Bulletin of Environmental Contamination and Toxicology (2020) 105:790–797
https://doi.org/10.1007/s00128-020-03010-9

The Forms, Distribution, and Risk Assessment of Sulfonamide Antibiotics in the Manure–Soil–Vegetable System of Feedlot Livestock

Caixia Jin[1] · Shan Wei[1] · Ruilian Sun[3] · Wei Zou[1] · Xingli Zhang[1] · Qixing Zhou[4] · Rui Liu[2] · Liuqun Huang[1]

Received: 2 July 2020 / Accepted: 23 September 2020 / Published online: 10 October 2020

Abstract

Manure, soil, and vegetable samples were collected from different-sized livestock farms in Xinxiang, China. The residues of sulfadiazine, sulfamonomethoxine, and sulfamethoxazole were analyzed by high-performance liquid chromatography. The results indicated that the concentration ranges of the three total sulfonamides in manure, soil, and vegetables were 10.13–566.23 μg kg^{-1}, 7.60–176.26 μg kg^{-1}, and 0–32.70 μg kg^{-1}, respectively. The mean concentrations were 219.71 μg kg^{-1}, 70.73 μg kg^{-1}, and 7.08 μg kg^{-1} for manure, soil, and vegetables, respectively. The mean concentrations in soil were lower than the ecotoxic effect trigger value (100 μg kg^{-1}), indicating a low risk for organisms in soil. The concentrations of the three sulfonamides varied significantly in different types of vegetables and all were lower than the acceptable daily intake value (50 μg (kg day)$^{-1}$). However, the potential ecotoxicity and danger to human and animal health via accumulation of the antibiotic in the food chain cannot be ignored.

Keywords Sulfonamides · Manure · Soil · Vegetable · Residue · Risk assessment

With the rapid development of livestock and poultry breeding, many antibiotics are used in the livestock industry as veterinary drugs or feedstuff additives (Karci and Balcioglu 2009; Li et al. 2010). Antibiotics usually cannot be absorbed completely in the gut of animals; therefore, approximately 30% to 90% of the parent compounds or metabolites are excreted into the environment via feces and urine (Li et al. 2015; Yang et al. 2017). Kumar et al. (2005) reported that the concentration of antibiotics ranged from 0 to 200 mg kg^{-1} in the manure of animals. Previous studies have shown that antibiotics cannot be degraded effectively during the composting process (Gou et al. 2018); therefore, the practice of placing animal manure on vegetable fields used to produce food poses a risk of contaminating the soil. The retention of antibiotics in the environment ranged from days to months or even years, and the antibiotic residue in fertilized soil also exceeded 16 mg kg^{-1} (Guardo et al. 2008). Once the active ingredients enter the upper soil layer, they can accumulate and be absorbed by vegetables; and it can be absorbed by plants such as wheat, corn, lettuce, etc. from the soil through the root system and accumulated in the plant body (Franklin et al. 2016). At last antibiotics in the soil or plant caused genetic selection of resistant bacteria, thus causing genetic selection of resistant bacteria (Sarmah et al. 2006). Eventually, human and animal health can be affected by the residues of veterinary medicines in crops and vegetables via the food chain.

Sulfonamides are a class of derivatives with p-aminobenzene sulfonamide as the basic structure. Inhibition of bacterial dihydrofolate synthase makes it unable to make full use of p-aminobenzoic acid to synthesize tetrahydrofolate, thus affecting DNA synthesis. They have wide antibacterial action against gram-negative and gram-positive bacteria,

✉ Caixia Jin
jincaixia@htu.cn

✉ Rui Liu
starliurui@126.com

[1] Key Laboratory for Yellow River and Huai He River Water Environment and Pollution Control, Ministry of Education, Henan Key Laboratory of Environmental Pollution Control, School of Environment, Henan Normal University, 46 Jian-She Road, Xinxiang, China

[2] Key Laboratory of Pollution Ecology and Environmental Engineering, Institute of Applied Ecology, Chinese Academy of Sciences, 72 Wen-hua Road, Shenyang, China

[3] Environment Research Institute, Shandong University, Qingdao, China

[4] Key Laboratory of Pollution Processes and Environmental Criteria (Ministry of Education), College of Environmental Science and Engineering, Nankai University, Tianjin, China

and they can also act on some rickettsia and protozoa (e.g., *Toxoplasma*, *Coccidia*). Due to their low price and broad-spectrum resistance to various bacteria, they are widely used in animal husbandry. Sulfonamides are the most commonly consumed antibiotics in China and worldwide. Because of these factors, residual sulfonamides have been frequently detected in wastes from livestock farms and environmental matrices, such as soil, river water, and groundwater (Karci and Balcioglu 2009). The residue of sulfa antibiotics in livestock and poultry feces varies from 0.02 to 46.37 mg kg^{-1} (An et al. 2015). Sulfonamide antibiotic residue in the soil can be absorbed by plants in aquaculture and grazing animals or indirectly absorbed during the application of manure or slurry. Thus, the consumption of contaminated plants with antibiotics at subtherapeutic concentrations may be another health risk for animals and humans. However, there is limited data of the relevant sulfonamide antibiotic residues in livestock farms.

Thus, in the present study we investigated the residue levels and spatial pattern of three sulfonamides in the manure–soil–vegetable system of 13 different livestock farms in Xinxiang, China. The environmental risk of sulfonamides was also evaluated in different environmental media, such as manure, soil, and vegetables.

Materials and Methods

Sulfadiazine (SD), sulfamonomethoxine (SMM), and sulfamethoxazole (SMZ) with purities of > 99.0% were purchased from Sigma-Aldrich Corp. (St. Louis, Missouri, USA). Fluorescamine (purity > 99.0%) was purchased from Alfa Aesar Corp. (Beijing, China). High-performance liquid chromatography-grade acetonitrile, methanol, and acetone were obtained from Sigma-Aldrich Corp. All other reagents were of analytical grade and water was of high purity.

Samples were collected from 13 different livestock farms, including 7 swine farms, 4 dairy farms, and 2 chicken farms in Xinxiang, Henan, China in September and October 2016. The 13 farms are in different regions, and thus reflect the average concentration of three Sulfonamide antibiotics in manure, soil and vegetables in Xinxiang. The sampling sites are considered representative and are described in Fig. 1. 3–6 soil subsamples (depth 0–20 cm) were collected along an S-shaped line in the vegetable field, avoiding the field edge, crop roots, and sites newly fertilized. The soil samples were placed into valve bags and stored in the refrigerator. Fresh manure samples were collected and placed in plastic containers and transferred to the laboratory. Depending on the status of the vegetable field, 12 kinds of vegetables (green vegetables, leaf lettuce, Chinese cabbage, green Chinese onion, Chinese chives, spinach, chrysanthemum, lettuce, Chinese radish, water spinach, caraway and garlic bolts), including roots, stems, and leaves were randomly collected. All samples were freeze-dried at – 60°C and sieved before testing for antibiotics. They were kept at – 20°C until subsequent analysis.

A subsample of 2.0 g of the freeze-dried soil sample was placed in a 50-mL polyethylene centrifuge tube and extracted with 10 mL of methanol–1% acetic acid. The tubes were vortexed for 2 h and extracted in an ultrasonic bath for 10 min. Then, the extracts were centrifuged at 16,000×*g* for 10 min. The process was repeated twice. The supernatants were combined and concentrated to approximately 15 mL at 45°C using a rotary evaporator (RE-2000A, Shanghai yarong). The supernatants were extracted with dichloromethane and the upper layer was removed after liquid–liquid partitioning. Then, the lower layer was concentrated at 45°C using a rotary evaporator. The residue was dissolved in 0.1 mol

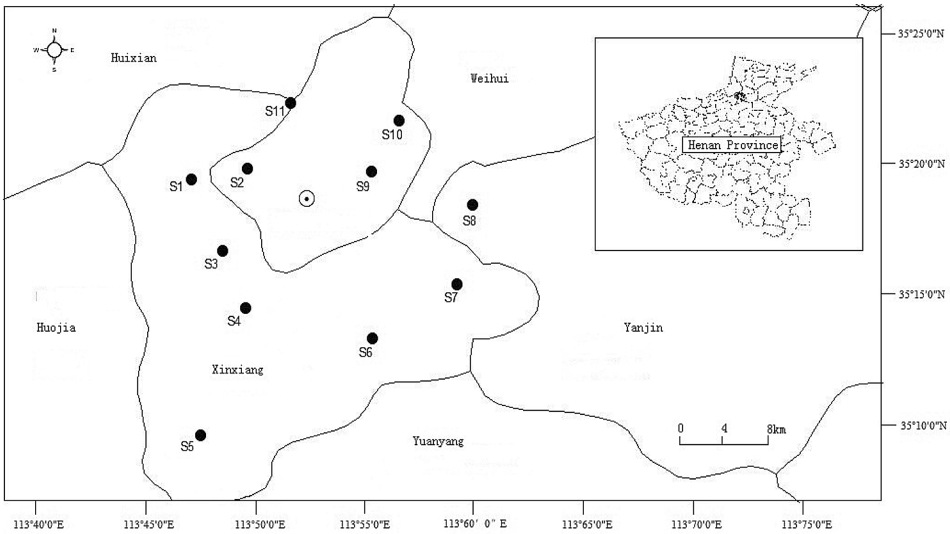

Fig. 1 Sampling sites of the study area in Xinxiang City

L^{-1} HCl and filtered through a 0.22 μm glass fiber filter to remove particulates for analysis.

The forms of organic pollutants in soils were fractionated into water-soluble, organic solvent-extractable, and bound residues. The soil samples were shaken and extracted using sonication in each successive solvent. 0.01 mol L^{-1} $CaCl_2$ was chosen to simulate the water-soluble residues, and methanol, acetonitrile, acetone, and dichloromethane were chosen for the organic solvent-extractable residues. 2 mol L^{-1} NaOH was chosen to extract the bound residues in soils.

A 1.0 g manure sample or 0.2 g freeze-dried vegetable sample was weighed and placed in a 50-mL polyethylene centrifuge tube and 10 mL of acetonitrile, 0.1 g of Na_2EDTA, and 0.1 mL of acetic acid was added. The tubes were vortexed for 2 h, ultrasonicated for 10 min, and centrifuged at 16,000×*g* for 10 min. The extraction was repeated twice. The supernatants were collected and defatted twice with 5 mL of hexane. The upper layer was removed after liquid–liquid partitioning. Then, the lower layer was concentrated at 45°C using a rotary evaporator. The residue was dissolved in 0.1 mol L^{-1} HCl and filtered through a 0.22 μm glass fiber filter to remove particulates for analysis. Then, the analytes were derivatized with fluorescamine.

A total of 10 mg of each antibiotic was weighed accurately and dissolved in a small amount of methanol. The solution was then diluted to 100 mL with deionized water and stored at − 4℃ until subsequent use. The measurement of sulfonamide antibiotics was conducted using high-performance liquid chromatography (Waters 2695, Waters, Singapore) equipped with a fluorescence detector (Waters 2475, Waters). The derivatized compounds were detected at Ex = 405 nm and Em = 495 nm. Chromatographic separation was achieved using an Agilent Eclipse Plus-C18 column (150 mm × 4.6 mm, 3.5 μm). Mobile phase A was acetonitrile and mobile phase B was 0.5% acetic acid. The gradient elution was set as follows: 0 min, 30% A and 10 min 55% A. The column temperature was 35°C with a 20-μL injection volume. The flow rate was 1 mL min^{-1}. The average recovery rate was 86.5% to 94.6% with the coefficient of variation (CV) less than 6.4% at 0.5–10 mg kg^{-1}. The limit of detection was 1.0–2.3 μg kg^{-1}. For routine determination, extracted samples were analyzed in triplicate and the average values were used.

Exploratory analysis of the sulfonamides data was based on descriptive statistics techniques. A nonparametric Kolmogorov–Smirnov (K–S) test was used to test the normality of the sample distributions with the significance level set at $p < 0.05$. Exploratory sulfonamides analysis was performed using the commercial software program SPSS 13 for Windows. Geostatistics were used to describe the spatial variation (spatial dependence) of antibiotics in the surface soil.

Results and Discussion

The occurrence of the three sulfonamides in manure is presented in Table 1 and Fig. 2. SD, SMM and SMZ are widely used in veterinary medicine to treat a variety of bacterial diseases in the livestock industry. Tested sulfonamides were detected in at least one of the manure samples and the frequencies of detection of SD, SMZ, and SMM were 93.8%, 87.5%, and 25.0%, respectively. The concentration ranges of the individual compounds in manure were 0–516.05 μg kg^{-1}, 0–195.71 μg kg^{-1}, and 0–157.23 μg kg^{-1} for SD, SMZ, and SMM, respectively. In all manure sample, the concentration of SD was the highest, the second highest was that of SMZ and SMM had the lowest concentration. Generally, compared with the other two sulfonamides, SD was detected at a considerably higher concentration and detection frequency.

The CV was 86.04%, implying that the residual levels among different farms varied greatly. The sulfonamide concentrations of 37.5% of the samples were higher than 200 μg kg^{-1} in all samples, and 37.5% of the samples contained sulfonamide concentrations between 100 and 200 μg kg^{-1}. In total, 31.3% contained concentrations that were lower than 100 μg kg^{-1}. The K–S test (significance level $p < 0.05$) was used to determine whether the dataset had a normal distribution. The distributions of SD, SMM, and SMZ concentrations were found to be abnormally distributed. Spearman's rank correlation coefficients were

Table 1 Concentrations and detection of 3 sulfonamides in manure and soil samples

Sample types	Sulfonamides	Range (μg kg^{-1})	Mean (μg kg^{-1})	Median (μg kg^{-1})	Detection ratio (%)
Manure	SD	ND–516.05	167.80 ± 7.98	102.92 ± 5.57	93.8
	SMM	ND–157.23	19.86 ± 2.13	0	25.0
	SMZ	ND–195.71	32.05 ± 3.25	14.44 ± 1.23	87.5
	∑SAs	10.13–566.23	219.71 ± 10.20	125.54 ± 8.49	100.0
Soil	SD	ND–104.63	54.17 ± 4.39	62.13 ± 4.31	92.9
	SMM	ND–93.97	11.02 ± 2.73	0	21.4
	SMZ	ND–22.57	5.54 ± 0.88	4.71 ± 0.56	72.4
	∑SAs	7.60–176.26	70.73 ± 5.64	75.01 ± 5.67	100.0

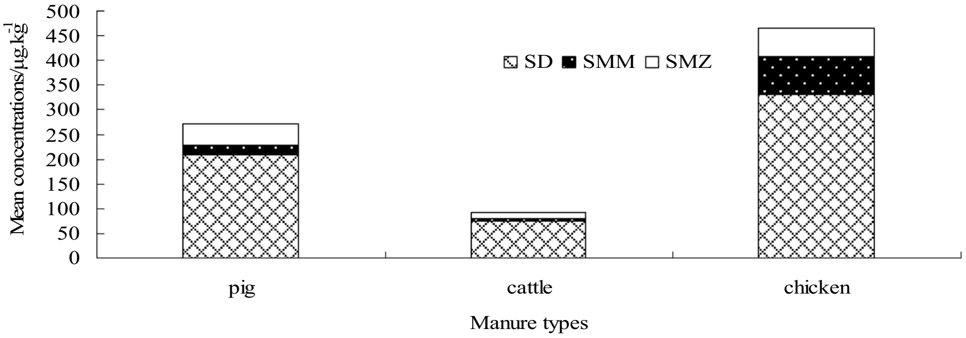

Fig. 2 Mean concentrations of 3 sulfonamides in manure samples

calculated to determine the correlation between the different antibiotics, with significant correlations found between SMM and SMZ (r = 0.5051, $p < 0.05$), whereas no significant correlation was found between SD and SMM (r = 0.2221) and between SD and SMZ (r = 0.3360).

As the prevalent veterinary antibiotic, sulfonamide antibiotics have frequently been detected in animal manure in previous studies. Zhao et al. (2010) investigated the residue levels of sulfonamides in animal manure samples from large-scale livestock and poultry feedlots in eight provinces. Their results showed that the concentrations of all sulfonamides were below 10 mg kg^{-1} and only SD was observed at a maximum concentration of 6.04 mg kg^{-1} in chicken manure. SMZ exhibited both a very low detection frequency and low residue levels in all animal manures. Hua et al. (2017) showed that the maximum residues of SD and SMZ were 46.37 mg kg^{-1} and 37.32 mg kg^{-1} in China, respectively. Followed by SMZ and sulfamerazine, with a maximum residual concentration of 18.00 mg kg^{-1} and 16.50 mg kg^{-1}. The concentrations of the three sulfonamides in the present study were lower than those in previous studies. The reason could attribute to low usage amount of antibiotic in Xinxiang, which was reported that the ratio of usage of antibiotics referred to instructions for use was 66.67% (Shen 2014). In the experiment, the detection frequencies of SD in chicken and pig manure were higher than those of cow manure. Meanwhile, the concentration of SD was the highest in chicken manure, lowest in cow manure, and pig manure had intermediate values. It can conclude that the usage of antibiotics for different livestock and poultry farms is in the order: chicken farms > swine farms > dairy farms. The consumption of antibiotics of chicken farms was the highest for high aquaculture density in order to reduce the chicken morbidity. The variation of the concentrations and detection frequencies of the three sulfonamides in different districts varied greatly, which could be attributed to drug administration strategies and operational experience.

The residue concentration of the three sulfonamides also varied in different types of animals. As shown in Fig. 2, the total concentration of the three sulfonamides was 464.92 μg kg^{-1}, 271.40 μg kg^{-1} and 93.18 μg kg^{-1} for chicken, pig and cow manure samples, respectively. The concentrations of sulfonamides in chicken manure were higher than those in pig and cattle, which was the same as the result reported by Karci (2009) and Hu et al. (2008). The reason was the different absorptive capability of the same types of animals and different feed consumption for the same antibiotics (Winckler and Grafe 2001). Higher antibiotic residue levels were not found in manure from large-scale breeding farms compared to normal-scale breeding farms. On the contrary, smaller farms showed higher residue levels. This was related to the animal operation, administration of medicine, breeding performance, and prescription pattern among the farms.

Sulfonamide antibiotics are usually excreted into the environment and end up in soils from the application of animal manures that are used as agricultural fertilizer. The occurrence of the three sulfonamides in manure-fertilized soils is presented in Table 1. The detection rates were 92.9%, 72.4%, and 21.4% for SD, SMZ, and SMM, respectively. The mean concentrations of the three sulfonamides varied from 5.54 to 54.17 μg kg^{-1} in the order of SD > SMM > SMZ. The detection frequencies and concentrations of sulfonamides in soils were generally consistent with those in manure, i.e., SD was the dominant contaminant over SMM and SMZ. The concentration ranges of SD, SMM, and SMZ in soils were ND–104.63 μg kg^{-1}, ND–93.97 μg kg^{-1}, and ND–22.57 μg kg^{-1}, respectively. The concentration range and mean value of the total sulfonamide concentration was 7.60–176.26 μg kg^{-1} and 70.73 μg kg^{-1}, respectively, with a CV of 61.6%, implying that the residual levels among different farms varied greatly. The highest and lowest concentrations of the three sulfonamides were 176.26 and 7.60 μg kg^{-1}, respectively. Approximately 21.4% of the samples were above 100 μg kg^{-1}, 42.9% of the samples were between 50 and 100 μg kg^{-1}, and 35.7% of the samples were below 50 μg kg^{-1}. A K–S test ($p < 0.05$) was used to determine whether the dataset had a normal distribution. The distributions of SD, SMM, and SMZ were found to be non-normal. Spearman's rank correlation coefficients were calculated to determine the correlation between the different antibiotics, with no significant correlations among the different antibiotics found.

The residual concentrations of sulfonamides in soils were related to the residue level in the manure. The residues of sulfonamides in manure-fertilized soils were much lower than those in animal manure. The main reasons for this phenomenon were the dilution, degradation, leaching, and uptake by vegetables after manure was incorporated into the soils. The concentrations of sulfonamides in manure-fertilized soils were highest in chicken farms and lowest in cattle farms, which was consistent with the residual levels in these animal manures.

The forms of organic pollutants in soils can be fractionated into water-soluble, organic solvent-extractable, and bound residues. The availability and toxicity of the three forms differed greatly. The organic solvent-extractable and water-soluble residues had high biological activity and might have a direct influence on organisms and the environment. On the contrary, the bound residues increased the persistence of organic pollutants in soils and reduced availability. When environmental conditions are changed, the bound residues can be transferred into other forms of high biological activity and may cause risks. As shown in Fig. 3, the average percentages of organic solvent-extractable, bound residues, and water-soluble were 57.4%, 28.8%, and 12.6%, respectively, for SMM, which was the same for SD and SMZ. Thus, the organic solvent-extractable residues were the main forms of sulfonamides and might be the most important reservoir of sulfonamides in the soil. A variety of factors can influence the forms of organic pollutants in soils, such as manure types, volume, frequency, vegetable species, and physicochemical properties of the soil (Zhou and Hu 2017).

Vegetables planted in manure-fertilized soil may absorb sulfonamide antibiotics. Therefore, sulfonamides in the soil could enter the food chain and have a potential risk on human health. As shown in Table 2, the detected frequency of sulfonamide antibiotics in plants was 58.1% of all vegetable samples in the 13 different farms. The range of sulfonamide concentrations was ND–32.70 μg kg^{-1} in vegetables, with an average value of 7.08 μg kg^{-1}.

The concentrations of SD and SMZ in vegetables were significantly higher than that of SMM ($p < 0.05$). The concentration ranges of SD, SMM, and SMZ in vegetables were ND–28.99 μg kg^{-1}, ND–10.69 μg kg^{-1}, and ND–6.54 μg kg^{-1}, respectively. Among the three sulfonamides, SMZ was detected with the highest detection frequency (51.6%), with a mean concentration of 1.30 μg kg^{-1}. SD was detected with the highest residue concentration of 5.29 μg kg^{-1}; however, its detection frequency was not the highest among the three sulfonamides. Both the detection frequency and concentrations of SMM were very low. Among all vegetable samples, 64.5% of the samples were below 5 μg kg^{-1}, 16.1% were above 20 μg kg^{-1}, and 19.4% were between 5 μg kg^{-1} and 20 μg kg^{-1}. The distributions of SD, SMM, and SMZ in vegetables were found to be non-normal based on the results of K–S tests at a significance level of 0.05. From Spearman's rank correlation coefficient, significant correlation existed among the three sulfonamide antibiotics. The sulfonamide residues in vegetables were much lower

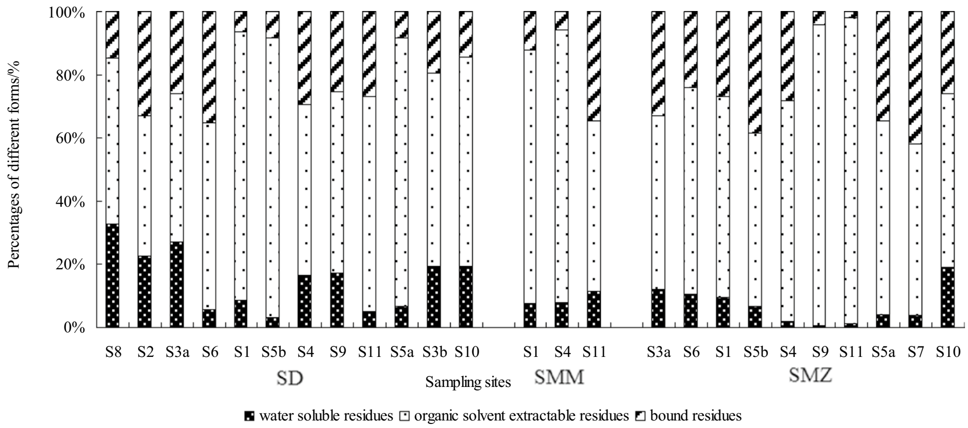

Fig. 3 Percentages of different forms in soils

Table 2 Concentrations and detection of 3 sulfonamides in vegetable samples

SAs	Range (μg kg^{-1})	Mean (μg kg^{-1})	Median (μg kg^{-1})	Detection (%)
SD	ND–28.99	5.29 ± 0.95	0	29.0
SMM	ND–10.69	0.49 ± 0.04	0	6.5
SMZ	ND–6.54	1.30 ± 0.21	0.79 ± 0.18	51.6
∑SAs	ND–32.70	7.08 ± 1.12	1.42 ± 0.35	58.1

than those in the contaminated soils. One reason for this phenomenon is that the uptake by vegetables from soils is very limited. The other reason is that the adsorption of sulfonamides by organic matter or mineral substances in soils can hinder the uptake and accumulation process of vegetables.

In the study, the residual concentrations of the three sulfonamides varied greatly among the different types of vegetables, with the highest mean concentration (green vegetables) being 23 times higher than the lowest mean concentration (Chinese radish). The highest mean concentration of the three sulfonamides was 23.80 μg kg^{-1} in green vegetables. The content of sulfonamides in the following sequence: green vegetables > leaf lettuce > Chinese cabbage > green Chinese onion > Chinese chives > spinach > chrysanthemum > lettuce > Chinese radish. No sulfonamide antibiotic residues were found in water spinach, caraway, and garlic bolts. The residual levels of SD in green vegetables and leaf lettuce were the highest at concentrations of 22.26 μg kg^{-1} and 17.48 μg kg^{-1}, respectively. SMM was detected only in Chinese cabbage and Chinese chives at concentrations of 0.73 μg kg^{-1} and 2.14 μg kg^{-1}, respectively, and the frequency of SMZ detection was 51.6% in the vegetables, which was the highest among the three sulfonamides. The root exudates produced by the different types of vegetables changed the microbial communities and enzyme activity in the rhizosphere, which affected uptake by the vegetables. Moreover, other factors affect the uptake ability of plants, such as the growth stages of the vegetable as well as physicochemical properties and the environment of the soils, which can also play important roles in the accumulation of pollutants from the environment (Hu et al. 2010; Zhou et al. 2011).

The capacities of accumulating antibiotics for different types of vegetables vary greatly in the environment. The bio-concentration factor (BCF) was used to describe the ability of different vegetables to accumulate sulfonamide antibiotics from the soil (based on dry weight). The formula for BCF is expressed as follows:

$$\mathrm{BCF} = \frac{C_1}{C_0}$$

where BCF is the bio-concentration factor and C_1 and C_0 are the concentrations of sulfonamides in vegetable and soil, μg kg^{-1}, respectively. As shown in Table 3, the BCFs of all vegetables were not greater than 1; therefore, the capacities of all vegetables for accumulating antibiotics from the soil were low. Leaf lettuces and green vegetables had a relatively high bioaccumulation ability for SD. Thus, the ability of the same type of vegetables to accumulate sulfonamides was different. The ability to accumulate sulfonamides might be related to the types of vegetables, growth stages, properties of soils and other factors.

Table 3 Bio-concentration factors for different vegetables

Types of vegetables	BCF		
	SD	SMM	SMZ
Chinese cabbages	0.27	0.11	0.40
Green Chinese onions	0.37	–	0.57
Leaf lettuces	0.45	–	0.09
Green vegetable	0.46	–	0.55
Chinese chives	–	0.11	0.78
Spinach	–	–	0.73
Chrysanthemums	–	–	0.72
Chinese radish	–	–	0.21
Lettuces	–	–	0.68

The mean concentrations of SD in vegetables were the highest; however, the BCF values were lower than those for SMZ and the mean concentrations of SMZ in the soil were also low. Thus, the adsorption ability of the different types of sulfonamides was different, and the ability to transfer SMZi from the soil to the plant might be stronger than for the other two sulfonamides. It has been reported previously that SMZ was easily absorbed by pakchoi cabbage (Li et al. 2013). In the study, the same conclusions were drawn from a field investigation, with the ability to transfer the various sulfonamides found to be different. This ability to transfer sulfonamides might be related to the K_{ow} values of the chemical species. This was also confirmed by Tanoue et al. (2012) who found that the plant uptake abilities were related to the log K_{ow} value of pharmaceuticals by studying the adsorption of sulfonamides from contaminated soil. The log of SD and SMZ K_{ow} are − 0.09 and 0.89, respectively. Thus, plants easily absorb more SMZ than SD and SMM and the concentration of SMZ was not the highest among the sulfonamides. The residues and BCF of SMM in vegetables were the lowest of the three sulfonamides. The residues and detection of SMM in vegetables corresponded to those in soils.

The biological availability of different forms of sulfonamide residues in the soil varied greatly. A partial correlation was found between the forms of sulfonamide residues in the soil and sulfonamide concentration in the plants. The results showed that the relationship between the content of sulfonamides in the plant and water-soluble residues was positive. And there was a negative relation between sulfonamides in plants and organic solvent-extractable residues ($p < 0.05$). Thus, no relative relationship was found between bound residues and the content of sulfonamides in plants. Therefore, it is not reasonable to assess the ecological risk of pollutants to humans, animals, plants, and the microbiology in the environment by assessing the total content, rather it should be based on the content of available forms in the environment.

If the concentration of sulfonamides is greater than the safety threshold value, then sulfonamides will affect the function of organisms in the environment. Referring to the trigger value of sulfonamide antibiotics (100 μg kg^{-1}) in soils set by the Steering Committee of the Veterinary International Committee on Harmonization based on the ecotoxic effects of antibiotics on a range of organisms (Qiao et al. 2012), most of the residual concentrations of sulfonamides in the soils obtained in the present study were below the reference value, implying that the three sulfonamides have a low risk for organisms in the soils of Xinxiang. However, antibiotic-resistant bacteria could develop at low concentrations in soils by the synergistic effect of different antibiotics.

Sulfonamides have several potential adverse effects on humans and animals, such as allergic reactions and chronic toxic effects (Kim et al. 2010). However, sulfonamides could be absorbed by plants even at lower concentrations of sulfonamides in the soil. Therefore, antibiotic resistance in the environment could spread to humans via the food chain, which would lead to a threat to the human health. The risk assessment of vegetables was made based on the maximum residue limit and acceptable daily intake. The acceptable daily intake value of sulfonamides is 50 μg (kg day)$^{-1}$, which was established by the Joint FAO/WHO Expert Committee on Food Additives (2010). The maximum residue limit (MRL) for vegetables (100 μg kg^{-1}) in animal feed was set by the Ministry of Agriculture. If the body weight of an adult is 60 kg and a typical adult consumes approximately 500 g of vegetables and 100 g of animal-based food a day, the MRL for vegetables is 20 μg kg^{-1}. The results from the present study indicated that in all the vegetable samples, four samples exceeded the MRL and the concentration of sulfonamides in green vegetables was the highest. Thus, further studies are required regarding the safety of the residues of antibiotics in edible vegetables. The number of sulfonamides accumulated by any of the vegetables was below the (Acceptable Daily Intake) AID, implying that the residue levels in vegetables pose a very low risk for humans. However, the cumulative effects of consuming vegetables every day by humans should be considered.

Acknowledgements This work was supported by the National Natural Science Foundation of China (Nos. 41907349, 21906043, 31170478 and 41601333), the Technology Department of the Henan Science and Technology Fund Project (No. 202102310597).

References

An J, Chen H, Wei S, Gu J (2015) Antibiotic contamination in animal manure, soil, and sewage sludge in Shenyang, northeast China. Environ Earth Sci 74(6):5077–5086

Franklin AM, Williams CF, Andrews DM et al (2016) Uptake of three antibiotics and an antiepileptic drug by wheat crops spray irrigated with wastewater treatment plant effluent. J Environ Qual 45(2):546–554

Gou M, Hu HW, Zhang YJ et al (2018) Aerobic composting reduces antibiotic resistance genes in cattle manure and the resistome dissemination in agricultural soils. Sci Total Environ 612:1300–1310

Guardo AD, Calamari D, Benfenati E et al (2008) Pharmaceuticals as environmental contaminants: modeling distribution and fate. Pharmaceuticals in the environment. Springer, Berlin, Heidelberg, pp 121–132

Hua W, Chu Y, Fang C (2017) Occurrence of veterinary antibiotics in swine manure from large-scale feedlots in Zhejiang Province, China. Bull Environ Contam Toxicol 98(4):472–477

Hu XG, Luo Y, Zhou QX et al (2008) Determination of thirteen antibiotics residues in manure by solid phase extraction and high performance liquid chromatography. Chin J Anal Chem 36(9):1162–1166

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. Environ Pollut 158(9):2992–2998

Joint FAO/WHO Expert Committee on Food Additives (2010) Residue Evaluation of Certain Veterinary Drugs, Meeting 2010-Evaluation of data on ractopamine residues in pig tissues. FAO JECFA Monographs; Food and Agriculture Organization of the United Nations: Rome, www.fao.org/docrep/012/i1618e/i1618e00.pdf (accessed 24 Nov 2013)

Karci A, Balcioglu IA (2009) Investigation of the tetracycline, sulfonamide and fluoroquinolone antimicrobial compounds in animal manure and agricultural soils in Turkey. Sci Total Environ 407(16):4652–4664

Kim SC, Yang JE, Carlson K (2010) Dissolved and colloidal fraction transport of antibiotics in soil under biotic and biotic conditions. Water Qual Res J Can 45:275–285

Kumar K, Gupta SC, Chander Y et al (2005) Antibiotic use in agriculture and its impact on the terrestrial environment. Adv Agron 87:1–54

Li C, Chen JY, Wang JH et al (2015) Occurrence of antibiotics in soils and manures from greenhouse vegetable production bases of Beijing, China and an associated risk assessment. Sci Total Environ 521–522(1):101–107

Li XD, Yu HX, Xu SS et al (2013) Uptake of three sulfonamides from contaminated soil by pakchoi cabbage. Ecotox Environ Safe 92:297–302

Li XD, Xian QM, Liu HL et al (2010) Simultaneous determination of three sulfonamides residues in vegetable by high performance liquid chromatographic method with fluorimetric detection. Chin J Anal Chem 38(3):429–431

Qiao M, Chen W, Su J et al (2012) Fate of tetracyclines in swine manure of three selected swine farms in China. J Environ Sci 24(6):1047–1052

Sarmah AK, Meyer MT, Boxall ABA (2006) A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere 65(5):725–759

Shen XW (2014) The actualities investigation of the veterinary drugs use in the region of Xinxiang. Dissertation, Henan Agricultural University

Tanoue R, Sato Y, Motoyama M et al (2012) Plant uptake of pharmaceutical chemicals detected in recycled organic manure and reclaimed wastewater. J Agric Food Chem 60(41):10203–10211

Winckler C, Grafe A (2001) Use of veterinary drugs in intensive animal production: evidence for persistence of tetracycline in pig slurry. J Soils Sedim 1(2):66–70

Yang Q, Tian T, Niu T, Wang P (2017) Molecular characterization of antibiotic resistance in cultivable multidrug–resistant bacteria from livestock manure. Environ. Pollut. 229:188–198

Zhao L, Dong YH, Wang H (2010) Residues of veterinary antibiotics in manures from feedlot livestock in eight provinces of China. Sci Total Environ 408(5):1069–1075

Zhou QX, Cai Z, Zhang ZN, Liu WT (2011) Ecological remediation of hydrocarbon contaminated soils with weed plants. J Res Ecolo 2(2):97–105

Zhou QX, Hu XG (2017) Systemic stress and recovery patterns of rice roots in response to graphene oxide nanosheets. Environ Sci Technol 51(4):2022–2030