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Residues and risks of veterinary antibiotics in protected vegetable soils following application of different manures

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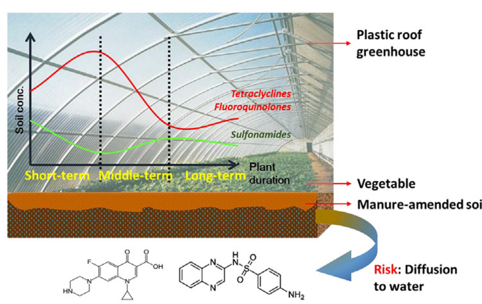
HIGHLIGHTS

- Intensive land manure application elevated antibiotics contamination in soil.
- Short-term planting affected tetracyclines and fluoroquinolones accumulation mostly.
- Manure sources impact levels and types of residual antibiotics in the farmland soils.
- Organic farming has less antibiotics residue than conventional greenhouse farming.
- Ciprofloxacin and sulfachinoxalin have higher migration risk than other antibiotics.

GRAPHICAL ABSTRACT



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ABSTRACT

The protected vegetable farming is a style of high frequent rotation farming which requires a huge amount of fertilizers to maintain soil fertility. A total of 125 surface soils covering from east to west of China were sampled for the analysis of 17 antibiotics in order to identify antibiotics contamination caused by long-term manures application. The results indicate that the agricultural land has accumulated a statistically significantly higher antibiotics concentration than conventional open croplands. The maximum oxytetracycline concentration was 8400 μg kg^{-1}, the highest level that has ever been reported for oxytetracycline in soils. The residual concentration is decided by both plant duration and manure type. Short-term (<5 years) planting shows the highest residues of tetracyclines and fluoroquinolones in the soils. The organic farming characteristic of applying commercial compost as a single fertilizer in planting shows the lowest antibiotics residue in the soils on the whole. Principal component analysis suggests that the various combinations of antibiotic compounds in the soil may be used to trace the manure source. The antibiotics in soil may threaten water quality through contamination by diffusion.

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Ciprofloxacin and sulfachinoxalin are calculated to be a higher migration risk to surface waters, hence their environmental fate requires further study.

1. Introduction

Manures are commonly applied to agricultural land in China to recycle their plant nutrients. The annual loading of manures is estimated to be up to 150 t ha^{-1} in protected vegetable farming with characteristically high frequency of vegetable rotation even in low temperature areas and this represents ten times the amount applied to crops in open fields (Qin et al., 2002). Therefore, the residue of veterinary antibiotics in soil might occur after long-term application of manure in farmland since most of the manure has been observed to be contaminated with antibiotics. We have found that all the 17 veterinary antibiotics analyzed were detected in 50 manure and compost samples from 8 Chinese provinces, and the oxytetracycline concentration was up to 417 mg kg^{-1} in a chicken manure sample (Zhang et al., 2015). Ubiquitous residues of fluoroquinolones, sulfonamides and tetracyclines in animal faeces have also been reported in several other studies (Zhao et al., 2010; Pan et al., 2011; Chen et al., 2012; Li et al., 2013b).

The veterinary antibiotics may enter soils through land-application of manures in the farmland and further release into water by runoff or leaching. The veterinary antibiotics contamination in farmland soil has been concerned of worldwide (Kumar et al., 2005; Hu et al., 2010; Li et al., 2011; Hou et al., 2015; Wei et al., 2016). However, the fate of antibiotics in soil varied with compounds. Sulfonamide antibiotics do not sorb strongly to soil and thus have been detected frequently in surface water, ground water, soil pore water (Wegst-Uhrich et al., 2014). While another antibiotics such as tetracyclines or fluoroquinolones, may persist for several months to years in soil (Jechalke et al., 2014). Meanwhile, antibiotics may accumulate in soil over time when the input rates exceed dissipation rates. A study on the sulfamethoxazole and ciprofloxacin contamination in Mexican soils demonstrated that these two chemicals could accumulate in the soils as a sequestered form over a period of 20 years during long-term irrigation with untreated wastewater (Dalkmann et al., 2012). The veterinary antibiotics may also transfer from soil to crops and posed a potential health hazardous to human being (Kumar et al., 2005; Hu et al., 2010). Soil contamination of antibiotics has also contributed to the spread of antibiotic resistant genes (ARGs) in the environment, which might result to an even more serious risk to human health (Martinez, 2008; Pruden et al., 2013; Zhu et al., 2013). A close relationship between the antibiotic use and ARGs abundance has been found for sulfonamide and tetracycline of the pig farms and cattle waste lagoons both in China (Zhu et al., 2013) and the United States (McKinney et al., 2010). An increase abundance of tetracycline resistance has been found in the arable soil with long-term application of fresh manure and compost, meanwhile, the dominant tetG genotypes shared strong homology with pathogenic bacteria (Peng et al., 2015).

Therefore, it was assumed that soil contamination by veterinary antibiotics in the protection vegetable farms became more and more seriously with the development of this intensive farming type. It has been estimated that the total cropping area of protected vegetables in China had reached 4.67 million ha by the end of 2010, double the area in 2004 (Yu, 2011). Hence, antibiotics contamination in such high frequency vegetable rotation farming systems has increasingly been of concern in recent years (Li et al., 2013a; Fang et al., 2014; Hou et al., 2015; Ur Rehman et al., 2015; Zhang et al., 2015; Wei et al., 2016), and many of these studies have focused on soil contamination by the antibiotics. In this study, soil samples with varied manure applications and vegetable planting durations were collected to investigate the veterinary antibiotics residue in the soils of intensive vegetable land, and to evaluate the potential environmental risk based on the current residual level. The data could provide a new insight on the antibiotics contamination in the Chinese intensive vegetable farming system and its relationship with the management of manure application.

2. Materials and methods

2.1. Soil sampling

A total of 125 soil surface (0–20 cm) samples were collected from the protected vegetable farm lands situated at 7 areas in Jiangsu province, Shanghai and Yunnan province, China in 2012 (Fig. s1). In addition, a total of other 39 surface soil samples (0–20 cm) were collected from the open farmland as a comparison in these areas, which included 13 samples from cereal crops lands and 26 samples from vegetable lands. The detail information of the sampling area could be found in the references (Wang et al., 2013; Yang et al., 2014). Briefly, the protected vegetable farmlands were selected based on the application of organic fertilizer, planting duration, management, and soil properties.

As shown in Table 1, the highest amount of manure application was in the protected vegetable farmland of Tongshan, Xuzhou (TS) in Jiangsu province. It has reached up to 150 t/ha yr^{-1} and the type of the applied organic fertilizer was dominated by livestock manure. The application amount in the other six sampling areas were all below 100 t/ha yr^{-1}, and the lowest amount was nearly 20 t/ha yr^{-1} in Pulangke (PLK) and Suoshi (SS), Nanjing. The planting duration also varied among the regions, spanning four to 30 years (Table 1). The vegetable farms in Tongshan (TS), Xuzhou have the longest duration of planting while the farms in Hushu (HS), Nanjing, have the shortest duration. Differences in field management correspond largely to the application of organic fertilizers or manures. Two types of management have been identified based on field investigations. One is conventional management such as TS and GL (Guli in Nanjing), HS, SS, and JN (Jinning in Kunming) and the other is an organic farming system such as PLK and QP (Qingpu in Shanghai). The former is typical of the combined use of manures and inorganic compound fertilizers during vegetable planting, and farmyard manures are the primary organic nutrient sources. The latter is characteristic of single using well manufactured organic compost (commercial compost) in planting (Zhang et al., 2015).

The soils are mainly comprised of two soil types based on FAO soil classification: Fluvic Cambisols and Stagnic Anthrosols (IUSS Working Group WRB, 2015). The soils of TS in Xuzhou, QP in Shanghai and JN in Kunming are all comprised of Fluvic Cambisols but developed from different parent materials. The TS soil and QP soil are developed from alluvial material of the Yellow River and the Yangtze River, respectively (Gong, 2003). The soil texture is characteristic of sandy loam to silt loam. While the JN soil is developed from local loamy alluvium. Soils of the four sampling areas in

Table 1
Application of organic amendments and management of the protected vegetable areas at the sites investigated.

Study area	Organic amendments		Planting duration[a] (yr)	Management[b]
	Types	Loading (t/ha yr^{-1})		
Tongshan, Xuzhou (TS)	Livestock manures	~150	20–30	Conventional farm
Guli, Nanjing (GL)	Mixed compost	~25	8–12	Conventional farm
Husu, Nanjing (HS)	Chicken manure	~35	4–5	Conventional farm
Pulangke, Nanjing (PLK)	Commercial compost	~20	>10	Organic farm
Suoshi, Nanjing (SS)	Chicken manure	~20	>10	Conventional farm
Qingpu, Shanghai (QP)	Commercial compost	~45	5	Organic farm
Jinning, Kunming (JN)	Cattle manures	~80	12–15	Conventional farm

[a] The planting duration is an approximate value based on farmer interview and the data represent planting duration for most of the farmlands in this area.
[b] The differences in management were mainly reflected by the application of fertilizers or manures. The conventional farms usually apply both organic materials and inorganic compound fertilizers for planting, while the organic materials contained mainly farmyard manures and composite with unsophisticated farm composts. In contrast, organic farming usually refers to farming types with careful management and application of well manufactured organic composts (Zhang et al., 2015).

Nanjing are all comprised of Stagnic Anthrosols developed from loess parent material with clay loam texture.

Each sample was placed in a plastic container, chilled immediately to near freezing, transported to the laboratory and stored at −20 °C. The samples were freeze-dried in a freeze drying system (Labconco, Kansas City, MO) and homogenized by sieving through a 0.15 mm mesh before extraction. Soil properties were measured based on the method of Sparks (1996). Briefly, soil pH was measured by pH meter (LP115, Mettler-Toledo, Switzerland) in a suspension with a soil:water ratio of 1:2.5. Soil organic matter (SOM) content was measured by wet oxidation using $K_2Cr_2O_7$. Total nitrogen content was determined by Kjeldahl method. Cation exchange capacity (CEC) was determined by extraction with ammonium acetate. Soil texture was described based on the classification of USDA method. Soil types were described based on Chinese Soil Taxonomy (Gong, 2003). All the soil properties are shown in Table 2.

2.2. Chemicals and standards

The 17 target antibiotics were comprised of four tetracyclines (TCs), eight sulfonamides (SAs), four fluoroquinolones (FQs) and one macrolide. The four TCs include tetracycline (TC), oxytetracycline (OTC), chlortetracycline (CTC) and doxycycline (DOC). The eight SAs include sulfadiazine (SDZ), sulfamethoxazole (SMX), sulfamethazine (SMZ), sulfamonomethoxine (SMM), sulfachinoxalin (SCX), sulfadimethoxine (SDM), sulfameter (SM) and sulfaclozine (SCZ). The four FQs include norfloxacin (NFC), ofloxacin (OFC), ciprofloxacin (CFC) and enrofloxacin (EFC). Roxithromycin (RTM) was selected as a representative of macrolide. The internal standards were used to quantification of the antibiotics concentration, which included deuterated antibiotics, tetracycline-D6, enrofloxacin-D5, sulfamethazine-D4 and sulfadimethoxine-D6. Another two deuterated antibiotics, ciprofloxacin-D8 and sulfamethoxazole-D4, and demeclocycline were selected as surrogate standards to check the recovery of each analysis. All the standards were purchased from Dr. Ehrenstorfer GmbH, Germany, except for tetracycline-D6 which was obtained from Toronto Research Chemicals Inc., Canada. Methanol and acetonitrile (HPLC grade) were purchased from Merck Company (Darmstadt, Germany), formic acid and oxalic acid and ammonium acetate were purchased from Tedia Company (Fairfield, OH) and Sigma-Aldrich (St. Louis, MO), respectively. Other analytical grade chemicals were obtained from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China).

Stock solutions of 100.0 mg L^{-1} were made by dissolving each standard in methanol and storing at −20 °C in a black volumetric flask. Working solutions and internal standard (1 mg L^{-1}) were made fresh by diluting the stock solution with methanol and storing at 4 °C in black volumetric flasks.

The extraction solvent is a mixture comprising of a EDTA–sodium phosphate buffer with acetonitrile:$Mg(NO_3)_2$–$NH_3 \cdot H_2O$ (v/v, 3:1), which was developed by previous study (Huang et al., 2013). Briefly, the Sodium phosphate buffer (SPB) was prepared by mixing 10.56 g of NaH_2PO_4 and 0.82 mL of H_3PO_4 in 1 L water; and the $Mg(NO_3)_2$–$NH_3 \cdot H_2O$ solution was prepared by mixing the 50% $Mg(NO_3)_2$ and 2.5% $NH_3 \cdot H_2O$ at 96:4 (V:V). Ultrapure water was produced by using a Milli-Q apparatus (Millipore, Bedford, MA).

2.3. Sample preparation and analysis

Aliquots (0.2 g) of soil samples were weighed into 50 mL polytetrafluoroethene (PTFE) centrifuge tubes and spiked with the surrogate standards and internal standards (100.0 μg kg^{-1}). Then 20 mL extraction solvent was added to each tube and placed in the dark overnight. Ultra-sonication was used to extract the antibiotics from the soil. Ultra-sonic extraction was conducted three times in total for 15 min each time. All the extracts were sequentially combined, purified and concentrated through an oasis hydrophilic–liphophilic balance (HLB) cartridge, followed by elution with methanol (10 mL, containing 0.1% formic acid) and the leachates were collected for analysis.

Table 2
Description of the soil properties in the protected vegetable farms of the selected areas.

Sampling area	pH	SOM (g kg^{-1})	t-N (g kg^{-1})	CEC (cmol kg^{-1})	Soil texture	Soil type[a]
Tongshan, Xuzhou (TS)	7.6 (6.6–8.1)	30.8 (17.9–108.8)	1.8 (1.3–4.2)	24.1 (12.6–37.5)	Sandy loam	Fluvic Cambisols
Guli, Nanjing (GL)	4.9 (3.9–6.4)	30.9 (26.4–40.8)	1.6 (1.1–1.9)	21.2 (15.5–24.8)	Clay loam	Stagnic Anthrosols
Husu, Nanjing (HS)	5.3 (4.1–6.6)	35.2 (27.5–63.9)	1.8 (1.4–2.5)	25.9 (22.5–29.2)	Clay loam	Stagnic Anthrosols
Pulangke, Nanjing (PLK)	6.6 (5.7–7.5)	39.0 (20.8–55.5)	2.2 (1.8–2.9)	16.3 (10.5–22.4)	Clay loam	Stagnic Anthrosols
Suoshi, Nanjing (SS)	5.4 (4.2–7.1)	26.6 (12.4–39.3)	1.8 (1.6–2.3)	17.7 (13.0–34.7)	Clay loam	Stagnic Anthrosols
Qingpu, Shanghai (QP)	6.6 (5.4–7.5)	27.0 (14.1–39.3)	1.8 (1.1–2.4)	16.0 (14.7–19.2)	Silt loam	Fluvic Cambisols
Jinning, Kunming (JN)	6.1 (5.0–7.2)	31.6 (23.3–49.3)	2.2 (1.6–2.8)	13.8 (11.0–15.8)	Loam	Fluvic Cambisols

SOM: soil organic matter; t-N: total soil nitrogen content; CEC: cation exchange capacity.
[a] Soil types are described based on the FAO soil classification (IUSS Working Group WRB, 2015).

Analysis of the antibiotics was conducted using an LC–MS/MS system consisting of a Shimadzu LC-20AD and triple quadripole mass spectrometer (API 3200, AB-Sciex, Framingham, MA). Separations were performed by gradient elution on a Kromasil C18 column (5 μm, 250 × 4.6 mm, Akzo Nobel, Sweden) at a constant temperature of 35 °C. The elution consists of water containing 0.1% formic acid (mobile phase A) and methanol (mobile phase B). The program was set as follows: 0–1 min: 15% B, 1–2 min: 15–30% B, 2–5 min: 30–40% B, 5–10 min: 40–50% B, 10–14 min: 50–70% B, 14–16 min: 70–100% B, and 100% B held for 4 min. The total flow rate was 0.5 mL min^{-1} and the sample injection volume was 10 μL. The mass spectrometer was set up in positive electrospray ionization (ESI+) mode. Nitrogen gas was used as the drying and collision gas, the electrospray settings were optimized by infusion of separate standard solutions of 1.0 mg L^{-1} into the ESI+ source at a flow rate of 10 μL min^{-1}. Details of the optimized MS operating parameters of the antibiotics are described by Huang et al. (2013). Quantification was performed by internal standard calibration.

2.4. Method validation

The recoveries were investigated by adding standard mixture into a relatively clean sample exposed by UV-light. The samples were divided into two equal aliquots (subsamples A and B). Subsample A was spiked with the standard mixture and subsample B (nonspiked) was used to determine the background concentrations of the analytes and the recovery was calculated as described in Eq. (1).

$$\text{Recovery}(\%) = (C_a - C_b) \Big/ C_{\text{spiked}} \times 100 \quad (1)$$

where C_a and C_b are the measured concentrations of the analyte in subsamples A and B, respectively, and C_{spiked} is the spiked concentration of the analyte. Here, the spiked concentrations were 50, 100 and 300 μg kg^{-1} with three replicates (n = 3) of each. The recoveries of all the target antibiotics ranged from 76.8 to 121.0% on average (Table s1). The limit of detection (LOD) and limit of quantification (LOQ) were calculated with signal to noise ratios of 3 and 10, respectively (IUPAC criterion), which ranged from 0.09 to 3.16 μg L^{-1} and 0.49 to 10.5 μg L^{-1}, respectively. Determination of the method detection limit (MDL) was based on the United States Environmental Protection Agency (USEPA) method using the variability of seven replicate samples spiked with the native standard mixtures at a level five times the estimated MDL (USEPA, 1999). In order to ensure high quality of the extraction procedure, each sample was spiked with three surrogates (demeclocycline, ciprofloxacin-D8 and sulfamethoxazole-D4) and aged overnight before extraction.

2.5. Calculation of the predicted environmental concentration for surface water

Veterinary antibiotics in soil might enter surface water by runoff or diffusion and eventually lead to water contamination and potential risks (Jechalke et al., 2014). The risk can be assessed by calculating the predicted environmental concentration for surface water ($PEC_{surface\ water}$) based on the measured antibiotics concentration in soil and the partition coefficient (K_d) of antibiotics between soil and soil pore water (EMEA, 2006). The calculation was presented as Eq. (2):

$$PEC_{surface\ water} = \frac{PEC_{pore\ water}}{DF} \quad (2)$$

where $PEC_{pore\ water}$ (μg L^{-1}) is predicted environmental concentration for soil pore water based on Eq. (3) and DF is dilution factor which is a constant and usually fixed to 3 based on the method of EMEA (2006).

$$PEC_{pore\ water} = \frac{C_{soil}}{K_d} \quad (3)$$

In Eq. (3), where C_{soil} (μg kg^{-1}) is the measured concentration of antibiotics in soil. The K_d (L kg^{-1}) was calculated by using Eq. (4) as recommended in the reference of Doretto et al. (2014).

$$K_d = K_{oc} \times f_{oc}/100 \quad (4)$$

where K_{oc} is an organic carbon normalized octanol–water partition coefficients, which is sourced from published papers (Tolls, 2001; Zhang and Dong, 2007; Doretto et al., 2014; Kong et al., 2014). The f_{oc} is the content of soil organic carbon.

2.6. Statistical analysis

Principal component analysis (PCA) was performed using SPSS 13.0 for windows. All the detected antibiotics values were included in the analysis. The principal components were considered if their Eigenvalues were >1. The Varimax with orthogonal rotation method was selected for rotation in the PCA. The principal component scores were plotted to show the relationship among the individual compounds. Linear fitting and correlation analysis were checked by Pearson correlation coefficient at a significant level of 0.05.

3. Results and discussion

3.1. Antibiotics concentration in the soils of protected vegetable farmland

Veterinary antibiotics were detected ubiquitously in the soils of protected vegetable farmland. As shown in Table 3, almost all the antibiotics (such as TCs, FQs and RTM) have a high detectable ratio over 90%. Five of the seventeen antibiotics including TC, NFC, OFC, EFC, and RTM are detected in all soil samples and hence are the most important target antibiotics in the soils. This observation is similar to the results of Li et al. (2011) who found that most FQs had a detectable ratio of 100% and TC had a detectable ratio of 97%. The investigation of Hou et al. (2015) on soils amended with livestock manure also indicated a high detectable ratio of 100% for TCs. Although the SAs have a lower detectable ratio compared with other three types of antibiotics in general, SCZ, SCX and SDM are the three SAs that had a relatively higher detectable ratio. There were detectable ratios of these three SAs of up to 100% in the soil samples of Guli (GL), Suoshi (SS) and Qingpu (QP).

Concentrations of the different antibiotics ranged widely in the soil samples from 0.1 to 8400 μg kg^{-1}. In terms of the average concentration, OTC showed the highest concentration followed by EFC and OFC. The average concentration of OTC in the TS soil was 397.6 μg kg^{-1} and the highest concentration was 8400 μg kg^{-1} (Table 2). The OTC concentration was much higher than previously reported in vegetable farmland soils in China (Hu et al., 2010; Li et al., 2011; Wei et al., 2016). When compared to the traditional open farmlands, concentration of the antibiotics in the protected vegetable land is significantly ($p < 0.05$) higher as shown in Fig. 1. This was probably attributed to the higher loading amount of organic amendments in the protected vegetable land than that in the open farmland as a result of high frequent vegetable rotation (Qin et al., 2002). Wei et al. (2016) reported that a high detectable frequency of 13 veterinary antibiotics was found in animal manure-

Table 3
Description of the occurrence and concentration of antibiotics in the soil of protected vegetable farms.

VA	TS (n = 33)		GL (n = 18)		HS (n = 17)		PLK (n = 18)		SS (n = 15)		QP (n = 11)		JN (n = 13)	
	Conc. (μg kg^{-1})	DR%	Conc. (μg kg^{-1})	DR%	Conc. (μg kg^{-1})	DR%	Conc. (μg kg^{-1})	DR%	Conc. (μg kg^{-1})	DR%	Conc. (μg kg^{-1})	DR%	Conc. (μg kg^{-1})	DR%
TC	27.4(1.3−249)	100	3.2(1.2−11.9)	100	5.4(1.0−47.6)	100	1.3(1.1−1.8)	100	2.6(1.0−22.1)	100	1.2(1.0−1.5)	90.9	2.0(0.9−2.9)	100
OTC	397.6 (1.0−8400)	100	48.8(13.3−238.5)	100	21.6(3.2−96.6)	94.1	10.7(2.3−41.1)	100	7.3(1.7−36.6)	100	13.4(2.0−47.3)	100	12.5(2.8−47.0)	100
CTC	8.3 (1.3−98.0)	93.9	9.8(1.4−101.5)	94.4	3.6(1.4−25.6)	100	2.4(1.3−4.3)	94.4	2.1(1.4−4.9)	100	1.5(1.3−1.8)	81.8	2.4(1.9−3.4)	100
DOC	27.5(1.1−256)	100	2.7(1.2−9.9)	100	5.0(0.8−46.3)	100	1.0(0.8−1.5)	100	2.5(0.8−21.5)	93.3	1.0(0.8−1.9)	100	3.0(1.1−6.7)	92.3
SDZ	0.6(1.5−20.1)	90.9	0.7(0.5−2.2)	77.8	0.7(0.5−1.5)	88.2	0.6(0.5−0.8)	94.4	0.6(0.5−0.9)	73.3	0.7(0.6−0.9)	100	ND	0
SMX	0.6(0.4−1.9)	81.8	0.7(0.4−2.9)	88.9	0.7(0.4−3.1)	100	0.6(0.4−0.9)	83.3	0.5(0.4−0.6)	80	0.5(0.4−0.6)	90.9	ND	0
SMZ	1.0(0.5−4.3)	84.8	0.9(0.6−2.4)	83.3	0.9(0.6−1.4)	88.2	0.9(0.7−1.3)	72.2	0.9(0.5−1.6)	86.7	0.8(0.7−1.0)	63.6	0.1(0.3−1.1)	76.9
SMM	0.4(0.2−2.3)	72.7	0.3(0.2−1.0)	88.9	0.5(0.2−1.1)	94.1	0.6(0.2−1.3)	94.4	0.9(0.5−1.5)	100	0.3(0.2−0.4)	63.6	ND	0
SCX	1.0(0.8−2.8)	93.9	1.2(0.8−3.0)	100	1.4 (0.8−3.0)	100	17.8(0.8−38.4)	94.4	26.5(20.3−34.1)	100	0.9(0.8−1.2)	100	ND	0
SDM	0.8(0.6−3.0)	97.0	0.6(0.6−0.9)	100	0.7(0.6−1.4)	94.1	0.9(0.6−1.3)	100	1.0(0.9−1.3)	100	0.6(0.6−0.7)	100	ND	0
SM	1.0(0.7−2.9)	57.6	0.8(0.7−1.3)	83.3	0.9(0.7−1.4)	100	2.0(0.8−3.7)	61.1	2.0(0.8−3.4)	93.3	0.8(0.7−1.0)	81.8	ND	0
SCZ	1.2(0.9−2.2)	84.8	5.0(1.6−24.0)	100	3.4(1.0−12.4)	94.1	1.6(1.1−6.5)	100	3.9(1.1−11.0)	100	3.0(1.0−10.3)	100	0.1(0.1−0.2)	84.6
NFC	15.4(1.5−102)	100	6.1(2.3−20.7)	100	4.4(1.3−9.5)	100	3.8(1.5−9.2)	100	4.9(1.0−15.3)	100	22.4(1.4−83.4)	100	4.6(1.7−14.1)	100
OFC	9.5(1.1−66.5)	100	14.8(4.9−28.1)	100	13.2(4.5−56.8)	100	6.5(1.9−16.6)	100	9.6(1.5−49.8)	100	13.7(2.7−41.5)	100	5.5(1.9−22.4)	100
CFC	15.3(0.6−136)	100	6.9(1.0−37.1)	100	21.7(0.7−145.5)	100	5.8(0.3−17.5)	100	8.4(0.5−18.9)	100	17.8(0.5−75.5)	90.9	3.4(0.8−12.0)	100
EFC	13.9(0.2−92)	100	10.7(0.4−75)	100	61.1(0.4−333.5)	100	11.7(2.3−30.2)	100	10.0(0.4−61.0)	100	8.6(0.5−34.7)	100	1.7(0.9−4.0)	100
RTM	1.3(0.1−4.8)	100	0.3(0.1−0.9)	100	0.5(0.1−3.2)	100	0.4(0.1−0.9)	100	0.3(0.1−1.4)	100	1.1(0.1−5.0)	100	1.1(0.8−1.4)	100

Concentration data are presented as mean (minimum−maximum); VA: Veterinary antibiotic; DR: Detectable ratio; n: sample number; ND: not detected.

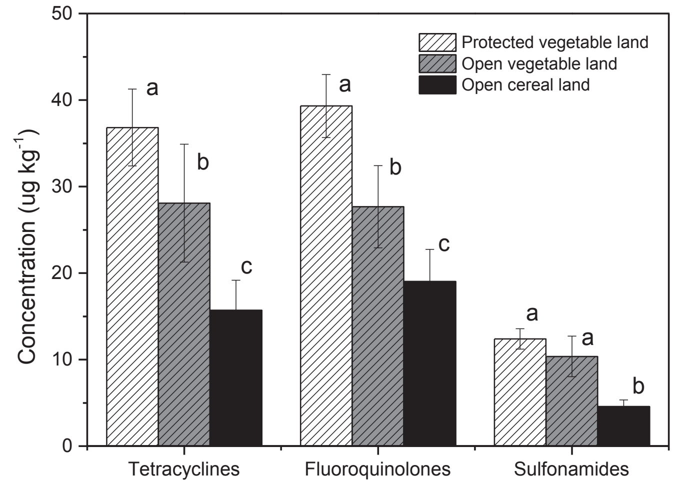

Fig. 1. Antibiotic concentrations in the soils with different cultivation models; The protected vegetable land represents a new style of high frequency of planting rotation, while the open vegetable land and open rice land are the two traditional farming patterns for vegetable and rice cultivation, respectively. Different letters in each antibiotic type indicate a significant difference at the 0.05 level.

amended soils in East China. Ur Rehman et al. (2015) reviewed the pharmaceutical contamination from highly populated developing countries and pointed out that livestock manures are a major source of veterinary drugs to the environment.

Farming management system in relation to manure application was supposed to make an important role in the residual level of antibiotics as that impacted the contamination of heavy metals (Yang et al., 2014) and phthalic acid esters (Wang et al., 2013) in the soils of green house. As shown in Tables 1 and 3, the concentrations of TCs in the soils of organic farms (PLK and QP soils) are comparatively lower than that in the conventional farms (TS, GL, HS soils) in general. However, in terms of FQs, the organic farm (QP) had the highest residual concentration. In contrast to the application of fresh manures with a large amount in the conventional protected farm, refined commercial compost is the single organic fertilizer applied in the organic farm. The difference in manures application between the two types of vegetable land might be the main explanation for the range of antibiotic residues in the soils. In a previous study (Zhang et al., 2015), we have reported that refined commercial compost had the lowest antibiotic residues as a whole and the residues of FQs in the commercial compost reached a magnitude of thousands of μg kg^{-1}. Therefore, the high persistence of FQs in the commercial compost may result in its particularly high residues in soils receiving the compost. Furthermore, the strong adsorption of FQs to soils, especially to clay minerals, might be another important mechanism leading to the high occurrence of FQs in commercial compost-amended soils (Tolls, 2001; Thiele-Bruhn, 2003). A significant ($p < 0.05$) positive correlation was obtained between FQs concentrations and soil CEC content in the soils as shown in Fig. s2,a.

3.2. Relationship between the antibiotics residue and planting duration

Planting duration of protected vegetable land also impacts the antibiotics residue in soils because it impacts the balance between continuous input and degradation of antibiotics in the farmland (Li et al., 2013a; Peng et al., 2015). Fang et al. (2014) examined a dynamic persistence of antibiotics in a manure-amended soil in which chlortetracycline (CTC) was introduced successively, and their results presented a trend of initial suppression and followed by recovery for the introduced antibiotic. This implied that the antibiotics might accumulate in the soil initially during the continuous input and then dissipate gradually.

The characteristics of antibiotics in the soils varying with planting duration seemed to justify such a hypothesis. All the samples were classified into three groups based on the planting duration of short-term (<5 years), middle-term (6–10 years) and long-term (>10 years). A significant ($p < 0.05$) difference could be observed between short-term and middle-term, long-term (Fig. 2). In the case of TCs and FQs, the average residual concentrations are much higher in the short-term planting land than that in the middle and long-term planting land. The trends are comparable to the observations of Fang et al. (2014) in their laboratory incubation experiment with CTC. However, the highest residual concentration of SAs occurred in the middle-term planting land. There might be two mechanisms to govern the fate of SAs in the soil over time. One aspect is the high water solubility of SAs, which may lead to the easy migration of SAs from soil to water and show less persistence than TCs and FQs (Jechalke et al., 2014). The other aspect is the high affinity of some SAs (such as SDZ) with soil organic matter (Fig. s2,b), which might mitigate the reduction of SAs from the soil and be persistent for a longer time (Hou et al., 2015).

3.3. Soil antibiotics relevance to the types of manures

Livestock manure and poultry manure have been found contamination with various antibiotics (Zhao et al., 2010; Zhou et al., 2013; Zhang et al., 2015). Hence, land application of manure has been recognized as a major source to the antibiotics contamination in soils (Ur Rehman et al., 2015; Wei et al., 2016). A principal component analysis (PCA) was performed to examine the relationship between the dominant antibiotics in the soils and the types of manures and composts. A total of four types of manures were contained in the PCA analysis. The samples with cattle manure application were not included because of the limited available data.

The PCA results are presented in Fig. 3. The antibiotics could be classified into various groups in each organic fertilizer amended soil. In the case of livestock manure amended soil, the three dominant components are discriminated generally based on chemical properties of the antibiotics, each component corresponding to each antibiotic type. Meanwhile, the second and third components were composed of the FQs and TCs with high concentration, respectively. Higher contents of TCs and FQs coupled with lower levels of SAs were also reported for the soils of organic vegetable systems amended with livestock manures in North China (Hu et al., 2010). Hence, both TCs and FQs are the predominant antibiotics in soil amended with livestock manures. In the case of chicken manure-amended soil, SCX as well as NFC and CFC are grouped into the first component, and another four SAs comprised the second component. SCX was identified to be a dominant SA in poultry manures in previous investigations by Zhang et al. (2015). It was present in the highest concentrations among 17 antibiotics in chicken manure-amended soils from Suoshi, Nanjing (SS) (Table 3). In the investigation conducted by Wei et al. (2016), SAs were observed at a higher residual level in soil amended with poultry manure than in soil amended with cow manure or other livestock manure. Hence, SAs are assumed to be compounds characteristic of poultry manure-contaminated soils. In the commercial compost-amended soil the dominant antibiotics in the first and second components are combined with TCs, SAs and FQs. The commercial compost is usually not made from a single type of manure, and the concentrations of most antibiotics in the compost have declined substantially during the composting process (Zhang et al., 2015). As for the mixed compost amended soil, SAs, EFC and CFC dominate in the first and second component. This is different to the other three manures amended soils. Therefore, various combinations of the antibiotics observed in the soils could be used to trace the source of antibiotics in the farmland being loaded with different manures.

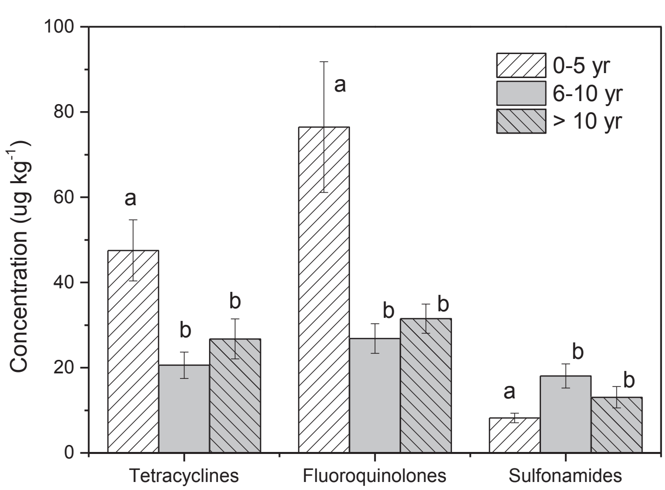

Fig. 2. Antibiotic concentrations in the soil of protected vegetable lands with different planting duration; Different letters in each antibiotic group indicate a significant difference at the 0.05 level.

3.4. Potential environmental risk to waters

Most antibiotics are water soluble although the solubility varies greatly based on their molecular structures and physic-chemical properties (Kemper, 2008). In addition to the water dissolution straightly, some antibiotics like TCs have a strong binding with dissolved organic matter (DOM) via ionic interaction and hydrogen bonds, hence are susceptible to migration from soil to water with DOM (Tolls, 2001). The antibiotics migration from soil to water has been proved by field investigation in which a diffuse contamination of surface water by antibiotics was caused through leaching from agriculture soils (Alder et al., 2001). Moreover, the soil texture in some soils of the study areas is characteristic of silt loam to loam (Table 2) and this facilitates the leaching of antibiotics from soil to groundwater. Therefore, a predicted environmental concentration in surface water ($PEC_{\text{surface water}}$) was calculated to evaluate a potential risk of the vegetable land soils contaminated by veterinary antibiotics. A threshold value (0.1 μg/L) recommended by Steering Committee of Veterinary International Committee (VICH) was used to compare with the $PEC_{\text{surface water}}$ values for risk assessment (EMEA, 2006). The results are shown in Fig. 4 based on the sampling areas.

The $PEC_{\text{surface water}}$ values for most of the soil samples are below the threshold value, suggesting the low environmental risk of current antibiotics residue in the soils. However, it should notice that the $PEC_{\text{surface water}}$ value varied greatly among the sampling areas and individual compounds. Oxytetracycline presents an environmental risk in a few samples of TS soil although it has the highest average concentration. As same as the OTC, the $PEC_{\text{surface water}}$ values of the NFC, OFC and EFC are all lower than the threshold value although their detectable ratios and concentrations are higher in these soils. Ciprofloxacin was the unique FQs which shows a potential risk in the soils of GL, HS, SS, TS and QP. In particular of the TS, HS and QP soils, the mean values of $PEC_{\text{surface water}}$ are all above the threshold, suggesting a severe risk of the ciprofloxacin to the surface water. This deserves to be concerned

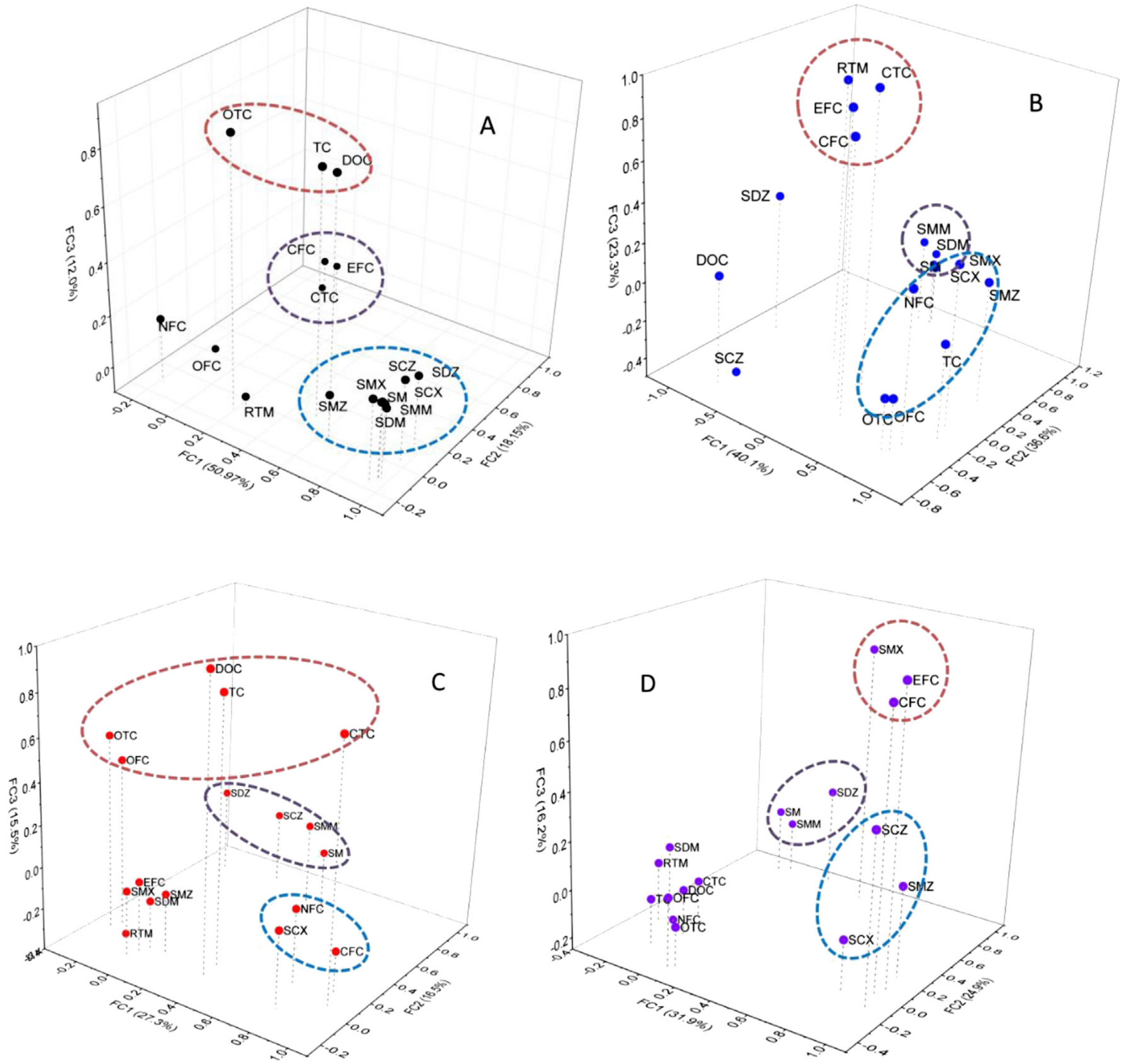

Fig. 3. PCA component plots of the soil antibiotics in the protected vegetable lands with application of different organic amendments; A: livestock manure, B: commercial compost, C: chicken manure, D: mixed compost.

about since the ciprofloxacin is the most widely prescribed FQs in the world (Pico and Andreu, 2007), and it has been recognized as a dominated antibiotics and been detected in various composts and manures (Zhang et al., 2015).

In contrast to the TCs and FQs, the SAs present a relatively higher environmental risk although most of the SAs are detected comparatively lower concentration in the soils (Fig. 4). This might be resulted from the higher water solubility and lower sorption coefficient (K_d or K_{oc}) of the SAs. The K_{oc} value is usually 2–3 magnitude lower than that of TCs and FQs (Tolls, 2001). In the case of SCX and EFC in the SS soil, although the average soil concentration of SCX is approximately twice of the EFC, the calculated average $PEC_{\text{surface water}}$ of the SCX is nearly 70 times of the EFC. Soil properties such as pH, soil organic matter and CEC have an important role in the mobility of antibiotics from soil to water in addition to the influence of chemical properties on the value of $PEC_{\text{surface water}}$. High pH and low soil organic matter content will increase the migration potential to groundwater (Wang et al., 2015). However, a significant correlation between SOM and SDZ as shown in Fig. s2 might suggest an overestimate of the risk for SDZ in soil with a high SOM content.

4. Conclusions

Veterinary antibiotics are detected ubiquitously in the soils of protected vegetable farmland, and the concentrations are significantly higher than that in the open farmlands. Oxytetracycline, enrofloxacin, ofloxacin and sulfachinoxalin are the four dominant antibiotics in the soils in terms of detectable ratio and concentration. The highest concentration of 8400 μg kg^{-1} of oxytetracycline occurred in a protected farmland soil in Tongshan of Xuzhou, Jiangsu Province. Residue of the antibiotics in the farmland soils is assumed to be impacted by management system, plant duration and soil properties etc. In terms of the management system, the soils in the organic farmland have less antibiotics residue on the whole. The planting duration affects the antibiotics residue mainly through the balance of input and dissipation, short-term planting are found the highest residual levels of TCs and FQs on average. Soil organic matter and CEC might impact the persistence of SAs and FQs in the soils, respectively. Land-application of organic amendments as a major source to antibiotics contamination in the vegetable soils could be identified by forming various combinations of characteristic compounds in the soils. Furthermore, a higher

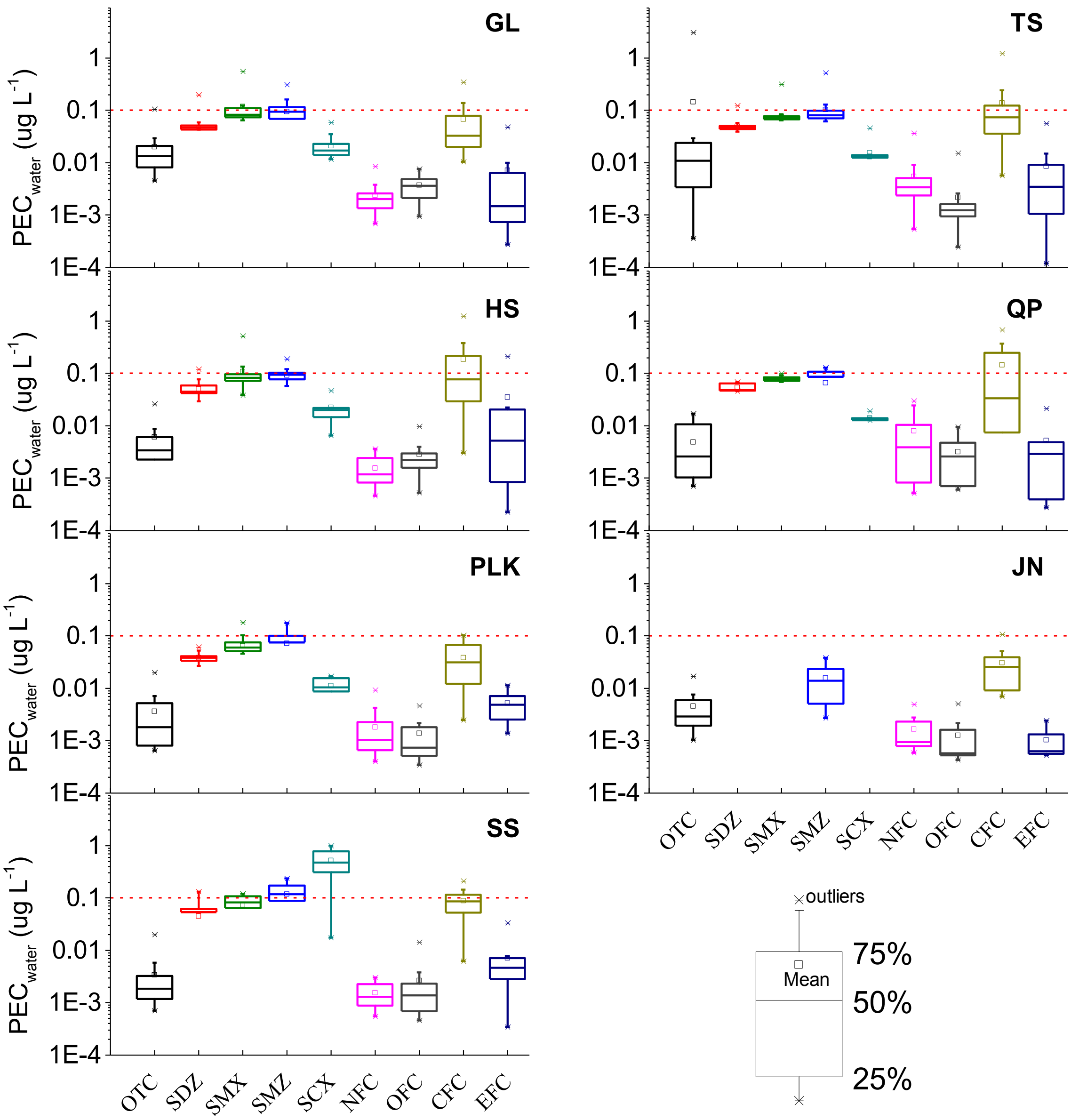

Fig. 4. Box plots indicating the predicted antibiotic concentrations in water with the soil concentration in the different sampling areas, PEC_{water} > 0.1 μg/L suggesting the potential risk of antibiotics in water.

environmental risk caused by ciprofloxacin and sulfachinoxalin should be concerned of based on the predicted environmental concentration, however, further study should be focused on the risk assessment of antibiotics in the soil impacted by different soil properties.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2016.02.111.

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