Long-term monitoring of sulfonamides and tetracyclines in manure amended soils and leachate samples - A follow-up study

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ABSTRACT

Antibiotics can be detected in manure and digestate samples worldwide. As manure is a frequently used fertilizer, antibiotics are found in soil and leachate samples. Only little is known about the long-term fate of antibiotics in the soil environment. One shortcut is the lack of appropriate monitoring studies. Here we present the results of an unequalled soil monitoring study over 18 years from an agricultural field site in Lower Saxony (Germany). Sulfonamides and tetracycline are mainly fixed in the upper soil layer. Contents showed a sharp decrease below sampling depth of 30 cm (plough depth). Sulfaguanidine and sulfanethazine (SMZ) were detected down to 90 cm. Water samples taken below the field site revealed the transfer of sulfonamides into leachate. High variances were observed between sampling points emphasizing the need for sampling strategies for environmental studies. In addition, field lysimeters with defined input of sulfonamides enabled a long-term monitoring and mass balance of antibiotic transfer into leachate over 10 years. SMZ showed the highest mobility with concentrations up to 65 ng L⁻¹. Less than 0.5% of the applied SMZ was transferred into the leachate. Data of lysimeter and field water samples support the theory of a steady state process with a continuous input of sulfonamides such as SMZ into leachate. Soils contaminated with antibiotics can be a long-term source for the input of antibiotic active compounds into deeper soil layers and groundwater.

1. Introduction

Due to its high nutrient content, easy applicability and availability, manure is used as soil fertilizer (Weiland, 2010). According to the Federal Statistical Office, more than 200 Mio. m⁻³ (200 billion liter) liquid manure and 20 Mio. tons (200 Mio. Mg) dung were spread on agricultural land in Germany in 2015 (Statistisches Bundesamt). However, besides nutrients, manure can contain (micro) pollutants such as synthetic hormones, heavy metals or pharmaceuticals (Hu et al., 2017). Pharmaceuticals such as antibiotics are used worldwide in animal husbandry with upward trend (Van Boeckel et al., 2015). Due to incomplete absorption and poor metabolism, antibiotics are partially excreted by the treated animal. Excretion rates can vary remarkably ranging from 10 to 90% depending on the compound (Sarmah et al., 2006; Spielmeyer, 2018). In 2018, 722 tons antibiotics were sold to German veterinarians (Wallmann et al., 2019). The main part of the active ingredients (37.5%, 271 tons) belonged to the beta-lactams. These compounds are known to possess a reduced stability in manure matrices due to the alkaline pH (Tsui et al., 1978; Berendsen et al., 2018). Assuming a mean excretion rate of 50% for the other ingredient classes, this would make up 225 tons of antibiotics that were transferred into manure in 2018. Taken into account the mass of manure produced in Germany per year (see above), this would correspond to an average antibiotic content in manure of 1 mg kg⁻¹. As there are hotspots for the distribution of antibiotics (Wallmann et al., 2019), remarkable higher contents can be found in manure and dung samples in Germany (Wohde et al., 2016; Spielmeyer, 2018). Zhou et al. (2013) calculated that 3244 tons of antibiotics might be transferred into manure in Chinese cattle and swine breeding operation systems. Although being estimations, these numbers illustrate the potential dimension of the excretion of antibiotic active compounds by treated animals.

As manure is frequently utilized as soil fertilizer, antibiotics enter the environment via this pathway. Especially in regions with a high density of animal breeding, soils are vulnerable towards contamination with antibiotics (de la Torre et al., 2012). The question whether the contents found in manure or soil can enhance the formation of antibiotic resistances is not finally clarified.
Antibiotics are detected in soil and leachate samples worldwide. There are several monitoring studies dealing with the occurrence of antibiotics in those matrices; however, long-term studies are rare (e.g. Kivits et al., 2018). A long-term approach is essential to gain insight into the fate of antibiotics in the soil environment concerning potential elimination pathways, compound stability and mobility within soil matrices. In 2017, we demonstrated the transfer of sulfonamides into leachate samples in field lysimeters over six years (Spielmeyer et al., 2017). Here, the results of the subsequent sampling years (2016–2019) are presented.

This recent work also contained results for leachate samples from an agricultural field site in Lower Saxony, Germany. From the same site, data regarding the antibiotic content in soil samples are available (Hamscher et al., 2002, 2005). Both data sets were extended with data until 2019 enabling statements about the behavior of sulfonamides and tetracyclines in soil and leachate samples. Compared to the initial publications, the range of compounds being analyzed was enhanced by sulfadimethoxine (SMX) and sulfamethoxazole (SMX) as well as chlortetracycline (CTC), doxycycline (DC), iso-chlortetracycline (isoCTC), and abiotic transformation products of chlortetracycline. IsoCTC was not investigated by Hamscher et al. (2002, 2005) as their study focused on the initial compounds, not the transformation products. SGU and 4-OH-SDZ were not included in the previous publication by Spielmeyer et al. (2017) due to an uncertain or the lack of an unequivocal compound identification. In this study, identification was enabled by high resolution mass spectrometric analyses (HR-MS). Additionally, we were able to obtain an isolated standard of 4-OH-SDZ for comparison of MS spectra and analytical retention time. Thus, our studies performed over a period of almost two decades provide clear evidence of a permanent contamination of agricultural fields and corresponding leachate with veterinary antibiotics and some of their metabolites. Several of the compounds under investigation are among the best-selling veterinary drugs worldwide and therefore, the time has come, to rethink their current applications.

2. Material and methods

2.1. Reagents and materials

Eleven sulfonamides and five tetracyclines were investigated, including sulfacloropyridazine (SCY), sulfadiazine (SDZ), 4-hydroxy sulfadiazine (4-OH-SDZ), sulfadimethoxine (SDM), sulfaguanidine (SGU), sulfamerazine (SMR), sulfamethazine (SMZ), sulfamethoxazole (SMX), sulfamethoxydiazine (SMPD), sulfapyridine (SPY), sulfathiazole (STZ) as well as chlortetracycline (CTC), doxycycline (DC), iso-chlortetracycline (isoCTC), oxytetracycline (OTC), tetracycline (TC). An overview regarding structures, molecular weights, utilized precursor and product ions for analysis is provided in the Supplemental Material (Table A1). Standards for analysis and manure fortification were purchased in VETRANAL™ grade from Sigma-Aldrich (Taufkirchen, Germany). IsoCTC, the main transformation product of CTC, was obtained with ten sulfonamides (SCY, SDZ, SDM, SGU, SMPD, SMR, SMX, SMZ, SPY, STZ) applied to two field lysimeters in Lower Saxony (Germany). A detailed description of the soil parameters, manure application and sampling procedure is provided by Spielmeyer et al. (2017). Leachate samples at field site A (bedrock derived of limestone) were taken in 1 m depth, at field site B (sandy soil) in 2 m depth. Samples were stored at -18 °C before analysis.

For site B, data about the lysimeter seepage rate (L m$^{-2}$ d$^{-1}$) were utilized to set up a mass balance. Based on concentrations determined for SMZ, an estimation of the remaining sulfonamide proportion within the soil column was performed. In October 2018, soil samples were taken from the same lysimeter to investigate the remaining antibiotic content.

2.4. Sample preparation and analysis - water samples

The procedure is described in detail in Spielmeyer et al. (2017) and Bailey et al. (2016). Briefly, an internal standard consisting of $^{13}$C$_6$ SDZ, SMX and SMZ was added to 30 mL water sample to reach a final concentration of 100 ng L$^{-1}$. Samples were automatically applied to SPE using the system GX-271 ASPEC™ (Gilson, Limburg-Offenheim, Germany). Blank samples and standards (50 ng L$^{-1}$) were prepared using 30 mL tap water.

For LC-MS/MS analysis, a Dionex Ultimate RS3000 UHPLC (Dionex Thermo Fisher Scientific, Germering, Germany) connected to an ABSciex QTrap 3200 triple quadrupole mass spectrometer (ABSciex, Darmstadt, Germany) was utilized. Column and gradient details are provided in the Supplemental Material (Section A2). MS data acquisition was conducted in multiple reaction monitoring mode (MRM) recording two characteristic fragment ions. Details are provided in the Supplemental Material (Table A1).

2.5. Sample preparation and analysis - soil samples

One g soil was weighed in a 15 mL polypropylene centrifuge tube. The repeatability of selecting 1 g portion of 1 kg sample was not tested. Each sample was fortified with the internal standard consisting of $^{13}$C$_6$ SDZ, SMX and SMZ to reach a final content of 70 μg kg$^{-1}$. Samples were incubated at room temperature in the dark for seven days to allow an interaction of the internal standard with the soil matrix. This interaction respective the incubation time was found to influence the recovery rates of the investigated antibiotics (see Table A4 in the Supplemental Material). The chosen procedure (7 d incubation of internal standard before extraction) was considered as suitable compromise between the
simulation of long-term storage of soil samples and time spent for sample preparation. The first extraction step was conducted with 3 ml 1 M citrate buffer (pH 4.7) which contained 0.05 M EDTA. After addition, the samples were vigorously shaken for 10 min and phases were separated by centrifugation (3 min, 3000 g). The supernatant was removed and stored in a 50 ml polypropylene centrifuge tube at 10 °C before further treatment. The solid residue was resuspended in 2 ml citrate buffer and 4 ml methanol and the mixture was shaken on a horizontal shaker at room temperature in the dark for 24 h. Phases were separated by centrifugation (3 min, 3000 g) and the supernatant was combined with the solution of the first extraction step. For reducing the organic fraction, the extract was incubated in a stream of nitrogen at 40 °C for 30 min. Afterwards, samples were diluted to 27.5 ml with deionized water and applied to SPE. SPE was performed via an automatic SPE system (GX-271 ASPEC™, Gibson, Limburg-Offheim, Germany). Cartridges were preconditioned with each 3 ml of methanol + 0.1% formic acid, methanol and citrate buffer. After application of 25 ml sample volume, elution was performed with two times 1 ml methanol + 0.1% formic acid and two times 1 ml methanol. Eluates were reduced to dryness (rotary evaporator) and the residue was reconstituted in two times 100 μl methanol/water (1:1, v/v). Samples were stored at -20 °C before analysis.

For quantitative analysis, a system consisting of a Dionex Ultimate RS3000 UHPLC (Dionex Thermo Fisher Scientific, Germering, Germany) was connected to an ABSciex QTrap 3200 triple quadrupole mass spectrometer (ABSciex, Darmstadt, Germany). UHPLC conditions are given in the Supporting Information (Section A2). MS analyses were performed using electrospray ionization (ESI) in positive mode. Capillary voltage and temperature were set to 5000 V and 500 °C, respectively. Data acquisition was conducted via multiple reaction monitoring mode (MRM) recording two fragment ions (except iso-chlortetracycline, only one intense fragment is formed) (Table A1). Scan time for each transition was set to 100 ms.

Details concerning method validation are provided in the Supplemental Material (Section A3, Table A3-A5). For quantification, a solvent standard was utilized with a concentration of 500 μg L⁻¹ (internal standard 350 μg L⁻¹) which corresponds to 100 μg kg⁻¹ (internal standard 70 μg kg⁻¹) in case of 100% recovery. Results are presented as μg antibiotic per kg soil dry matter. Results were corrected by recovery rates obtained after 14 d analyte and 7 d internal standard incubation on soil matrix (Table A4). Data below MLOQ are often presented as 0.5 MLOQ. However, this was not applicable in our case. For several compounds, MLOQ and MLOQ differed by less than factor 2 (Table A3) which lead to (theoretical) contents below the limit of detection. Therefore, contents below MLOQ were set to median value of MLOD and MLOQ (Table A3).

2.6. HR-MS measurements

To verify the presence of SGU and 4-OH-SDZ in water and soil samples, extracts were additionally analyzed using a Shimadzu Nexera X2 UHPLC (Shimadzu, Darmstadt, Germany) connected to an ABSciex TripleToF 5600 + quadrupole - time of flight mass spectrometer. Both full scan as well as product ion data acquisition were conducted. Detailed method parameters are provided in the Supplemental Material (Section A2). Calibration of the MS system was performed before measurements with sodium formate (Juo et al., 2014).

3. Results and discussion

3.1. Soil and leachate samples from livestock farming area

The retrospective soil study includes samples from 2001, 2005, 2009, 2015 and 2018 and, thus, covers a period of 18 years. Depth profiles down to 90 cm were available for the years 2001 and 2015.

3.1.1. Sulfonamides

Of all sulfonamides investigated, four compounds were detected in the soil samples, namely SDZ, 4-OH-SDZ, SGU, SMZ. They were found in all samples taken between 0 and 30 cm (Figure 1 A-D). Presence of SGU and 4-OH-SDZ were verified by HR-MS measurements (data provided in Table A6). In Germany, SDZ and SMZ are the most applied sulfonamides in veterinary medicine making up 80% of the distributed sulfonamides in 2015 (Wallmann et al., 2016). Thus, their detection in soil samples in areas of intense livestock farming can be expected whereas the detection of other sulfonamides is less likely.

4-OH-SDZ is a metabolite of SDZ formed e.g. by pigs treated with the sulfonamide. As no complete conversion takes place, both SDZ and 4-OH-SDZ are excreted in pig manure with a ratio of approximately 2:1 (Lamshöft et al., 2007). The investigated field site is frequently fertilized with manure from a pig breeding farm. In one sample from 2018, 4-OH-SDZ was present with a content of 2.3 mg kg⁻¹ fw whereas SDZ was surprisingly not found. Thus, the metabolite was (and might be still) introduced in the soil via manure application. Another possibility might be the formation of 4-OH-SDZ in the soil itself (see Section 3.2).

SDZ and 4-OH-SDZ showed comparable contents within the upper soil (0–30 cm, maximum contents of 5.8 and 8.0 μg kg⁻¹ dry matter for SDZ and 4-OH-SDZ, respectively). It should be taken into account that for all matrices investigated the quantification of 4-OH-SDZ was performed using a SDZ standard assuming similar ionization of both compounds. Comparison of the isolated 4-OH-SDZ with an analytical standard of SDZ implies that the metabolite gives an up to factor 5 less intense MS signal than the parent compound. Thus, contents of 4-OH-SDZ presented here might be actually higher. Due to uncertainties concerning compound purity and matrix effects for 4-OH-SDZ in soil and leachate extracts (isolated compound was not available during method validation), no correction of the analytical results was performed.

In case of samples taken below 30 cm, samples showing detectable amounts of SDZ did not show the presence of 4-OH-SDZ (Figure 1A and B). On the one hand, this point is attributed to the low analyte content (contents close to MLOD). Another explanation might be different sorption processes of the compounds. 4-OH-SDZ was frequently detected in water samples taken below the field site (88 out 116 samples), while positive results for SDZ were found only sporadically (less than ten samples). 4-OH-SDZ might be transferred more easily to deeper soil layers than SDZ leading to non-detectable amounts of the metabolite in the soil column. This example underlines that analyses of soil or leachate samples only would provide an incomplete picture of the fate of antibiotics in environmental matrices. Based on soil sample results, one might consider 4-OH-SDZ to be retained in the upper soil layer. However, leachate samples prove the transfer of this compound within the soil column. Whenever possible, both sample types should be investigated to obtain a deeper insight in the mobility of compounds in the soil environment.

A sharp decrease of the antibiotic content and detection frequency was observed below 30 cm for all analytes (Figure 1). For all years, there was a clear separation between samples taken in the upper soil layer (A-horizon, 0–30 cm) and the subsoil (B-horizon, 30–90 cm). The A-horizon corresponds to the plough layer and is visually distinguishable from the other layers due to its high content of organic matter (2.01% organic carbon vs. 0.64% organic carbon in the Bhs- and 0.2% organic carbon in other layers due to its high content of organic matter (2.01% organic carbon vs. 0.64% organic carbon in the Bhs- and 0.2% organic carbon in all samples taken between 0 and 30 cm (Figure 1 A–D). Presence of SGU and 4-OH-SDZ were verified by HR-MS measurements (data provided in Table A6). In Germany, SDZ and SMZ are the most applied sulfonamides in veterinary medicine making up 80% of the distributed sulfonamides in 2015 (Wallmann et al., 2016). Thus, their detection in soil samples in areas of intense livestock farming can be expected whereas the detection of other sulfonamides is less likely.

In contrast to SDZ, detectable and quantifiable amounts of SMZ were observed in the subsoil although both compounds were present in
Figure 1. Contents of (A) SDZ, (B) 4-OH-SDZ, (C) SGU, (D) SMZ, (E) TC in soil samples taken on a monitoring field site in Lower Saxony (Germany) over the course of 18 years; contents are given in μg antibiotic per kg dry matter, contents were corrected by recovery rate (see Table A4); values are given as arithmetic mean ± standard deviation (n = 4; except 2015, only 3 samples were available for depths below 40 cm); numbers indicate the number of positive samples with content above MLOD, negative samples were set to 0 for mean calculation; dotted and solid line represent the MLOD and MLOQ, respectively (due to scaling dotted line not visible in case of TC); * - one value eliminated as outlier.
comparable contents in the upper soil layer. One explanation can be the lower MLOD and MLOQ for SMZ (Figure 1, Table A3). SDZ contents were already close to the MLOQ in the A-horizon, making a detection of the compound less likely in the layers below. Another reason can be a different mobility of SDZ and SMZ within the soil matrix. In several studies investigating the fate of sulfonamides in soil columns, SMZ was found to possess a higher mobility than other investigated compounds (Kurwadkar et al., 2011; Vithanage et al., 2014; Kivits et al., 2018). This assumption is supported by the results of the leachate samples. Although SMZ, SDZ and 4-OH-SDZ possessed similar contents in the upper soil layer (Figure 1), only SMZ and 4-OH-SDZ were detected in the leachate with concentrations up to 97 and 37 ng L$^{-1}$, respectively (Table 1).

Leachate values of SMZ for the different sampling points confirm the results of previous studies both in tendency and order of magnitude (Hamscher et al., 2005; Spielmeyer et al., 2017). Although being collected under the same field, leachate samples of the four suction cups showed distinct differences concerning the SMZ concentration. Similar tendencies were found for SGU and 4-OH-SDZ (Table A7 and A8). This can be attributed to an inhomogeneous distribution of SMZ in the soil matrix. Within the upper soil layer samples, relative standard deviations up to 40% were obtained per sampling day (Figure 1 D). Another explanation can be an inhomogeneous soil matrix itself, for instance slightly varying contents of humic matter or different proportions of soil constituents such as sand or loam. These factors are known to influence the mobility of sulfonamides in soil matrices (Unold et al., 2009; Bailey et al., 2016; Pan and Chu, 2017). Leachate samples at S3 and S4 showed a more intense color (yellow to yellow-brown) than samples at S1 and S2 (slightly yellow), which is probably due to an enhanced transfer of soil ingredients such as humic matter. This could favor the wash out of other compounds present in the soil such as sulfonamides consequently leading to maximum values of SMZ in the leachate at S3 and S4 (Table 1).

Interestingly, soil and leachate samples showed different patterns concerning the distribution of minimum and maximum antibiotic contents. In most cases, soil samples taken at S4 delivered the lowest SMZ content while samples taken at S2 showed the maximum value (Table A9). The opposite was found for the leachate samples with lowest concentrations at S2 (<MLOQ up to 18 ng L$^{-1}$) and highest values at S4 (35–97 ng L$^{-1}$) (Table 1). As stated before, inhomogeneities within the soil matrix can influence the mobility of compounds such as sulfonamides within the soil column. These results underline the need for selecting several sampling points within one field site to gain a comprehensive insight in the fate and transfer of contaminants in natural matrices. Taking water samples at S1 or S2 only would have implicated a low potential of sulfonamides being transferred into leachate whereas results for sampling point S4 only might have overestimated the mobility (Table 1, Table A7 and A8).

### Table 1. SMZ, SGU and 4-OH-SDZ in leachate samples below an agriculture field within an area of intensive livestock farming.

| sampling date | SMZ | SMZ | SMZ | SMZ | SGU | 4-OH-SDZ |
|---------------|-----|-----|-----|-----|-----|----------|
|               | S1  | S2  | S3  | S4  | S4  | S4       |
| October 27, 2016 | 15  | <MLOQ | 29  | 92  | 106 | 12       |
| November 10, 2016 | 13  | <MLOQ | 41  | 79  | 91  | 11       |
| November 24, 2016 | 15  | 12   | 40  | 81  | 234 | 18       |
| December 10, 2016 | 15  | 10   | 40  | 82  | 150 | 16       |
| December 19, 2016 | <MLOQ | <MLOQ | 27  | 78  | 186 | 13       |
| January 02, 2017  | <MLOQ | <MLOQ | 41  | 78  | 180 | 17       |
| January 16, 2017  | <MLOQ | <MLOQ | 23  | 73  | 200 | 15       |
| February 07, 2017 | 14  | <MLOQ | 36  | 44  | 111 | <MLOQ    |
| February 25, 2017 | 12  | <MLOQ | 37  | 43  | 111 | 10       |
| March 05, 2017    | <MLOQ | <MLOQ | 27  | 40  | 95  | <MLOQ    |
| March 26, 2017    | <MLOQ | <MLOQ | 41  | 35  | 92  | <MLOQ    |
| April 01, 2017    | <MLOQ | <MLOQ | 49  | 36  | 124 | 10       |
| December 19, 2017 | 27  | <MLOQ | 51  | 61  | 99  | 16       |
| January 01, 2018  | 19  | <MLOQ | 46  | 50  | 104 | 13       |
| January 15, 2018  | 16  | <MLOQ | 60  | 47  | 80  | 11       |
| February 13, 2018 | 10  | <MLOQ | 34  | 40  | 72  | <MLOQ    |
| February 25, 2018 | 11  | <MLOQ | 49  | 48  | 86  | 10       |
| March 17, 2018    | 12  | <MLOQ | 53  | 48  | 100 | 11       |
| April 02, 2018    | 17  | <MLOQ | 56  | 52  | 126 | 12       |
| April 17, 2018    | 16  | <MLOQ | 54  | 57  | 143 | 14       |
| May 02, 2018      | 19  | <MLOQ | 59  | 65  | 145 | 16       |
| December 18, 2018 | 11  | 14   | 16  | 86  | 107 | 33       |
| December 31, 2018 | 33  | 11   | 35  | 97  | 105 | 28       |
| January 10, 2019  | 24  | <MLOQ | 27  | 89  | 132 | 31       |
| January 20, 2019  | 14  | 18   | 15  | 82  | 168 | 37       |
| February 12, 2019 | <MLOQ | 10   | <MLOQ | 45  | 139 | 30       |
| February 27, 2019 | 18  | 15   | 22  | 42  | 85  | 18       |
| March 13, 2019    | 16  | 12   | 28  | 43  | 93  | 20       |
| March 28, 2019    | <MLOQ | <MLOQ | 24  | 44  | 95  | 17       |

Concentrations given in ng L$^{-1}$.  
<MLOQ - concentrations below 10 ng L$^{-1}$.

MLOQ - method limit of quantification, 4-OH-SDZ - 4-hydroxy-sulfadiazine, SGU - sulfaguanidine, SMZ - sulfamethazine; for description of sampling sites S1 to S4 see Hamscher et al. (2002).

Data for S1 to S3 for SGU and 4-OH-SDZ are provided in Table A7 and Table A8, respectively. * Values for MLOQ were taken from sulfadiazine (Table 2).
SGU showed the highest soil contents of all sulfonamides investigated (Figure 1C). Like SMZ, it revealed a high mobility within the soil column and samples down to 90 cm were found to contain SGU. Consequently, this sulfonamide was also detected in leachate possessing the highest concentration of the compounds analyzed (Table 1). SGU is the most polar sulfonamide investigated in this study and comparatively low Kd values were previously determined (Białek-Bielińska, 2012; Maszkowska et al., 2013). Both factors might favor the transfer of the compound in the soil column.

In contrast to SDZ and SMZ, SGU is not authorized as active ingredient in human and veterinary medicine in Germany (VETDATA). According to data from the German Federal Office of Consumer Protection and Food Safety, SGU was not distributed to German veterinarians from 2013-2015 (afterwards, no detailed data available) (Wallmann et al., 2014, 2015; 2016). Analysis of soil and manure samples in Germany, Spain and in the USA did not reveal SGU in any sample (Hailer et al., 2002; shelver et al., 2010; Biel-Maso et al., 2017). Also manure and digestate samples from the respective field site did not contain SGU in amounts above the MLOD. This raises the question about the source of this antibiotic in the soil.

As a permanent input via manure can be excluded, the formation of SGU within the soil might be an explanation. SDZ, SGU and SMZ possess the same substructure units. A cleavage of the diazine respective methazine ring would lead to the guanidine subunit present in SGU. Sur and Stork (2003) found a similar transformation pathway for imidacloprid in the same substructure units. A cleavage of the diazine respective methid in human and veterinary medicine in Germany (VETDATA). According to data from the German Federal Office of Consumer Protection and Food Safety, SGU was not distributed to German veterinarians from 2013-2015 (afterwards, no detailed data available) (Wallmann et al., 2014, 2015; 2016). Analysis of soil and manure samples in Germany, Spain and in the USA did not reveal SGU in any sample (Hailer et al., 2002; shelver et al., 2010; Biel-Maso et al., 2017). Also manure and digestate samples from the respective field site did not contain SGU in amounts above the MLOD. This raises the question about the source of this antibiotic in the soil.

One explanation might be the high persistence of sulfonamides in soil due to sequestration processes (Forster et al., 2009). SGU might derive from an application which occurred decades ago. At first sight, the depth profile analysis and results of the leachate sample (Figure 1C, Table 1) contradict this theory. If positive SGU findings were the result of a former application, one should expect a decrease of the soil SGU content over a period of 18 years. This was not observed for the values within the upper soil layer (0–30 cm, Figure 1C). As discussed in detail for the lysimeter study (Section 3.2), even comparable high leachate concentrations of 200 ng L⁻¹ over several years correspond to less than 1% of the initial soil content being washed out. Thus, the positive findings of SGU might be the result of a former application of the antibiotic even though soil contents remain on a comparable level over years. This would underline the high stability of sulfonamides in soil matrices and their potential for a long-term transfer into leachate samples.

Thus, the origin of SGU cannot be clarified so far. Studies investigating the presence and fate of sulfonamides in soil and leachate samples should include the analysis of SGU to check whether this specific sulfonamide can only be found in places where SDZ and SMZ are present. This might help to clarify whether SGU could be potentially formed in soil matrices. However, studies performed with ¹⁴C-labelled SDZ applied to soil did not report a formation of SGU so far (Unold et al., 2009; Kasteel et al., 2010; Sittig et al., 2014). A detection of SGU only or in higher amounts than SDZ and SMZ would give a hint about a potential SGU usage in the study area before it was replaced by sulfonamides such as SDZ and SMZ. This would deliver useful information about the stability of sulfonamides in soil matrices and a potential long-term transfer of the antibiotics into leachate.

3.1.2. Tetracyclines

TC was detected in all samples taken between 0 and 30 cm (Figure 1E). CTC, DC and OTC were not found. Hamscher et al. (2002, 2005) reported traces of CTC for the same field site with contents below 10 μg kg⁻¹. As the MLOD of the method presented here was 25 μg kg⁻¹ (Table A3), negative results are probably due to analytical shortcomings. In contrast to this study (triple-quadrupole mass spectrometer), Hamscher et al. (2002, 2005) utilized an ion trap. Ion traps enable the accumulation of ions potentially leading to lower MLOD. CTC was detected in a manure sample from an associated pig fattening farm (2.3 mg kg⁻¹ fw). Thus, an input of this antibiotic in the soil occurs. In manure, CTC undergoes an isomerization to its main transformation product isoCTC. This abiotic reaction occurs at pH values above 7.5 (Halling-Sørensen et al., 2002). The presence of isoCTC could be proven in both manure (5.1 mg kg⁻¹) and soil samples (contents up to 23.5 μg kg⁻¹, Table A10). Thus, the detection of isoCTC might serve as indirect proof for CTC.

Tetracyclines are known to show strong sorption to soil matter due to physiosorption processes like chelate complex formation with multivalent cations (Eichhorn and Ago, 2004). A transfer to groundwater samples below the investigated field site was not observed within previous studies (Hamscher et al., 2002, 2005). Laboratory studies did not find a transfer of TC along an artificial, trenched soil column (Pan and Chu, 2017). Within this study, both TC and isoCTC were detected in sampling depth of 0–30 cm which corresponds to the plough depth. In few cases, TC was detected in samples taken between 30 and 40 cm (Figure 1E). This would suggest a mobility of the antibiotic within the soil matrix. However, the positive findings are mainly ascribed to sampling issues. During separation of the soil column into 10 cm segments, a partial mixture of sample material above and below 30 cm probably occurred. Moreover, the plough depth is not exactly 30 cm everywhere in the field and may be up to 35 cm, thus, topsoil material will be included in the layer 30–40 cm. A transfer due to leaching processes should have led to positive findings in samples below 40 cm, as it was observed for sulfonamides (see discussion in Section 3.1.1), but this was not the case.

Over the course of the years, TC contents did not show a clear trend which would imply an accumulation or an elimination of the compound (Figure 1E). A pig manure sample from 2018 possessed a TC content of 0.81 mg kg⁻¹ fw. In previous manure samples, TC was detected as well ranging from 0.5 to 9.7 mg kg⁻¹ fw (Hamscher et al., 2002, 2005; Spielmeyer et al., 2017). This indicates a permanent input of the antibiotic into the soil and an increase of the TC soil content would be expected. Based on a plough depth of 30 cm, a soil density of 1,500 kg per m³ (taken from “Guideline on environmental impact assessment for veterinary medicinal products in support of the VICH guidelines GL6 and GL38”) and a manure application of 45 m³ fresh manure per hectare and year (Hamscher et al., 2002), a TC content of 1 mg kg⁻¹ in manure would correspond to an annual increase of the soil content by 10 μg kg⁻¹ in soil after ploughing. Based on the variability of the four samples per sampling point (Figure 1E), this increase could not be represented by the analytical method. Within the method validation, an interday precision of 3.2% for TC was determined (Table A3). Depending on the initial soil content, an addition of 10 μg kg⁻¹ might not be distinguishable from the analytical variation (3.2% of 300 μg kg⁻¹ corresponds to 9.6 μg kg⁻¹). Furthermore, the four sampling points themselves provide a variation as already discussed for sulfonamides (see Section 3.1.1) (Figure 1E). Consequently, a remarkable increase of the TC soil content over the study period can only be observed when the TC content of the utilized manure is continuously higher than 1 mg kg⁻¹ or when more than 45 m³ are applied. Both factors cannot be excluded. However, the observed fluctuations between sampling years are probably the result of an inhomogeneous distribution of the antibiotic within the matrix. In addition, 1 g sample material was used for sample preparation and this amount is supposed to represent an area of 256 m². Samples were homogenized before usage, but the risk of hot spots within the material cannot be completely eliminated. This is especially emphasized by the results for 2009. Ploughing should lead to a homogenization of the soil within the first 30 cm. However, samples from 10–20 cm showed a more than two times higher TC content than the surrounding segments (Figure 1E). Similar inhomogeneities were reported in previous studies from the same field site (Hamscher et al., 2002,
2005). This underlines the need for developing sampling strategies before taking field samples to reduce errors to a minimum.

The constant TC content could also represent a pseudo-persistence, a term first utilized for contaminants in sewage effluents (Daughton, 2002; Mackay et al., 2014). On the one hand, sequestration processes would lead to a reduction of the TC content. On the other hand, the application of contaminated manure leads to a permanent input of TC into the soil. If both processes occurred in the same order of magnitude, an (apparent) constant TC content would be the result. For sulfonamides, microwave extraction with elevated temperatures (e.g. 150 °C for 15 min) can be employed to enhance the recovery of the analytes from (aged) soil samples (Förster et al., 2008). However, tetracyclines possess a reduced temperature stability (Loflin et al., 2008), so these enhanced extraction methods are not applicable in their case.

### 3.2. Lysimeter studies

The results presented here are a follow-up survey of a previously published study (Spielmeyer et al., 2017). To avoid redundancy, the discussion presented here is limited to new aspects based on the results obtained for the years 2016–2019.

Ten sulfonamides were applied to the lysimeters at site A and B from autumn 2009 until autumn 2011. Within the years of manure application, all sulfonamides except STZ were detected in the leachate at site A (bedrock soil) (see Table 2 in Spielmeyer et al. (2017)). From 2015-2019, all sulfonamides except STZ were detected in the leachate at site A. In other studies, these detections were limited to SDZ, SGU, SMX, and SMZ and most results (bedrock soil) (see Table 2 in Spielmeyer et al. (2017)). From 2015-2019, the majority of the samples (Figure 2B). No permanent increase of the

Table 2. Detection frequency of sulfonamides in lysimeter leachate.

|    | site A | 2015 (6) | 2016 (8) | 2017 (16) | 2018 (10) | 2019 (10) |
|----|--------|----------|----------|----------|----------|----------|
|    | MLOD | MLOQ | >MLOD | >MLOQ | >MLOD | >MLOQ | >MLOD | >MLOQ | >MLOD | >MLOQ |
| SCY | 7.5  | 20   | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
| SDZ | 2    | 10   | 0      | 0      | 0      | 2      | 0      | 2      | 0      | 0      |
| 4-OH-SDZ | 2* | 10* | n.a.  | n.a.  | n.a.  | 0      | 0      | 0      | 0      | 0      |
| SDM | 5    | 10   | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
| SGU | 10   | 60   | 2      | 1      | 4      | 1      | 16     | 0      | 10     | 1      |
| SMR | 5    | 10   | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
| SMZ | 2    | 10   | 6      | 1      | 8      | 0      | 15     | 2      | 10     | 1      |
| SMX | 2    | 7.5  | 6      | 0      | 4      | 0      | 5      | 0      | 0      | 0      |
| SMPD| 2    | 10   | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
| SPY | 5    | 10   | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
| STZ | 7.5  | 20   | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
| site B | 2015 (8) | 2016 (12) | 2017 (31) | 2018 (18) | 2019 (20) |
| SCY | 7.5  | 20   | 0      | 0      | 0      | 0      | 0      | 0      | 1      | 1      |
| SDZ | 2    | 10   | 0      | 0      | 0      | 4      | 0      | 0      | 0      | 1      |
| 4-OH-SDZ | 2* | 10* | n.a.  | n.a.  | n.a.  | 20     | 0      | 8      | 16     | 0      |
| SDM | 5    | 10   | 0      | 0      | 1      | 0      | 0      | 0      | 0      | 0      |
| SGU | 10   | 60   | 2      | 1      | 8      | 2      | 30     | 19     | 18     | 20     |
| SMR | 5    | 10   | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
| SMZ | 2    | 10   | 8      | 8      | 12     | 12     | 30     | 28     | 18     | 20     |
| SMX | 2    | 7.5  | 8      | 11     | 0      | 21     | 0      | 18     | 0      | 15     |
| SMPD| 2    | 10   | 8      | 9      | 0      | 18     | 0      | 9      | 0      | 12     |
| SPY | 5    | 10   | 7      | 0      | 8      | 0      | 24     | 6      | 17     | 1      |
| STZ | 7.5  | 20   | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 5      |

MLOD/Q - method limit of detection/quantification, n.a. - not analyzed, SCY - sulfachloropyridazine, SDZ - sulfadiazine, 4-OH-SDZ - 4-hydroxy sulfadiazine, SDM - sulfadimethoxine, SGU - sulfaguanidine, SMR - sulfamerazine, SMZ - sulfamethazine, SMX - sulfamethoxazole, SMPD - sulfamethoxypyridazine, SPY - sulfapyridine, STZ - sulfathiazole; 2015 (6) - sampling year and number of investigated samples.

Data from 2015 taken from Spielmeyer et al. (2017) (except data for SGU); data from 2016 from Spielmeyer et al. (2017) were enhanced by data from second half of 2016 investigated within the study presented here.

* Due to the lack of a pure standard substance, values from SDZ were adapted
4-OH-SDZ derives from the transformation of the applied SDZ in the soil. It appears highly unlikely that the positive content corresponded to less than 0.5% of the SDZ content. Thus, it anticipated the manure utilized for sulfonamide application, but the 4-OH-SDZ content was also detected in the leachate samples. This metabolite of SDZ was also detected in the application of the antibiotic into the soil.

The consequence of a steady state process leading to a continuous transfer of sulfonamides into soil matrix which is reflected by decreasing recovery rates with prolonged sample storage (Table A4) (Hamscher et al., 2005; Müller et al., 2013). They could be build up by covalent bond formation between sulfonamides and humic substances (Gulkowska et al., 2013). Gulkowska et al. (2013) distinguished between desorbable, labile and stable SMZ within their incubation setup with synthetic humic acids. The constant release of a small amount of SMZ might be due to desorable and labile SMZ still present in the soil matrix. Another explanation would be the permanent input of the sulfonamide into the leachate even after stopping the application of the antibiotic into the soil.

Beside the intentionally applied sulfonamides, 4-OH-SDZ was detected in the leachate samples. This metabolite of SDZ was also detected in the manure utilized for sulfonamide application, but the 4-OH-SDZ content corresponded to less than 0.5% of the SDZ content. Thus, it appears highly unlikely that the positive findings in the leachate derive from the initial content in the applied manure only. In fact, the results support previous findings that 4-OH-SDZ cannot only be formed within the treated organism, but in soil samples as well. Several studies investigated the fate of SDZ in soil. They found a transformation of the antibiotic leading to different transformation products such as 4-OH-SDZ (Unold et al., 2009; Kasteel et al., 2010; Sittig et al., 2014). At field site B, one sample of the upper soil was taken from each lysimeter to check which portion of the sulfonamides can still be detected. Analyses revealed the presence of 4-OH-SDZ and contents were five times lower than those of SDZ (Table A1). This would support the assumption that 4-OH-SDZ derives from the transformation of the applied SDZ in the soil.

Based on the amount of fortified manure applied to the soil, a theoretical content of 133 μg kg⁻¹ would be expected for each of the applied sulfonamides. In fact, contents of all analytes were between 1.6 and 13.4 μg kg⁻¹ (Table A11). Due to the results of the leachate analyses, a wash-out and extended transfer into deeper soil layers can be excluded. A degradation due to sunlight exposure can be neglected as well as the soil has been ploughed after manure application. The lysimeter setup does not enable a horizontal diffusion of the compounds. Consequently, most of the added sulfonamides still have to be present in the soil column, most likely as so-called non-extractable residues (NER) due to sequestration processes (Höltge and Kreuzig, 2007; Förster et al., 2009; Gulkowska et al., 2013; Müller et al., 2013). NER are formed within few hours after application of sulfonamides to soil matrix which is reflected by decreasing recovery rates with prolonged sample storage (Table A4) (Hamscher et al., 2005; Müller et al., 2013). They could be build up by covalent bond formation between sulfonamides and humic substances (Bialk and Pedersen, 2008; Gulkowska et al., 2013).

For site B, the seepage volume of the lysimeter was utilized for performing a mass balance. Since the first manure application in autumn 2009, 3300 L per m² passed through the soil column. If an average SMZ concentration of 40 ng L⁻¹ was assumed (low concentrations at the beginning, high concentrations towards the end of the study period), this would correspond to a transfer of 120 μg SMZ into the leachate over 10 years. Between 2009 and 2011, 50 mg per sulfonamide were applied to the study area. Within 10 years, less than 0.5% of the initially applied SMZ were transferred into the leachate. Assuming a steady state process, this would correspond to transfer of sulfonamides into the leachate for several decades even after stopping application of contaminated manure.
4. Conclusion

It was clearly demonstrated that especially long-term lysimeter and field studies accompanied by sophisticated analytical methods allow for a detailed investigation of the environmental fate of worldwide used veterinary drugs and their metabolites. Tetracyclines were shown to persist in higher concentrations (>100 μg kg⁻¹) in top soil, no leaching in deeper soil segments or groundwater occurred. Although certain sulfonamides have not been applied since several years to agricultural soils, low concentrations (ng L⁻¹) of the active drugs and one metabolite were transferred to leachate. We conclude that some veterinary drugs are even more persistent than expected and present in the soil environment even decades after their application. In regard of threshold values for the induction of antibiotic resistance, even low amounts may have an impact on the formation of antimicrobial resistance in the environment. This underlines the necessity of a precautionary limit for antimicrobial active compounds in manure and digestate.

Declarations

Author contribution statement

Astrid Spielmeyer: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Madeleine S. Petri: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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References

Bailey, C., Spielmeyer, A., Hamscher, G., Schlötrumpf, H., Frings, R.M., 2016. The veterinary antibiotic journey: comparing the behaviour of sulfadiazine, sulfamethazine, sulfamethoxazole and tetracycline in cow excrement and two soils. J. Soil Sediments 16, 1690–1704.
Berendsen, B.J.A., Lahr, J., Nibbeling, C., Jansen, I.J.M., Borgers, I.E.A., Wipfler, E.L., van de Schans, M.G.M., 2018. The persistence of a broad range of antibiotics during calve, pig and broiler manure storage. Chemosphere 204, 267–276.
Bialk-Bielinska, A., 2012. Sulframethoxazone and sulfaguanidine: their sorption potential on natural soils. Chemosphere 86, 1059–1065.
Bialk, H.M., Pedersen, J.A., 2008. NMR investigation of enzymatic coupling of sulfonamide antimicrobials with hemic substances. Environ. Sci. Technol. 42, 1025–1030.
Bialk, H.M., Simpson, A.J., Pedersen, J.A., 2005. Cross-coupling of sulfonamide antimicrobial agents with model hemic constituents. Environ. Sci. Technol. 39, 4752–4757.
Biel-Maerz, M., Corada-Fernández, C., Lara-Martin, P.A., 2017. Determining the distribution of pharmaceutically active compounds (PhACs) in soils and sediments by pressurized hot water extraction (PHWE). Chemosphere 185, 1001–1010.
Doughton, C.G., 2002. Environmental stewardship and drugs as pollutants. Lancet 360, 1025–1029.
de la Torre, A., Iglesias, I., Carballo, M., Ramírez, P., Muñoz, M.J., 2012. An approach for mapping the vulnerability of European Union soils to antibiotic contamination. Sci. Total Environ. 414, 672–679.
Doretto, K.M., Peruchi, L.M., Rath, S., 2014. Sorption and desorption of sulfamethoxazole, sulfadimethoxime and sulfamethazine in Brazilian soils. Sci. Total Environ. 476, 406–414.
Eichert, P., Aga, D.S., 2004. Identification of a photosensitization product of chlorotetracycline in hog lagoons using LC/ESI-ion trap MS and LC/ESI-time-of-flight-MS. Anal. Chem. 76, 6002–6011.
Figueroa-Diva, R.A., Vanuevan, D., Mackay, A.A., 2010. Trends in soil sorption coefficients within common antimicrobial families. Chemosphere 79, 786–793.
Forster, M., Laabs, V., Lamshöft, M., Groeneweg, J., Zühlke, S., Spitteler, M., Krauss, M., Kaupenjohann, M., Amelung, W., 2009. Sequestration of manure-applied sulfadiazine residues in soils. Environ. Sci. Technol. 43, 1824–1830.
Forster, M., Laabs, V., Lamshöft, M., Putz, T., Amelung, W., 2008. Analysis of aged sulfadiazine residues in soils using microwave extraction and liquid chromatography tandem mass spectrometry. Anal. Bioanal. Chem. 391, 1029–1038.
Gulkowska, A., Sander, M., Holländer, J., Krausz, M., 2013. Covalent binding of sulfamethazine to naturally and synthetic humic acid macromolecules using laccase catalysis and covalent bond stability. Environ. Sci. Technol. 47, 6916–6924.
Haller, M.Y., Muller, S.R., McDernell, C.S., Alder, A.C., Suter, M.J.F., 2002. Quantification of veterinary antibiotics (sulfonamides and trimethoprim) in animal manure by liquid chromatography-mass spectrometry. J. Chromatogr. A 952, 111–120.
Halleng-Børgesen, B., Sengelov, G., Tjorholt, J., Høyer, H., Nau, H., 2005. Different behavior of tetracyclines and sulfonamides in sandy soils after repeated fertilization with liquid manure. Environ. Toxicol. Chem. 24, 861–868.
Hamacher, G., Pawelzik, H.T., Høyer, H., Nau, H., 2005. Determination of persistent tetracycline residues in soil fertilized with liquid manure by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. Anal. Chem. 74, 1509–1518.
Hölger, S., Kreuzig, R., 2007. Laboratory testing of sulfamethoxazole and its metabolite acetyl-sulfamethoxazole in soil. Clean 35, 104–110.
Hu, Y., Cheng, H., Tao, S., 2017. Environmental and human health challenges of industrial livestock and poultry farming in China and their mitigation. Environ. Int. 107, 111–120.
Juo, C.G., Chen, C.L., Lin, S.T., Fu, S.H., Chen, Y.T., Chang, Y.S., Yu, J.S., 2014. Mass accuracy improvement of reversed-phase liquid chromatography/electrospray ionization mass spectrometry based urinary metabolic analysis by post-run calibration using sodium formate cluster ions. Rapid Commun. Mass Spectrom. 28, 1813–1820.
Kasteel, R., Mboh, C.M., Unold, M., Groeneweg, J., Vanderborght, J., Vereecken, H., 2010. Transformation and sorption of the veterinary antibiotic sulfadiazine in two soils: a short-term batch study. Environ. Sci. Technol. 44, 4651–4657.
Krivis, T., Broers, H.P., Beetje, H., van Vliet, M., Griffison, J., 2018. Presence and fate of veterinary antibiotics in aged-groundwater in areas with intensive livestock farming. Environ. Pollut. 241, 988–998.
Kurkdjian, S.T., Adams, C.D., Meyer, M.T., Kolpin, D.W., 2011. Comparative mobility of veterinary antibiotics (sulfonamides and trimethoprim) in two soils: a short-term extractability of sulfadiazine after application to soils. Environ. Pollut. 172, 1733–1745.
Löfing, K.A., Adams, C.D., Meyer, M.T., Suramwalli, B., 2008. Effects of ionic strength, temperature, and pH on degradation of selected antibiotics. J. Environ. Qual. 37, 378–386.
Macay, D., Hughes, D.M., Romano, M.L., Bonnell, M., 2014. The role of persistence in chemical evaluations. Integrated Environ. Asses. Manag. 10, 588–594.
Maszkowska, J., Kolodzięska, M., Bialk-Bielinska, A., Mrózik, W., Kumirska, J., Stepnowski, P., Palavinskas, R., Kruger, O., Kalbe, U., 2013. Column and batch tests of sulfanilamide leaching from different types of soil. J. Hazard Mater. 260, 468–474.
Müller, T., Rosendahl, I., Focks, A., Siemens, J., Klasmeier, J., Matthies, M., 2013. Short-term extractability of sulfadiazine after application to soils. Environ. Pollut. 172, 180–185.
Pan, M., Chan, L.M., 2017. Leaching behavior of veterinary antibiotics in animal manure-applied soils. Sci. Total Environ. 579, 466–473.
Sarmah, A.K., Meyer, M.T., Boxall, A.B., 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere 65, 725–755.
Sheveler, W.L., Hakk, H., Larsen, G.L., DeSatter, T.M., Casey, F.X.M., 2010. Development of an ultra-high-pressure liquid chromatography–tandem mass spectrometry multi-
residue sulfonamide method and its application to water, manure slurry, and soils from swine rearing facilities. J. Chromatogr. A 1217, 1273–1282.
Sittig, S., Kasteel, R., Groeneweg, J., Hofman, D., Thiele, B., Koppchen, S., Vereecken, H., 2014. Dynamics of transformation of the veterinary antibiotic sulfadiazine in two soils. Chemosphere 95, 470–477.
Spielmeyer, A., 2018. Occurrence and fate of antibiotics in manure during manure treatment: a short review. Sustain. Chem. Pharm. 9, 76–86.
Spielmeyer, A., Ahlborn, J., Hamscher, G., 2014. Simultaneous determination of 14 sulfonamides and tetracyclines in biogas plants by liquid-liquid-extraction and liquid chromatography tandem mass spectrometry. Anal. Bioanal. Chem. 406, 2513–2524.
Spielmeyer, A., Häper, H., Hamscher, G., 2017. Long-term monitoring of sulfonamide leaching from manure amended soil into groundwater. Chemosphere 177, 232–238.
Statistisches Bundesamt, Die Hälfte der Landwirte düngt mit Gülle.https://www.destatis.de/DE/Themen/Branchen-Unternehmen/Landwirtschaft-Forstwirtschaft-Fische/Produktionsmethoden/aktuell-duengen.html (last access 07.08.2020).
Sur, R., Stork, A., 2003. Uptake, translocation and metabolism of imidacloprid in plants. Bull. Insectol. 56, 35–40.
Tsuiji, A., Nakashima, E., Hamano, S., Yamana, T., 1978. Physicochemical properties of amphoteric beta-lactam antibiotics I: Stability, solubility, and dissolution behavior of amino penicillins as a function of pH. J. Pharmacol. Sci. 67, 1059–1066.
Unold, M., Kasteel, R., Groeneweg, J., Vereecken, H., 2009. Transport and transformation of sulfadiazine in soil columns packed with a silty loam and a loamy sand. J. Contam. Hydrol. 103, 38–47.
Van Boeckel, T.P., Brower, C., Gilbert, M., Grenfell, B.T., Levin, S.A., Robinson, T.P., Teillat, A., Laxminarayan, R., 2015. Global trends in antimicrobial use in food animals. Proc. Natl. Acad. Sci. U. S. A. 112, 5649–5654.
VETIDATA, University Leipzig, Veterinärmedizinischer Informationsdienst für Arzneimittelanwendung, https://www.vetidata.de (latest access: 07.08.2020).
Vithanage, M., Rajapaksha, A.U., Tang, X.Y., Thiele-Bruhn, S., Kim, K.H., Lee, E.S., Ok, Y.S., 2014. Sorption and transport of sulfamethazine in agricultural soils amended with invasive-plant-derived biochar. J. Environ. Manag. 141, 95–103.
Wallmann, J., Bender, A., Hanack, R., Reimer, I., Heberer, T., 2014. Abgabemengenerfassung antimikrobiell wirksamer Stoffe in Deutschland 2013. Dtsch. Tierarzneblatt 1234–1237.
Wallmann, J., Bender, A., Reimer, I., Heberer, T., 2015. Abgabemengenerfassung antimikrobiell wirksamer Stoffe in Deutschland 2014. Dtsch. Tierarzneblatt 1260–1264.
Wallmann, J., Bode, C., Heberer, T., 2019. Abgabemengenerfassung von Antibiotika in Deutschland 2018. Dtsch. Tierarzneblatt 67, 1082–1090.
Wallmann, J., Reimer, I., Heberer, T., 2016. Abgabemengenerfassung antimikrobiell wirksamer Stoffe in Deutschland 2015. Dtsch. Tierarzneblatt 1650–1657.
Weiland, P., 2010. Biogas production: current state and perspectives. Appl. Microbiol. Biotechnol. 85, 849–860.
Wohde, M., Berkner, S., Junker, T., Konradi, S., Schwarz, L., Döring, R.-A., 2016. Occurrence and transformation of veterinary pharmaceuticals and biocides in manure: a literature review. Environ. Sci. Eur. 28, 23.
Zhou, L.J., Ying, G.G., Liu, S., Zhang, R.Q., Lei, H.J., Chen, Z.F., Pan, C.G., 2013. Excretion masses and environmental occurrence of antibiotics in typical swine and dairy cattle farms in China. Sci. Total Environ. 444, 183–195.