when needed. And all solutions were sterilized by filtration for use in biological experiments. The stock solutions of SMZ and TMP for sorption–desorption tests were freshly prepared in 10 mM sodium chloride (NaCl) solution at 20 mg/L and 4 mg/L, respectively.

2.3. Antibacterial activity tests of antibiotics in aqueous solution

According to previous methods (Peng et al., 2014; Suarez et al., 2007), *E. coli* ATCC 25922 was used as indicator bacteria to determine the antibacterial activity of TMP, SMZ, and TMP and SMZ mixture at a mass ratio of 1:5 based on their normal use ratio (Huovinen et al., 1995; Giguère et al., 2013). In this bioassay, 10 mM MOPS buffer (pH 7.3) was applied to prepare sets of 2:1 serially diluted standard solutions with concentrations in the range of 4 mg/L to 1×2^{-9} mg/L for TMP and 20 mg/L to 5×2^{-9} mg/L for SMZ, while 2 mg/L TMP + 10 mg/L SMZ to 1×2^{-10} mg/L TMP + 5×2^{-10} SMZ for their mixtures. 1.5 ml of a 2 × Mueller-Hinton broth *E. coli* ATCC 25922 culture containing approximately 1×10^6 colony forming units (CFU) was added to the test tubes containing 1.5 ml antibiotic solution, yielding concentrations varying from 2 mg/L to 1×2^{-10} mg/L for TMP, 10 mg/L to 5×2^{-10} mg/L for SMZ, and 1 mg/L TMP + 5 mg/L SMZ to 1×2^{-11} mg/L TMP + 5×2^{-11} for their mixtures. Negative

Table 1
Properties of three representative soils used in the experiments.

Soil	Location	Soil type	pH[a]	OM[b] (%)	CEC[c] (cmol kg^{-1})	Texture[d] (%)			
						<0.002 mm	0.002–0.02 mm	0.02–0.063 mm	0.063–2 mm
A	Shandong	silt clay loam	7.6 ± 0.2	1.04 ± 0.04	6.4 ± 0.2	21.7 ± 4.2	39.7 ± 2.7	28.8 ± 9.9	9.8 ± 4.0
B	Guangdong	silt loam	3.5 ± 0.1	4.27 ± 0.13	7.4 ± 0.2	8.5 ± 3.9	42.3 ± 8.4	7.6 ± 2.5	41.6 ± 6.7
C	Hunan	loam	4.3 ± 0.1	1.34 ± 0.07	25.7 ± 0.1	10.3 ± 1.7	42.9 ± 10.6	22.9 ± 3.9	23.9 ± 9.3

[a] pH value measured in a slurry of 1:5 soil/0.01 M $CaCl_2$ solution.
[b] Organic matter content determined by dry combustion.
[c] Cation exchange capacity determined by ammonium acetate method.
[d] Particle size measured by the hydrometer method.

controls were prepared by adding 1.5 ml of the 1×10^6 CFU/ml inoculum or 1.5 ml sterile 2 × MH broth into culture tubes containing 1.5 ml 10 mM MOPS buffer, respectively. Subsequently, the test tubes were incubated for 8 h at 37 °C on a shaker plate rotating 200 rpm in the dark. After completion of the incubation periods, culture solution absorbance at 600 nm was measured to determine the antibacterial activity.

2.4. Sorption and desorption experiments

Batch equilibrium tests were applied in sorption and desorption experiments for TMP and SMZ individually as single solutes and in combination as co-solutes by soils from an aqueous solution of 10 mM NaCl. Based on the antibacterial activity of antimicrobials in aqueous solution, water solubility and limits of quantitation for HPLC determination, sorption isotherms were measured using six initial concentrations ranging from 0.1 to 4 mg/L for TMP and from 0.5 to 20 mg/L for SMZ. A soil mass to solution volume ratio of 2 g/20 ml for Soil B and Soil C while 10 g/30 ml for Soil A were chosen so that the solution concentrations were above the limits of quantitation for the analytes. Sorption and desorption experiments were performed in 50 ml polytetrafluoroethylene (PTFE) centrifuge tubes. The centrifuge tubes were wrapped with aluminum foil to avoid photolysis. The soil mixtures in tubes were equilibrated by rotating end-over-end at 20 rpm for 24 h at 25 °C. After equilibration, tubes were centrifuged (9310g for 10 min) and supernatant (1 ml) was passed through a 0.22 μm polyethersulfone filter into 2 ml amber glass vial, and subsequently analyzed by high performance liquid chromatography (HPLC). For blank samples (without soil), the chemical loss was found less than 5%. Control experiments (without antibiotic) showed that change of pH for the soil suspension before and after 24 h in equilibration with soil was less than 0.1. Moreover, before and after replacement of the sorption equilibration supernatant with fresh background solution, pH values for soils A, B and C was 7.70, 3.92, 4.69, and 8.32, 4.34, 4.97, respectively. The same approach as the single solute systems was employed in co-solute batch sorption equilibrium experiments except that TMP and SMZ were co-administered in 1:5 ratio for co-solute batch experiments. All experiments were conducted in triplicate.

After the removal of 16 ml or 26 ml supernatant (based on the soil–water ratio), the same volume of 10 mM NaCl solution used as desorption solution was added. The resulting soil mixtures were then equilibrated and processed as described previously.

2.5. Antibacterial activity tests of soil-adsorbed antibiotics

Antibacterial activity experiment was carried out by following our previous method (Peng et al., 2014). Briefly, after completion of sorption experiments, the 24 h desorption process was repeated until the concentrations of antibiotics in supernatants below their detection limits which were the concentrations with no observable growth inhibition to *E. coli* ATCC 25922. The initial solution concentrations for antibacterial activity experiment were as follows: For TMP, 0, 0.1, 0.2, 0.4, 1 and 2 mg/L for Soil B and Soil C, and 0, 0.4, 1, 2 and 4 mg/L for Soil A; for SMZ, 0, 10 and 20 mg/L for all soils; while for TMP and SMZ in combination as co-solutes, 0, 0.1 mg/L TMP + 0.5 mg/L SMZ, 0.2 mg/L TMP + 1 mg/L SMZ, 0.4 mg/L TMP + 2 mg/L SMZ and 1 mg/L TMP + 5 mg/L SMZ for Soil B and Soil C, and 0, 0.4 mg/L TMP + 2 mg/L SMZ, 1 mg/L TMP + 5 mg/L SMZ, 2 mg/L TMP + 10 mg/L SMZ and 4 mg/L TMP + 20 mg/L SMZ for Soil A. After removal of the supernatant from each tube, 0.5 ml of MH broth containing 0.1 ml of 0.5 McFarland bacterial suspension was added into the tube for Soil B and Soil C while 1 ml of MH broth containing 0.2 ml of 0.5 McFarland bacterial suspension was added for Soil A. Then the tubes were incubated at 37 °C for 24 h with shaking at 200 rpm in the dark. After incubation, the bacterial number in soil of each tube was measured by the plate counting method for determination of the growth inhibition of *E. coli* ATCC 25922.

2.6. Chemical analysis by HPLC

The concentrations of SMZ and TMP in supernatant solution were measured by Agilent Series 1200 HPLC system with an ultraviolet detector (260 nm) and an Agilent Zorbax Eclipse XDB-C18 column (4.6 × 150 mm, 5 μm) with the column temperature set at 30 °C. The injection volume was 100 μl. Mobile phase A was acetonitrile. Mobile phase B was an aqueous solution containing 0.01 M ammonium acetate and 1‰ acetic acid. Gradient elution was programmed at a flow rate of 0.8 ml/min as follows: 10% A increased to 40% A in 6 min, then to 90% A in 1 min, kept at 90% A for 1 min and then returned to 10% A in 1 min, and re-equilibrated for 5 min. The calibration curves were

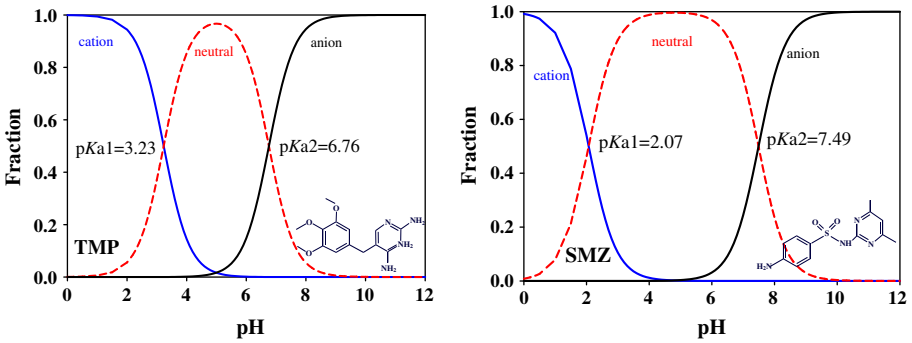

Fig. 1. Chemical structures of trimethoprim (TMP) and sulfamethazine (SMZ) and their speciation at different pH values.

significantly linear in the concentration range of 0.01–4 mg/L for TMP and 0.05–20 mg/L for SMZ ($p < 0.05$). The limits of quantitation (LOQ) were 2.16 μg/L for TMP and 4.23 μg/L for SMZ.

2.7. Data analysis

Sorption and desorption data were fitted to the Freundlich and Langmuir models (1 and 2).

$$\text{Freundlich model,}\quad C_s = K_f C_e^{\,1/n} \tag{1}$$

$$\textit{Langmuir model,}\ C_s = \frac{KLQmCe}{1 + KLCe} \tag{2}$$

where C_s is the adsorbed concentration (mg/kg), C_e represents the equilibrium concentration in solution phase (mg/L), K_f and n are thermodynamic parameters representing sorption/desorption capacity and intensity, respectively. Q_m is the maximum adsorption capacity (mg/kg), and K_L is the Langmuir coefficient (L/mg).

The bacterial growth inhibition (I) can be calculated according to Eq. (3).

$$I = \frac{A\max - A}{A\max} \tag{3}$$

where A represents sample absorbance, A_{max} is the maximum absorbance obtained in the control samples (with no antibiotics dosed), and the values of I range from 0 to 1.

The data quantifying the relationship between bacterial growth inhibition and the test concentrations of each antibiotic in aqueous solution were fitted with a four-parameter logistic regression (4) by SigmaPlot 10.0 (Peng et al., 2014; Suarez et al., 2007).

$$y = y_0 + \frac{a}{1 + (x/x_0)^b} \tag{4}$$

where x is the antibiotic concentration, y is the growth inhibition value, y_0 represents the minimum values of growth inhibition, and x_0 corresponds to the concentration of the antibiotic that yields 50% growth inhibition relative to the sample with no inhibition.

Antibacterial activity of soil-adsorbed antibiotics was calculated following the Eq. (5) reported by previous studies (Chander et al., 2005; Peng et al., 2014).

$$\%\ \text{decline in CFU} = [\text{CFU(without antibiotic)} - \text{CFU(with antibiotic)}]/\text{CFU(without antibiotic)} \times 100. \tag{5}$$

Hyperbola I function (6) was used to fit the curve obtained by plotting inhibition of bacterial growth vs. the soil phase concentrations of each compound by SigmaPlot 10.0.

$$y = ax/(1 + bx) \tag{6}$$

where x is the soil-phase concentration of each compound, y is the percent decline in CFU; a and b are empirical constants.

One-way analysis of variance (ANOVA) was used to calculate statistical significant difference of sorption between the soils by SPSS (version 13.0). In all case, a probability of $p \leq 0.05$ was considered as statistically significant difference.

3. Results

3.1. Inhibition of E. coli ATCC 25922 by antibiotics in aqueous solutions

Dose–response curves (% *E. coli* ATCC 25922 growth inhibition vs. in vitro TMP concentration) for TMP and TMP in combination with SMZ at a ratio of 1:5 are depicted in Fig. 2. As shown in Fig. 2, for TMP, the minimum inhibitory concentrations (MIC) (=the lowest concentration that completely inhibits growth) was higher than 2 mg/L, while EC_{10} and EC_{50} values were 0.01 mg/L and 0.096 mg/L, respectively. Comparing the growth inhibition of *E. coli* ATCC 25922 exposure to TMP (Fig. 2, empty circles) versus exposure to the mixture of TMP and SMZ (Fig. 2, solid circles), the curve shifted slightly to the left in the presence of SMZ, with a smaller EC_{50} value of 0.045 mg/L. This suggests that the existence of SMZ can strengthen the antibacterial activity of TMP. However, when exposure to SMZ as a single chemical was tested, the growth of *E. coli* ATCC 25922 was almost not inhibited, even at its highest exposure concentration of 10 mg/L considered in this study, only with an inhibition of 15%. Therefore, no fitted curve was obtained for SMZ. Accordingly, Salmon et al. (1995) found that MICs for *E. coli* isolates from swine in the United States, Canada, and Denmark ranged from 16 to 512 mg/L for SMZ and from 0.03 to 16.0 mg/L for TMP-sulfadiazine. Moreover, when comparing the obtained MIC values to those in guidelines of the CLSI (256 mg/L and 8 mg/L), *E. coli* ATCC 25922 used in the present study was quite sensitive to both SMZ and TMP.

3.2. Single solute sorption and desorption

The sorption–desorption isotherms for TMP and SMZ are presented in Fig. 3 and Fig. 4, respectively, and the calculated sorption and desorption parameters are summarized in Table 2. The sorption isotherms from the sorption experiment were well fitted with the Freundlich model for TMP and with the Langmuir model for SMZ in all soils with their coefficients (r) being higher than 0.99 (Table 2). As shown in Table 2, the Freundlich sorption coefficients K_f values, representing sorption capacity, for TMP in Soils A, B and C were 5.88, 20.6 and 21.8, respectively; whereas the K_f values for SMZ were 0.599, 4.84 and 2.09 for the three soils, which were smaller than those for TMP ($p < 0.05$). This suggests a higher tendency for TMP than SMZ to adsorb on to the soils. Furthermore, the K_f values clearly suggested that both TMP and SMZ had comparable sorption in Soil B and Soil C, but had relatively smaller sorption in Soil A ($p < 0.05$). This is consistent with the high soil pH value, low soil organic matter content and CEC value in Soil A. Linear sorption behavior (n values were closer to 1) was observed for TMP while non-linear behavior (n values deviated from 1) for SMZ in the present study, which were similar to what has been reported earlier for other sulfonamides (Lertpaitoonpan et al., 2009; Srinivasan et al., 2013; Schwarz et al., 2012). For example, sorption of sulfamethoxazole in three pastoral soils of New Zealand was found to display non-linear sorption dependency (Srinivasan et al., 2013).

Desorption isotherms (Fig. 3 and Fig. 4), representing the amount of compound still adsorbed on soil related to equilibrium concentration

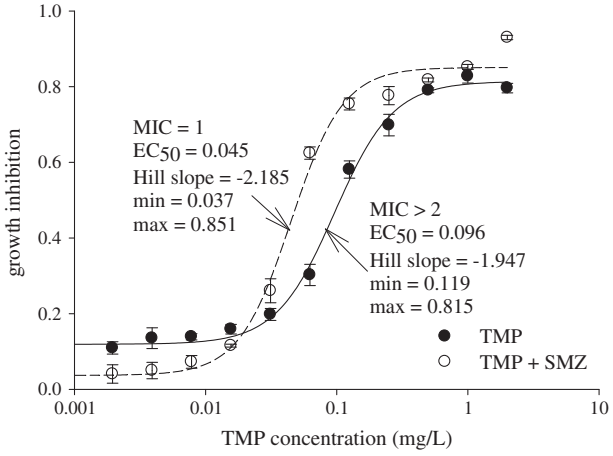

Fig. 2. Dose–response relationships between solution concentrations of the two antibiotics (TMP and SMZ) and bacterial growth inhibition of *E. coli*.

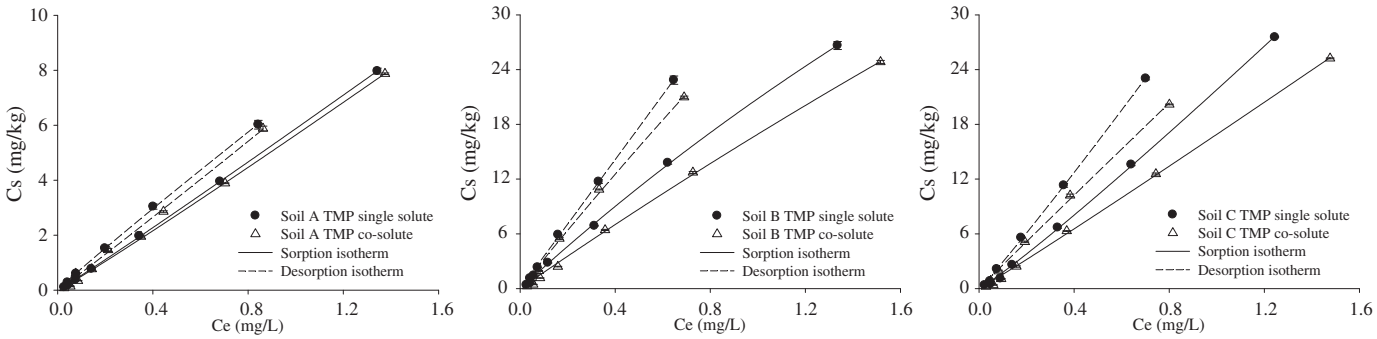

Fig. 3. Sorption and desorption isotherms for trimethoprim (TMP) as single solute and co-solute in the three typical soils (Soil A, Soil B and Soil C) using the Freundlich model.

after one desorption cycle, were obtained according to the procedure used for adsorption isotherms. The fitted parameters are given in Table 2. Positive hysteresis phenomenon was observed for the two compounds in the three soils (Fig. 3 and Fig. 4). The desorption K_f values were consistently higher than those obtained for the sorption, indicating that TMP or SMZ remains retained on all three soils after one desorption cycle.

3.3. Co-solute sorption and desorption

Isotherms generated to assess the effect of co-solutes on sorption and desorption of single solutes were described well by the Freundlich model for TMP (Fig. 3) and Langmuir model for SMZ (Fig. 4). The comparison of K_f values between single solutes and co-solutes (Table 2) revealed a subtle reduction in sorption affinity of TMP when in combination with SMZ with a mass ratio of 1:5, while the presence of TMP increased the sorption of SMZ, but with no statistical difference ($p > 0.05$). Additionally, the presence of co-solute did not affect the degree of linearity for isotherms.

In terms of desorption, the $K_{f,\,des}$ values for TMP as co-solute in the three soils are smaller than those for TMP as single solute with no observed significant difference ($p > 0.05$). However, $K_{f,\,des}$ for SMZ had two patterns, higher values were observed compared with SMZ as single solute in Soil B and Soil C, while an opposite trend was found in Soil A ($p > 0.05$).

3.4. Antibacterial activity of soil-adsorbed antibiotics

To assess the impact of soil adsorbed antibiotics on the growth of *E. coli* ATCC 25922, the % decline in CFU (*E. coli* growth inhibition) was plotted against chemical concentrations in soil (C_s), which are shown in Fig. 5. Bacterial growth inhibition was first examined for soil-adsorbed TMP or SMZ to see whether they as single solute were still biologically active, followed by the combined effect of soil-adsorbed mixture (TMP and SMZ). Obvious inhibition of bacterial growth was observed for soil-adsorbed TMP in the three soils at relative low concentrations (1–6 mg/kg). The growth inhibition for soil-adsorbed TMP with and without the presence of SMZ for the three soils were in the same order of Soil A > Soil C > Soil B. Essentially, the presence of SMZ appeared to intensify the antibacterial activity of soil-adsorbed TMP (Fig. 5). And the results implied that soil-adsorbed antibiotics could inhibit bacterial growth at certain concentrations. However, for soil-adsorbed SMZ, under the highest initial concentration of 20 mg/L, no obvious inhibition effect could be found in the three soils. Increased antibacterial activity was observed with increased soil-adsorbed antibiotic concentrations for the mixture of TMP and SMZ.

4. Discussion

The present study investigated single solute and co-solute sorption process and their antibacterial activity in soils. For single solute sorption, the observed sorption with higher adsorption capacity for TMP than SMZ was comparable to those reported previously (Chu et al., 2013; Kurwadkar et al., 2007; Lertpaitoonpan et al., 2009; Thiele-Bruhn et al., 2004). The present study also demonstrated that soil properties showed significant influences on sorption behaviors. Both TMP and SMZ presented relative higher sorption capacity in Soil B and Soil C than in Soil A. This can be attributed to the lower pH values and higher soil organic matter contents and CEC values for Soil B and Soil C than Soil A (Table 1). The pH values for Soil B and Soil C were far below 7, resulting in the soil mixture of Soil B and Soil C evaluated in this study had pH values around 4–5. However, the pH value of sorption system was about 8 for Soil A. Since both TMP and SMZ are amphoteric molecules with two pK_a values (Fig. 1); thus they mainly existed as neutral form in Soil B and Soil C, and as anionic form in Soil A, resulting in electrostatic repulsion from negative surface charge of soil particles and less sorption in Soil A (Chu et al., 2013; Kurwadkar et al., 2007). Previous studies also demonstrated that sorption capacity for sulfonamides decrease with increasing pH values (Białk-Bielińska et al., 2012; Doretto et al., 2014; Kurwadkar et al., 2007; Lertpaitoonpan et al., 2009; Srinivasan et al., 2013). Besides, Soil B had the highest organic matter content and Soil C had the largest cation exchange capacity. Doretto et al. (2014) demonstrated that sulfonamides have relatively high

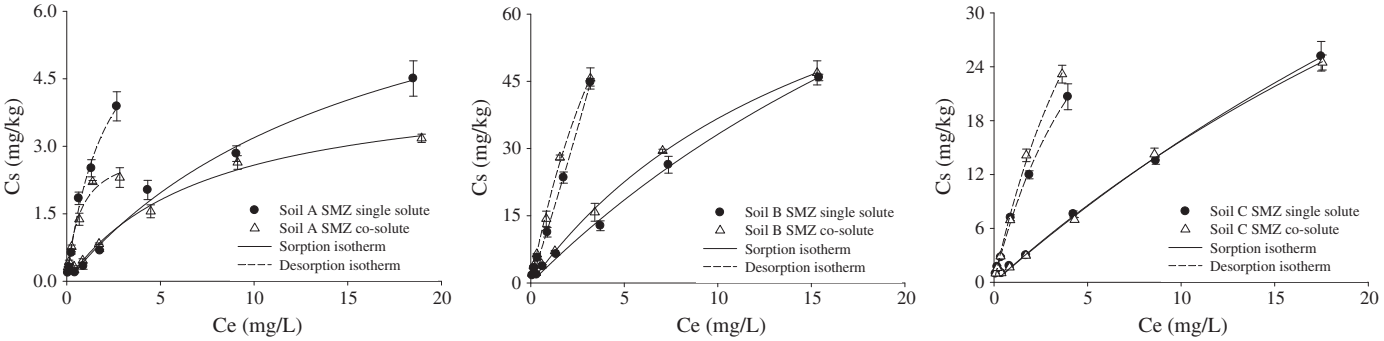

Fig. 4. Sorption and desorption isotherms for sulfamethazine (SMZ) as single solute and co-solute in the three typical soils (Soil A, Soil B and Soil C) using the Langmuir model.

Table 2
Parameters of the fitted sorption–desorption models for trimethoprim (TMP) and sulfamethazine (SMZ) tested as single solutes and co-solutes in three soils.

Sorbent	Solute	Sorption						Desorption					
		Freundlich model			Langmuir model			Freundlich model			Langmuir model		
		K_f	n	R	Q_m/mg · kg^{-1}	K_L	R	K_f	n	R	Q_m/mg · kg^{-1}	K_L	R
A	TMP single solute	5.88	1.035	0.999	5.62	−0.040	0.999	7.12	0.957	0.999	7.87	0.120	0.999
	TMP co-solute	5.65	1.048	0.999	5.32	−0.052	0.999	6.81	1.028	0.999	6.40	−0.069	0.999
	SMZ single solute	0.599	0.698	0.991	0.512	0.060	0.996	2.05	0.673	0.991	3.30	0.482	0.996
	SMZ co-solute	0.684	0.542	0.984	0.603	0.133	0.996	1.57	0.461	0.967	4.34	1.449	0.990
B	TMP single solute	20.6	0.915	0.999	23.8	0.145	0.999	35.7	1.013	0.999	35.3	−0.004	0.999
	TMP co-solute	16.7	0.967	0.999	18.0	0.063	0.999	30.7	0.994	0.998	32.4	0.089	0.998
	SMZ single solute	4.84	0.824	0.999	4.19	0.026	0.998	13.5	1.027	0.999	12.9	−0.024	0.999
	SMZ co-solute	6.78	0.715	0.998	5.84	0.059	0.999	18.1	0.812	0.998	21.3	0.151	0.999
C	TMP single solute	21.8	1.087	0.999	19.5	−0.097	0.999	33.7	1.062	0.999	30.0	−0.124	0.999
	TMP co-solute	16.9	1.036	0.999	16.4	−0.029	0.999	25.4	0.991	0.999	26.7	0.068	0.999
	SMZ single solute	2.09	0.869	0.999	1.83	0.016	0.999	7.29	0.763	0.999	8.70	0.170	0.999
	SMZ co-solute	2.12	0.858	0.999	1.88	0.002	0.999	8.27	0.813	0.997	9.73	0.142	0.999

sorption in soils with high organic carbon content and high cation exchange capacity. SMZ sorption in soil could be influenced by organic carbon, soil surface area and soil pH (Lertpaitoonpan et al., 2009; Chu et al., 2013). And hydrophobic partitioning and hydrogen bonding were the main mechanisms for non-ionized form of sulfonamides adsorbed to organic matter (Chu et al., 2013; Lertpaitoonpan et al., 2009; Schwarz et al., 2012).

For co-solute sorption, the mixture of SMZ and TMP was applied at a mass ratio of 5:1, which implies that the presence of SMZ might affect the sorption of TMP as SMZ could occupy some active sorption sites in soil. In line with Srivastava et al., (2009) previous report for co-sorption of sulfadimethoxine and ormetoprim, the present study on co-solute sorption of TMP and SMZ found that the presence of SMZ slightly decreased the sorption capacity of TMP in soils, but no significant difference ($p > 0.05$) between single solute and co-solute sorption was observed (Fig. 3). In addition, with increase of concentrations for antibiotics, SMZ caused a more evident reduction in sorption affinity of TMP in soil, which could be attributed to the limits of active sorption sites for antibiotics in soil. Similar results were observed by a previous study investigating the sorption of two different sulfonamides in pasture soils (Srinivasan et al., 2013). They found the presence of 17β-estradiol seemed to have a subtle influence on sulfamethoxazole sorption. Desorption testing from the present study showed positive hysteresis for both compounds, suggesting that whatever amount of these two compounds were adsorbed to soil, they would not desorb readily after a single desorption cycle. Similar hysteresis was also reported for other organic compounds (e.g., Weber et al., 1998; Sukul et al., 2008; Peng et al., 2014). And organic matter in soil could play an important role in hysteresis phenomena from desorption isotherms as observed in previous studies (Carroll et al., 1994; Mersie and Seybold, 1996; Weber et al., 1998; Sukul et al., 2008).

The present study found that soil-adsorbed TMP retained its bioactivity, showing growth inhibition of *E. coli* in the soils, while soil-adsorbed SMZ did not show any bacterial growth inhibition effects. This is expected due to more pronounced effectiveness of TMP than SMZ towards *E. coli* ATCC 25922 used in the present study. However, the presence of SMZ enhanced the bioactivity of soil-adsorbed TMP (Fig. 5), which is due to the synergistic effect between TMP and sulfonamides (Aarestrup and Jensen, 1999; EUCAST, 2003). Tappe et al. (2008) also found that the presence of TMP can increase the sensitivity of *Pantoea agglomerans* to SMZ by an order of magnitude. Previous studies also reported that some soil-adsorbed antibiotics such as tetracycline, tylosin, oxytetracycline and ofloxacin retained antibacterial activity (Chander et al., 2005; Goetsch et al., 2012; Peng et al., 2014). But Subbiah et al. (2011) found that β-lactams and florfenicol antibiotics remain bioactive in soils while ciprofloxacin, neomycin, and tetracycline are neutralized. This indicates that residual antibiotics in soil do not necessarily exert a selective pressure, and the degree to which antibiotic remains bioactive depends on the physiochemical properties and biological potency of this chemical.

The different bioactivity among soils (Soil A > Soil C > Soil B) (Fig. 5) for TMP can be attributed to the different sorption–desorption hysteresis. As can be seen from Fig. 3, the sorption–desorption hysteresis for TMP under low supernatant equilibrium concentration was in the following order: Soil B > Soil C > Soil A, which is opposite to the order for antibacterial activity of adsorbed antibiotic among soils. To inhibit bacterial growth, TMP and SMZ must be taken up into the cell to interfere with folate metabolism. As a result, soil-adsorbed antibiotic must be desorbed before entering bacterial cells. It has been reported that sulfonamide of neutral species is preferably transported across cell membrane systems (Trapp and Horobin, 2005) and the anionic species is the reactive form once in the cell (Henry, 1943). Consequently, the

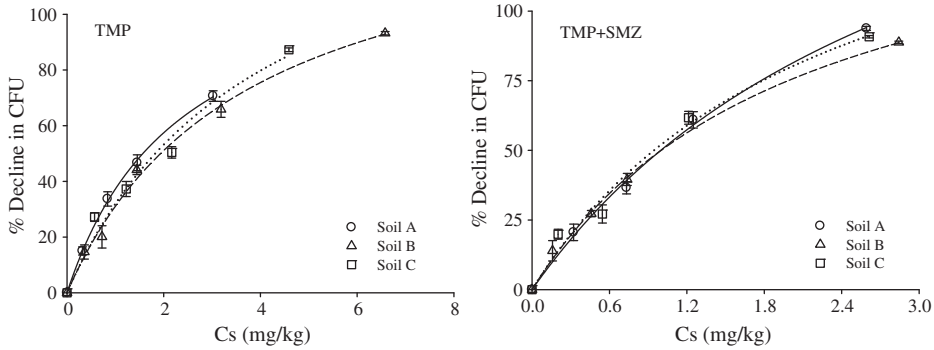

Fig. 5. Percent decline in colony forming units (CFU) of *Ecxx coli* by the soil-adsorbed antibiotics (TMP and SMZ).

easier antibiotic desorbed, the stronger bioactivity exhibited. Mengelers et al. (1997) observed that the degree of ionization plays the dominant role in antibacterial activity of sulfonamides. Thus, factors that influence ionization or sorption–desorption processes of an antibiotic are important in determining the antibiotic activity of the soil-adsorbed antibiotic.

The present study showed that the soil-adsorbed TMP could affect soil bacterial growth in the worst-case scenario of contaminated soils, and the co-presence of SMZ and TMP can jointly impose antibacterial effects on soil bacteria. This co-presence of various antibiotics could enhance the selective pressure on soil microbial community, resulting in the development of antibiotic resistant bacteria. A previous study found that antibiotic residues such as ceftiofur are a significant contributor to the proliferation of antibiotic resistant bacteria in animal farming systems (Call et al., 2013). However, more research is needed in order to understand the role of antibiotic residues in the amplification and transmission of antibiotic resistant bacteria.

5. Conclusions

The results from the present study showed that co-solute sorption of TMP and SMZ was not considerably different from the single solute sorption of the two antibiotics, and hysteresis phenomenon was observed in both systems. The soil pH, CEC and organic matter content are important factors affecting sorption and desorption of the two antibiotics in the three soils. The indicator bacterium *E. coli* ATCC 25922 was found to be more sensitive to TMP than to SMZ in both dissolved form and soil-adsorbed form. Soil-adsorbed TMP at environmentally relevant concentrations still retained antibacterial activity, while co-presence of SMZ could enhance antibacterial activity of the soil-adsorbed TMP. Joint antibacterial activity of TMP and SMZ suggests that various soil antibiotic residues may impose higher selective pressure on soil microbial community, possibly causing development of antibiotic resistant bacteria in the soil environment.

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