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Occurrence of veterinary antibiotics in arable soil with different fertilisation modes: a field study

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ABSTRACT

Context. Antibiotics contained in animal manure can contaminate arable soils and even groundwater. Systematic studies on the presence of veterinary antibiotics (VAs) in soils under different fertilisation modes are urgently needed to provide ample evidence for preventing and controlling VAs pollution. **Aims.** This study tried to illustrate the effect of different fertilisation modes on the occurrence of soil VAs in a long-term field experiment. **Methods.** Soil samples were collected from a 30-year fertilisation experiment with four fertiliser application modes: (1) no fertiliser (CK); (2) chemical fertiliser (TR1); (3) cattle manure (TR2) and; (4) combination of chemical fertiliser and cattle manure (TR3). **Key results.** Results showed that tetracyclines and sulfonamides were ubiquitously detected in soil samples with the concentration from not detectable (ND) to 6.95 µg kg^{-1} and ND to 3.85 µg kg^{-1}, respectively, which were significantly lower than those of cattle manure (5.13–1628 µg kg^{-1}). In addition, the long-term fertilisation generally improved soil properties and increased levels of VAs. The combined application of chemical fertiliser and cattle manure could significantly improve contents of soil nutrients. The correlation coefficient showed that the concentration of antibiotics and soil properties, such as soil organic matter, total nitrogen and available phosphorus, had positive correlation ($P < 0.05$). **Conclusions.** This study indicated that different fertilisation methods had significant effects on the occurrence of antibiotics in arable soil. **Implications.** The combination of inorganic and organic fertiliser application was a reasonable fertilisation mode to improve the soil fertility and control antibiotics contamination.

Keywords: agricultural soil, arable soil, cattle-manure, long-term fertilisation, soil nutrients, sulfonamides, tetracyclines, veterinary antibiotics.

Introduction

Antibiotics are a type of natural or synthetic chemical capable of inhibiting or killing microorganisms, that have existed and substantially benefited public health for decades (Tasho and Cho 2016). Improper or excessive use of antibiotics could cause large amounts of undigested antibiotic residues in urine and faeces of humans and animals as maternal drugs or active pharmaceuticals (Sarmah *et al.* 2006). In addition, antibiotics and their metabolites have been excreted into various environments along with sewage, sludge or manure of hospitals and farms (Kumar *et al.* 2012). Numerous studies reported that antibiotics had been detected in different environmental mediums, such as animal manure (Martínez-Carballo *et al.* 2007; Hu *et al.* 2010; Hou *et al.* 2015; Guo *et al.* 2016; Pan and Chu 2018; Zhou *et al.* 2020), sewage treatment plants (Wu *et al.* 2016), water (Zhang *et al.* 2015; He *et al.* 2018; Liu *et al.* 2018), soil (Hu *et al.* 2010; Li *et al.* 2015; Guo *et al.* 2016; Tasho and Cho 2016; Sun *et al.* 2017), and even in groundwater (Hu *et al.* 2010; Ma *et al.* 2015). China is one of the largest producers and consumers of antibiotics. For example, in 2013, approximately 92 700 tons of antibiotics were used in China. However, an estimated 54 000 tons of them were excreted and finally released into environment (Zhang *et al.* 2015).

Veterinary antibiotics (VAs) are widely used for treating bacterial diseases and promoting growth in animals (Sarmah *et al.* 2006; Kumar *et al.* 2012; Zhou *et al.* 2013).

However, large amounts of agricultural soils in China had the problem of accumulation of residual VAs, caused by the application of animal manure as fertiliser because of farmers' limited knowledge of VAs treatment and regulation (Chen et al. 2018). Recently, the accumulation of VAs in agricultural soils became an increasing concern over the world. Previous work suggested that livestock and poultry manure was one of the main sources of VAs in agricultural soil (Ho et al. 2014), with residual VAs being absorbed by vegetables and subsequently polluting the food chain (Hu et al. 2010). VA residues could also cause the enrichment of harmful bacteria and increasing antimicrobial resistance (Martínez 2008; WHO 2014), posing serious threats to global public health. In addition, VA residues also induced toxic effects on vegetable crops; e.g. wheat, Chinese cabbage and tomato (Jin et al. 2009). It is noticed that such toxic effects could occur at very low levels because of long-term exposure (Kuppusamy et al. 2018). For food safety and health, it is mandatory to monitor and solve the antibiotics contamination problems.

The occurrence, fate and risks of VAs in agricultural soils had been a hot issue in recent years. VAs contamination was a common phenomenon in agricultural soils (Hu et al. 2010; Chen et al. 2014; Ho et al. 2014; Tang et al. 2015; Guo et al. 2016; Wei et al. 2019; Cai et al. 2020). Antibiotics commonly used in livestock husbandry include macrolides, β-lactams, cephalosporins, sulfonamides, tetracyclines and fluoroquinolones. These can be detected in agricultural soils ranging from not detectable (ND) to mg kg^{-1} levels (Kuppusamy et al. 2018), reflecting the diversification and abuse of antibiotics. The detected residual concentrations of individual antibiotics were up to 2.68 mg kg^{-1} in Tianjin (Hu et al. 2010), 8.40 mg kg^{-1} in Xuzhou (Zhang et al. 2016), 2.01 mg kg^{-1} in Tianjin (Wei et al. 2016), and 4.72 mg kg^{-1} in Yangtze River Delta (Sun et al. 2017). Moreover, Gros et al. (2019) demonstrated that VAs were detected in manure-amended soils with average concentrations ranging from 0.078 to 150 μg kg^{-1}. Hu et al. (2010) reported that the concentration ranges of all the selected antibiotics in the manure and manure-amended soil were 0.1–184 mg kg^{-1} and 0.1–2683 μg kg^{-1} in northern China, respectively. Concentrations of tetracyclines and quinolones antibiotics in organic farm soils in Guangzhou were 2.32–305 μg kg^{-1} (Xiang et al. 2016) and 0.46–55.2 μg kg^{-1} (Wu et al. 2014), respectively. Hence, the tetracyclines antibiotics were usually measured as predominant antibiotics with high levels of both residual concentrations and detected frequencies in agricultural soils throughout the world, resulting in a potential threat to both plants and soil organisms (Kuppusamy et al. 2018).

To date, many researchers have focused on the fate and sources of VAs in agricultural soils worldwide (Kumar et al. 2012; Tasho and Cho 2016; Kuppusamy et al. 2018; Qiao et al. 2018). In consideration of the extension of resource utilisation of animal manure in China, here we present the occurrence of typical VAs in arable soils under different fertilisation modes (no fertiliser, chemical fertiliser, cattle manure, and combined fertiliser) from a 30-year fertilisation experiment, and reveal the relationships between the VAs and soil properties under exclusive application of cattle-manure. We also provide a reference for the control of antibiotics.

Materials and methods

Chemicals and standards

Four types of tetracyclines (TCs) including: (1) tetracycline (TC); (2) oxytetracycline (OTC); (3) chlortetracycline (CTC); and (4) doxycycline (DC), as well as four types of sulfonamides (SAs) including: (1) sulfameter (SM); (2) sulfamethazine (SMZ); (3) sulfathiazole (STZ); and (4) sulfadiazine (SDZ) were used in this study (Dr. Ehrenstorfer GmbH, Germany). To quantify the concentrations of antibiotics, the deuterated antibiotics such as tetracycline-D6 (Toronto Research Chemicals Inc., Canada), sulfamethazine-D4 and sulfadimethoxine-D6 (Dr. Ehrenstorfer) were used as the internal standards. Sulfamethoxazole-D4 and demeclocycline (Dr. Ehrenstorfer), another two deuterated antibiotics, were performed as the surrogate standards to assay the recovery of each analysis. Other analytical grade chemicals were from Sinopharm Chemical Reagent Co. Ltd, China, except methanol, formic acid, oxalic acid and acetonitrile (HPLC grade) were from Tedia Company Inc., USA, and ammonium acetate from Sigma-Aldrich, USA. Ultrapure water was produced by a Milli-Q apparatus (Millipore, Bedford, MA).

The stock solution of each standard (1.0 mg L^{-1}) was dissolved in methanol, and stored at −20°C in dark. Working solutions were prepared freshly by methanol dilution, and stored at 4°C in dark. The extraction solvent was a mixture of EDTA-sodium phosphate buffer (SPB, pH = 4) prepared based on Huang et al. (2013). Briefly, SPB was prepared by mixing NaH$_2$PO$_4$ (10.56 g) and H$_3$PO$_4$ (0.82 mL) in 1 L water. EDTA-SPB (pH = 4) was obtained by dissolving Na$_2$EDTA (80 g) in 1 L SPB.

Experimental design and sampling

Soil samples were collected at October 2015, from a 30-year fertilisation experiment at the Soil Fertility and Fertilisation Efficiency Monitoring Base, located in the Agricultural Science Institute of Xuzhou, Jiangsu Province (34°16′N, 117°17′E). Four fertiliser application modes were designed: (1) no fertiliser (CK); (2) chemical fertiliser (combination of nitrogen, phosphorus and potassium, TR1); (3) exclusive application of cattle manure (TR2); and (4) combination of chemical fertiliser and cattle manure (TR3). Each treatment contained four replicated districts. Chemical fertiliser contained pure N (8 g kg^{-1}), P$_2$O$_5$ (4 g kg^{-1}) and K$_2$O (6 g kg^{-1}). Cattle manure contained pure N (6.3 g kg^{-1}), P$_2$O$_5$ (5.1 g kg^{-1}) and K$_2$O (7.4 g kg^{-1}). The annual amount

of fertiliser in TR1, TR2 and TR3 was 37 500 kg hm^{-2} from 1984 until now.

A total of 16 surface soil (0–20 cm) samples were collected by five-point sampling method from the area of each district (33 m^2) using a pre-cleaned stainless soil auger and transferred into cloth bags. Stones and residual roots were removed. All samples were immediately transferred to the laboratory, and stored at −20°C in dark. Soil samples were sieved through a stainless steel sieve (0.25 mm), freeze-dried, and then sealed in brown glass bottles for the antibiotic extraction. Physicochemical properties of soil are in Table 1.

Extraction and analytical procedures

Antibiotics in manure and soil samples were analysed as described by Wei *et al.* (2019). Briefly, each 0.5 g manure or 5 g soil sample was mixed with 2.0 mL 150 mg L^{-1} Na$_2$EDTA solution, and then mixed with 5 mL acetonitrile/methanol (65/35, v/v), and finally mixed with 5 g anhydrous Na$_2$SO4 and 0.5 g NaCl. The supernatant was pipetted and centrifuged (3000g, 10 min, room temperature) using a d-SPE sorbent consisting of 12.5 mg C$_{18}$, 12.5 mg primary secondary amine and 225 mg Na$_2$SO4.

Purified samples were analysed using a liquid chromatography-tandem mass spectrometry system equipped with a C$_{18}$ column (3 μm × 150 mm × 4.6 mm) at 40°C, and the injection volume was 20 mL. The mobile phase was methanol-acetonitrile (1:1, v/v) and 0.3% formic acid solution with 0.1% ammonium formate (v/v). Mobile phase A and mobile phase B were 0.1% formic acid (v/v), and methanol-acetonitrile (1:1, v/v).

Results

Variations of soil physico-chemical characteristics

With more than 30 years of different fertilisation modes, soil physico-chemical characteristics in the current study are

listed in Table 2. Soil nutrients under different fertilisation modes are significantly diverse. The pH of the top soil samples in different treatments are weakly alkaline. The fertilisation significantly decreases soil pH, but increases the total organic matter (OM) and cation exchange capacity (CEC). The cattle manure could increase soil total N (TN) and active phosphate (AP), but decrease active potassium (AK). Compared with the control treatment, fertilisation raises soil TN, AK, AP, OM about 34.6–121%, 55.4–145%, 95.7–1184% and 29.8–97.8%, respectively.

Distributions of selected antibiotics in soils and cattle manure

Eight typical VAs were detected ubiquitously in soils of the long-term experiment with different fertilisation modes. Detection frequencies for all tested antibiotics reached 100% in fertilisation experiments (TR1, TR2, TR3), and only 62.5% in the control treatment (CK). It is noticed that the residue of TCs in the soil was significantly higher than that of SAs ($P < 0.05$) as shown in Table 3.

Concentrations of antibiotics in current arable soils were at a fairly low level ranging from ND to 6.95 μg kg^{-1}. Average concentrations for SAs in order were SDZ > SM > STZ > SMZ, and for TCs were CTC > TC > DC > OTC. Thus, SDZ (2.38 ± 1.44 μg kg^{-1}) or CTC (2.72 ± 2.50 μg kg^{-1}) were the dominant SAs or TCs under different treatments.

Distributions of SAs and TCs in cattle manure are shown in Fig. 1. Concentrations of TCs are 2-fold higher than that of SAs. CTC is the dominant antibiotic (1628 μg kg^{-1}), followed by TC (296.8 μg kg^{-1}), DC (152.5 μg kg^{-1}) and OTC (36.7 μg kg^{-1}). For SAs antibiotics in cattle manure, the concentration of SM is the highest with a mean value of 18.5 μg kg^{-1}, while SMZ (5.13 μg kg^{-1}) is the lowest.

Occurrence of selected antibiotics in different treatments

Different long-term fertilisation treatments affected soil antibiotics residues. Residues of four types of TCs and SAs

Table 1. The physico-chemical properties of soil with the mean values.

	pH	Total N (g kg^{-1})	Total P (g kg^{-1})	Organic matter (g kg^{-1})	Physical clay content (mg kg^{-1})	Clay content (mg kg^{-1})	Cation exchange capacity (cmol kg^{-1})
Soil	8.01	0.66	0.74	10.8	0.01	59.8	20.4

Table 2. Soil properties with different fertilisation treatments.

Treatment	pH	TN (g kg^{-1})	AK (g kg^{-1})	AP (g kg^{-1})	Soil OM (g kg^{-1})	CEC (cmol kg^{-1})	C/N
CK	8.08 ± 0.02a	0.81 ± 0.03b	27.67 ± 2.08d	9.41 ± 0.14d	14.57 ± 1.10d	14.80 ± 0.44b	10.47 ± 0.59a
TR1	7.74 ± 0.06b	1.09 ± 0.05b	52.00 ± 2.00b	18.42 ± 1.41c	18.91 ± 1.21c	15.72 ± 0.53b	10.04 ± 0.77a
TR2	7.98 ± 0.05a	1.51 ± 0.03a	43.00 ± 1.00c	120.86 ± 7.49a	24.68 ± 1.31b	18.69 ± 0.96ab	9.48 ± 0.37ab
TR3	7.79 ± 0.03b	1.79 ± 0.05a	67.67 ± 0.58a	108.54 ± 5.70b	28.82 ± 2.06a	20.65 ± 0.93a	9.16 ± 0.43b

The values of soil samples with different lowercase letters were significantly different from each other ($P < 0.05$).

C

Table 3. The concentrations of veterinary antibiotics in the soil samples (µg kg⁻¹).

| Antibiotics		Minimum	Maximum	Mean ± s.d.	Median
Sulfonamides	SM	ND	2.92	1.64 ± 1.00	2.04
	SMZ	0.32	1.73	0.90 ± 0.53	0.78
	STZ	0.48	2.98	1.31 ± 0.90	0.99
	SDZ	0.49	3.85	2.38 ± 1.44	2.93
Tetracyclines	TC	0.22	4.14	1.54 ± 1.53	0.91
	OTC	0.19	3.58	1.51 ± 1.10	1.40
	CTC	ND	6.95	2.72 ± 2.50	1.59
	DC	ND	3.90	2.05 ± 1.28	1.56

ND, not detected.

in different treatments are shown in Figs 2, 3, respectively. Residual VA concentrations of TR1 treatment are below 0.5 µg kg⁻¹, and similar to that of CK. In TR2, concentrations of TCs (2.2–6.95 µg kg⁻¹) and SAs (1.4–3.85 µg kg⁻¹), are higher than those of the other three treatments, indicating that the pure cattle manure could enhance significantly soil antibiotics level. Hence, pure manure might be the primary polluted source of antibiotics in soils. These results suggest that the combined application of organic–inorganic fertiliser could reduce concentrations of TCs in soils, especially for CTC. However, it is not the case for SAs because there was no significant difference among all concentrations. As shown in Fig. 4, the occurrence of total concentrations of TCs and SAs under different treatments is

similar to that of the individual antibiotics. Compared with TR2, the concentration of TCs and SAs in TR3 is reduced about 58.9% and 28.8%, respectively. Typically, as the major compounds in soil, the concentration of CTC and SDZ in TR3 decreased about 73.6% and 20.6%, respectively. However, the total concentration of SAs is higher than that of TCs in TR3, suggesting that a higher decreasing tendency for TCs antibiotics might be owing to the compound fertiliser.

Correlations between antibiotics and soil properties

Table 4 lists correlation coefficients between antibiotics and soil properties in different fertilisation treatments. Strong positive correlations among different classes of antibiotics are observed. Concentrations of TC, OTC, CTC, DC are significantly ($P < 0.05$) correlated with those of SM, SMZ, SDZ and STZ. Generally, the chemicals in the branch of the same group have a close correlation, and show their positive correlations in soils. It indicates that the residues of soil antibiotics would increase along with the application dosages. Additionally, AP has an extremely strong positive correlation with TCs and SAs; CEC, TN and OM are positively correlated with antibiotics. Notably, OTC, SM and SDZ have extremely strong correlations with soil properties except AK, whereas TC and CTC are weakly correlated with soil properties. Therefore, soil properties, such as AP, TN, SOM and CEC, played a vital role in the residual of antibiotics.
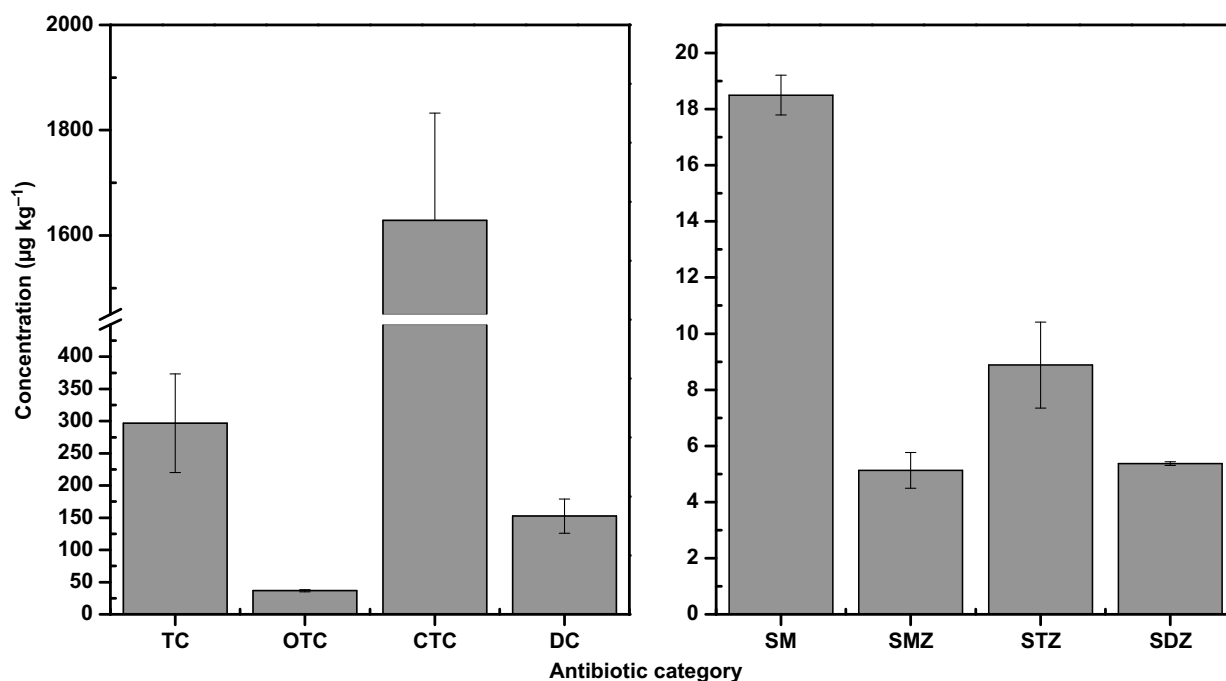
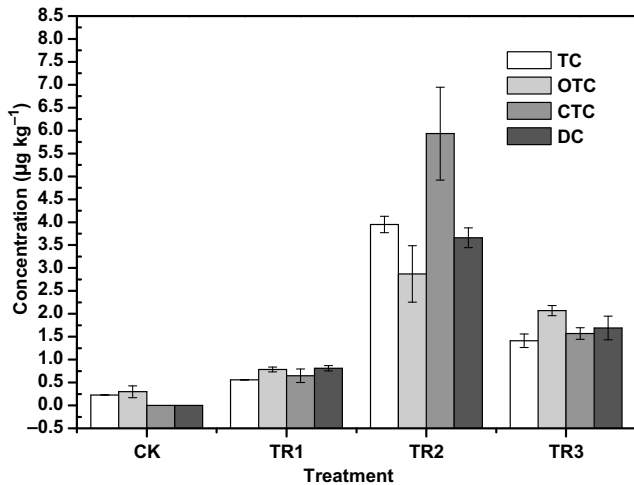**Fig. 1.** Antibiotics in manure.

D

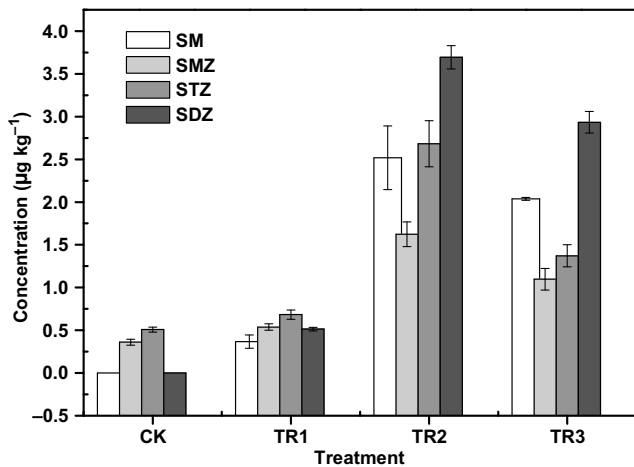**Fig. 2.** TCs in soil with different fertiliser treatments.

Fig. 3. SAs in soil with different fertiliser treatments.
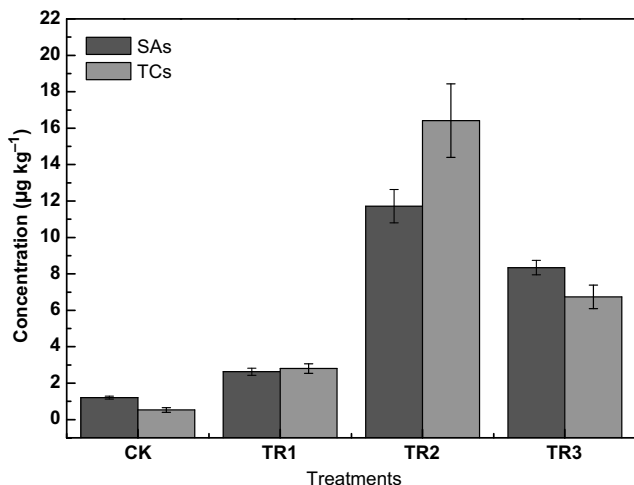

Fig. 4. Total antibiotics in soil with different fertiliser treatments.

Discussion

Occurrence of typical VAs in soil–manure system

As shown in Fig. 3, both fertilisation modes and antibiotics residues of manure could affect directly the accumulation of the antibiotics in soil. Both detection frequencies and residual concentrations of SAs were lower than those of TCs. CTC was the major individual antibiotic in the present field with the maximum concentration of 1628 μg kg^{-1} and 6.95 μg kg^{-1} in cattle manure and arable soils, respectively. According to standards of veterinary medicines from the Food and Drug Administration, if residual levels of antibiotics are lower than the eco-toxic effect trigger value of 100 μg kg^{-1} in soil, this would pose a low environmental risk.

The levels of eight typical antibiotics in the applied cattle manure and manure-amended soil of the present work were much lower than those of the previous results from various agricultural soils around the world, even for soils continuously manured over 30 years (Hamscher *et al.* 2002; Martínez-Carballo *et al.* 2007; Karcı and Balcıoğlu 2009; Ho *et al.* 2014; Hou *et al.* 2015; Guo *et al.* 2016). A large number of studies had shown that the residual VAs that came from agricultural soil fertilised with manure was a common phenomenon. An investigation undertaken in Austria revealed that 46 mg kg^{-1} of CTC, 29 mg kg^{-1} of OTC, 23 mg kg^{-1} of TC and 20 mg kg^{-1} of sulfadimidine were detected in pig manure (Martínez-Carballo *et al.* 2007). Zhao *et al.* (2010) also found that OTC (0.15–59.6 mg kg^{-1}), CTC (0.16–27.6 mg kg^{-1}), SMZ (0.1–0.66 mg kg^{-1}) or SDZ (0–3.12 mg kg^{-1}) were detected in chicken, pig and cattle manure samples. In manure samples, a higher range of concentrations of TCs (54.1 ± 5776 μg kg^{-1}) was detected than that of SAs (3.2 ± 5.2 μg kg^{-1}) in Jiangsu Province (Guo *et al.* 2016), similar to those reported in other cities of China (Hou *et al.* 2015; Qian *et al.* 2016; Xie *et al.* 2016; Zhou *et al.* 2020). However, the concentrations of SAs (0.1–35.5 mg kg^{-1}) were higher than those of TCs (ND–0.5 mg kg^{-1}) in manure collected from Turkey (Karcı and Balcıoğlu 2009).

The concentration of antibiotics varies greatly across soils from different origins. Our result is consistent with previous conclusions; i.e. TCs were commonly examined as a class of predominant antibiotic in the livestock and poultry manure with a broad concentration range from ND to hundreds of ppm. After the application of these polluted manures to the field, the soil would act as an antibiotics pool. Residual TCs and SAs in agricultural soils have been detected worldwide. Hu *et al.* (2010) reported that the OTC was the predominant antibiotic in amended soil with concentrations of 124–2683 μg kg^{-1}, followed by CTC (1–1029 μg kg^{-1}), TC (0.1–105 μg kg^{-1}), and SMZ (0.03–0.9 μg kg^{-1}). An investigation in the vegetable bases in Beijing revealed that TCs and SAs in soils were detected with concentrations of 6.1–430 μg kg^{-1} and ND–13 μg kg^{-1}, respectively

Table 4. The relationships between antibiotics and soil properties.

	OTC	CTC	DC	SM	SMZ	STZ	SDZ	CEC	AK	AP	SOM	TN
TC	0.93*	1.00*	0.98*	0.88*	0.96*	0.99*	0.88*	0.57	0.10	0.84*	0.58	0.59
OTC		0.90	0.97*	0.99*	0.99*	0.95*	0.99*	0.83*	0.44	0.98*	0.84*	0.85*
CTC			0.98*	0.84*	0.94*	0.98*	0.85*	0.52	0.05	0.80*	0.53	0.54
DC				0.92*	0.98*	0.99*	0.93*	0.67	0.26	0.89*	0.69	0.70
SM					0.98*	0.92*	1.00*	0.90*	0.50	0.99*	0.89*	0.90*
SMZ						0.98*	0.98*	0.78	0.34	0.95*	0.78*	0.79
STZ							0.92*	0.64	0.16	0.88*	0.65	0.65
SDZ								0.89*	0.49	0.99*	0.89*	0.89*
CEC									0.77*	0.92*	0.99*	0.99*
AK										0.52	0.82*	0.81*
AP											0.91*	0.92*
SOM												1.00*

*Significant at $P < 0.05$.

(Li *et al.* 2015). In addition, the occurrence of several typical antibiotics was described, including OTC (ND–1620 µg kg^{-1}), TC (ND–275 µg kg^{-1}), DC (ND–814 µg kg^{-1}), SMZ (ND–0.98 µg kg^{-1}), SDZ (ND–1.21 µg kg^{-1}), and SDM (ND–35.4 µg kg^{-1}) from soils fertilised by manure on a national scale (Zeng *et al.* 2019). Apparently, the concentration of TCs tended to be higher than that of SAs. The same tendency was confirmed by other researchers (Wu *et al.* 2014; Zhang *et al.* 2015; Huang *et al.* 2016; Qian *et al.* 2016; Xie *et al.* 2016; Sun *et al.* 2017; Wei *et al.* 2019). In contrast, similar concentrations were observed in vegetable soils of the Pearl River Delta, including OTC (ND–80 µg kg^{-1}), TC (ND–74 µg kg^{-1}), CTC (ND–105 µg kg^{-1}), SMZ (ND–74 µg kg^{-1}), SDZ (ND–86 µg kg^{-1}), SM (ND–120 µg kg^{-1}), SMX (ND–55 µg kg^{-1}), and SDM (ND–40 µg kg^{-1}) (Li *et al.* 2011) in the wastewater irrigation fields in Beijing and Tianjin, including SDZ (ND–97 µg kg^{-1}), SMX (ND–90 µg kg^{-1}), OTC (ND–112 µg kg^{-1}), and CTC (ND–5.2 µg kg^{-1}) (Chen *et al.* 2014). However, in a paddy soil adjacent to a composting facility in Korea, the concentration of TCs (4.07–7.02 µg kg^{-1}) was lower than that of SAs (24.4–38 µg kg^{-1}) (Ok *et al.* 2011). Ji *et al.* (2012) also reported that the highest level of antibiotic existed in SAs (5.85–33.4 µg kg^{-1}) rather than TCs (4.54–24.7 µg kg^{-1}) in soils near a poultry farm in Shanghai. Moreover, the residual concentrations of eight selected antibiotics in the study area above were as low as 0.1% of those in cattle manure, which could result from leaching, photodegradation, biodegradation and uptake by vegetables (Blackwell *et al.* 2009; Hu *et al.* 2010).

The occurrence and fate of antibiotics in soil varied with compounds. There was a variation between concentrations of TAs and SAs in present field experiments, which depended on types and dosages of the antibiotics, as well as their molecular structures and physico-chemical properties,

such as water solubility, volatility, and adsorption capacity (Hu *et al.* 2010; Albero *et al.* 2018). Although eight antibiotics were at low levels, they were still likely to persist for several months to years in soils (Jechalke *et al.* 2014). Thus the potential risk of the emergence and spread of the bacterial resistance via vegetables must be taken into consideration.

Occurrence of typical VAs in different treatments

In this study, compared with CK treatment, concentrations of soil antibiotics and nutrients were slightly increased in TR1, suggesting that no significant accumulation of antibiotics occurred in 30 years of chemical fertiliser application. Moreover, compared to the combined application in TR3, AP significantly increased under the application of exclusive manure fertiliser in TR2; in contrast, SOM and TN decreased. Our results indicate that the effect of combined application on soil nutrients was better than that of pure manure additive. Although the application amount of manure in TR3 was half of TR2, the content of antibiotics was more than half that of TR2. This might be explained by the change of soil properties under different fertilisation patterns, which might further affect the adsorption and degradation of antibiotics. Soil properties have been reported to play an essential role in the occurrence and metabolic fate of antibiotics (Tasho and Cho 2016). In this study, AP was the primary property that affected residues of VAs in all soil samples. OTC, SM, and SDZ showed significant positive correlations with AP, SOM, CEC, and TN (Table 4) suggesting that these properties might be the key factors, which caused an excess of accumulation of OTC, SM and SDZ in TR3. Previous studies also reported that pH had a negative influence on the adsorption of antibiotics, whereas SOM and CEC were positively

associated with the adsorption of antibiotics (Thiele-Bruhn 2003; Jones *et al.* 2005; Zhang *et al.* 2010; Leal *et al.* 2013), which was consistent with our results. Further, residues of VAs might be affected by their physico-chemical properties and management system (Ho *et al.* 2014; Tasho and Cho 2016). Yin *et al.* (2012) suggested that the content of soil antibiotics was sensitive to environmental and anthropogenic factors. Sorption has been considered as a major process determining the metabolic fate of soil antibiotics. Previous studies had revealed the adsorption of SAs (ter Laak *et al.* 2006; Leal *et al.* 2013; Wang *et al.* 2015) and TCs in soils (Sassman and Lee 2005; Zhang *et al.* 2010; Fernández-Calviño *et al.* 2015), while the adsorption capacity of TCs was stronger than that of SAs in manure and soil (Kong *et al.* 2012; Pan and Chu 2016; Tasho and Cho 2016). SAs had the lower K_d values than TCs, indicating that SAs had stronger water solubility, and thus had easier migration from manure or soil into groundwater and surface water (Doretto *et al.* 2014). Hence, numerous studies suggested that SAs occurred in soil–manure system at low concentrations. It also confirmed that TCs had much stronger adsorption to the soil by exploring the leaching behaviour of antibiotics (Blackwell *et al.* 2009). Besides, TCs had multiple polar and ionisable functional groups, which might involve strong absorption of soil particles through cation exchange and complexation (Pan and Chu 2016). The other explanation was that SAs were more easily hydrolysed and degradable by soil microorganisms than TCs (Thiele-Bruhn 2003). Thus, TCs could be more easily accumulated in soils and should be paid more attention.

Water management of fields, such as drying–rewetting cycles, has shown a great effect on the persistence of antibiotics (Jechalke *et al.* 2014; Albero *et al.* 2018). Previous studies suggested that the antibiotic dissipation rate under soil with frequent drying–rewetting cycles was lower than that under continuous irrigation (Jechalke *et al.* 2014). Frequent drying–rewetting cycles might cause a higher accessibility of polar sorption sites of SOM. Drying–rewetting cycles could increase AP as well, particularly in manure-fertilised soil (Sun *et al.* 2018). According to our results, it might further disturb the occurrence of antibiotics. For decades, many studies claimed that the combined, but not exclusive, fertiliser application could significantly promote soil nutrients (Hartmann *et al.* 2015; Francioli *et al.* 2016). Our results showed that the combined application of organic–inorganic fertiliser could dramatically improve soil properties. Long-term usage of organic manure became non-point source pollution and lead to the accumulation of antibiotics in soil. Such pollution also enriched bacterial resistance and accelerated greenhouse effect. The accumulation of antibiotics in arable soils would make irreversible changes to soil physico-chemical properties and micro-ecological function. As a non-renewable resource, the potential risk of leaching, absorbtion by vegetables, and horizontal resistant gene transfer in arable soil should be paid much more attention. The findings in this study shed light on a better understanding of the occurrence of VAs in arable soil, and would help to establish more regulations and strategies to reduce the improper use of antibiotics from different sources.

Conclusions

In this study, the occurrence of eight typical VAs was detected in manure and soil samples from a 30-year field experiment with four types of fertilisation modes. TCs and SAs were ubiquitously detected in soil samples with concentrations of ND–6.95 µg kg^{-1} and ND–3.85 µg kg^{-1}, respectively, which were even 2-fold to 3-fold less than those of other agricultural soils. Total concentrations of antibiotics in agricultural soils were significantly lower than those in cattle manure (5.13–1628 µg kg^{-1}). In addition, the long-term fertilisation generally increased soil properties and levels of VAs. The combined application of organic–inorganic fertiliser could significantly improve concentrations of soil nutrients compared with the exclusive application, whereas it reduced the residual VAs from manure fertiliser into soil. Furthermore, concentrations of antibiotics had a positive correlation with soil properties, including available phosphorus, CEC, SOM and total N. This study suggested that fertilisation modes had significant effects on the occurrence of antibiotics in arable soils. Hence, the combined application of organic and inorganic fertiliser was an effective fertilisation mode to improve the soil fertility and control antibiotics contamination.

References

Albero B, Tadeo JL, Escario M, *et al.* (2018) Persistence and availability of veterinary antibiotics in soil and soil-manure systems. *Science of The Total Environment* **643**, 1562–1570. doi:10.1016/j.scitotenv.2018.06.314

Blackwell PA, Kay P, Ashauer R, *et al.* (2009) Effects of agricultural conditions on the leaching behaviour of veterinary antibiotics in soils. *Chemosphere* **75**, 13–19. doi:10.1016/j.chemosphere.2008.11.070

Cai Y, Yu J, Du Z (2020) Analysis on the treatment of livestock and poultry manure and its influencing factors in family farm and combination of planting and breeding farm. *Ecological Economics* **36**, 178–185.

Chen C, Li J, Chen P, *et al.* (2014) Occurrence of antibiotics and antibiotic resistances in soils from wastewater irrigation areas in Beijing and Tianjin, China. *Environmental Pollution* **193**, 94–101. doi:10.1016/j.envpol.2014.06.005

Chen X, Wu L, Xie X (2018) Assessing the linkages between knowledge and use of veterinary antibiotics by pig farmers in rural China. *International Journal of Environmental Research and Public Health* **15**, 1126. doi:10.3390/ijerph15061126

Doretto KM, Peruchi LM, Rath S (2014) Sorption and desorption of sulfadimethoxine, sulfaquinoxaline and sulfamethazine antimicrobials in Brazilian soils. *Science of The Total Environment* **476-477**, 406–414. doi:10.1016/j.scitotenv.2014.01.024

Fernández-Calviño D, Bermúdez-Couso A, Arias-Estévez M, *et al.* (2015) Kinetics of tetracycline, oxytetracycline, and chlortetracycline adsorption and desorption on two acid soils. *Environmental Science and Pollution Research* **22**, 425–433. doi:10.1007/s11356-014-3367-9

Francioli D, Schulz E, Lentendu G, *et al.* (2016) Mineral vs. organic amendments: microbial community structure, activity and abundance of agriculturally relevant microbes are driven by long-term

fertilization strategies. *Frontiers in Microbiology* **7**, 12–25. doi:10.3389/fmicb.2016.01446

Gros M, Mas-Pla J, Boy-Roura M, *et al.* (2019) Veterinary pharmaceuticals and antibiotics in manure and slurry and their fate in amended agricultural soils: findings from an experimental field site (Baix Empordà, NE Catalonia). *Science of The Total Environment* **654**, 1337–1349. doi:10.1016/j.scitotenv.2018.11.061

Guo XY, Hao LJ, Qiu PZ, *et al.* (2016) Pollution characteristics of 23 veterinary antibiotics in livestock manure and manure-amended soils in Jiangsu province, China. *Journal of Environmental Science and Health, Part B* **51**, 383–392. doi:10.1080/03601234.2016.1142743

Hamscher G, Sczesny S, Höper H, *et al.* (2002) Determination of persistent tetracycline residues in soil fertilized with liquid manure by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. *Analytical Chemistry* **74**, 1509–1518. doi:10.1021/ac015588m

Hartmann TE, Yue SC, Schulz R, *et al.* (2015) Yield and N use efficiency of a maize–wheat cropping system as affected by different fertilizer management strategies in a farmer's field of the North China Plain. *Field Crops Research* **174**, 30–39. doi:10.1016/j.fcr.2015.01.006

He S, Dong D, Zhang X, *et al.* (2018) Occurrence and ecological risk assessment of 22 emerging contaminants in the Jilin Songhua River (Northeast China). *Environmental Science and Pollution Research* **25**, 24003–24012. doi:10.1007/s11356-018-2459-3

Ho YB, Zakaria MP, Latif PA, *et al.* (2014) Occurrence of veterinary antibiotics and progesterone in broiler manure and agricultural soil in Malaysia. *Science of The Total Environment* **488-489**, 261–267. doi:10.1016/j.scitotenv.2014.04.109

Hou J, Wan W, Mao D, *et al.* (2015) Occurrence and distribution of sulfonamides, tetracyclines, quinolones, macrolides, and nitrofurans in livestock manure and amended soils of Northern China. *Environmental Science and Pollution Research* **22**, 4545–4554. doi:10.1007/s11356-014-3632-y

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**, 2992–2998. doi:10.1016/j.envpol.2010.05.023

Huang B, Wang B, Ren D, *et al.* (2013) Occurrence, removal and bioaccumulation of steroid estrogens in Dianchi Lake catchment, China. *Environmental International* **59**, 262–273. doi:10.1016/j.envint.2013.06.018

Huang H, Chen Y, Zheng X, *et al.* (2016) Distribution of tetracycline resistance genes in anaerobic treatment of waste sludge: the role of pH in regulating tetracycline resistant bacteria and horizontal gene transfer. *Bioresource Technology* **218**, 1284–1289. doi:10.1016/j.biortech.2016.07.097

Jechalke S, Heuer H, Siemens J, *et al.* (2014) Fate and effects of veterinary antibiotics in soil. *Trends in Microbiology* **22**, 536–545. doi:10.1016/j.tim.2014.05.005

Ji X, Shen Q, Liu F, *et al.* (2012) Antibiotic resistance gene abundances associated with antibiotics and heavy metals in animal manures and agricultural soils adjacent to feedlots in Shanghai; China. *Journal of Hazardous Materials* **235-236**, 178–185. doi:10.1016/j.jhazmat.2012.07.040

Jin C, Chen Q, Sun R, *et al.* (2009) Eco-toxic effects of sulfadiazine sodium, sulfamonomethoxine sodium and enrofloxacin on wheat, Chinese cabbage and tomato. *Ecotoxicology* **18**, 878–885. doi:10.1007/s10646-009-0349-7

Jones AD, Bruland GL, Agrawal SG, *et al.* (2005) Factors influencing the sorption of oxytetracycline to soils. *Environmental Toxicology and Chemistry* **24**, 761–770. doi:10.1897/04-037R.1

Karcı A, Balcıoğlu IA (2009) Investigation of the tetracycline, sulfonamide, and fluoroquinolone antimicrobial compounds in animal manure and agricultural soils in Turkey. *Science of The Total Environment* **407**, 4652–4664. doi:10.1016/j.scitotenv.2009.04.047

Kong W, Li C, Dolhi JM, *et al.* (2012) Characteristics of oxytetracycline sorption and potential bioavailability in soils with various physical–chemical properties. *Chemosphere* **87**, 542–548. doi:10.1016/j.chemosphere.2011.12.062

Kumar RR, Lee JT, Cho JY (2012) Fate, occurrence, and toxicity of veterinary antibiotics in environment. *Journal of the Korean Society for Applied Biological Chemistry* **55**, 701–709. doi:10.1007/s13765-012-2220-4

Kuppusamy S, Kakarla D, Venkateswarlu K, *et al.* (2018) Veterinary antibiotics (VAs) contamination as a global agro-ecological issue: a critical view. *Agriculture, Ecosystems & Environment* **257**, 47–59. doi:10.1016/j.agee.2018.01.026

Leal RMP, Alleoni LRF, Tornisielo VL, *et al.* (2013) Sorption of fluoroquinolones and sulfonamides in 13 Brazilian soils. *Chemosphere* **92**, 979–985. doi:10.1016/j.chemosphere.2013.03.018

Li Y-W, Wu X-L, Mo C-H, *et al.* (2011) Investigation of sulfonamide, tetracycline, and quinolone antibiotics in vegetable farmland soil in the Pearl River Delta area, southern China. *Journal of Agricultural and Food Chemistry* **59**, 7268–7276. doi:10.1021/jf1047578

Li C, Chen J, Wang J, *et al.* (2015) Occurrence of antibiotics in soils and manures from greenhouse vegetable production bases of Beijing, China and an associated risk assessment. *Science of The Total Environment* **521-522**, 101–107. doi:10.1016/j.scitotenv.2015.03.070

Liu X, Lu S, Guo W, *et al.* (2018) Antibiotics in the aquatic environments: a review of lakes, China. *Science of The Total Environment* **627**, 1195–1208. doi:10.1016/j.scitotenv.2018.01.271

Ma Y, Li M, Wu M, *et al.* (2015) Occurrences and regional distributions of 20 antibiotics in water bodies during groundwater recharge. *Science of The Total Environment* **518-519**, 498–506. doi:10.1016/j.scitotenv.2015.02.100

Martínez JL (2008) Antibiotics and antibiotic resistance genes in natural environments. *Science* **321**, 365–367. doi:10.1126/science.1159483

Martínez-Carballo E, González-Barreiro C, Scharf S, *et al.* (2007) Environmental monitoring study of selected veterinary antibiotics in animal manure and soils in Austria. *Environmental Pollution* **148**, 570–579. doi:10.1016/j.envpol.2006.11.035

Ok YS, Kim S-C, Kim K-R, *et al.* (2011) Monitoring of selected veterinary antibiotics in environmental compartments near a composting facility in Gangwon Province, Korea. *Environmental Monitoring and Assessment* **174**, 693–701. doi:10.1007/s10661-010-1625-y

Pan M, Chu LM (2016) Adsorption and degradation of five selected antibiotics in agricultural soil. *Science of The Total Environment* **545-546**, 48–56. doi:10.1016/j.scitotenv.2015.12.040

Pan M, Chu LM (2018) Occurrence of antibiotics and antibiotic resistance genes in soils from wastewater irrigation areas in the Pearl River Delta region, southern China. *Science of The Total Environment* **624**, 145–152. doi:10.1016/j.scitotenv.2017.12.008

Qian M, Wu H, Wang J, *et al.* (2016) Occurrence of trace elements and antibiotics in manure-based fertilizers from the Zhejiang Province of China. *Science of The Total Environment* **559**, 174–181. doi:10.1016/j.scitotenv.2016.03.123

Qiao M, Ying G-G, Singer AC, *et al.* (2018) Review of antibiotic resistance in China and its environment. *Environment International* **110**, 160–172. doi:10.1016/j.envint.2017.10.016

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**, 725–759. doi:10.1016/j.chemosphere.2006.03.026

Sassman SA, Lee LS (2005) Sorption of three tetracyclines by several soils: assessing the role of pH and cation exchange. *Environmental Science & Technology* **39**, 7452–7459. doi:10.1021/es0480217

Sun J, Zeng Q, Tsang DCW, *et al.* (2017) Antibiotics in the agricultural soils from the Yangtze River Delta, China. *Chemosphere* **189**, 301–308. doi:10.1016/j.chemosphere.2017.09.040

Sun D, Bi Q, Li K, *et al.* (2018) Significance of temperature and water availability for soil phosphorus transformation and microbial community composition as affected by fertilizer sources. *Biology and Fertility of Soils* **54**, 229–241. doi:10.1007/s00374-017-1252-7

Tang X, Lou C, Wang S, *et al.* (2015) Effects of long-term manure applications on the occurrence of antibiotics and antibiotic resistance genes (ARGs) in paddy soils: evidence from four field experiments in south of China. *Soil Biology and Biochemistry* **90**, 179–187. doi:10.1016/j.soilbio.2015.07.027

Tasho RP, Cho JY (2016) Veterinary antibiotics in animal waste, its distribution in soil and uptake by plants: a review. *Science of The Total Environment* **563-564**, 366–376. doi:10.1016/j.scitotenv.2016.04.140

ter Laak TL, Gebbink WA, Tolls J (2006) The effect of pH and ionic strength on the sorption of sulfachloropyridazine, tylosin, and

oxytetracycline to soil. *Environmental Toxicology and Chemistry* **25**, 904–911. doi:10.1897/05-232R.1

Thiele-Bruhn S (2003) Pharmaceutical antibiotic compounds in soils – a review. *Journal of Plant Nutrition and Soil Science* **166**, 145–167. doi:10.1002/jpln.200390023

Wang N, Guo X, Xu J, *et al.* (2015) Sorption and transport of five sulfonamide antibiotics in agricultural soil and soil-manure systems. *Journal of Environmental Science and Health, Part B* **50**, 23–33. doi:10.1080/03601234.2015.965612

Wei R, Ge F, Zhang L, *et al.* (2016) Occurrence of 13 veterinary drugs in animal manure-amended soils in Eastern China. *Chemosphere* **144**, 2377–2383. doi:10.1016/j.chemosphere.2015.10.126

Wei R, He T, Zhang S, *et al.* (2019) Occurrence of seventeen veterinary antibiotics and resistant bacterias in manure-fertilized vegetable farm soil in four provinces of China. *Chemosphere* **215**, 234–240. doi:10.1016/j.chemosphere.2018.09.152

WHO (2014) Antimicrobial resistance: global report on surveillance. Available at http://www.who.int/drugresistance/documents/surveillancereport/en/

Wu X-L, Xiang L, Yan Q-Y, *et al.* (2014) Distribution and risk assessment of quinolone antibiotics in the soils from organic vegetable farms of a subtropical city, Southern China. *Science of The Total Environment* **487**, 399–406. doi:10.1016/j.scitotenv.2014.04.015

Wu M-H, Que C-J, Xu G, *et al.* (2016) Occurrence, fate and interrelation of selected antibiotics in sewage treatment plants and their receiving surface water. *Ecotoxicology and Environmental Safety* **132**, 132–139. doi:10.1016/j.ecoenv.2016.06.006

Xiang L, Wu X-L, Jiang Y-N, *et al.* (2016) Occurrence and risk assessment of tetracycline antibiotics in soil from organic vegetable farms in a subtropical city, south China. *Environmental Science and Pollution Research* **23**, 13984–13995. doi:10.1007/s11356-016-6493-8

Xie W-Y, Yang X-P, Li Q, *et al.* (2016) Changes in antibiotic concentrations and antibiotic resistome during commercial composting of animal manures. *Environmental Pollution* **219**, 182–190. doi:10.1016/j.envpol.2016.10.044

Yin LM, Edwards MA, Li J, *et al.* (2012) Roles of hydrophobicity and charge distribution of cationic antimicrobial peptides in peptide-membrane interactions. *Journal of Biological Chemistry* **287**, 7738–7745. doi:10.1074/jbc.M111.303602

Zeng Q, Sun J, Zhu L (2019) Occurrence and distribution of antibiotics and resistance genes in greenhouse and open-field agricultural soils in China. *Chemosphere* **224**, 900–909. doi:10.1016/j.chemosphere.2019.02.167

Zhang G, Liu X, Sun K, *et al.* (2010) Sorption of tetracycline to sediments and soils: assessing the roles of pH, the presence of cadmium and properties of sediments and soils. *Frontiers of Environmental Science & Engineering in China* **4**, 421–429. doi:10.1007/s11783-010-0265-3

Zhang Q-Q, Ying G-G, Pan C-G, *et al.* (2015) Comprehensive evaluation of antibiotics emission and fate in the river basins of China: source analysis, multimedia modeling, and linkage to bacterial resistance. *Environmental Science & Technology* **49**, 6772–6782. doi:10.1021/acs.est.5b00729

Zhang H, Zhou Y, Huang Y, *et al.* (2016) Residues and risks of veterinary antibiotics in protected vegetable soils following application of different manures. *Chemosphere* **152**, 229–237. doi:10.1016/j.chemosphere.2016.02.111

Zhao L, Dong YH, Wang H (2010) Residues of veterinary antibiotics in manures from feedlot livestock in eight provinces of China. *Science of The Total Environment* **408**, 1069–1075. doi:10.1016/j.scitotenv.2009.11.014

Zhou L-J, Ying G-G, Liu S, *et al.* (2013) Excretion masses and environmental occurrence of antibiotics in typical swine and dairy cattle farms in China. *Science of the Total Environment* **444**, 183–195. doi:10.1016/j.scitotenv.2012.11.087

Zhou X, Wang J, Lu C, *et al.* (2020) Antibiotics in animal manure and manure-based fertilizers: Occurrence and ecological risk assessment. *Chemosphere* **225**, 127006. doi:10.1016/j.chemosphere.2020.127006

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