Water Air Soil Pollut (2018) 229: 104
https://doi.org/10.1007/s11270-018-3710-8

Hairy Vetch Incorporated as Green Manure Inhibits Sulfathiazole Uptake by Lettuce in Soil

**Jiff Randy Caban · Saranya Kuppusamy ·
Jang Hwan Kim · Young-Eun Yoon · Song Yeob Kim ·
Yong Bok Lee**

Received: 23 October 2017 / Accepted: 23 January 2018 /

Abstract Veterinary antibiotics like sulfonamides are frequently detected in arable lands and they can potentially contaminate food crops. It is thus of great importance to identify strategies to reduce food crops' uptake of antibiotics. For the first time, using a pot culture experiment, sulfathiazole (STZ) uptake by lettuce (*Lactuca sativa* L.) grown in antibiotic-contaminated soils (10 and 100 mg STZ kg^{-1} soil) and treated with (in)organic amendments, namely chemical fertilizer (NPK), compost, and hairy vetch, was investigated. Subsequent enhanced plant growth was witnessed when using hairy vetch treatment. The amount of antibiotic uptake was significantly reduced to 5 and 33% with hairy vetch application compared to compost or NPK application at 10 and 100 mg kg^{-1} STZ, respectively. The total amounts of accumulated STZ in plant parts increased as the levels of STZ contaminated in soils were increased. STZ was much more abundant in the roots than the leaves. Within 30 days, the extractable STZ in the treated soils—especially with hairy vetch—diminished considerably to concentrations that are frequently detected in arable soils. We conclude that utilization of green manure (cover crop—hairy vetch) is a viable strategy for safer crop production in antibiotic-contaminated soils.

Keywords Sulfonamides · Plant accumulation · Soil amendment · NPK fertilizers · Hairy vetch · Compost

1 Introduction

Antibiotics are widely used in agriculture for therapeutic purposes to treat or control infectious diseases in animals (Ahmed et al. 2015), and this is about four to five times greater than that in human use. In veterinary practice, antibiotics are also used to increase feed efficiency and to promote growth in food-producing animals at subtherapeutic levels (Schwarz et al. 2001; Wassenaar 2005; Kim et al. 2016). In 2010, it was estimated that a total of 63,200 t of antibiotics was utilized for the livestock industry worldwide and that amount is projected to increase to 105,600 t by 2030 (Van Boeckel et al. 2015). In various countries, 11–23% of these antibiotics were sulfonamides (SAs), due to their broad spectrum antibacterial and anticoccidial activity (De Liguoro et al. 2007). In South Korea, SAs are one of the most widely administered groups of antibiotics in animal husbandry and its consumption amounted to 98,845 kg in 2015 with 32,269, 27,677, and 13,068 kg for sulfathiazole, sulfamethoxazole, and sulfamethazine, respectively (Kim et al. 2011, 2017). However, SAs exhibit a high excretion rate (90%) by animals as parent compounds or as acetylated metabolites in urine and feces (Kim et al. 2017). Therefore, SAs

---

J. R. Caban · J. H. Kim · Y.-E. Yoon · Y. B. Lee (✉)
Division of Applied Life Science (BK21 Plus), Gyeongsang National University, Jinju 52828, Republic of Korea
e-mail: yblee@gnu.ac.kr

S. Kuppusamy · S. Y. Kim · Y. B. Lee
Institute of Agriculture and Life Science, Gyeongsang National University, Jinju 52828, Republic of Korea

may enter agricultural soils directly through grazing livestock and/or indirectly through the spreading of manure (Li et al. 2013). In soil, SAs persist for months at relatively high concentrations (Stoob et al. 2006). Residues of SAs have been found in soils and adjacent compartments (Thiele-Bruhn 2003; Du and Liu 2012; Hu et al. 2010; Jechalke et al. 2014). Released antibiotics in the soil can be taken up by plants, which may pose a threat to people's health when they consume antibiotic-contaminated plants (Rajapaksha et al. 2014). Therefore, it is necessary to understand the potential risk of antibiotic uptake by food crops. Notably, most studies focus on sulfamethazine while those analyses associated with sulfathiazole—which has a high usage in South Korea—are scarce.

Exposure to low-level antibiotics in soil has raised significant concerns of the toxic effects, as well as the transfer and spread of antibiotic-resistant genes among microorganisms (Boxall et al. 2002; Schmitt et al. 2006). It is thus of great importance to develop efficient, cost-effective strategies for remediating antibiotic-contaminated soils. Several studies have focused on remediating such contaminated soils using different sorbents like clay minerals (Gao and Pedersen 2005), carbon nanotubes (Ji et al. 2009), and biochar (Rajapaksha et al. 2014). Among the sorbents, this study hypothesized that application of green manure would effectively enhance biodegradation and reduce antibiotic availability for plant uptake. The use of organic matter such as green manure/cover crop is especially environmental friendly rather than chemical fertilizers and is recommended for sustainable agriculture. Because the availability of carbon substrates largely controls microbial growth in soil (Elfstrand et al. 2007), green manure amendments stimulate soil microbial growth and activity (Tejada et al. 2008). Moreover, incorporation of cover crops in a crop production system may impact on both soil biological and chemical processes, affecting the subsequent fate of agrochemicals (Zablotowicz et al. 1998). Previous studies have reported the effect of green manures on weed control, soil chemical/biological properties, crop growth, and yield (Tejada et al. 2008; Muchanga et al. 2016). However, the impact of green manure on plants grown in the antibiotic-contaminated soil is not available. In South Korea, legume-based green manures, notably hairy vetch (*Vicia villosa*) which is an important N source is widely used in sustainable agriculture. In the above context, for the first time, this study aims to determine sulfathiazole uptake by plants in the soils exposed to hairy vetch. Compost which is one of the other commonly used organic amendments in agriculture including mineral fertilization (NPK) is used for treatment comparisons.

2 Materials and Methods

2.1 Chemicals

Analytical grade sulfathiazole (STZ), simatone, disodium ethylene diaminetetraacetic acid (Na_2EDTA), ammonium hydroxide (NH_4OH), high-performance liquid chromatography (HPLC) grade water, methanol (MeOH), acetonitrile (ACN), formic acid (CH_2O_2), and hydrochloric acid (HCl) with 99% purity were obtained from Sigma-Aldrich (St. Louis, USA). Corning™ Falcon™ conical centrifuge tubes (Thermo Fisher Scientific, USA) and Advantec sterile syringe filters of 0.2 μm (Cole-Parmer, USA) were used. Solid phase extraction (SPE) cartridges, 3 mL per 60 mg of HLB (hydrophilic-lipophilic-balance), were purchased from Water Oasis Co. (Milford, MA). Stock solution (500 mg L^{-1} STZ) was prepared with methanol in amber color bottles (to prevent degradation of STZ) and stored at 4 °C. Working solutions of 10 and 100 mg L^{-1} STZ were prepared immediately before the experiment by diluting the stock solution. Distilled water was used when ultra-pure water was required.

2.2 Test Soil

A fine silty mixed mesic Typic Haplaquepts soil collected in 2016 from the Gyeongsang National University Crop Research Farm, Jinju, South Korea, was used in the study. This particular soil was selected because it was previously free from antibiotics. To ensure homogeneity, following collection, the soil was air-dried and passed through a 2-mm screen and mixed prior to use in uptake studies. Soil physicochemical properties were measured according to standard analytical procedures (Daquiado et al. 2016; Kuppusamy et al. 2017). The soil contained pH 6.7 ± 0.2, EC 36.3 μS cm^{-1}, organic matter 14.6 ± 0.9 g kg^{-1}, total nitrogen 1.8 ± 0.4 g kg^{-1}, and available phosphorous 79 ± 2.9 mg kg^{-1}. Exchangeable cations ($cmol^{+}$ kg^{-1}) like Ca^{2+}, Mg^{2+}, and K^{+} of the soil amounted to 9.2 ± 0.4, 1.2 ± 0.02, and 1.2 ± 0.03, respectively.

2.3 Organic Amendments

Two organic amendments—firstly, compost and secondly, green manure and a control (inorganic fertilizer—NPK, but no manure)—were tested in this study. Urea, super phosphate, and potassium chloride were the sources of inorganic N, P, and K, respectively. Physicochemical properties of compost and hairy vetch were determined according to the methods described in Huang et al. (2004) study. Compost used was a livestock manure variety that contained 31.1% C, 2.2% N, 0.5% S, and 14.4 C:N ratio. Field-grown dry and homogenized hairy vetch (*Vicia villosa*) with 44.1% C, 4.3% N, 0.3% S, and 10.4 C:N ratio served as the source of green manure. In the case of inorganic fertilizer input, the amendment rates were 35, 30, and 18 kg ha^{-1} for N, P, and K, respectively. Compost treatment received 10 t ha^{-1} compost while 6 t ha^{-1} hairy vetch was incorporated into the green manure treatment.

2.4 Pot Experiment

Homogenized soil samples were placed in 20 L polyvinyl chloride pots (4 kg of soil was filled pot^{-1}). The treatments were employed as follows: (1) NPK amended control soil without STZ; (2) 10 mg kg^{-1} STZ-contaminated NPK amended soil; (3) 100 mg kg^{-1} STZ-contaminated NPK amended soil; (4) compost amended control soil without STZ; (5) 10 mg kg^{-1} STZ-contaminated compost amended soil; (6) 100 mg kg^{-1} STZ-contaminated compost amended soil; (7) hairy vetch amended control soil without STZ; (8) 10 mg kg^{-1} STZ-contaminated hairy vetch amended soil; (9) 100 mg kg^{-1} STZ-contaminated hairy vetch amended soil. STZ stock solution was added to 4 kg of each air-dried soil to give nominal substance concentrations of 10 and 100 mg kg^{-1} of dry weight. Respective fertilizers were added to the top of each pot and the soil was mixed thoroughly. Seeds of lettuce (*Lactuca sativa*) (lettuce seeds used were the commercially available variant readily available in the Korean market) were germinated and grown in moist vermiculite for 2 weeks and then transferred to the treated soils. One seedling was transplanted per pot and continued to grow in a greenhouse (day time temperature = 30–35 °C; night time temperature = 20–25 °C) located at the Gyeongsang National University, Jinju, South Korea. Each pot was maintained at 70% water-holding capacity. Triplicate pots were prepared for each treatment and were randomized in the greenhouse side-by-side. Subsamples of soil were collected before transplanting (0th day) and 30 days after transplanting. Plant samples (roots and leaves) were harvested after 30 days of transplanting when the lettuce entered full growth stage and then rinsed with ultra-pure water to remove any adhering soil particles. Both plant and soil samples were then freeze-dried and stored in sealed plastic bags at − 20 °C until required for analysis.

2.5 Chemical Analysis

Antibiotic concentrations in the soil and plant samples were measured following the methodology of Kim and Carlson (2007). First, 1 g of soil was transferred into 50 mL falcon tube. About 20 mL Mcllavine buffer (USDA 2003) was added followed by simatone (50 μL of 240 μg/L) and 200 μL of 5% Na_2EDTA and the sample was vigorously mixed in a shaker (Koencon, South Korea) for 20 min at 400 rpm. The sample was then centrifuged (Hanil Science, South Korea) at 4000 rpm for 15 min followed by filtration using 0.2 μm sterile filters. The filtered sample was decanted to another 50 mL falcon tube and kept at 4 °C. Extraction was repeated again and the supernatants were combined for an SPE cleanup procedure. For the SPE cleanup procedure, initially, the pH of the aqueous sample was adjusted to the2.0–2.5 range to increase the hydrophobicity of STZ in the SPE cartridge. The cartridge was pre-conditioned with 3 mL MeOH and 3 mL of 0.5 N HCl followed by 3 mL water. About 3 mL of MeOH was used to extract STZ. After elution, approximately 30 μL of 10% CH_2O_2 was added to the extracted sample, mixed, and filtered. Then the sample was transferred into amber vials fitted with auto sampler inserts for LC assay. Referring to the plant sample, 0.5 g of the sample was extracted with 10 mL of ACN:ultra-pure water (70:30) and 150 μL of 5% Na_2EDTA in a shaker for 20 min at 200 rpm, centrifuged for 10 min at 4000 rpm, supernatant filtered and stored at 4 °C. The sample was re-extracted and then the aqueous samples were pooled and subjected to SPE cleanup. Further SPE cleanup protocol and LC analyses are the same as that of soil.

Concentration of STZ in soil and plant sample extracts were determined with an AB Sciex API 4000 LC-MS/MS (AB Sciex, Singapore) equipped with an Agilent 1290 HPLC system (Agilent Technologies, USA). An XBridge C8 (Waters, USA) 2.1 × 100 mm (3.5 μm pore size) column was used for chromatographic separation. ESI (electrospray ionization) was utilized to detect STZ and a positive mode was adapted. Mobile phase A was composed of 0.1% CH_2O_2 in HPLC grade water, and the mobile phase B was 0.1% CH_2O_2 in HPLC grade MeOH. The column temperature was set at 40 °C and the gradient was ramped from 95% mobile phase A and 5% mobile phase B to 5% mobile phase A and 65% mobile phase B in the first min and held isocratically for 8 min. Injection volume was 10 μL and the flow rate was 0.3 mL min^{-1}. Selective ion monitoring mode helped to detect simatone and STZ. The calibration's reliability was checked periodically by injecting known standards and solvent into the column. The detection limit of the LC system was 0.7 μg L^{-1}. For quality control, experiments on recovery were carried out by spiking a known concentration of STZ standard (5 and 10 mg L^{-1}) in untreated and uncontaminated soil. The results showed a significant recovery of 91 ± 8.5%. The accuracy and precision of the whole chromatographic operation was checked every ten samples by injecting known standards and a solvent blank.

2.6 Statistical Analysis

Statistical analyses were undertaken using SAS software version 9.4 (SAS Institute Inc.). Duncan's Multiple Range Test (DMRT) was executed at the 0.05 probability level for making treatment mean comparisons (n = 3). Graphs were generated through SigmaPlot software version 10.0 (Systat Software Inc.)

3 Results and Discussion

3.1 Effect on Plant Growth

In this study, a negative effect on the plant growth was observed as reduced fresh weight revealed the most pronounced outcome for increasing antibiotic concentration (Fig. 1), which is in agreement with previous studies (Mikes and Trapp 2010; Ahmed et al. 2015). The highest spiking concentration of antibiotics (100 mg kg^{-1} STZ), which is well above what can be typically expected in agricultural soil (Stoob et al. 2006; Martínez-Carballo et al. 2007; Karcı and Balcıoğlu 2009), had the worst effect on the growth of lettuce and presented the lowest values (0.2 ± 0.02 to 0.4 ± 0.08 g). This negative effect can be attributed to the higher bioavailability of SAs for plants because of their higher mobility in soils (Boxall et al. 2002) which makes them toxic to plant growth. Sartorius et al. (2009) also discovered that plants' growth decreased due to the effects of toxic alteration of SAs on root morphology. However, Ferro et al. (2010) and Michelini et al. (2012) stated that influences on enhancing or inhibiting plant growth depend on the antibiotic exposure level. Meanwhile, at an environmentally relevant soil concentration of 10 mg kg^{-1} STZ, the highest values were obtained for hairy vetch amended soil (19.8 ± 1.2 g), which was ×5–8 greater than the NPK or compost amended soils. Notably, with hairy vetch amendment, fresh weights for the 10 mg kg^{-1} antibiotic-treated lettuce were not negatively affected and statistically equal to those of the control lettuce. A non-inhibitory effect of STZ after adding hairy vetch may be caused by the improvement in the soil's biological properties as well as in the nutrition (Tejada et al. 2008).

3.2 Lettuce Uptake of STZ

The cultivation experiment revealed that lettuce grown in soils contaminated with 10 mg kg^{-1} STZ took up about 12.7% of the applied STZ with NPK application. However, the uptake amount was significantly reduced to 5.1% with hairy vetch application, followed by 7.4% with compost application compared to the NPK application (Table 1). This reduction of STZ uptake by lettuce with hairy vetch can be due to the biodegradation of STZ in the soil. Incorporating fresh organic residues such as hairy vetch, which can be readily mineralized by microbes greatly increases soil microbial and enzymatic activities (Villalobos and Fereres 2017), which may have favored the microbial transformation of STZ. Meanwhile, reduced transport of STZ to lettuce in compost-mixed soils may possibly be attributed to the compost's high potential to adsorb STZ (Kahle and Stamm 2007). The determined STZ uptake in lettuce was clearly smaller at the soil spiking concentration of 10 mg kg^{-1}, compared to STZ uptakes of 68.2, 32.5, and 46.8% in lettuce after 30 days' exposure to 100 mg STZ kg^{-1} soil treated with NPK, hairy vetch, and compost, respectively. This agrees with previous findings (Dolliver et al. 2007; Migliore et al. 2010), where the total amounts of accumulated SAs to plant parts increased as the levels of SAs contaminated in soils were increased. Given its low molecular weight

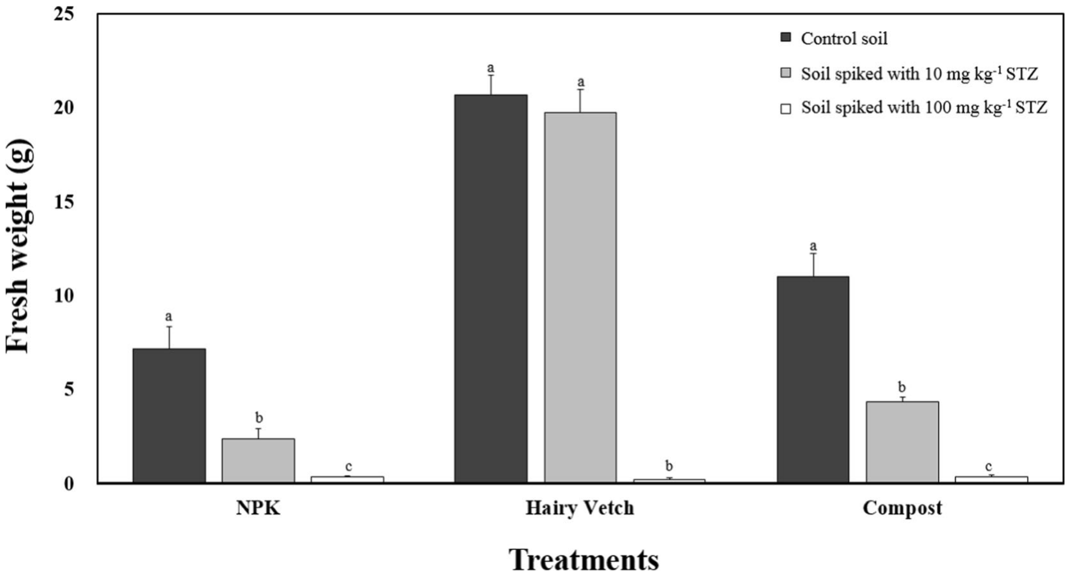

Fig. 1 Fresh weight of lettuce samples under different treatments at 30 days after transplanting. Values are means of triplicate samples. Error bars indicate SE. Means with different matching letter(s), in the same treatment ($P > 0.05$), are significantly different according to DMRT

(255.3 g mol^{-1}), hydrophilicity, and low affinity to soil particles ($K_{ow} = 0.05$; log pKa = 2.2 and 7.24) and at soil pH 6.7 of this study, it is expected that the majority of STZ may exist in neutral form and a certain percentage will be in the anionic state. Because clay particles and humic substances carry a predominantly negative charge, it is predicted that electrostatic repulsion may have occurred, rendering STZ more freely dissolved in the soil pore water which resulted in its high plant uptake (Carter et al. 2014; Miller et al. 2016). Hence, increasing the concentration of antibiotics in soil will increase the potential health risk.

Further, in lettuce samples, the antibiotic was much more abundant in the roots than the leaves. Likewise, Michelini et al. (2012) observed an increased accumulation of SAs inside plant roots than at the rhizoplane level. According to Gao et al. (2005), many legacy pollutants have low translocation to aboveground plant materials. Various studies (Fantke et al. 2011; Li et al. 2013) reported that roots have a high potential to accumulate lipophilic organic compounds like antibiotics owing to their adsorption on lipophilic root solids. STZ is expected be subject to ion trapping in root cells that may had limited its translocation out of the lettuce roots owing to a decline in their membrane permeability (Trapp 2004; Miller et al. 2016). Cultivation of leaf vegetables rather than root vegetables in the antibiotic-contaminated agricultural soils may therefore greatly reduce the potential risk of antibiotic uptake by food crops.

Table 1 Concentration (mg kg^{-1}, dry weight) of STZ recorded in the soil and lettuce samples under different treatments at 30 days after transplanting

Treatments	Control soil			Soil contaminated with 10 mg kg^{-1} STZ			Soil contaminated with 100 mg kg^{-1} STZ		
	S	L	R	S	L	R	S	L	R
NPK	nd	nd	nd	0.4 ± 0.005^{c}	0.07 ± 0.008^{a}	1.2 ± 0.02^{a}	17.8 ± 0.8^{a}	29.5 ± 5.1^{a}	38.7 ± 8.2^{a}
Hairy vetch	nd	nd	nd	0.2 ± 0.003^{c}	0.01 ± 0.01^{c}	0.5 ± 0.4^{c}	13.8 ± 0.4^{b}	14.2 ± 2.9^{c}	18.3 ± 6.8^{c}
Compost	nd	nd	nd	0.3 ± 0.005^{c}	$0.04 \pm 0.009a^{b}$	0.7 ± 0.3^{b}	17.3 ± 0.4^{a}	17.3 ± 4.8^{b}	29.5 ± 3.4^{b}

Values are means of triplicate samples ± SE. Means with different matching letter(s), in the same treatment ($P > 0.05$), are significantly different according to DMRT

nd not detected, *S* soil, *L* lettuce leaves, *R* lettuce roots

3.3 STZ Content in Soil

STZ was not detected in the control soils (Table 1). Within 30 days, the extractable STZ in the treated soils diminished to concentrations that are frequently detected in arable soils (Stoob et al. 2006; Michelini et al. 2012). This could be possibly due to sorption, diffusion, degradation, and/or plant uptake of this compound over time. SAs are polar and non-volatile, so degradation and plant uptake can be the major removal mechanisms (Wu et al. 2010). Notably, in this study, at 30 days of transplanting, the total fresh weight of lettuce from hairy vetch-treated pots at an environmentally relevant STZ concentration (10 mg kg^{-1}) averaged 19.8 g, while the amount of STZ mass accumulated in the plant was as small as 0.5 μg kg^{-1}. Consequently, loss by plant uptake from the soil is negligible compared with the amount present in the soil (0.2 mg kg^{-1}). The extractable fractions of STZ from soil planted with lettuce equaled 14% of the high-spiking concentration (100 mg kg^{-1} STZ). Conversely, for lettuce, the value was 33%. Therefore, more than 65% of the applied STZ might have undergone biodegradation, diffusion and/or sorption in the hairy vetch soil matrix.

4 Conclusion

This study suggests that a practical strategy for farmers is to incorporate organic manures and especially green manures (a cover crop like hairy vetch) rather than inorganic fertilizers as a source of plant nutrients. This will allow the manure to sit in the soil for as long as possible before planting food crops. Having this extra time will help degrade the antibiotics and/or sorb them to the soil matrix and thus reduce their concentration for plant uptake. In future, investigations elucidating antibiotic uptake mechanisms and pathways in agricultural soils treated with organic amendments are warranted. Further studies are recommended to assess the responses of different plant species to diverse antibiotic groups in the presence of hairy vetch.

Funding Information This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT (NRF-2017R1A2B4007092).

References

Ahmed, M. B. M., Rajapaksha, A. U., Lim, J. E., Vu, N. T., Kim, I. S., Kang, H. M., Lee, S. S., & Ok, Y. S. (2015). Distribution and accumulative pattern of tetracyclines and sulfonamides in edible vegetables of cucumber, tomato, and lettuce. *Journal of Agricultural and Food Chemistry, 63*(2), 398–405.

Boxall, A. B., Blackwell, P., Cavallo, R., Kay, P., & Tolls, J. (2002). The sorption and transport of a sulphonamide antibiotic in soil systems. *Toxicology Letters, 131*(1), 19–28.

Carter, L. J., Harris, E., Williams, M., Ryan, J. J., Kookana, R. S., & Boxall, A. B. (2014). Fate and uptake of pharmaceuticals in soil–plant systems. *Journal of Agricultural and Food Chemistry, 62*(4), 816.

Daquiado, A. R., Kuppusamy, S., Kim, S. Y., Kim, J. H., Yoon, Y. E., Kim, P. J., Oh, S. H., Kwak, Y. S., & Lee, Y. B. (2016). Pyrosequencing analysis of bacterial community diversity in long-term fertilized paddy field soil. *Applied Soil Ecology, 108*, 84–91.

De Liguoro, M., Poltronieri, C., Capolongo, F., & Montesissa, C. (2007). Use of sulfadimethoxine in intensive calf farming: evaluation of transfer to stable manure and soil. *Chemosphere, 68*(4), 671–676.

Dolliver, H., Kumar, K., & Gupta, S. (2007). Sulfamethazine uptake by plants from manure-amended soil. *Journal of Environmental Quality, 36*(4), 1224–1230.

Du, L., & Liu, W. (2012). Occurrence, fate, and ecotoxicity of antibiotics in agro-ecosystems. A review. *Agronomy for Sustainable Development, 32*(2), 309–327.

Elfstrand, S., Båth, B., & Mårtensson, A. (2007). Influence of various forms of green manure amendment on soil microbial community composition, enzyme activity and nutrient levels in leek. *Applied Soil Ecology, 36*(1), 70–82.

Fantke, P., Charles, R., de Alencastro, L. F., Friedrich, R., & Jolliet, O. (2011). Plant uptake of pesticides and human health: dynamic modeling of residues in wheat and ingestion intake. *Chemosphere, 85*(10), 1639–1647.

Ferro, S., Trentin, A. R., Caffieri, S., & Ghisi, R. (2010). Antibacterial sulfonamides: accumulation and effects in barley plants. *Fresenius Environmental Bulletin, 19*(9b), 2094–2099.

Gao, J., & Pedersen, J. A. (2005). Adsorption of sulfonamide antimicrobial agents to clay minerals. *Environmental Science & Technology, 39*(24), 9509–9516.

Gao, Y., Zhu, L., & Ling, W. (2005). Application of the partition-limited model for plant uptake of organic chemicals from soil and water. *Science of the Total Environment, 336*(1), 171–182.

Hu, X., Zhou, Q., & Luo, Y. (2010). Occurrence and source analysis of typical veterinary antibiotics in manure, soil, vegetables and groundwater from organic vegetable bases, northern China. *Environmental Pollution, 158*(9), 2992–2998.

Huang, G. F., Wong, J. W. C., Wu, Q. T., & Nagar, B. B. (2004). Effect of C/N on composting of pig manure with sawdust. *Waste Management, 24*(8), 805–813.

Jechalke, S., Heuer, H., Siemens, J., Amelung, W., & Smalla, K. (2014). Fate and effects of veterinary antibiotics in soil. *Trends in Microbiology, 22*(9), 536–545.

Ji, L., Chen, W., Duan, L., & Zhu, D. (2009). Mechanisms for strong adsorption of tetracycline to carbon nanotubes: a comparative study using activated carbon and graphite as adsorbents. *Environmental Science & Technology, 43*(7), 2322–2327.

Kahle, M., & Stamm, C. (2007). Sorption of the veterinary antimicrobial sulfathiazole to organic materials of different origin. *Environmental Science & Technology, 41*(1), 132–138.

Karcı, A., & Balcıoğlu, I. A. (2009). Investigation of the tetracycline, sulfonamide, and fluoroquinolone antimicrobial compounds in animal manure and agricultural soils in Turkey. *Science of the Total Environment, 407*(16), 4652–4664.

Kim, S. C., & Carlson, K. (2007). Temporal and spatial trends in the occurrence of human and veterinary antibiotics in aqueous and river sediment matrices. *Environmental Science & Technology, 41*(1), 50–57.

Kim, K. R., Owens, G., Kwon, S. I., So, K. H., Lee, D. B., & Ok, Y. S. (2011). Occurrence and environmental fate of veterinary antibiotics in the terrestrial environment. *Water, Air, & Soil Pollution, 214*(1–4), 163–174.

Kim, S. Y., Kuppusamy, S., Kim, J. H., Yoon, Y. E., Kim, K. R., & Lee, Y. B. (2016). Occurrence and diversity of tetracycline resistance genes in the agricultural soils of South Korea. *Environmental Science and Pollution Research, 23*(21), 22190–22196.

Kim, J. H., Kuppusamy, S., Kim, S. Y., Kim, S. C., Kim, H. T., & Lee, Y. B. (2017). Occurrence of sulfonamide class of antibiotics resistance in Korean paddy soils under long-term fertilization practices. *Journal of Soils and Sediments, 17*(6), 1618–1625.

Kuppusamy, S., Yoon, Y. E., Kim, S. Y., Kim, J. H., & Lee, Y. B. (2017). Long-term inorganic fertilization effect on the micronutrient density in soil and rice grain cultivated in a South Korean paddy field. *Communications in Soil Science and Plant Analysis, 48*(13), 1603–1615.

Li, X., Yu, H., Xu, S., & Hua, R. (2013). Uptake of three sulfonamides from contaminated soil by pakchoi cabbage. *Ecotoxicology and Environmental Safety, 92*, 297–302.

Martínez-Carballo, E., González-Barreiro, C., Scharf, S., & Gans, O. (2007). Environmental monitoring study of selected veterinary antibiotics in animal manure and soils in Austria. *Environmental Pollution, 148*(2), 570–579.

Michelini, L., Reichel, R., Werner, W., Ghisi, R., & Thiele-Bruhn, S. (2012). Sulfadiazine uptake and effects on Salix fragilis L. and Zea mays L. plants. *Water, Air, & Soil Pollution, 223*(8), 5243–5257.

Migliore, L., Rotini, A., Cerioli, N. L., Cozzolino, S., & Fiori, M. (2010). Phytotoxic antibiotic sulfadimethoxine elicits a complex hormetic response in the weed lythrum salicaria L. *Dose-Response, 8*(4), 414–427.

Mikes, O., & Trapp, S. (2010). Acute toxicity of the dissociating veterinary antibiotics trimethoprim to willow trees at varying pH. *Bulletin of Environmental Contamination and Toxicology, 85*(6), 556–561.

Miller, E. L., Nason, S. L., Karthikeyan, K. G., & Pedersen, J. A. (2016). Root uptake of pharmaceuticals and personal care product ingredients. *Environmental Science & Technology, 50*(2), 525–541.

Muchanga, R. A., Hirata, T., & Araki, H. (2016). Hairy vetch becomes an alternative basal N fertilizer in low-input fresh-market tomato production in plastic high tunnel. In III International Symposium on Organic Greenhouse Horticulture 1164 (pp. 127–134).

Rajapaksha, A. U., Vithanage, M., Lim, J. E., Ahmed, M. B. M., Zhang, M., Lee, S. S., & Ok, Y. S. (2014). Invasive plant-derived biochar inhibits sulfamethazine uptake by lettuce in soil. *Chemosphere, 111*, 500–504.

Sartorius, M., Riccio, A., Cermola, M., Casoria, P., Patriarca, E. J., & Taté, R. (2009). Sulphadimethoxine inhibits Phaseolus vulgaris root growth and development of N-fixing nodules. *Chemosphere, 76*(3), 306–312.

Schmitt, H., Stoob, K., Hamscher, G., Smit, E., & Seinen, W. (2006). Tetracyclines and tetracycline resistance in agricultural soils: microcosm and field studies. *Microbial Ecology, 51*(3), 267–276.

Schwarz, S., Kehrenberg, C., & Walsh, T. R. (2001). Use of antimicrobial agents in veterinary medicine and food animal production. *International Journal of Antimicrobial Agents, 17*(6), 431–437.

Stoob, K., Singer, H. P., Stettler, S., Hartmann, N., Mueller, S. R., & Stamm, C. H. (2006). Exhaustive extraction of sulfonamide antibiotics from aged agricultural soils using pressurized liquid extraction. *Journal of Chromatography A, 1128*(1), 1–9.

Tejada, M., Gonzalez, J. L., García-Martínez, A. M., & Parrado, J. (2008). Effects of different green manures on soil biological properties and maize yield. *Bioresource Technology, 99*(6), 1758–1767.

Thiele-Bruhn, S. (2003). Pharmaceutical antibiotic compounds in soils—a review. *Journal of Plant Nutrition and Soil Science, 166*(2), 145–167.

Trapp, S. (2004). Plant uptake and transport models for neutral and ionic chemicals. *Environmental Science and Pollution Research, 11*(1), 33–39.

USDA. (2003). *Qualitative identification of tetracyclines in tissues*. USA: United States Department of Agriculture (USDA), Food Safety and Inspection Service.

Van Boeckel, T. P., Brower, C., Gilbert, M., Grenfell, B. T., Levin, S. A., Robinson, T. P., Teillant, A., & Laxminarayan, R. (2015). Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences, 112*(18), 5649–5654.

Villalobos, F. J., & Fereres, E. (2017). *Principles of agronomy for sustainable agriculture* (pp. 23–24). Springer. https://doi.org/10.1007/978-3-319-46116-8.

Wassenaar, T. M. (2005). Use of antimicrobial agents in veterinary medicine and implications for human health. *Critical Reviews in Microbiology, 31*(3), 155–169.

Wu, C., Spongberg, A. L., Witter, J. D., Fang, M., & Czajkowski, K. P. (2010). Uptake of pharmaceutical and personal care products by soybean plants from soils applied with biosolids and irrigated with contaminated water. *Environmental Science & Technology, 44*(16), 6157–6161.

Zablotowicz, R. M., Locke, M. A., & Smeda, R. L. (1998). Degradation of 2, 4-D and fluometuron in cover crop residues. *Chemosphere, 37*(1), 87–101.