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Mobility of Veterinary Drugs in Soil with Application of Manure Compost

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Abstract Sulfonamides and tetracyclines are pharmaceuticals widely used to treat human and animal diseases. They are of considerable concern in Korea because of the potential risk of residues in aquatic and terrestrial environments. This study investigated the mobility of veterinary drugs in the soil column with the application of manure compost to assess the risk of groundwater contamination by leaching in the Korean agricultural environment. The degree of sulfonamides and tetracyclines mobility, measured by the concentration of leachates from silty loam soil for 9 days, was observed being on the first day of this study, in the order sulfathiazole, sulfamethazine > sulfamethoxazole > chlortetracycline > oxytetracycline, and the sulfonamides concentrations were about ten times higher than the tetracyclines concentrations with continuous leaching. The results indicate that sulfonamides pose a high risk of ground and surface water contamination and tetracyclines have the potential to persist in soils with bioactive epimers.

Keywords Mobility · Sulfonamides · Tetracyclines · Soil column · Leaching

The Korean animal farming industry uses very intensive rearing systems, but farmers still self-prescribe and treat their animals with drugs without a veterinarian's direction. Resistance to antibiotics is increasing annually, and environmental contamination through the excretion of animal feces and urine and the subsequent dispersion of contaminated manure onto land are of great concern in Korea. Currently, veterinary drugs are recognized as not only medicines but also as 'newly emerging contaminants' in the environment. In recent years, many studies have examined the occurrence and fate of antibiotics in aquatic environments in various countries, including Korea. More than 30 antibiotic substances have been found in sewage influent and effluent samples, surface waters, and even ground and drinking water (Kümmerer 2001; NIER 2007). When considering agricultural activities, administered drugs and their metabolites or degradation products reach the aquatic environment via the application of manure compost or liquid fertilizer to agricultural lands, or by pasture-reared animals excreting on the land directly, followed by surface run-off and drifting or leaching in deeper layers of soil. Most antibiotics are water-soluble and about 90% of one dose can be excreted in urine and up to 75% in animal feces (Halling-Sørens 2001). Regarding antibiotics used in animal husbandry, the largest source of antibiotic contamination of aquatic environments is animal waste, including feces and urine, and the soil acts as a reservoir and plays roles in decomposition and filtration. Therefore, for environmental sustainability, it is important to investigate the fate and behavior of antibiotics in soil, including their physicochemical properties, such as the molecular structure, size, shape, solubility, and hydrophobicity of the chemicals, and their sorption, fixation, and mobility in various soil types under different climates. There is still a lack of fundamental data on the occurrence, fate, and effects of antimicrobials in the environment and a need for conducting accurate risk assessment and risk management both in terms of human and environment health. In the agricultural environment, veterinary drugs are present mainly in combination with manure or manure compost. The presence of manure compost or slurry may affect the

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physicochemical properties of soil, including the water-holding capacity, pH, and microflora. In this respect, studies have examined the fate of veterinary drugs in manure in soil (Kay et al. 2005a, b, c). Laboratory-scale soil column leaching studies have been commonly used to investigate the mobility of chemicals and suggest their behavior in the environment (Lynch 1995). This study investigated the mobility of veterinary drugs in a soil column to which manure compost was applied to assess the potential for groundwater contamination in Korean arable soils, reflecting the Korean agricultural environment.

Materials and Methods

Chlortetracycline (CTC) and its epimers 4-epi-chlortetracycline (ECTC) and 4-epi-anhydrochlotetracycline (EACTC); oxytetracycline (OTC) and its epimer 4-epi-oxytetracycline (EOTC); and three sulfonamides (SAs): [sulfamethoxazole (SMTZ), sulfamethazine (SMT), and sulfathiazole (STZ), and VETRANAL® analytical standard] were purchased from Fluka (Buchs, Switzerland). All solvents were ULTRA RESI-ANALYZED™ grade purchased from J.T. Baker (Phillipsburg, NJ, USA). All other chemicals were of analytical grade and commercially available, unless otherwise stated.

The soil column design and leaching experiments (Fig. 1) were a modification of the method described by the

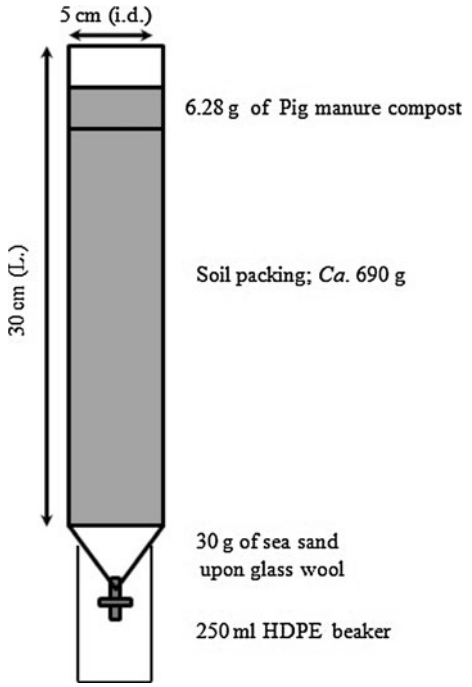

Fig. 1 Schematic design of the soil column experiments with manure compost

United States Environmental Protection Agency (US EPA 1985). Arable soil was collected from the Pesticide Dissipation Test Fields of ChungBuk National University, where no manure compost containing veterinary drugs had been applied for at least 10 years. These test fields are located in central Korea and the soils are typical for Korea. The soil was a silty loam with the following characteristics: organic carbon 0.6%, cation exchange capacity (cmol(+)/kg soil) 8.4, and pH (H_2O, 1:5) 6.1–6.3. Approximately 690 g of soil were packed into a glass column (5 cm i.d. × 30 cm long, with a column surface area of 19.625 cm^2) for each veterinary drug in duplicate, and the columns were aged for 1 month while maintaining more than 40% of the field moisture capacity with distilled water. Solid manure compost was collected from an organic animal farm, accredited by the government as animal-drug free. For application as a fertilizer, the application amount was calculated following the Standard Fertilizer Application (Rural Development Administration 2007) Guidelines for Chinese cabbage culture (pre- and post-application; 32.0 N kg/10 acres, ~3.2 kg/m^2), and 6.28 g of manure compost were applied to the 19.625 cm^2 area at the top of each soil column after the soil aging period. Then, 2 mL of CTC, OTC, SMTZ, SMT, or STZ standard solution (100 μg mL^{-1}) in water were added to the column. The soil columns were irrigated with distilled water. The amount of irrigation was based on the average daily precipitation over 10 years in the Cheongju region; considering the 9.34 mm average and the soil column surface area, each column was irrigated with 18.5 mL of water daily for 9 days. During the experiment, the soil columns were covered with black textile to avoid photodegradation at room temperature. To analyze the leachates, all of the leachates were filtered through a GF/C filter with 1 g of Celite 545 under a vacuum. Then, 10 mL of each subsample from the soil leachates were subject to column clean-up with a 1 g SPE C18 cartridge. For activation and pretreatment, 5 mL of methanol (MeOH) and 5 mL of EDTA-McIlvaine buffer/MeOH (10:90, v/v, pH 4.0) were eluted, and then the extract was eluted on the column. After eluting the extract, 10 mL of water were eluted and discarded, and then 20 mL of methanolic 0.01 M oxalic acid were eluted and collected. The collected sample was concentrated under N_2 gas, and then 1 mL of MeCN:water (10:90 *v*/*v*) was added for the analysis. For the high-performance liquid chromatography tandem mass spectroscopy (HPLC–MS-MS) analysis, we used an ACQUITY UPLC System coupled to a TQD and XEVO triple quadrupole mass spectrometer from Waters (Milford, MA, USA) using an electrospray source. The HPLC mobile phase used a C18 ACQUITY UPLC BEH (100 × 2.1 mm; particle diameter 1.7 μm) from Waters, which was programmable with 20 mM NH_4HCO_2/20% MeOH and

Table 1 MS/MS parameters for the two tetracyclines and their epimers and the three sulfonamides

Generic name	MRM transition Precursor ion → Product ion (*m/z*)	Cone voltage (V)	Collision energy (eV)
CTC	479 > 444, 479 > 462	36	18, 18
ECTC	479 > 444, 479 > 462	36	18, 18
EACTC	461 > 154, 461 > 444	36	18, 18
OTC	461 > 426, 461 > 443	34	18, 14
EOTC	461 > 426, 461 > 444	34	18, 14
SMT	279 > 124, 279 > 186	40	26, 16
SMTZ	254 > 108, 254 > 156 (99)	36	24, 16
STZ	256 > 108, 255 > 156 (92)	34	20, 14

20 mM NH_4HCO_2/95% MeOH. Each multiple reaction monitoring (MRM) transition (*m/z*) of precursor ion to product ion of the target analytes is described Table 1. The data were processed using MassLynx 4.1 from Waters. The mean recovery ratios were 110.27%, 97.32%, and 94.43% for CTC, ECTC, and EACTC, respectively. To clarify the capability to determine each analyte in each matrix while avoiding confusion arising from the various definitions of the limits of detection (LOD) and quantification (LOQ), the practical quantitation and reporting limits were determined using the following equation:

$$\text{Amount (ng)} \times [\text{dilution volume (mL)/injection volume } (\mu\text{L})] \times [1/\text{sample (g)}],$$

where the amount is primary/secondary ion transition <20%; the primary transition is the level at which a 10:1 peak-to-peak signal-to-noise ratio (SNR) is observed for the analyte quantitation ion transition, and a 3:1 peak-to-peak SNR is observed for the analyte secondary ion transition, which is greater than the instrument detection limit and lowest calibrated level. For this study, the practical quantitation limit for each veterinary drug in water was 0.01 ng mL^{-1}. The recovery ratios were as follows: CTC 107.75, ECTC 78.23, EACTC 76.64%, OTC 79.44%, EOTC 82.43%, SMT 100.67%, SMTZ 89.53%, and STZ 81.23%.

Results and Discussion

Various mobility patterns of the SAs were observed in the column experiments, and the concentrations of SMT and SMTZ in the leachates were consistently lower than that of STZ, but they were detected continuously. The level of STZ was about twice that of the other SAs (Fig. 2). SAs are a large group of structurally related antibiotics that are N- substituted derivatives of sulfanilamide. SAs are fairly water soluble and are polar compounds that ionize depending on the pH of the medium (Thiele-Bruhn et al. 2004). Their mobility in soil is closely related to sorption. The sorption of most antibiotics is not characterized solely by their hydrophobic properties, but also by other mechanisms, such as ion exchange, cation binding at clay surfaces, surface complexation, and hydrogen bonding, which also play a significant role (Tolls 2001). For CTC, steady concentrations were eluted after irrigation with the continuous detection of its epimers. Relatively less EACTC formed than ECTC. The ECTC concentration was highest at day 5 and then decreased; ECTC, except on day 2, was below the practical quantification limit (PQL) and reached the concentration of CTC at day 9 (Fig. 3). OTC was eluted from day 4, was highest on day 8, and was then detected at the PQL. EOTC was eluted only at and below the PQL (Fig. 4). Comparing the leachate concentrations of TCs and SAs, those of the TCs were almost half those of the SAs.

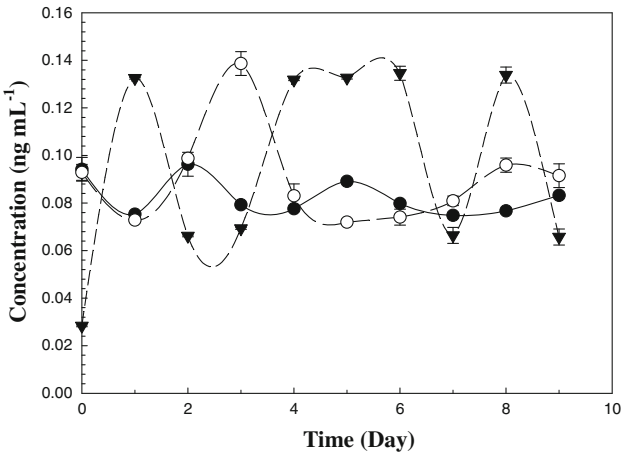

Fig. 2 Leachate concentrations of SMTZ (*Filled circle*), SMT (*Circle*), and STZ (*Filled inverted triangle*)

Various patterns of mobility were found, but the SAs were generally more mobile than the TCs. Sorption behavior is normally represented as the sorption coefficient (K_d), which is defined as the ratio of the concentrations of a compound in the sorbent and aqueous phases at equilibrium. The concentration in the aqueous phase can attain only the concentration of the freely dissolved compound.

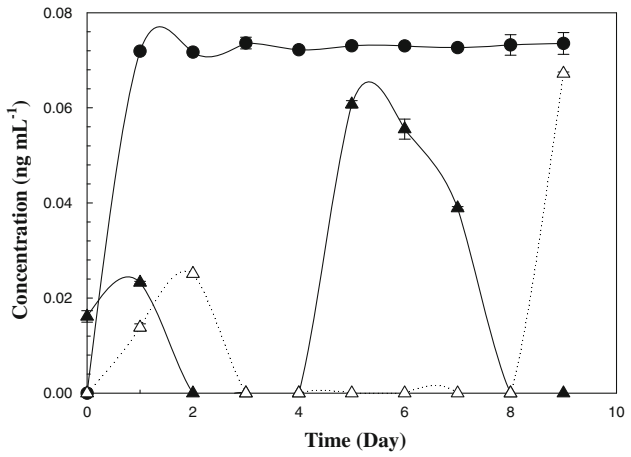

Fig. 3 Leachate concentrations of CTC (*Filled circle*), ECTC (*Filled triangle*), and EACTC (*Triangle*)

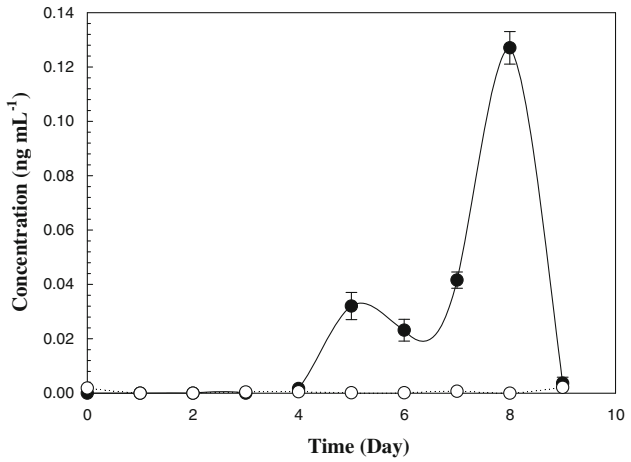

Fig. 4 Leachate concentrations of OTC (*Filled circle*) and EOTC (*Circle*)

The total concentration in the soil solution cannot be considered, as it includes fractions that are associated with dissolved organic matter (DOM) and are adsorbed to suspended particles. Reported values of the K_d (L kg^{-1}) and K_{oc} (L kg^{-1}) of STZ are 4.9 and 200, respectively, and 3.1 and 124.6 for SMT in loamy sand (pH 5.2); the K_d and K_{oc} of OTC are 420–1,030 and 27,800–93,300 from various soils (Tolls 2001). The frequently reported occurrence of SAs in water and soil analyses makes it possible to speculate on the mobility differences of SAs and TCs (Hirsch et al. 1999; Martínez-Carballo et al. 2007). Kay et al. (2005a, b, c) reported that pig slurry application to clay soil induced increasing soil water pH but had no significant impact on the mobility of OTC in the soil column, suggesting that as the pH increases, both acidic and basic compounds are more mobile as sorption decreases. Acidic compounds have a negative charge at higher pH and thus will sorb less to soil, which also has a negative charge. Basic compounds exist as positive ions at lower pH; as the sorption of positive ions is greater, the mobility will increase with the pH. The formation of CTC epimers is dependent on the pH (Halling-Sørensen et al. 2002), and ECTC and EACTC are the major degradation products when CTC is under acidic conditions. It is likely that the pH changes caused by applying manure compost induced faster mobility of SAs than TCs and affected the formation of ECTC and EACTC, although no pH change in the leachates was observed. A low degradation rate for SAs in slurry has been observed, and it is interesting that the excreted acetyl conjugates of SAs are again converted to the parent compounds (Berger et al. 1986; Langhammer and Büning-Pfaue 1989). Sukul and Spiteller (2006) proposed that when manure slurry is applied to a field as fertilizer at a maximum dose rate of 50 m^3/ha, sulfonamide residues in soil could reach 1 kg/ha, which is the same order of magnitude as the application rate of modern pesticides. Considering the usage pattern of SAs and TCs in humans and animals, the source of SA residues in water and groundwater is considered to be the point-source discharge of animal feeding operations and agricultural activities, e.g., the application of liquid fertilizer and poorly fermented animal compost on arable land. Manure and manure compost are also sources of SA contamination and may trigger accelerated leaching of SAs in the soil by changing the pH. We investigated the mobility of SAs and TCs in small-scale soil column experiments, reflecting the Korean arable environment. The mobility, measured by the concentration of leachates from silty loam soil over 9 days, was in the order SAs (STZ and SMT > SMTZ) > TCs (CTC > OTC). We postulate that the changes in soil pH following manure compost application promote the elution of chemicals. SAs pose a high risk of ground and surface water contamination and TCs have the potential to persist as bioactive epimers in soils. More studies of the residual concentration in the soil layer, pH measurement of leachates, and long-term processes are needed for a more definite assessment.

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