Dissolved and Colloidal Fraction Transport of Antibiotics in Soil under Biotic and Abiotic Conditions

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Veterinary antibiotics, leached from contaminated manure that is applied as fertilizer, could be transported to surface water as runoff or infiltrated to groundwater after a significant rainfall. The main objective of this study was to evaluate the transport behaviour of ten antibiotics belonging to four different groups in soil through the column leaching study under biotic and abiotic conditions. Among compared antibiotic groups, tetracyclines (TCs) were found to have no residual in the leachate and mainly remained sorbed to the fixed column soil ranging 34~81% of initially applied mass. In contrast, sulfamethazine (SMTs), one of the sulfonamides (SAs) group, was mostly recovered in the leachate at the ranges of 74~83% of the total mass applied. Also, colloidal-facilitated transport of antibiotics was observed. Depending on the antibiotics, recovered mass of colloidal-facilitated transport was 0.4~22.7% for biotic and 0.2~62.5% for abiotic treatment as compared to total mass applied in soil. In general, a less mass was recovered in biotic leachate and soil columns indicating that microorganism activity might be a minor attenuation mechanism. Results indicated that phase partitioning of antibiotics would be a major factor to be assessed in evaluation of fate and transport of antibiotics in soil.

Key words: antibiotics, mobility, colloidal-facilitated transport, sorption

Introduction

Human and animal-derived antibiotics have been found in several different environmental compartments due to the widespread use of these compounds (Mellon et al. 2001). The antibiotics are only partially metabolized and the parent compounds along with the metabolites can be released to the environment (Heberer 2002a; Diaz-Cruz et al. 2003; Kay et al. 2005a). Consequently, there is an increased concern that released antibiotics can lead to the development of antibiotic resistant microorganisms via either horizontal or vertical resistance gene transfer mechanisms (Chee-Sanford et al. 2001; Ohlsen et al. 2003; Sengeløv et al. 2003; Rooklidge 2004).

Among several suggested transport pathways of antibiotics from source to the environment, the major release pathway of human-used antibiotics has been assumed to be wastewater treatment plants (WWTPs) due to incomplete treatment efficiency. Several previous studies have reported the residuals of released antibiotics in the effluent of a WWTP or streams near the WWTP (Hirsch et al. 1999; Heberer 2002b; Miao and Koenig 2002; Barreiro and Lores 2003; Mc Ardell et al. 2003; Stamatelatou et al. 2003; Göbel et al. 2004). In addition, veterinary antibiotics can be introduced in soil when manure containing residual of antibiotics is applied in the field as fertilizer (Hamscher et al. 2002; Christian et al. 2003; De Liguoro et al. 2003; Blackwell et al. 2004; Jacobsen et al. 2004); and after rainfall, the introduced antibiotics can be transported to surface water as runoff or leach to the sub-surface (Burkhardt et al. 2005; Pedersen et al. 2005; Davis et al. 2006). Previous study showed that annual consumption of veterinary antibiotics in European countries including France, Sweden, and UK ranged from 14–3,902 tons, and concentration of antibiotics in agricultural soil was measured up to 900 μg/kg depending on antibiotic compounds (Thiele-Bruhn 2003). As a result, field and laboratory studies have been conducted to verify the fate and transport of released veterinary antibiotics. Sorption experiments were carried out to examine the partitioning behaviour of veterinary antibiotics in solids (e.g. soil or manure) under various conditions and these studies concluded that several mechanisms including hydrophobicity, cation exchange, surface bridging, and hydrogen bonding are involved in the sorption process (Thiele-Bruhn et al. 2004; Kolz et al. 2005; Mackay and Canterbury 2005; Sassman and Lee 2005). Indeed, pH might be the most important parameter impacting sorption and desorption of veterinary antibiotics in soil (Sassman and Lee 2005).

Lysimeter and column leaching experiments are alternative methods to observe the fate and transport of veterinary antibiotics in a soil environment. In general, less sorptive antibiotics tend to leach into the sub-surface causing contamination in groundwater and strongly sorptive antibiotics have the affinity to bind with soil particles. With this hypothesis, Rabølle (2000) investigated the leaching behaviour of four antibiotics and
documented that the most strongly sorbed compound, oxytetracycline (OTC), was not recovered in the leachate from the soil column. Tylosin (TYL) was only observed in the top portion of the soil columns and the least sorptive two compounds, metronidazole and olaquindox, were almost completely recovered in the leachate. Another study also evaluated the leaching behaviour of three veterinary antibiotics and similar results showed that the more strongly sorbed compounds were less likely to leach to the groundwater (Kay et al. 2005a; Kay et al. 2005b).

One of the pitfalls in previous leaching experiments was that the role of microorganisms was not considered. Although, microorganism activity may not be the major dissipation mechanism in most soil systems, the biotic effect should not be ignored. Thus, the first objective of our study was to evaluate the leaching behaviour of selected antibiotics in the leachate and soil column with inhibition of microorganism activity as a variable.

The second objective of this study was to examine whether mobile colloids might be able to facilitate the transport of antibiotics in the subsurface. In fact, the colloidal-facilitated transport mechanism of other organics including pesticides plays an important role in transport of these compounds to groundwater (Chaplain and Mills 1992; de Jonge et al. 1998; Worrall et al. 1999; Sprague et al. 2000; Grolimund and Borkovec 2005). However, colloidal related transport of antibiotics has not been studied and only one of the recent studies proposed that partitioning of TYL in colloidal matter of manure was as strong as the solid (Kolz et al. 2005). Thus, the result of our study evaluating the effect of colloids as carriers to leach into the sub-surface will increase the understanding of fate and transport of antibiotics.

**Experimental Methods**

**Materials**

All HPLC (high performance liquid chromatograph) grade organic solvents and chemicals purchased from Sigma-Aldrich Co. (St. Louis, Mo., USA) were used in this experiment unless otherwise stated except for analytical grade chemicals of formic acid (88%, HCOOH, F.W. 46.03), citric acid-monohydrate (C\(_6\)H\(_8\)O\(_7\)\(\cdot\)H\(_2\)O, F.W. 210.14), sodium phosphate-dibasic anhydrous (Na\(_2\)HPO\(_4\), F.W. 141.96), disodium ethylene diamine tetra-acetic acid (Na\(_2\)EDTA, F.W. 372.24), sodium chloride (NaCl, F.W. 58.44), and calcium chloride dehydrate (CaCl\(_2\)\(\cdot\)2H\(_2\)O, F.W. 147.02). Ten antibiotics shown in Table 1 were purchased from Sigma-Aldrich Co. (St. Louis, Mo., U.S.A.) and stock solutions (100 μg/mL) were prepared in methanol every month and stored at 4°C until use. Solid phase extraction (SPE) cartridges, 3 mL/60 mg of HLB (Hydrophilic-Lipophilic-Balanced),

### TABLE 1 Properties of studied antibiotics

<table>
<thead>
<tr>
<th>Groups</th>
<th>Substances</th>
<th>Acronym</th>
<th>Log (\text{Kow})</th>
<th>(K_d) (L/kg)</th>
<th>Degradation (texture/%/time[d])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracyclines</td>
<td>Tetracycline</td>
<td>TC</td>
<td>-1.2</td>
<td>1140–1620</td>
<td>Manure/65/84</td>
</tr>
<tr>
<td>(TCs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Soil+manure/100/14</td>
</tr>
<tr>
<td></td>
<td>Chlortetracycline</td>
<td>CTC</td>
<td>-0.6 (\dagger)</td>
<td>22–164973</td>
<td>Manure/24/84</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Soil+manure/88/30</td>
</tr>
<tr>
<td></td>
<td>Oxytetracycline</td>
<td>OTC</td>
<td>-1.2</td>
<td>78–3020</td>
<td>Soil+manure/0/180</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sediment/50/44</td>
</tr>
<tr>
<td>Sulfonamides</td>
<td>Sulfathiazol</td>
<td>STZ</td>
<td>0.1</td>
<td>3–5</td>
<td>NA</td>
</tr>
<tr>
<td>(SAs)</td>
<td>Sulfamethazine</td>
<td>SMT</td>
<td>0.9</td>
<td>1–3</td>
<td>NA</td>
</tr>
<tr>
<td>Macrolides</td>
<td>Erythromycin-H(_2)O (\dagger)</td>
<td>ETM-H(_2)O</td>
<td>3.1</td>
<td>NA</td>
<td>Soil+manure/2/5/30</td>
</tr>
<tr>
<td>(MLs)</td>
<td>Tylosin</td>
<td>TYL</td>
<td>3.5 (\dagger)</td>
<td>8–128</td>
<td>Slurry+sand/50/4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Manure/50/5/3</td>
</tr>
<tr>
<td>Ionophores</td>
<td>Monensin</td>
<td>MNS</td>
<td>5.4–8.5 (\dagger)</td>
<td>NA</td>
<td>Manure/30/40/70</td>
</tr>
<tr>
<td>(IPs)</td>
<td>Salinomycin</td>
<td>SLM</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Narasin</td>
<td>NRS</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

\(\dagger\) Dehydrated form of erythromycin was used.
\(\dagger\) Reference from (Tolls 2001) if stated otherwise.
\(\dagger\) Reference from Thiele-Bruhn (2004).
\(\dagger\) Reference from Jacobsen et al. (2004).
\(\dagger\) Reference from (Thiele-Bruhn 2003); value for polyether group is shown and individual substance value cannot be found.
\(\dagger\) Reference from (Tolls 2001; Thiele-Bruhn 2003; Sassman and Lee 2005); values are ranged depending on different soil texture and pH; detailed information can be found in references.
\(\dagger\) Reference from Thiele-Bruhn (2003)
NA: information is not available.
were purchased from Water Oasis Co. (Milford, Mass., USA). Milli-Q water (18.3 MΩ cm) from a Millipore (Billerica, Calif., USA) purification system was used for all of chemical analyses.

Column System

The soil was collected from the surface (0–10 cm) at the Agricultural Research, Development, and Educational Center at Colorado State University located north of Fort Collins, Colo., USA. The collected soil was air-dried in the dark at room temperature to prevent losses of antibiotics due to photolysis and sieved (<2 mm) to remove any debris. The texture of soil was sandy clay loam and the properties are summarized in Table 2.

The general procedure of the experiment followed the Organisation for Economic Co-operation and Development guidelines (OECD 2004). Small portions of soil were manually packed into glass columns (35 cm × 5 cm, height × internal diameter) under gentle vibration to obtain uniform filling up to a height of approximately 30 cm. A glass wool plug (pore size of 8 µm) and 50 g of acid washed sand were placed at the bottom of columns to prevent any leakage of soil and a glass sinter disk was positioned at the top of the columns to ensure equal distribution of the artificial rain (0.01 M CaCl₂). Before antibiotics were applied to the columns, pre-wetting was conducted with artificial rain from the bottom of the columns to avoid air bubbles and channelling in the soil columns (Rabølle 2000). After pre-wetting, artificial rain was drained with gravity and equilibrated for 24 h. Schematic diagram of column system is shown in Fig. 1.

The amount of antibiotics (65 μg) applied on top of the soil columns was calculated according to OECD guidelines and prepared in water by dilution from stock solution (100 μg/mL). To feed continuous artificial rain from top to bottom, a peristaltic pump was used and the flow rate was set to 0.11 mL/min corresponding to a rate of 84 mm over a period of 24 h. During the study period, all columns were covered with aluminum foil at room temperature to prevent any possible photodegradation of antibiotics in soil column. Leachate was collected in 500 mL amber glass bottles placed at the bottom of each column every 3 days to measure antibiotic concentration, and measurements of antibiotic concentration in leachate lasted 42 days.

Column Experiments

Two sets of column experiments were conducted and the experimental setup is summarized for each in Table 3. The first set of experiments was focused on the mobility of antibiotics in the dissolved phase and sorption to soil with and without microbial activity. Abiotic conditions were achieved by adding NaN₃ (10 mg/L) in the feeding tank as an inhibitor. The second set of experiments studied the mobility of antibiotics in the colloidal phase.

Extraction of Antibiotics in Water and Soil

The concentration of antibiotics in the dissolved and colloidal fractions was analyzed according to procedures

![Fig. 1. Schematic diagram of column system.](image)

**TABLE 2. Properties of soil used in column study**

<table>
<thead>
<tr>
<th>Soil texture</th>
<th>pH</th>
<th>CEC b</th>
<th>EC c</th>
<th>O.M. d</th>
<th>NO₃-N</th>
<th>P</th>
<th>Bulk density MT/m³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sandy Clay Loam</td>
<td>7.6</td>
<td>24.2</td>
<td>1.1</td>
<td>21</td>
<td>16.3</td>
<td>4.0</td>
<td>1.1</td>
</tr>
</tbody>
</table>

* Percentages of sand, silt and clay were 55, 16 and 29, respectively.

b Cation exchange capacity
c Electrical conductivity
d Organic matter
Kim et al. described previously (Lindsey et al. 2001; Yang and Carlson 2004; Yang et al. 2004; Kim and Carlson 2007). Briefly, SPE was conducted using 3 mL/60 mg HLB cartridges to clean up the sample. Soil samples were sectioned with 3 cm intervals and each segment was carefully homogenized manually. A sub-sample (1 g) from each segment was used for analysis. The procedure of soil extraction was modified from the United States Department of Agriculture guidelines (USDA 2003). Two different buffer solutions, McIlvaine buffer solution (pH 4.0) for chlortetracycline (CTC), OTC, TC, sulfathiazol (STZ), SMT and 1M of ammonium hydroxide buffer solution (pH 10.0) titrated with formic acid for erythromycin-H2O (ETM-H2O), TYL, monensin (MNS), salinomycin (SLM), narasin (NRS), were applied to extract antibiotics from the solid to liquid phase. After 20 mL of buffer solution and 200 μL of 5% Na₂EDTA (1 mmol in solution) to chelate metals was added, the sample was mixed for 20 min at 400 rpm (Model No-4626, Lab-line instrument, Needham Heights, Mass., USA), followed by 10 min of centrifuging at 4000 rpm (IEC Clinical Centrifuge, International Equipment Co., Needham Heights, Mass., USA). Finally, the liquid phase was filtered with 0.2 μm glass fibre filters and decanted into another 40 mL vial and kept at 4°C. Extraction was repeated once in the same manner as described above and supernatants were combined to make a total volume of 40 mL for the SPE cleanup procedure. The SPE conditions were the same as for aqueous samples. Adequate concentration of the internal standard, simatone (50 μL of 0.24 mg/L), was used to quantify the antibiotics. Recoveries and limits of quantification are summarized in Table 4. All measured concentrations in the experiment were corrected for recoveries.

**Separation and Detection of Antibiotics**

To separate the antibiotics, a HP 1100 HPLC equipped with an auto sampler and variable UV detector was used. The analytical column was an XTerra MS C₁₈ (Waters,

### TABLE 3. Column experimental setup

<table>
<thead>
<tr>
<th>Column ID</th>
<th>Applied Antibiotics</th>
<th>Treatment</th>
<th>Measured matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Set</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Column 1, 2</td>
<td>TC, CTC, OTC STZ, SMT</td>
<td>Biotic</td>
<td>Dissolved Phase</td>
</tr>
<tr>
<td>Column 3, 4</td>
<td>ETM-H₂O, TYL, MNS, SLM, NRS</td>
<td>Biotic</td>
<td>(&lt;0.2 μm) and Soil</td>
</tr>
<tr>
<td>Column 5, 6</td>
<td>TC, CTC, OTC STZ, SMT</td>
<td>Abiotic</td>
<td></td>
</tr>
<tr>
<td>Column 7, 8</td>
<td>ETM-H₂O, TYL, MNS, SLM, NRS</td>
<td>Abiotic</td>
<td></td>
</tr>
<tr>
<td>Column 9, 10</td>
<td>TC, CTC, OTC STZ, SMT</td>
<td>Abiotic</td>
<td></td>
</tr>
</tbody>
</table>

| Second Set |                      |           |                 |
| Column 1, 2 | TC, CTC, OTC STZ, SMT | Biotic    | Colloidal Phase |
| Column 3, 4 | ETM-H₂O, TYL, MNS, SLM, NRS | Abiotic   | (0.2μm < PS < 1.2μm) and 1.2μm < PS < 2.7μm) |

* Biotic and abiotic treatment indicate without NaN₃ and with NaN₃ in feeding solution respectively

* PS: pore size

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### TABLE 4. Recovery and limit of quantification (LOQ) study for water and soil

<table>
<thead>
<tr>
<th>Recovery (%) *</th>
<th>Limit of quantification (μg/L or μg/kg) b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
</tr>
<tr>
<td>TC</td>
<td>106</td>
</tr>
<tr>
<td>CTC</td>
<td>112</td>
</tr>
<tr>
<td>OTC</td>
<td>103</td>
</tr>
<tr>
<td>STZ</td>
<td>90</td>
</tr>
<tr>
<td>SMT</td>
<td>104</td>
</tr>
<tr>
<td>ETM-H₂O</td>
<td>102</td>
</tr>
<tr>
<td>TYL</td>
<td>102</td>
</tr>
<tr>
<td>MNS</td>
<td>115</td>
</tr>
<tr>
<td>SLM</td>
<td>100</td>
</tr>
<tr>
<td>NRS</td>
<td>105</td>
</tr>
</tbody>
</table>

* Average of three different concentration (0.1, 1 and 5μg/L for water and 1, 30 and 90 μg/kg for soil). Relative standard deviation (RSD) ranged between 1 and 11% for water and 13 and 31% for soil.

b Statistical calculation based on reference from (Zhu et al. 2001).
Transport of Antibiotics in Soil

Milliford, Mass., USA) (2.1 × 50 mm, 2.5 μm pore size, end-capped) with a C₁₈ guard column (Phenomenex, Torrence, Calif., USA) installed to filter any particles from the sample. For detection of antibiotics, a ThermoFinnigan LCQ Duo ion trap mass spectrometer (ThermoQuest, Woburn, Mass., USA) was used and the instrument was operated in the positive ion mode with electrospray ionization to perform the mass spectrometric analysis. Detailed information of the optimized HPLC/MS/MS parameters is shown in Table 5.

Statistical Analysis

The significance of difference was evaluated with one way analysis of variance (ANOVA) at the level of \( p < 0.05 \) (Kay et al. 2005a). As a post-statistical analysis to compare the difference of individual means, Tukey’s and HSU’s MCB method were evaluated with Minitab (MINITAB 2000).

Results and Discussion

Tracer Test

A tracer test was conducted to evaluate the hydraulic conductivity of the column system with sodium chloride (1 g/L) as the reference compound. The application pattern was static mode with continuous flow from the feed reservoir at the same flow rate as the antibiotics application (0.11 mL/min). As shown in Fig. 2, the ten columns were consistent with each other in breakthrough curve indicating that flow was uniform in each soil column and an estimated average retention time was 54–56 h. A tracer test of the second set of columns was examined in the same manner and the same result was obtained (data are not shown).

Fig. 2. The breakthrough curves for tracer test of the columns

Mobility of Dissolved Antibiotics and Sorption on Soil

In the first set of experiments, the mobility of ten antibiotics in the dissolved phase and sorption characteristics on soil were evaluated with soil column leaching system. The adapted column system was originally designed for screening tests to evaluate potential leaching behaviour of different substances (Worrall et al. 1999; Kay et al. 2005a). As shown in Fig. 3, the travel time of leachate in the dissolved phase varied depending on the class of veterinary antibiotics. While none of the three TCs or TYL was detected in the dissolved phase of the leachate

<p>| TABLE 5. Optimized parameters of high performance liquid chromatography (HPLC) and mass spectrometry (MS) |</p>
<table>
<thead>
<tr>
<th>Column temp. (°C)</th>
<th>Flow rate (mL/min)</th>
<th>Mobile phase condition a</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC, CTC, OTC, STZ, SMT</td>
<td>15</td>
<td>0.32</td>
</tr>
<tr>
<td>ETM-H₂O, TYL</td>
<td>45</td>
<td>0.32</td>
</tr>
<tr>
<td>MNS, SLM, NRS</td>
<td>15</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Optimized MS condition: Nitrogen gas for drying and nebulizing
Capillary voltage: 4.5V
Capillary voltage: 21V
Capillary temperature: 165°C

a Mobile phase A: 99.9% water + 0.1% formic acid, Mobile phase B: 99.9% acetonitrile + 0.1% formic acid, and Mobile phase C: 100% methanol.
During the study period (45 days), the SAs showed a relatively high mobility, followed by the SLM, NRS, MNS, and ETM-H2O under both biotic and abiotic conditions.

The recovered mass of SMT shown in Fig. 3 had the highest peak at 6 days after application of antibiotics and it followed a decreasing trend until the end of the experiment for both treatments. A similar trend was observed for STZ in the dissolved phase of leachate but ETM-H2O and NRS showed very different profiles. In case of NRS, the initial peak was observed at 3 days after application in the soil column and two additional peaks were measured at 7 and 16 days after the initial peak. The highest recovered mass was observed in the second peak. This result might be resulted from pH or redox change that caused increase mobility of NRS. Previous studies have shown that sorption of acidic and basic compounds can be decreased and mobility increased as pH changes (de Jonge et al. 1998; Kay et al. 2005a).

As shown in Fig. 4, the pH value varied between the range of 7.6~8.1 during the first thirty days of the experiment in the leachate and the recovered mass was lower as pH decreased except during the first high peak. Although the effect of pH was hard to judge in leachate of NRS due to the low mass recovery, this result showed agreement with previous studies that pH might play an important role in sorption and mobility of antibiotics (Sassman and Lee 2005).

The results of statistical analysis revealed that the recovered mass was significantly different (p = 0.04) with biotic treatment (without NaN₃ addition) while no significant difference was observed with abiotic treatment (with NaN₃ addition) among examined antibiotics in the dissolved phase of leachate. In case of solid matrix including colloid and soil, recovered mass of antibiotics was not significantly different between biotic and abiotic treatment. This result indicated that biodegradation might affect the attenuation of antibiotics in the dissolved phase but it is not the major removal mechanism when antibiotics are sorbed to colloid or soil. In fact, previous study conducted a screening test for twelve SAs in solid matrix and reported that all evaluated compounds were not readily biodegradable (Ingerslev and Halling-Sørensen 2000).

Since measuring the solid residual of antibiotics in the columns can provide information on sorption characteristics, the soil was sectioned after the first experiment. The percentages of mass recovery of studied antibiotics from each group are shown in Fig. 5. While none of the TCs was observed in the dissolved phase of leachate, the most abundant mass was detected in soil. Residuals of CTC, TYL, and NRS were found in all depths of soil and CTC was evenly distributed from top to bottom of the soil. For TYL and NRS, the highest mass was detected at the top of soil column (0~3 cm) and generally similar amounts of mass were present throughout. However, SMT was only detected at three sampling points through the soil column.

This result can be expected due to different physicochemical properties of studied antibiotics in each group (Table 1). In general, TCs have a high $K_d$ value resulting in a greater sorption and SAs have the lowest $K_d$ value. The different sorption results agree with the high rate of mass recovery in leachate for SAs and none for TCs.
Since TYL has a high $K_{ow}$ and a relatively high $K_d$ value (Table 1) and most of the recovered mass was observed in the top part of the soil (Fig. 5), hydrophobicity might have been the primary sorption mechanism. In comparison, the low $K_{ow}$ and high $K_d$ value (Table 1) of TCs indicate that hydrophobicity is not the major sorption mechanism of these substances in soil. Other studies investigated the sorption of TCs under various pH, clay content, and cation exchange capacity conditions and showed greater sorption of TCs at lower pH and higher ionic strength. The conclusion was that sorption behaviour of TCs was pH dependent and cation exchange might be responsible for sorption of TCs in soil (Figuerola et al. 2004; Sassman and Lee 2005). Although no information is available for sorption of ionophores (IPs), the high $K_{ow}$ (Fig. 4) might be able to explain the sorption characteristic of IPs on soil.

Among the studied antibiotics, the measured mass of TC ($p < 0.001$), CTC ($p = 0.01$), OTC ($p = 0.01$), and SLM ($p = 0.02$), showed significant differences in soil column residuals between biotic and abiotic treatment (Table 3). However, the difference was not significant for other antibiotics.

![Fig. 4](image1.png)

**Fig. 4.** Measured pH and percentage of applied mass recovery in leachate for NRS; Column condition is shown in Table 3.

![Fig. 5](image2.png)

**Fig. 5.** Percentage of mass recovery for four antibiotics in soil column after column leaching (A) CTC, (B) SMT, (C) TYL, and (D) NRS; Condition of each columns is summarized in Table 3; Note that scale of x-axis is different.
Effect of Mobile Colloid in Transport of Antibiotics

The second set of experiments was focused on the colloid-facilitated transport behaviour of antibiotics in the soil. Several researches revealed that colloid-facilitated transport increased the mobility of strongly sorbed organic compounds including pesticides (Chaplain and Mills 1992; de Jonge et al. 1998; Worrall et al. 1999; Sprague et al. 2000; Villholth et al. 2000; Grolimund and Borkovec 2005). In addition, a recent sorption study of TYL onto manure showed that the sorption of TYL on colloidal materials (<1.2 μm) was as high as on particulates (<2 mm) (Kolz et al. 2005). Since no significant difference was observed when separating or combining all antibiotics in a column (Fig. 3 and 5), all ten antibiotics were applied in the soil columns for this set of experiments. Again, sodium azide (NaN₃) was used to examine the impact of biotic and abiotic conditions.

Two different filter paper sizes (1.2 and 2.7 μm) were used to differentiate pore size. The extraction procedure was the same as used with the dissolved phase and the mass recovery of each particle size was calculated with subtraction from mass recovery of 2.7 μm to 1.2 μm and 1.2 μm to 0.2 μm, respectively.

As shown in Fig. 6, colloidal-facilitated transport occurred for all studied antibiotics. Among different groups, SAs were measured to have the highest mass recovery in the colloidal form followed by MLs and IPs and the least mass of TCs were detected in the colloidal phase. Results for the recovered colloidal mass of SAs and TCs indicated that the strongest sorbing compounds are the least likely to be transported in the colloidal form. Thiele-Bruhn et al. (2004) showed that the polar functional group of SAs had a high affinity to interact with the surface of clay particles or soil organic matter initially but the weak binding forces (van der Waals or hydrolysis) could lead to release. This information might be applied to our result because most of mass for SAs was recovered at the first two times of sampling trials and drastically decreased afterward (Fig. 6).

**Fig. 6.** Percentage of applied mass recovery in colloidal phase at the size of 1.2μm < pore size < 2.7μm (A) TCs, (B) SAs, (C) MLs, and (D) IPs; Condition of each columns is summarized in Table 3; Note that scale of y-axis is different.
For comparison of biotic and abiotic treatments, STZ showed the highest mass recovery in both treatments, followed by ETM-H₂O in biotic treatment and SMT in abiotic treatment (Table 6). Statistical analysis revealed that there were no significant differences (p > 0.05) between biotic and abiotic treatments for all studied compounds indicating that the bioavailability of organic compounds became minimized after partitioning to a colloidal phase.

Comparison of Transport Behaviour

To compare transport behaviour of tested antibiotics, the percentage of applied mass recovery in different fractions is summarized in Table 6. The range of total mass recovery was 17.5~83.0% for biotic treatment and 27.8~97.0% for abiotic treatment. Incomplete absolute mass recovery might be caused by either transformation of parent compounds to other metabolites or strong sorption on soil particles that cannot be extracted.

Diverse transport behaviour was observed across the ten studied compounds. Most of the applied mass was recovered in soil matrix for the three TCs and none of the residuals were observed in the dissolved phase of the leachate. In contrast, transport of SMT in the dissolved phase and colloidal-facilitated transport of STZ were apparent. For ETM-H₂O and TYL, colloid associated mobility was the most dominant transport mechanism in biotic treatment and sorption on soil was mainly observed in abiotic treatment. Although the reason that majority of recovered mass was measured in different fractions for ETM-H₂O and TYL is not clear, our result showing less recovered mass in colloid fraction with abiotic treatment indicated that microorganism activity cannot affect the removal of antibiotics once compounds are sorbed to colloid fraction. Generally, the least amount of total mass was recovered for IPs except for SLM in biotic treatment. Among recovered mass, the majority was remained in the soil (15.6~48.4% for biotic and 18.8~26.6% for abiotic) followed by in colloidal phase (0.4~22.7% for biotic and 0.2~62.5% for abiotic) and in dissolved phase (0.2~3.3% for biotic and 1.3~6.1% for abiotic) respectively.

Conclusion

Fate and mobility of ten antibiotics belonging to four different groups were evaluated by assessing phase partitioning (dissolved and colloidal fractions) and retention characteristics in soil under biotic and abiotic conditions through column test. Transport of different antibiotic was mainly dependent on their polarity and physicochemical properties. TCs were mostly sorbed on soil which accounts for 34 to 57% of applied mass in biotic treatment and for 55 to 81% in abiotic treatment. However, applied mass of SMT was mainly recovered in leachate, e.g., 74% in biotic and 83% in abiotic treatment. Fate of antibiotics in soil was less subject to microbial activity. In general, less mass was recovered in biotic treatment indicting that biodegradation in soil during experiment was minimal even though no statistical difference was shown between biotic and abiotic treatment in soil. Antibiotics in soil were mostly transported via colloidal sorption mechanism. For example, about 22% of applied mass of STZ and ETM-H₂O under biotic condition and 62% of applied mass of STZ under abiotic condition were recovered as colloidal fractions. However, the colloid-facilitated transport of TCs was minimal among four different antibiotic groups compared. Results of our study clearly demonstrated that the fate and transport of antibiotics in soil vary significantly depending on the chemical structure (polarity) and physicochemical characteristics (Kₐw and Kᵢ etc). Therefore, phase partitioning as well as inherent characteristics of antibiotics should be carefully considered for the environmental impact assessment of the residual antibiotics in soil.

<table>
<thead>
<tr>
<th>Matrix</th>
<th>PS (µm)</th>
<th>TC</th>
<th>CTC</th>
<th>OTC</th>
<th>STZ</th>
<th>SMT</th>
<th>ETM-H₂O</th>
<th>TYL</th>
<th>MNS</th>
<th>SLM</th>
<th>NRS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissolved</td>
<td>&lt; 0.2</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>11.4</td>
<td>73.9</td>
<td>0.2</td>
<td>0.0</td>
<td>0.2</td>
<td>3.3</td>
<td>2.3</td>
</tr>
<tr>
<td>Colloid</td>
<td>0.2~1.2</td>
<td>1.1</td>
<td>0.2</td>
<td>0.3</td>
<td>16.4</td>
<td>3.6</td>
<td>21.7</td>
<td>14.5</td>
<td>1.6</td>
<td>1.3</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>1.2~2.7</td>
<td>0.2</td>
<td>1.3</td>
<td>0.3</td>
<td>6.3</td>
<td>4.7</td>
<td>0.9</td>
<td>0.9</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Soil</td>
<td>33.6</td>
<td>56.7</td>
<td>47.2</td>
<td>5.0</td>
<td>0.8</td>
<td>17.2</td>
<td>10.9</td>
<td>15.6</td>
<td>48.4</td>
<td>18.8</td>
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<tr>
<td>Sum</td>
<td>34.8</td>
<td>58.1</td>
<td>47.8</td>
<td>39.1</td>
<td>83.0</td>
<td>40.0</td>
<td>26.4</td>
<td>17.5</td>
<td>53.1</td>
<td>21.4</td>
<td></td>
</tr>
</tbody>
</table>

(A) Mass recovery in biotic treatment (%)
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