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Joint antibacterial activity of soil-adsorbed antibiotics trimethoprim and sulfamethazine

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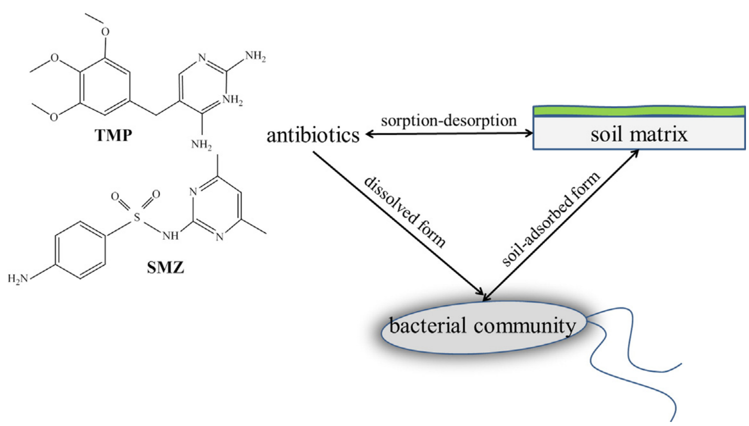
HIGHLIGHTS

- 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.

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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

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