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Short-term extractability of sulfadiazine after application to soils

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ABSTRACT

The long-term environmental fate of the veterinary antibiotic sulfadiazine (SDZ) in soils is determined by a reversible sequestration into a residual fraction and an irreversible formation of non-extractable residues (NER), which can be described as first-order rate processes. However, the concentration dynamics of the resulting fractions of SDZ in soil show an unexplained rapid reduction of extractability during the first 24 h. We therefore investigated the short-term extractability of SDZ in two different soils under different SDZ application procedures over 24 h: with and without manure, for air-dried and for moist soils. In all batches, we observed an instantaneous loss of extractability on a time scale of minutes as well as kinetically determined sequestration and NER formation over 24 h. Data evaluation with a simple kinetic model led to the conclusion that application with manure accelerated the short-term formation of NER, whereas sequestration was very similar for all batches.

1. Introduction

Fertilization of agricultural fields with manure is the main emission pathway of veterinary antibiotics into the environment (Kümmerer, 2008; Boxall, 2010). The parent compound administered to pigs is usually metabolized within the body (Thiele-Bruhn, 2003; Kümmerer, 2010). Therefore, manure of treated animals often contains the active antimicrobial ingredient as well as its metabolites. Sulfonamides, including sulfadiazine (SDZ), are among the most important substance classes of veterinary antibiotics in the European Union (Sarmah et al., 2006). They were detected in manure-amended agricultural soils (Christian et al., 2003; Hamscher et al., 2005), in surface runoff (Kreuzig et al., 2005; Stoob et al., 2007) and in deeper soil layers presumably due to leaching (Hamscher et al., 2005; Unold et al., 2009). The extractability of sulfonamides in soil was often observed to decrease over time with fast initial reduction followed by a second much slower decrease, which resulted in persisting low-level concentrations in soil (Stoob et al., 2007; Schauss et al., 2009; Zarfl et al., 2009). The loss of extractability primarily goes along with a reversible sequestration in soil (Förster et al., 2009) possibly due to sorption or physical entrapment (Stokes et al., 2005), and with the emergence of non-extractable residues (e.g. Kreuzig and Höltge, 2005; Schmidt et al., 2008; Förster et al., 2009; Junge et al., 2011). A comprehensive review of the environmental fate and effects of SDZ in soil was given by Schauss et al. (2009).

Förster et al. (2009) used a sequential extraction method to distinguish three different fractions in soils that constitute SDZ pools of decreasing bioaccessibility (Semple et al., 2004). Extraction with $CaCl_2$ and methanol as mild solvents resulted in an 'easily extractable fraction' (EAS; Zarfl et al., 2009). A subsequent microwave-assisted high-temperature extraction freed sequestered amounts of SDZ from soil, named 'residual fraction' (RES). A considerable amount could not be extracted and constitutes the 'non-extractable residues' fraction (NER). In the long-term, both the residual and the NER fraction slowly increased over time. After 218 days, less than 5% were easily extractable, 45–50% was still extractable by the harsh extraction and about 50% remained non-extractable. Remarkably, already 24 h after application of SDZ and its metabolites to soil, only 40–50% of the applied amounts were easily extractable, 30–35% was extractable by the microwave-assisted extraction and 15–25% was even non-extractable.

Zarfl et al. (2009) developed a conceptual kinetic model that could describe the long-term concentration dynamics of SDZ and its metabolites in the different fractions. The model considered transformation reactions, a reversible sequestration into the residual fraction and irreversible NER formation from the easily extractable fraction as first-order rate processes. However, the rapid loss in extractability in the first 24 h could not be included into the kinetic model, when initial conditions for RES and NER

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were assumed to be zero, as would have seemed likely at least for NER. Nevertheless, neglecting the first 24 h and starting the simulations at day 1 (first data point) gave good agreement with the data, hence corroborating the model assumption that NER formation and reversible sequestration are describable as first-order processes on the medium- and long-term time scale.

To elucidate the yet unexplained fast reduction of extractability within the first 24 h, we conducted short-term batch experiments with two different soils using various modes of SDZ application to soil. In particular, we examined the influence of SDZ application in manure as opposed to the addition of an aqueous SDZ solution of the same pH. To answer whether the water content of the soil during application affects the observed rapid decrease in extractability, soils were adjusted to two different water contents prior to the experiments. Further factors that may also influence soil–solute interactions (e.g. SDZ concentration dependency, pH effect, liquid/solid ratio, temperature) were not tested here.

2. Material and methods

2.1. Experiments

2.1.1. Chemicals

All solvents used were of HPLC-grade. Salts, acids and bases were pro analysis grade. Water was purified using a Millipore Synergy water treatment system (Schwalbach, Germany). Sulfadiazine (4-amino-N-pyrimidin-2-ylbenzenesulfonamide, purity > 99%) was purchased from Sigma–Aldrich (Seelze, Germany). Sulfadiazine (CAS: 68-35-9) has a molecular weight of 250.28 g mol^{-1}, an aqueous solubility of 77 mg l^{-1} (ChemIDplus, 2011), an octanol-water partition coefficient (K_{OW}) of 0.81 and two dissociation constants (pK_a) at pH 6.49 and 2.48 (Sakurai and Ishimitsu, 1980). $^{13}C_6$-sulfadiazine served as internal standard for the analysis and was provided by the Institute of Environmental Biology and Chemodynamics of the RWTH Aachen University.

2.1.2. Soils and manure

The soils originated from the top soil horizons of two sites in North Rhine-Westphalia in Germany: a loamy sand soil from a bathystagnic Cambisol near Kaldenkirchen, and a silt loam soil from a cutanic Luvisol near Merzenhausen (soil characteristics shown in the Supporting Information). Both soils were air-dried, sieved to 2 mm and stored at ambient temperatures. After drying, the loamy sand and the silt loam had water contents (w/w) of 0.8% and 1.3%, respectively, corresponding to 3% of the maximum water holding capacity (WHC_{max}). The liquid manure used in the experiments was collected from pigs that had not been treated with SDZ. The organic matter content of the slurry was 0.54%, its pH was 8.3.

2.1.3. Soil batch experiments

Four separate application treatments were used with each soil (*lS*: loamy sand, *sL*: silt loam), to investigate the effect of manure addition and of initial soil moisture on the rapid sequestration and NER formation:

- Sulfadiazine was added to the soil either dissolved in water or in liquid manure.
- Soil had different 'initial water contents', i.e. the experiments were performed with air-dried soil (3% WHC_{max}) and with moist soil samples, which were pre-adjusted to 25% WHC_{max} by equilibration for 24 h in an end-over-end shaker.

Sulfadiazine solutions of 10 mg l^{-1} in water and manure were prepared by dilution of an aqueous SDZ stock solution (100 mg l^{-1}). The pH of the stock solution and the aqueous solution was adjusted to match the pH of the manure. Both, manure and water containing SDZ solution were then mixed with air-dried and moist soil at a ratio of 1:25 (v/w) so that each batch had a nominal SDZ concentration of 400 μg kg^{-1} dry soil. Remember that water contents were different before SDZ application only, but were subsequently adjusted to 40% WHC_{max} in all treatments. Soil and water or soil and manure were mixed manually and were then divided into aliquots of 50 g, which were incubated in the dark at ambient temperature (20 °C). Samples were taken immediately (i.e. no later than 30 min) after mixing, after 6 h (6 h) and after one day (24 h). Three pseudo-replicates were taken from each aliquot and were extracted immediately. Additional samples were taken after 2 h and 48 h, but these samples were analysed only for one treatment (*sL*/air-dried/manure) to check concentration dynamics (see Supporting Information).

2.1.4. Sequential soil extraction and analysis

Soil was extracted consecutively with calcium chloride ($CaCl_2$), methanol (MeOH), and with acetonitrile/water in a microwave according to the protocol of Förster et al. (2009). In detail, 10 g of soil (d.w.) were shaken end-over-end with 25 ml of 0.01 M $CaCl_2$ for 24 h at ambient temperature; samples were centrifuged and an aliquot of the extract was transferred to an autosampler vial and spiked with 50 μl of an internal standard solution containing $^{13}C_6$-SDZ in a concentration of 1 ng μl^{-1}. The supernatant was decanted and soil was subsequently extracted with 25 ml of methanol by shaking end-over-end for 4 h. Likewise to the previous extraction, an aliquot was centrifuged; the supernatant was transferred to an autosampler vial and spiked with internal standard. For the third extraction, the soil was heated to 150 °C with 50 ml acetonitrile/water (20:80, v/v) for 15 min in a microwave oven (Förster et al., 2008). Again, the supernatant was transferred to a vial after centrifugation and spiked with the internal standard. All samples were stored at −25 °C until analysis via LC–MS/MS (see Supporting Information).

2.2. Data evaluation

According to Zarfl et al. (2009) the fractions extracted with $CaCl_2$ and MeOH were summed up for each replicate to give the 'easily extractable fraction' (EAS). Sulfadiazine extracted by the harsher microwave-assisted extraction forms the 'residual fraction' (RES). The difference between the nominal concentration (400 μg kg^{-1} soil) and the sum of EAS and RES fractions was regarded as the non-extractable residues fraction (NER). This mass balance approach was justified because mineralization or transformation reactions (e.g. hydroxylation) showed no significant influence on the time scale of 24 h in other batch experiments (Kreuzig and Höltge, 2005; Schmidt et al., 2008; Förster et al., 2009; Junge et al., 2011).

Data were tested for outliers in each set of replicates according to Dixon's *Q* test ($n = 3$, $\alpha = 0.05$; Sachs and Hedderich, 2006). The outliers were eliminated from further calculations. Further statistical analyses of the extraction data, e.g. ANOVA, were not justified because of the small sample size ($n = 3$).

2.3. Model approach

2.3.1. Simplified kinetic model

We analysed the concentration dynamics of SDZ in the two fractions EAS and RES during the first 24 h with a simplified kinetic fate model that only considers the two different sequestration processes, namely translocation from EAS into RES (sequestration) and an additional sink process out of EAS (NER formation). All other processes are of no relevance on the short time scale of one day:

$$\frac{\mathrm{dEAS}(t)}{\mathrm{d}t} = -(k+q)\cdot\mathrm{EAS}(t) \quad (1)$$

$$\frac{\mathrm{dRES}(t)}{\mathrm{d}t} = k\cdot\mathrm{EAS}(t) \quad (2)$$

with the kinetic rate constants *k* and *q* [d^{-1}] for translocation into RES and for NER formation, respectively.

2.3.2. Parameter estimation

The rate constants *k* and *q* for each treatment were estimated by fitting the model to the respective data set. Initial conditions for the simulated EAS and RES dynamics were not fixed within the model, but also estimated. The measured concentrations directly after mixing were assigned to the time point $t = 0$, although soil contact during mixing and sampling may actually have lasted for up to 30 min. The resulting uncertainty affected the optimization results only marginally (see Supporting Information). The model optimization was performed using the computer software Scientist (Version 2.01, Micromath Inc.), which also provides statistical information on the parameters such as standard errors and 95%-confidence ranges as well as goodness-of-fit values (details in the Supporting Information).

3. Results and discussion

Obviously, the decrease in extractability of SDZ during the first 24 h occurred on two different time scales: (1) an instantaneous loss of extractability directly after mixing SDZ with soil on a time scale of minutes, and (2) a kinetically controlled further reduction on a time scale of days (Fig. 1). The latter is enhanced in the presence of manure, but this result cannot be extrapolated to the long-term fate of SDZ without further experimental proof.

3.1. Instantaneous loss of extractability

Immediately after mixing the SDZ solution with soil, total extractability of SDZ was clearly reduced in comparison to the nominal concentration of 400 μg kg^{-1} in all experiments (Fig. 1). The initial extractability ($t = 0$) as the sum of the $CaCl_2$ and MeOH