



# Occurrence and source analysis of typical veterinary antibiotics in manure, soil, vegetables and groundwater from organic vegetable bases, northern China

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*The residues of typical veterinary antibiotics from manure were detected and migrated in soil, vegetables and groundwater of organic vegetable bases.*

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## ABSTRACT

The residue of antibiotics is becoming an intractable environmental problem in many organic vegetable bases. However, their residual levels and distribution are still obscure. This work systematically analyzed the occurrence and migration of typical veterinary antibiotics in organic vegetable bases, northern China. The results showed that there was no obvious geographical difference in antibiotic distribution between soil and manure. A simple migration model can be easy and quick to predict the accumulation of antibiotics in soil. Antibiotics were mainly taken up through water transport and passive absorption in vegetables. The distribution of antibiotics in a plant was in the sequence leaf > stem > root, and performed biological accumulation. The residues of antibiotics in all samples in winter were significantly higher than those in summer. Overall, this work can lay the foundation for understanding ecological risk of antibiotics and their potential adverse effects on human health by food chain.

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## 1. Introduction

Antibiotics have been widely used to prevent/treat animal diseases and promote animal growth in livestock production. It was estimated that about 9200 tons of antibiotics were produced for farming in the United States in 2003 (A.H.I., 2005), the average usage of veterinary antibiotics has reached approximately 6000 tons annually in China (Zhao et al., 2010). Nowadays production and utilization of antibiotics are still being swelled. Antibiotics cannot be absorbed or metabolized absolutely in vivo, and 30–90% of antibiotics are excreted into the environment via manure (Heberer, 2002; Bound and Voulvoulis, 2004). Some of antibiotics are still biologically active, after they enter into the environment, even after wastewater treatment (Zhou et al., 2006). The occurrence of antibiotic resistance genes (ARGs) in various environmental compartments including sediments, soil, wastewater and drinking water was found from different countries (Knapp et al., 2010). Therefore, the antibiotic residues will lead to serious

environmental problems including ecological risk and human health damage.

In recent years, the occurrence and fate of antibiotics have been a hot-spot issue in environmental science. For example, it was reported that the concentrations of tetracycline and chlortetracycline was 4.0 and 0.1 mg/kg in manure, respectively (Hamscher et al., 2002). Antibiotics are usually accumulated with the form of organic manure in soil. The concentrations of tetracycline and chlortetracycline were up to 86.2–198.7 and 4.6–7.3 µg/kg in the soil from Germany, respectively (Hamscher et al., 2002). Furthermore, antibiotics can be taken up by various plants, crops and soil animals on farmland. There was the evidence that sulfamethoxine had bioaccumulation in a laboratory experiment (Migliore et al., 1995). Finally, antibiotics will enter groundwater because of rainfall, irrigation, and other human activities. Some antibiotics have been detected in groundwater from Germany (Hirsch et al., 1999; Hamscher et al., 2000).

The contamination of antibiotics is an urgent problem in organic vegetable bases, because of food security crises (Smukler et al., 2008; Siderer et al., 2005). Surprisingly, little is known about the fate of antibiotics in organic vegetable bases, although antibiotics were separately documented from liquid manure, soil and groundwater. For laboratory simulation, there were evident

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eco-toxic effects of antibiotics on wheat, Chinese cabbage and tomato (Jin et al., 2009). The persistence and sorption of antibiotics in soil and their potential effects on soil microorganisms were also investigated (Accinelli et al., 2007). The transportation of antibiotics to surface and ground water, and uptake by plants from manure-amended cropland, were studied in a laboratory (Dolliver et al., 2007; Kuchta and Cessna, 2009). However, these researches were laboratory simulation on ideal conditions. Field research is closest to the reality. However, the knowledge about the occurrence and source analysis of typical veterinary antibiotics in a field, especially in organic vegetable bases, is still very scarce.

The first purpose of this study is to investigate the occurrence and seasonal changes of typical antibiotics from manure, soil, vegetables and groundwater in organic vegetable bases. The second purpose is to explore the migration of antibiotics from manure to soil, from soil to vegetables and groundwater. In short, this is the first systematic analysis of antibiotic residues in organic vegetable bases.

## 2. Materials and methods

### 2.1. Reagents and sampling

Sulfamethoxazole (SMZ), sulfadoxine (SDO), sulfachloropyridazine (SCP) and chloramphenicol (CAP) were purchased from Sigma (St. Louis, MO, USA). Oxytetracycline (OTC), tetracycline (TC), chlortetracycline (CTC), lincomycin (LIN), ofloxacin (OFL), ciprofloxacin (CIP) and pefloxacin (PEF) were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Acetonitrile, and methanol (HPLC grade) were purchased from Fisher (New Jersey, USA). Ultrapure water was prepared with a Milli-Q water purification system (Millipore, Billerica, MA, USA). HLB cartridges for hydrophilic-lipophilic balances were bought from Waters (Taunton, MA, USA). All other reagents were of analytical reagent grade.

In this pilot project, four livestock farms and four organic vegetable bases (Fig. 1 and Table S1) were selected in four sampling campaigns (November 2007, December 2007, May 2008 and July 2008) in Tianjin, China. The four organic vegetable bases are located in different regions, which can reflect the whole situation of organic vegetable bases in northern China. In addition, these vegetables are transported throughout northern China. Hence the sampling sites should

be representative. In recent years, the organic fertilizers (manure) applied to the four selected organic vegetable bases were produced from the above four livestock farms. Soil samples were collected at the depth of 0–15 cm below the soil surface by a small shovel. Five sampling sites were distributed as the S type in a vegetable greenhouse, and then were fully mixed into one sample. In the meantime, the soil samples were collected as the control in a routine vegetable field (free antibiotics) near the organic vegetable bases. Groundwater was sampled from local wells at different depths (10, 15, 20, 30 and 40 m) using a portable water sampler (WB-PM, Beijing Purity Instrument Co., Ltd). Radish, rape, celery, and coriander were sampled around the soil-sampling sites at the harvest time. The whole vegetable was collected, including leaves, stems and roots. The five vegetable samples from the same one vegetable greenhouse were mixed as one sample, which was similar to soil sampling. At the same time, the vegetable was also collected as the control from a routine vegetable field (free antibiotics) near the organic vegetable bases. These samples were immediately transported to a laboratory for analysis under cooled conditions.

### 2.2. Sample extraction and clean-up

Lyophilized liquid manure (0.5 g) was extracted with 30 ml 0.1 M EDTA-McIlvaine buffer solution (pH 4.0). The mixtures were put to a vortex agitator and agitated for 30 s. Then the extraction was carried out by MAE (15 min, 40 °C). MAE was performed with an Ethos (Milestone, Italy) system equipped with a twelve-vessel carousel, operated in the closed-vessel mode. The both temperature and pressure were monitored during operation. A fiber optic temperature sensor in the interior of the microwave oven was also used. Extracts were centrifuged at 4000 rpm for 15 min. The supernatant was passed through a Water Oasis HLB cartridge (previously conditioned with 10 ml of methanol and 15 ml of ultrapure water) at a speed of 3 ml min<sup>-1</sup>. The analytes were eluted from the cartridge with 10 ml methanol and then concentrated to dryness under nitrogen flows. Finally, the residue was dissolved in 1 ml methanol for LC-MS-MS analysis.

Lyophilized soil (1.0 g) was extracted by 30 ml acetonitrile and 0.1 M EDTA – McIlvaine (V:V = 1:1). The extract was vortexed for 30 s and then ultrasonic for 15 min. The mixture was centrifuged in air-cooled and concentrated to half probably using a rotary evaporator and then passed through HLB cartridges. The analytes were eluted from the cartridge with 10 ml methanol, and then reduced to dryness under nitrogen flows. Finally, the residue was diluted by adding 1 ml methanol for LC-MS-MS analysis.

Each 1 L groundwater sample passed through 0.7 µm membrane filter and was added 50 ml 0.1 M EDTA– McIlvaine buffer and 2.4 ml formic acid, and then was extracted using HLB cartridges. The analytes were eluted from the cartridge with 10 ml methanol, and then reduced to dryness under nitrogen flows. Finally, the residue was diluted by adding 1 ml methanol for LC-MS-MS analysis.

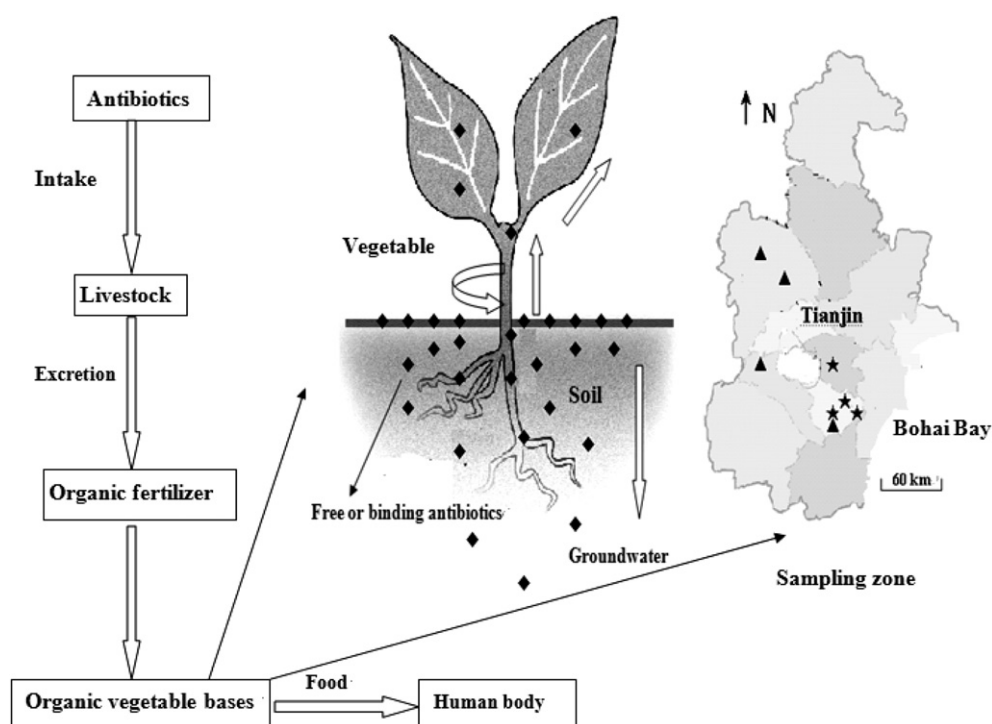


Fig. 1. The schematic of antibiotics in organic vegetable bases. ▲, location of four organic vegetable bases; ★, location of four livestock farms.

Lyophilized vegetable (1.0 g) was homogenized and added 30 ml methanol and hydrochloric acid (V:V = 1:1) to be extracted by ultrasonic for 15 min. The extract was vortexed for 1 min and centrifuged in air-cooled for 15 min. The residue was extracted with 20 ml acetone and centrifuged again. The two supernatants were mixed and extracted as the soil by HLB cartridges. Finally, the residue was diluted by adding 1 ml methanol for LC-MS-MS analysis.

### 2.3. Liquid chromatography mass spectrometry analysis

The LC-MS-MS system consisted of an Alliance 2695 HPLC (Waters, Manchester, UK) and a Waters Micromass<sup>®</sup> Quattro Micro<sup>™</sup> detector with electrospray ionization (ESI). Data acquisition and control were carried out using the Masslynx<sup>™</sup> NT software (Waters; Milford, MA, USA). An Agilent ZORBAX C<sub>18</sub> (150 mm × 2.1 mm; 3 μm particle size from Ireland) was used as the analytical column at a flow rate of 0.25 ml/min. Acetonitrile as mobile phase A and 0.5% formic acid as mobile phase B for gradient conditions set as follows: 0 min, 15 % A; 1 min, 20 % A; 10 min, 30 % A; 17 min, 15 % A; and 25 min, 15 % A. The column temperature was 25 °C, and the injection volume was 10 μL. Argon and nitrogen was used as the collision gas and nebulizer gas, respectively. For the MS detection, the instrument was operated in negative ion mode for CAP and positive for others. The electrospray source settings were optimized with the gas temperature adjusted to 350 °C, source temperature at 100 °C, capillary voltage for CAP at 3.0 kv and for others at 4.0 kv. Validations of the analytical method were listed in Tables S2 and S3.

## 3. Results and discussion

### 3.1. Antibiotics in manures

For two sampling campaigns (November 2007, and May 2008), all target compounds were detected in winter (Table 1). The highest concentration of antibiotics was up to 183.5 mg/kg for OTC and the lowest concentration was only 0.1 mg/kg for CIP. The concentrations of tetracyclines, sulfonamides and quinolones were equivalent with the previous result from different countries (Martinez-Carballo et al., 2007; Hamscher et al., 2005; Jacobsen and Halling-Sørensen, 2006; Karci and Balcioglu, 2009; Christian et al., 2003). In other words, the concentrations of various antibiotics were little different in various regions. This phenomenon was not suitable for water, soil and atmosphere, which were significantly influenced by environmental factors. Seasonal changes play an important role in the distribution of organic pollutants including antibiotics from sewage treatment plants (STPs) and hospitals, which had been abundantly documented (Pena et al., 2010). However, the relevant reports for seasonal changes of antibiotics in manure and soils were scarce. As shown in Table 1, antibiotic residues from manure in summer were significantly lower than those in winter, and the later was more 1–20 fold(s) than the former. Especially, it was not found the existence of PEF, CIP and LIN in summer manure. This result was attributed to the accelerated biodegradation of antibiotics at the

high temperature and strong activity of bacteria in summer. According to our investigation, it was proved that livestock was easy to get sick and needed more antibiotics for treating diseases in winter, which was also one key reason why the concentrations of antibiotics were higher in summer. Furthermore, this work investigated the usage of antibiotics in the four farms. OTC, TC and CTC were extensively used as feedstuff additives. SDO, OFL and LIN were also usually used for disease treatment and prevention. The result of the investigation was summarized in Table 1. According to Table 1, the amount of antibiotic usage was not completely identical with the antibiotic concentration, due to the difference from antibiotic microbial metabolic efficiency and chemical properties. For example, the half-life of LIN and OTC in manure was 15 and 100 days, respectively (Loftin et al., 2008), which explained the illogicality that the low residues went with the large usage. In short, the study of antibiotic distribution characteristics in manure is conducive to the rational use of antibiotics in animal husbandry.

### 3.2. Antibiotics in soils

Antibiotics may become persistent in the environment once it is released from manure into soil (Wang and Yates, 2008). It was found that the concentration range of antibiotics in soils was 0.1–2683 μg/kg and the concentration of OTC in soils was at the highest (2683 μg/kg), excepting that OFL and PEF were lower than the limit of detection (LODs). By the comparison of previous studies, it was found that the concentration of antibiotics varied greatly for soil in different regions (Brambilla et al., 2007; Hamscher et al., 2005; Jacobsen et al., 2004; Morales-Muñoz et al., 2004), which was sharp contrast to manure. The concentrations of antibiotics in organic vegetable bases were higher than those in conventional fields. A major reason for this phenomenon was the use of a large number of organic manure in organic vegetable bases. Many factors could affect the distribution of antibiotics in soils. The dilution with soil, degradation, leaching and uptake by vegetables were main reasons that the residue of antibiotics in soil was much lower than that in manure. As showed in Table 1, the residue of tetracyclines was significantly higher than that of sulfonamides. The difference in chemical properties of antibiotics was a major reason, besides the difference of usage. Tetracyclines had high  $K_d$  values (417–1026 ml/g) and strong combination with soil particles. Thus, they were relatively stable and not easily migrated in soils (Lunestad and Goksayr, 1990). On the contrary, the  $K_d$  values of sulfonamies were 0.9–18.1 ml/g, which suggested that sulfonamides had strong water-solubility and easy to move down

**Table 1**

The residues of antibiotics in manure (mg/kg) and soil (μg/kg) during winter and summer, and the usage of antibiotics for livestock.

	Antibiotic					
	OTC	TC	CTC	SMZ	SDO	SCP
Winter manure	5.3–183.5	8.3–43.5	0.4–26.8	2.0–5.7	0.1–32.7	0.3–2.4
Summer manure	0.08–1.21	0.11–29.3	0.14–1.87	0.23–2.0	0.15–1.42	0.21–2.76
Winter soil	124–2683	20.9–105	33.1–1079	0.1–0.9	1.2–9.1	1.3–2.5
Summer soil	<LOD	2.5	ND	0.03	ND	0.18
RR	9.37	2.30	1.43	0.38	1.63	0.09
Usage	****	***	****	**	***	**
	Antibiotic					
	CAP	OFL	PEF	CIP	LIN	
Winter manure	3.4–15.3	1.2–15.7	3.3–24.7	0.1–4.3	0.12–3.8	
Summer manure	0.14–14.3	0.23–7.8	<LOD	<LOD	<LOD	
Winter soil	0.1–1.1	<LOD	<LOD	10.3–30.1	1.1–11.7	
Summer soil	ND	0.6–1.6	ND	0.8	ND	
RR	0.86	0.84	1.24	0.19	0.18	
Usage	*	***	*	***	***	

\*Showed the amount of antibiotics used for livestock, and the more “” suggested the more usage of antibiotics; nd, not detected; RR (mg/kg) is residual rate.

from the surface soil (Boxall et al., 2002). This is consistent with the distribution of antibiotics in groundwater, which would be discussed later.

Moreover, the concentrations of antibiotics in winter were higher than those in summer, as shown in Table 1. The collected samples had very low antibiotic concentrations (0.6–2.5 µg/kg) in summer. The antibiotics were no detection in most of samples, and only TC, SMZ, SCP and OFL could be found in soils. The changing trend of antibiotics in soils was consistent with that in manure, as above mentioned. However, the result was in contrast with the previous report, which pointed out that the concentration in summer was higher than that in winter (Hamscher et al., 2000). The difference may be attributed to the organic vegetable base with its special particularities. Generally speaking, the manure is applied to arable lands in spring. However, the manure is usually fertilized to organic vegetable bases in winter. Referring the toxicity data from the literature (Baguer et al., 2000; Thiele-Bruhn and Beck, 2005), it could be sure that the current antibiotic residues had inhibited the microbial activity and increased microbial resistance in soils, but were far below the concentrations of toxic effects on soil animals.

### 3.3. Migration of antibiotics from manure to soil

Although there are wide researches on the antibiotic degradation process in a laboratory, the simulation is entirely different from the situation in a field. It is a complex process that antibiotics migrate to soil from manure, as described in Fig. 1. The concentrations of antibiotic residues were difficult to calculate in practical, after antibiotics from manure to soils. In this work, the residual rate was used to describe the residues of antibiotics in soil, and defined as the concentrations of antibiotics in soil on the harvest time of vegetables (Fig. 2). This work did not consider the intermediate processes during the antibiotic migration, such as degradation, plant uptake and runoff. The formula of the residual rate is expressed as follows:

$$RR = \frac{C_a V_m \rho_m}{A_a H_m \rho_s} \quad (1)$$

where RR is the residual rate (µg/kg ha yr);  $C_a$  is the concentration of antibiotics in manure (µg/kg);  $V_m$  is annual fertilizer volume (150 m<sup>3</sup>/ha yr);  $A_a$  is the area of applying manure (10 000 m<sup>2</sup>);  $H_m$  is the thickness of topsoil (0.15 m);  $\rho_m$  is the density of manure (1.1 g/cm<sup>3</sup>);  $\rho_s$  is the density of soil (1.15 g/cm<sup>3</sup>). The formula can estimate effects of manure on the soil in organic vegetable bases. The calculations showed that tetracyclines had the highest residual rate, followed by sulfonamides and quinolones. The residual rate for lincomycin and chloramphenicol was also high. The real concentrations of antibiotics were compared with RR for tetracyclines and the former was observably lower than the latter for the other antibiotics. It showed that the formula was suitable for persistent and low water-soluble antibiotics (tetracyclines) and not suitable for easily degradable and high water-soluble antibiotics (sulfonamides and quinolones). The formula provided a simple estimation method for the persistent antibiotics from manure to soil. If the safe concentrations of antibiotics in soil were known, the safe concentration in manure could be obtained by the inverse operation of the formula.

### 3.4. Antibiotics in vegetables

As shown in Table 2, most of the target antibiotics in vegetables were detected. The range of antibiotics was 0.1–532 µg/kg in vegetables, excepting that there was no detection in CIP in any vegetable sample. It was consistent with the previous result that

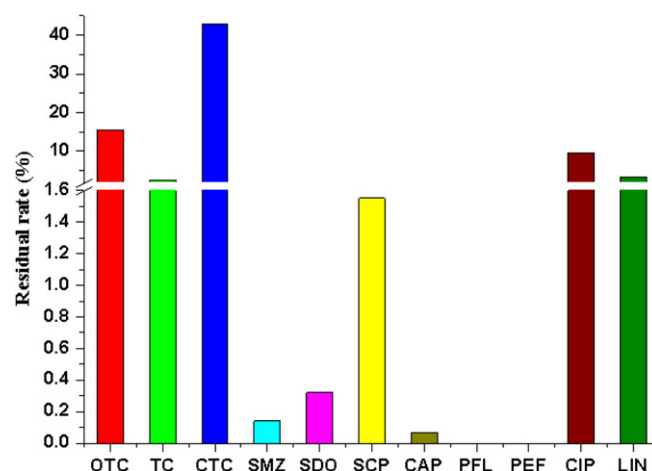


Fig. 2. The residual rate of antibiotics in organic vegetable bases.

the concentrations of tetracyclines and sulfamethazine in plant tissues were 1–50 µg/kg, and increased with time and the amount of antibiotics present in manure (Kumar et al., 2005; Migliore et al., 2010). The concentrations of OTC, SDO and LIN were all high in vegetables, indicating the physical and chemical properties (such as water solubility, and half-life) of these compounds had little effect on the uptake by vegetables, which further illustrating antibiotics were taken up by passive absorption. SDO and LIN occurred prevalently in the winter, but no antibiotics were detected in the summer. The concentration of antibiotics in vegetables from the organic vegetable bases was 4 folds higher than that from ordinary vegetables. In particular, the winter is a season with great environmental risks when the residue of antibiotics from manure, soil, vegetables and groundwater was high. Noticeably, it was found that CIP was detected in most of groundwater samples, but no CIP was detected in vegetable samples. The  $K_d$  value of CIP was very small (0.41–1.29 ml/g) (Uslu et al., 2008), so it had high mobility from soil to groundwater. Furthermore, CIP had strong sorption to fine particulate organic matter (FPOM) and was available for photodegradation (Belden et al., 2007), so it was not propitious for uptake by vegetables. Compared with the antibiotics in soil, the level of LIN in some vegetables exceeded that in soil, which suggested that antibiotics performed biological accumulation at the low concentration. The accumulation coefficients of LIN in celery leaves were 2.5–4.0, far below the value from laboratory simulation (Sarmah et al., 2006), so it was deduced that the accumulation coefficient would be greater under more serious pollution conditions. In order to distinctly evaluate the risk of antibiotics in vegetables, different organs of vegetable samples were collected in the vegetable harvest season. The analysis showed that the distribution of antibiotics in various tissues of the vegetables was leaves > stems > roots. On the contrary, Migliore et al. (1998) found that the concentration of antibiotics in the roots was higher than that in the leaves of wheat and corn. The types and growth stages of vegetables would affect the distribution of antibiotics in vegetables. About 15 days before the harvest, the immature rape was collected. It was discovered that the concentration of antibiotics in the roots of rape was higher than that in the leaves. Reference to the reported inhibitory concentration of plant growth (Kong et al., 2007; Migliore et al., 2000), it could be speculated that the current concentrations of antibiotic residues would not affect significantly on plant growth. Antibiotic-resistant bacteria or their corresponding resistance determinants are known to spread from animals and plants to humans via food chain (Boehme et al., 2004;



**Table 2**

The residues of antibiotics in different tissues of vegetables in the winter and the accumulation coefficient taken up by vegetables.

	Winter									Vegetable	Soil	R
	Radish <sup>a</sup>	Radish <sup>b</sup>	Radish <sup>c</sup>	Rape <sup>a</sup>	Rape <sup>c</sup>	Celery <sup>b</sup>	Celery <sup>c</sup>	Coriander <sup>a</sup>	Coriander <sup>c</sup>			
OTC	8.3	12.7	17–57	ND	56–187	ND	ND	35–205	78–330	76.8	1404	0.05
TC	ND	ND	1.4	ND	1.8	1.0–2.4	3.1	1.2–4.1	1.9–5.6	2.0	63	0.03
CTC	ND	9.4	6–18	ND	3.3	ND	12.6	92–481	54–532	79.3	556	0.14
SMZ	ND	ND	0.9–2.7	ND	0.2	ND	ND	ND	ND	0.5	0.5	1.00
SDO	0.1–0.4	0.2–0.5	0.2–0.6	0.1–0.5	0.3–1.2	0.1–0.3	0.2–0.6	0.1–0.5	0.2–1.0	0.4	5	0.08
SCP	ND	ND	0.1–0.5	ND	ND	ND	0.1	ND	0.3	0.2	1.9	0.09
CAP	ND	1.4–3.4	8–30	ND	0.7	ND	ND	ND	ND	2.9	0.6	4.75
OFL	ND	0.5	ND	ND	0.7–2.1	0.5–1.9	1.7–3.6	ND	0.7–3.5	1.5	0	+∞
PEF	ND	ND	0.8–1.6	ND	ND	ND	1.1	ND	ND	0.6	0	+∞
CIP	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.0	20.2	0
LIN	0.9–3.1	1.5–3.9	1.4–5.4	0.5–3.5	0.7–3.2	1.2–5.1	5–20	0.4–2.4	0.8–3.8	3.6	6.4	0.56

a, root; b, stalk; c, leaf (μg/kg); ND: not detected; R = CV/CS; CV, antibiotic residues in vegetable; CV, antibiotic residues in soil; antibiotic concentrations were below the limit of detection in summer samples.

Millar et al., 2001; Levy et al., 1976), but the no security concentration for human beings is unclear.

### 3.5. Accumulation models of antibiotics in vegetables

Previous studies reported accumulation models for some organic pollutants, such as the partition-limited model (Chiou et al., 2001). However, these models are usually inconvenient to operate due to complicated parameters. In practice, it is of significance to predict the accumulation of antibiotics in vegetables using some simple parameters. For example, Briggs et al. (1983) thought that  $K_{ow}$  is relevant to the plant concentration factor, and gave a simple model:

$$\text{Log SCF} = A \text{Log } K_{ow} - B \quad (2)$$

where SCF is the stem (or leaf) concentration factor and defined as the rate of antibiotic concentration in stems (or leaves) to that in soil.  $A$  or  $B$  is a constant (depending on plant species and growing environment). Although the model is simple, it is easy to operate. Briggs et al. (1983) gave the empirical formula for wheat ( $A$  for 0.95, and  $B$  for 2.0). In this study, this formula was applied to radish and rape growing in fields ( $A$  for 0.80,  $B$  for 0.81,  $R^2 = 0.60$ ,  $P < 0.001$ ), which showed SCF correlated with  $K_{ow}$ . Tetracyclines had the maximum  $K_{ow}$  and gone into soil with the highest concentrations, that is why the concentration of tetracyclines was highest among the target compounds in vegetables. It was indicated that the antibiotics with high log  $K_{ow}$  (the green circle in Fig. 3) were more suitable for the model than those with low log  $K_{ow}$  (the black circle in Fig. 3). The antibiotics with high log  $K_{ow}$  were mainly sulfonamides and quinolones, and those with low log  $K_{ow}$  were mainly tetracyclines. Moreover, the water solubility of sulfonamides and quinolones was stronger than that of tetracyclines, which means that there is a positive correlation between accumulation of antibiotics in vegetables and their water solubility. In the meantime, it showed that antibiotics were mainly taken up through water transport. There was the highest concentration of LIN with strong water solubility in vegetables, also presenting that antibiotics were mainly taken up through water transport and in passive absorption.

### 3.6. Antibiotics in groundwater

As shown in Table S4, only some antibiotics could be detected in groundwater and their level was only ng/L. According to Stolker et al. (2004), the range of SMZ in groundwater was 0.006–3.0 ng/L, which was basically consistent with this study. The concentration of antibiotics in groundwater was similar to that in drinking water (ng/L) and significantly lower than that in rivers and STPs (μg/L) (Ye et al., 2007a,b; Xu et al., 2006). The concentrations of

sulfonamides, chloramphenicol and lincomycin with high water-solubility were higher than those of tetracyclines with low water-solubility. Although the concentration of tetracycline was high in soil, it was only 5.2 ng/L in groundwater. The migration of tetracyclines from soil to groundwater was hindered because they could be strongly bound to soil particulars (Brambilla et al., 2007). Some previous studies suggested low level sorption of antibiotics with appreciable risk of run-off and leaching in absence of manure, and the presence of manure increased the sorption tendency of antibiotics significantly (Sukul et al., 2008). Hence, the organic manure with low concentrations of antibiotics would effectively reduce the contamination of groundwater.

The concentration of antibiotics in winter was far higher than that in summer. In particular, about 55% of the target antibiotics were detected in winter and only SDZ was detected in summer. In the organic vegetable bases, residues of antibiotics can be influenced by temperature and activity of bacteria. Furthermore, the winter was the most important season of antibiotics applied to organic vegetable bases by way of manure. Thus, it was not surprising that the residues of antibiotics in winter were higher than those in summer. This study also analyzed changes of antibiotics with different depths of groundwater. The changing curves of CIP and CAP with the depth of groundwater were depicted in Fig. 4. As shown in Fig. 4, the fluctuation in the concentrations of antibiotics was influenced by farming, and the average concentration was at 30 m. However, the trend was obscure when the concentrations

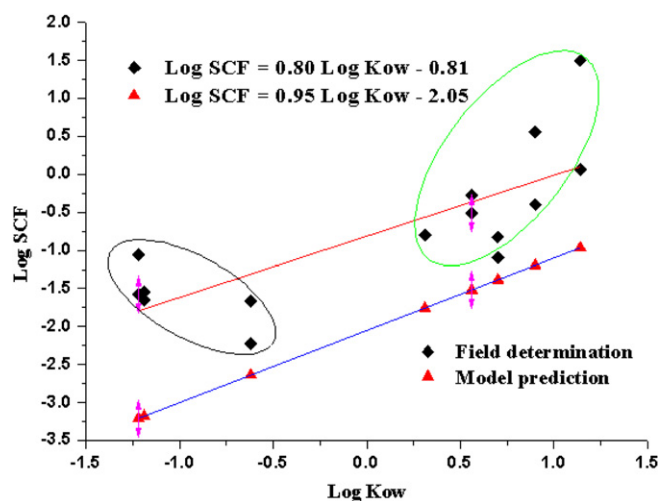


Fig. 3. The relationship between  $K_{ow}$  and SCF for field determination and model prediction. The values of antibiotic  $K_{ow}$  refer to Thiele-Bruhn (2005), Kuchta et al. (2009), Ye et al. (2007a,b) and Tolls (2001).

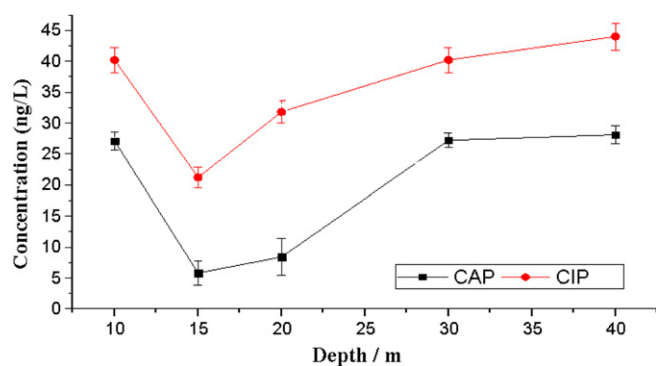


Fig. 4. The residues of CAP and CIP in groundwater from organic vegetable bases.

of antibiotics were low in groundwater. It is thus necessary to collect more samples in order to carry out a scientific evaluation for contamination and harmfulness of antibiotics in groundwater.

#### 4. Conclusions

With the application of manure containing antibiotics to organic vegetable bases, the residues of antibiotics in soil, vegetables and groundwater were widely detected. After varieties of antibiotics migrated from manure to soil, the physical and chemical properties significantly affected their occurrence. It was worthy of our attention that antibiotics performed the biological accumulation at the low concentration. Compared with antibiotics in summer, there were more antibiotic residues and higher environmental risks in winter. Antibiotic residues have undoubtedly a great pressure on the safety of ecosystems and human health in organic vegetable bases.

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#### Appendix. Supplementary data

There are three tables as background datasets for supplementary. Supplementary tables associated with this article can be found in the online version, at doi:10.1016/j.envpol.2010.05.023.

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