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Investigation of Sulfonamide, Tetracycline, and Quinolone Antibiotics in Vegetable Farmland Soil in the Pearl River Delta Area, Southern China

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ABSTRACT: Thirteen antibiotics in soil from vegetable farmlands of the Pearl River Delta, southern China, were investigated. At least three antibiotics were detected in each sample. Six antibiotics including four quinolones, tetracycline, and sulfamethoxazole were detected in >94% of the samples. The total contents of three tetracyclines, eight sulfonamides, and four quinolones were not detected−242.6, 33.3−321.4, and 27.8−1537.4 μg/kg, respectively. The highest antibiotic concentrations were observed mainly in vegetable farmlands affiliated with livestock farms. Chlortetracycline, sulfameter, and quinolones in some samples exceed the ecotoxic effect trigger value (100 μg/kg) set by the Steering Committee of Veterinary International Committee on Harmonization. The composition and concentration of antibiotics in soil were correlated with vegetable species. This study has revealed an alarming condition of antibiotics in vegetable farmland soil. Further investigation including environmental fate, plant uptake, and human exposure to antibiotics by plant-derived food should be conducted.

KEYWORDS: vegetable farmland, soil, antibiotics, quinolones, sulfonamides, tetracyclines, southern China

INTRODUCTION

Antibiotics have been widely used in disease treatment and prevention. They constitute >60% of human prescriptions and >70% of consumed veterinary medications. More than 70% of the antibiotics administered are released into the environment.[1] As much as 70 μg/kg of antibiotics were detected in pig excrement even 30 days after administration.[2] The reported concentration of sulfonamides in manure varied from 10 to 91 mg/kg.[3,4] Campagnolo et al.[5] detected antibiotics in pig manure with up to 100 μg/L for a single compound and 1000 μg/L total antibiotics. Oxytetracycline was still detected in manure after a 5 month maturation period.[5] Antibiotics have been also frequently found in river, coastal, and groundwaters,[6] and even tap water.[7] Various antibiotics were detected in the water and sediment of the Guangzhou section of the Pearl River, southern China.[8,9] Therefore, antibiotics have recently been considered as emerging organic pollutants that could lead to serious environmental issues such as ecological and human heath risk. Concerns regarding antibiotic discharge, presence, and effects on the environment are continually increasing.

Soil is the primary sink of antibiotics. Manure fertilization was one of the main and direct sources of antibiotics in arable soil.[10] Manure was commonly used as organic fertilizer in southern China. River water polluted with antibiotics was usually used as agricultural irrigation, which formed another important antibiotic source in arable soil. In addition, the use as pesticide of some antibiotics such as oxytetracycline in vegetable production could contribute to residues in soil.[11]

Various antibiotics have been increasingly detected in arable soils as analytical tools have improved. Up to 1000 μg/kg of oxytetracycline was detected in soil fertilized with antibiotic-contaminated pig manure, 2 orders of magnitude lower than the concentration in the manure.[12,13] In the soil from conventionally farmed land fertilized with manure, maximum concentrations of tetracycline, chlortetracycline, and sulfadimidine were detected at 199, 7, and 11 μg/kg, respectively.[14] The concentration of quinolones in agricultural soil was determined at approximately 6−52 μg/kg.[15] In northern Turkey, at least one antibiotic compound was detected in manured agricultural soil, oxytetracycline, at a maximum concentration of 500 μg/kg.[16] Theoretically, Winckler and Grafe[17] predicted that tetracycline concentrations in agricultural soil fertilized with manure ranged from 450 to 900 μg/kg on the basis of the regulation of manuring (i.e., 170 kg N/ha, 1500 kg/m^3 soil, distribution to 5 cm soil depth). The sulfonamide residues in soil were estimated to reach 1 kg/ha, the same order of magnitude as pesticide residue after fertilization with manure slurry at 50 m^3/ha.[18]

Antibiotics in soil can have adverse effects on terrestrial organisms, such as favoring the development of resistant bacteria. Soil phosphatase activity is significantly affected at concentrations of 1000 μg/kg sulfamethazine and 406 mg/kg tetracycline. Soil respiration is inhibited by sulfamethoxazole and sulfamethazine at 70 and 13 mg/kg, respectively. Soil microbe population structure and function can also be affected.[19] Moreover, if plants take up antibiotics, phytotoxicity can occur.[20]

Considering the increased detection of antibiotics in soil samples and their potential effects, it is crucial to investigate the concentration and distribution of antibiotics in soil. Several studies have examined antibiotic presence in soil, mostly focusing on soil fertilized with contaminated manure or sludge. Few studies have examined the occurrence of antibiotics in large-scale agricultural soil. Furthermore, compared with research in Europe and

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North America, there are few studies concerning antibiotic contamination of arable soil in China. Therefore, the purpose of this study is to investigate the level of antibiotics in large-scale agricultural soil of vegetable farmlands in the Pearl River Delta area of southern China, one of the most developed areas and also an important vegetable production area in China, to better understand their occurrence in a subtropical region and provide initial data for evaluating antibiotic pollution in large-scale agricultural soil.

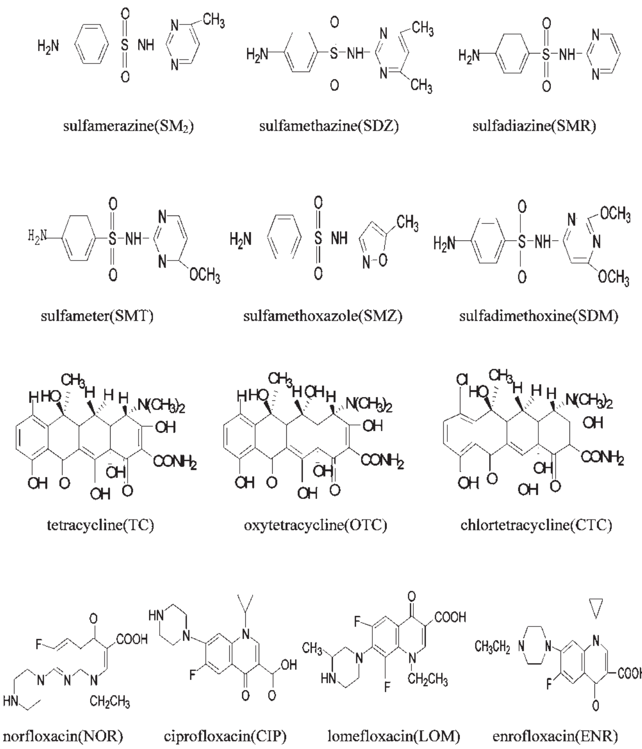

Figure 1. Molecular structures of the 13 antibiotics selected in this study.

Soil samples were analyzed using liquid chromatography coupled with ultraviolet and fluorescence detectors for the 13 antibiotics, including 6 sulfonamides, 3 tetracyclines, and 4 quinolones (Figure 1), which were most commonly used in both human therapy and animal cultivation and were frequently detected in the manure and surface water in the studied area.[7,8,21,22]

MATERIALS AND METHODS

Soil Sampling. Soil samples were collected from 21 vegetable farmlands of the Pearl River Delta area, southern China (Figure 2). Vegetable farmlands registered with the local Department of Agriculture were selected according to geographic location, scale, vegetable species grown, cultivation practices, and the surrounding environment. The local Department of Agriculture routinely monitors the vegetables from these farmlands for typical pollutants, mainly including pesticides, heavy metals, and nitrate. Therefore, the sampling sites selected in the present study should be representative. The areas of sampling farms ranged from 26 to 330 ha, about 10−75 km from one another. The investigated area covered 30000 km^2. The vegetable farmlands selected could be classified into four groups reflecting vegetable farming in the studied area: first, vegetable farmland affiliated with livestock farming and fertilized with untreated manure; second, traditional vegetable farmland, not certified by the Department of Agriculture and having no specific requirement for environmental quality of production sites and no restriction on the use of chemicals such as fertilizers and pesticides; third, pollution-free vegetable farmland, certified by the Provincial Agricultural Authority, with related requirement for environmental quality of production sites and restriction on the use of chemicals; fourth, green-food vegetable farmland, certified by the National Agricultural Authority, with much stricter related requirements for environmental quality of production sites and restrictions on the use of chemicals, compared with pollution-free vegetable farmland, and with more emphasis on the use of organic fertilizers such as manure. Eight to twelve composite soil samples distributed as the S type in a specific vegetable farmland were obtained according to farm size. Discrete soil subsamples (10−20) were fully mixed into one composite sample. Each subsample was collected at the depth of 0−20 cm below the soil surface by a small shovel. Two farmlands growing different species of vegetables were selected to

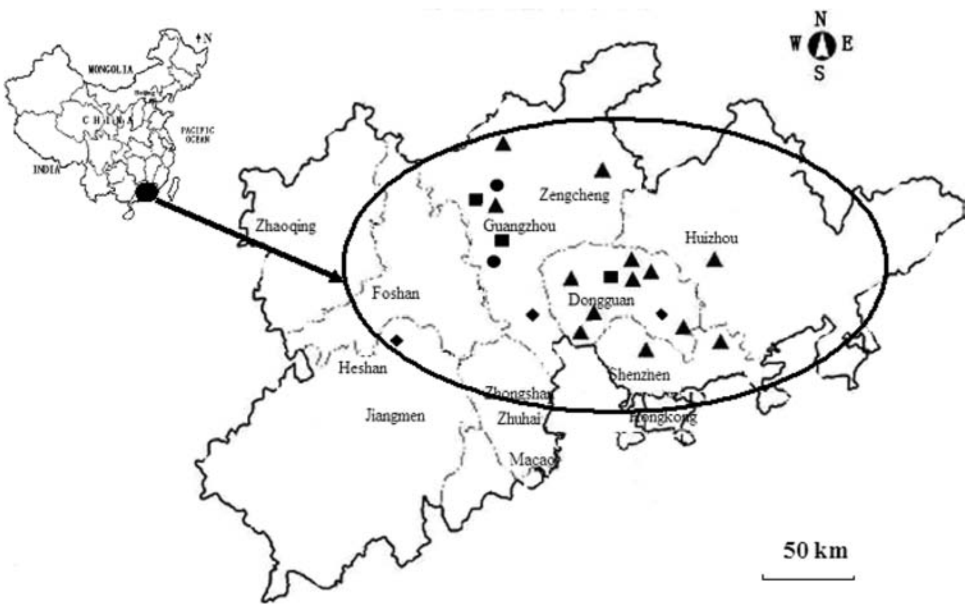

Figure 2. Soil sampling location in the Pearl River Delta, southern China: (▲) traditional vegetable farmland; (●) affiliated vegetable farmland of livestock farm; (■) pollution-free vegetable farmland; (◆) green-food vegetable farmland.

determine whether vegetable species influenced the soil antibiotic content. The soil samples were stored in amber glass bottles and were transferred to the laboratory as quickly as possible. The soil samples were freeze-dried and sieved before analysis, which included determining the basic soil characteristics and testing for antibiotics.

The soils were classified as sandy loam, sandy clay loam, or clay loam according to the international soil texture classification system. The clay content of the soils ranged from 7.3 to 35.0%. The organic matter (OM) content ranged from 13.5 to 71.6 g/kg, and the pH ranged from 5.0 to 7.2. The cation exchange capacity (CEC) ranged from 1.7 to 11.1 cmol/kg. The concentrations of the major nutrients were 0.83−4.09 g/kg total nitrogen (TN) and 74.2−386.4 mg/kg available nitrogen (AN); 0.03−1.24 g/kg total phosphorus (TP) and 12.7−366.1 mg/kg available phosphorus (AP); and 9.2−28.3 g/kg total potassium (TK) and 32.5−278.2 mg/kg available potassium (AK). The differences in basic characteristics of soils from various types of farmlands mentioned above were not significant.

Chemicals and Instruments. Six sulfonamides, including sulfamethazine, sulfadiazine, sulfamerazine, sulfamethoxazole, sulfameter, and sulfadimethoxine, and four quinolones, including norfloxacin, ciprofloxacin, lomefloxacin, and enrofloxacin, with purities of >98%, were purchased from Dr. Ehrenstorfer- Schäfers (Augsburg, Germany). Three tetracyclines, including oxytetracycline, tetracycline, and chlortetracycline, with purities of >98%, were obtained from the National Institute for the Control of Pharmaceutical Products (Beijing, China). HPLC-grade acetonitrile and methanol were obtained from Sigma-Aldrich (St. Louis, MO). All other reagents were of analytical reagent grade.

Individual stock solutions of the antibiotic standards were prepared by dissolving 100 mg L^{-1} antibiotic in acetonitrile. All stock solutions were stored at −20 °C in the dark for up to 1 month. Working standard solutions were prepared by diluting the stock solution with acetonitrile immediately before use. Mixed compound calibration solutions were prepared in acetonitrile and used as spiking solutions. An EDTA−McIlvaine buffer solution was prepared by dissolving 12.9 g of citric acid, 27.5 g of Na_2HPO_4, and 37.2 g of Na_2EDTA in 1 L of water. The extraction buffer was prepared by mixing the EDTA−McIlvaine buffer and methanol 50:50 (v/v).

All glassware was soaked in a K_2CrO_4−H_2SO_4 solution for 2 h, rinsed with ultrapure water, and dried at 250 °C before use. Ultrapure water was used throughout the analysis. The vacuum manifold and solid-phase extraction cartridges of LC-18 and LC-SAX used were purchased from Sigma-Aldrich. HLB cartridges for hydrophilic−lipophilic balances were bought from Waters (Taunton, MA).

All measurements were performed using a high-performance liquid chromatograph (Shimadzu, Kyoto, Japan) equipped with a gradient pump, autosampler, and programmable ultraviolet and fluorescence detectors. The column used was a 250 mm × 4.6 mm i.d., 5 m, Symmetry RP-18, with a 4 mm × 4 mm i.d. guard column of the same material (Waters).

Sample Extraction and Cleanup. *Sulfonamides and Tetracyclines.* The extraction and cleanup followed the method developed by Li et al.[23] with several changes. Each soil sample was pretreated in duplicate. After 2 g of soil had been transferred to a centrifuge tube, 10 mL of extraction buffer was added to each tube, which was then vortexed for 5 min. The centrifuge tubes were placed in an ultrasonic bath for 5 min. The samples were then centrifuged at 4000 rpm for 10 min. The supernatant was decanted into a 50 mL glass bottle. The extraction process was repeated two more times. The supernatant was pooled and concentrated to 1 mL using a nitrogen stream. An LC-18 cartridge in tandem with the LC-SAX cartridge used for the SPE was preconditioned sequentially with 6 mL of methanol and 6 mL of ultrapure water before the samples were extracted. Then, the concentrated supernatant was passed through the SPE tandem cartridges. The tandem cartridges were then rinsed with 6 mL of ultrapure water and vacuum-dried for 10 min. The LC-18 cartridge was eluted twice with 3 mL of methanol. The analytes were collected in a 10 mL brown glass vial, reduced to about 20 μL using nitrogen, and dissolved in 40% aqueous methanol to a final volume of 1 mL for the chromatographic analysis.

Quinolones. The extraction and cleanup procedures followed the method developed by Turiel et al.[24] with small changes. One gram of soil was placed in a 10 mL centrifuge tube and extracted with 5 mL of 50% $MgNO_3$ aqueous solution containing 4% aqueous ammonia. The centrifuge tubes were vortexed for 10 min and placed in an ultrasonic bath for 10 min. Next, the samples were centrifuged at 4000 rpm for 10 min. The supernatant was decanted into a 50 mL glass bottle. This process was repeated two more times. The supernatant was then pooled and concentrated to 1 mL using a nitrogen stream. The concentrated supernatant was purified in tandem using a glass cartridge containing 5 g of anhydrous sodium sulfate and an HLB cartridge. The tandem cartridges were eluted with a 3 mL mixed solution of acetonitrile and 0.067 M aqueous phosphoric acid at the ratio of 15:85 (v/v). The eluate was collected and concentrated to about 20 μL using nitrogen and then dissolved in 0.01 M phosphoric acid aqueous solution and acetonitrile (80:20 v/v) to a final volume of 1 mL for the chromatographic analysis.

Chromatographic Analysis. *Sulfonamides and Tetracyclines.* The liquid chromatography separation was performed at 25 °C with a 20 μL injection volume. The mobile phase consisted of 0.01 M phosphoric acid aqueous solution and acetonitrile (80:20 v/v) at a flow rate of 1 mL/min. Ultraviolet detection was conducted at 270 nm.

Quinolones. A fluorescence detector was used to analyze quinolones at an excitation wavelength of 280 nm and an emission wavelength of 450 nm with a 20 μL injection volume. The mobile phase consisted of 0.067 M phosphoric acid, with the pH adjusted to 3 using triethylamine, and acetonitrile (85:15 v/v) at a 1 mL/min flow rate.

Quality Assurance and Quality Control. Procedural blanks consisting of ultrapure water were analyzed as a control of procedural contamination. The method of standard addition was used to ensure identification of the target antibiotics in soil samples. Solvent was injected every 10 samples and after each sample containing potentially high levels of the target contaminants to avoid possible cross-contamination. A midrange calibration standard solution was run at the beginning, in the middle, and at the end of each sequence to monitor instrumental sensitivity and reproducibility. For routine determination, extract samples were performed in duplicate and the averages were used. Calibration curves of the target antibiotics were constructed by injecting mixed standard solutions for quantification.

RESULTS AND DISCUSSION

Method Validation. Calibration solutions from 0.002 to 1 μg/mL for sulfonamides and tetracyclines (chlortetracycline from 0.04 to 1 μg/mL) and from 0.5 to 500 ng/mL for quinolones in eight points were analyzed by the HPLC operating conditions to form the calibration curve. The correlation coefficients (r^2) of the calibration curve were >0.99, and the relative standard deviation (RSD) indicating the within-precision for all analytes was <5% for most antibiotics (all <10%).The limit of detection (LOD) based on a signal-to-noise ratio of >3 ranged from 0.6 to 23 μg/kg. The limit of quantification (LOQ) based on a signal-to-noise ratio of >6 ranged from 1.5 to 47.4 μg/kg. Recovery tests were performed by spiking a mixture of standards to the three types of soil mentioned above, using two different concentrations of 50 and 250 μg/kg for sulfonamides and tetracyclines and three concentrations of 50, 250, and 500 μg/kg for quinolones, followed by extraction of samples using the same extraction procedure as description above. Nine parallel samples of the

Table 1. Recoveries, Limits of Detection, and Limits of Quantification for Selected Antibiotics in Soils

group of antibiotics	antibiotics	recoveries ± RSD (%)			LOD (μg/kg)	LOQ (μg/kg)
		50 μg/kg	250 μg/kg (n = 9)	500 μg/kg		
tetracyclines	oxytetracycline	75.1 ± 4.7	79.0 ± 2.5		6.5	14.1
	tetracycline	60.6 ± 3.9	63.4 ± 2.4		1.2	3.3
	chlortetracycline	73.7 ± 5.1	77.8 ± 2.9		23.0	47.4
sulfonamides	sulfamerazine	82.1 ± 8.5	83.5 ± 8.3		2.8	6
	sulfamethazine	79.2 ± 5.7	82.0 ± 5.0		2.3	6.7
	sulfadiazine	70.6 ± 5.5	73.5 ± 9.4		2.0	5.5
	sulfameter	81.0 ± 6.2	81.0 ± 4.8		3.1	7.7
	sulfamethoxazole	71.6 ± 4.6	72.5 ± 9.4		5.5	12
	sulfadimethoxine	70.5 ± 7.3	71.5 ± 9.8		11.8	21.7
quinolones	norfloxacin	93.8 ± 2.8	94.3 ± 0.7	86.0 ± 1.7	0.6	1.5
	ciprofloxacin	83.1 ± 1.7	80.8 ± 0.5	76.6 ± 2.6	0.8	1.8
	lomefloxacin	67.8 ± 1.2	62.8 ± 1.6	61.3 ± 0.7	0.8	1.8
	enrofloxacin	75.7 ± 1.3	70.0 ± 1.6	63.2 ± 2.6	1.0	2.2

same analyte were examined, and the mean concentration was calculated as the mean recovery. The mean recoveries for these spiked antibiotics in soil are >60% (Table 1). Chromatograms of a standard sample and a soil sample are shown in Figure 3.

Occurrence of Antibiotics in Vegetable Farmland Soil. *Tetracyclines.* The detection rates of oxytetracycline, tetracycline, and chlortetracycline were 19, 97, and 36%, respectively. At least one of the tetracycline antibiotics was detected in each soil sample, and all three tetracycline compounds were detected in 13% of the samples. The maximum concentrations of oxytetracycline, tetracycline, and chlortetracycline were 79.7, 74.4, and 104.6 μg/kg, respectively, and 6.5% of the samples contained >100 μg/kg chlortetracycline. The mean values of oxytetracycline, tetracycline, and chlortetracycline concentrations were 9.6, 44.1, and 31.1 μg/kg, respectively (Table 2). The mean and maximum values of the total tetracycline concentrations were 84.8 and 242.6 μg/kg, respectively; 35% of the samples were above 100 μg/kg and 6% were above 200 μg/kg.

The use of antibiotic-containing manure as organic fertilizer was one of the main sources of antibiotic residues in soil. In Zhejiang Province, southeastern China, oxytetracycline, tetracycline, and chlortetracycline were respectively detected in 93, 88, and 93% of manure-fertilized soil samples, with concentrations up to 5172, 553, and 588 μg/kg, respectively.[25] The mean values of chlortetracycline, tetracycline, and oxytetracycline residues in manure-fertilized soils were 38, 13, and 12 times higher than in untreated soils, respectively.[26] Up to 2683 μg/kg of oxytetracycline and 1079 μg/kg of chlortetracycline were detected in organic vegetable farmland soil in Tianjin, northern China.[27] In Italy, the mean value of oxytetracycline concentration in soil fertilized with contaminated manure was 171 μg/kg, which was at least 8 times greater than levels in soil fertilized with uncontaminated manure.[13] In Austria, chlortetracycline was frequently detected in soil 4−8 weeks after manure application, whereas oxytetracycline and tetracycline were undetectable.[28] Conversely, <15 μg/kg of chlortetracycline and oxytetracycline was detected in soil 9 days after manure fertilization in Denmark.

Sulfonamides. The detection rates of the six sulfonamides ranged from 26% (sulfadimethoxine) to 94% (sulfamethoxazole). At least two sulfonamides were detected in each soil sample, and all six sulfonamides were detected in 10% of the samples. The mean concentrations of the six compounds varied from 4.9 to 51.4 μg/kg, in the order sulfameter > sulfamethoxazole > sulfamerazine > sulfadiazine > sulfamethazine > sulfadimethoxine. The maximum concentrations of the compounds ranged from 40.4 to 120.4 μg/kg, with 6.5% of the samples above 100 μg/kg, in the order sulfameter > sulfamerazine > sulfadiazine > sulfamethazine > sulfamethoxazole > sulfadimethoxine. The mean and maximum values of total concentrations of sulfonamides were 114.8 and 321.4 μg/kg, respectively; 58% of the samples were above 100 μg/kg, and 6% were above 200 μg/kg.

Few data on sulfonamide concentrations in soil were available. More than 15% of the sulfonamides applied were present in the soil 3 months after manure application, always exceeding 100 μg/kg. The sulfonamide concentrations in manure were generally higher, but the residual soil concentrations were relatively lower compared with tetracyclines. Sulfamethazine concentrations were 2 orders of magnitude lower in soil than in manure. The concentration of sulfamethoxazole in this study was significantly higher than in organic vegetable farmland soil in Tianjin, northern China.[27]

Quinolones. The detection rates were 97% for lomefloxacin and 100% for norfloxacin, ciprofloxacin, and enrofloxacin. All four compounds were detected in 97% of the soil samples. The mean concentrations of the four compounds ranged from 7.4 to 99.4 μg/kg, decreasing in the order enrofloxacin > norfloxacin > ciprofloxacin > lomefloxacin. The maximum concentrations of the four compounds ranged from 13.7 to 1347.6 μg/kg, with 15, 4, and 12% of the samples above 100 μg/kg for norfloxacin, ciprofloxacin, and enrofloxacin, respectively, decreasing in the order enrofloxacin > norfloxacin > ciprofloxacin > lomefloxacin, which was consistent with the order in manure dedected in the studied area.[21] The mean and maximum sum concentrations of quinolone compounds were 195.3 and 1527.4 μg/kg, respectively; 69 and 19% of the samples were above 100 and 200 μg/kg, respectively. The ciprofloxacin concentration in this study was generally equivalent to that in organic vegetable farmland soil in Tianjin, China.[27] Ma and Chen[2] reported 140.71 μg/kg

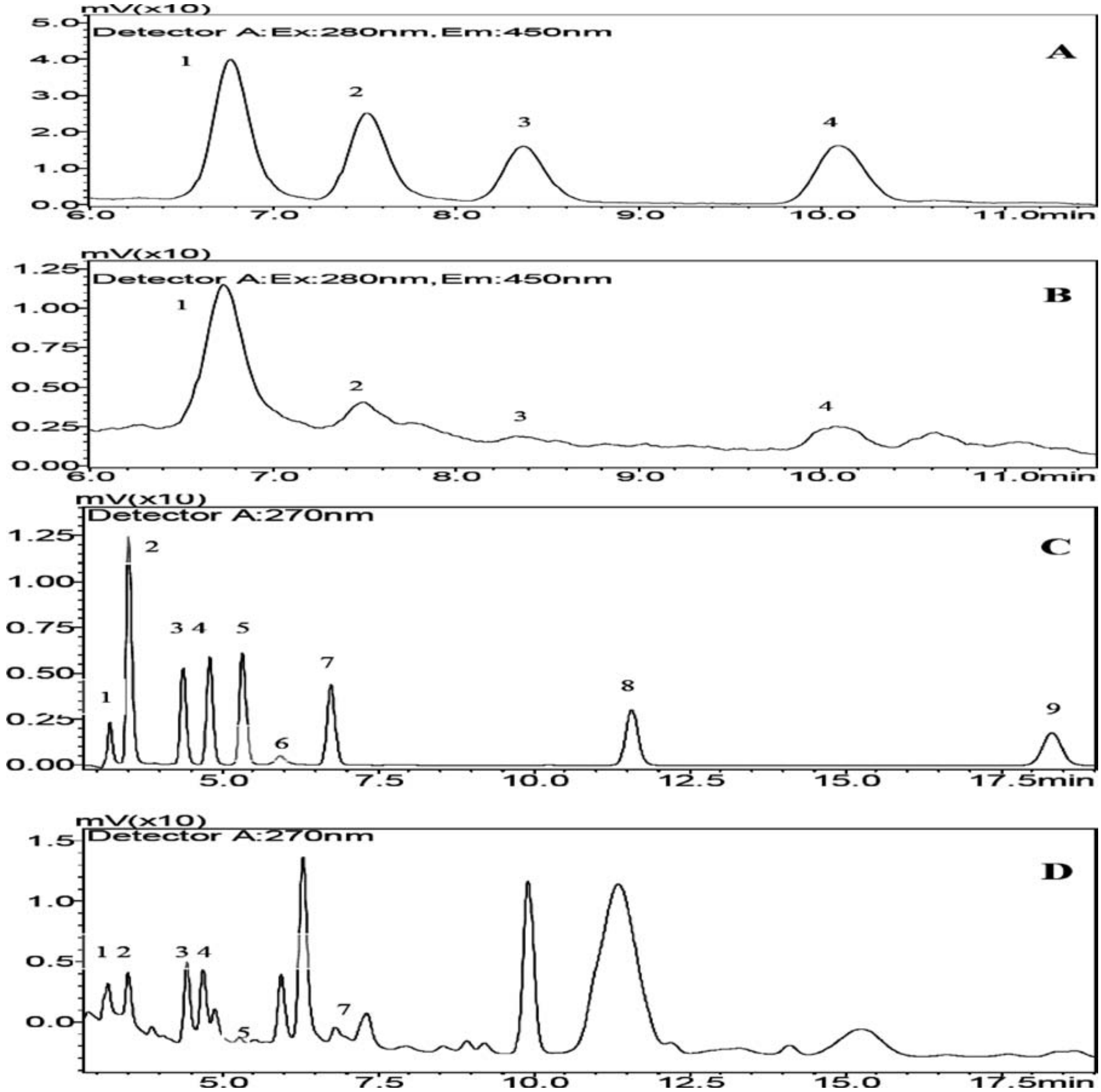

Figure 3. Chromatograms for the separation of antibiotics: (A) chromatogram of standard mixture of quinolones (0.5 μg/mL) (peaks: 1, norfloxacin; 2, ciprofloxacin; 3, lomefloxacin; 4, enrofloxacin); (B) chromatogram of a soil sample (peak identification is the same as in panel A); (C) chromatogram of standard mixture of sulfonamides and tetracyclines under the optimized conditions (1 μg/mL) (peaks: 1, oxytetracycline; 2, tetracycline; 3, sulfamerazine; 4, sulfamethazine; 5, sulfadiazine; 6, chlortetracycline; 7, sulfameter; 8, sulfamethoxazole; 9, sulfadimethoxine); (D) chromatogram of a soil sample (peak identification is the same as in panel C).

enrofloxacin and 75.64 μg/kg ciprofloxacin in the soil surrounding a hog farm in southern China. In Switzerland, quinolone concentrations in sludge-treated soil ranged from 270 to 300 μg/kg at 21 months after application, respectively.[29]

The regional differences in the above results among the antibiotic classes were caused by numerous factors, including soil properties,[3,16] cultivation conditions,[26] and the sources, persistence, and sorption of the antibiotic classes. For example, antibiotics degraded more rapidly in loamy sand soil than in sandy soil,[3] and higher soil temperature and moisture content favored antibiotic dissipation.[26] Repeated manure fertilization could increase soil antibiotic concentrations under certain conditions[14] and decrease concentrations under other conditions. In the present study, relatively lower contents of tetracyclines in agricultural soil compared with other areas in China[25,27] could be attributed to higher temperature and humidity in subtropical climate condition.

Characteristics of Antibiotics in the Soil of Different Types of Vegetable Farmlands. *Tetracyclines.* All three tetracycline compounds were detected in most soil samples from vegetable farmlands affiliated with livestock farms. Tetracycline and chlortetracycline were detected in most pollution-free vegetable farmland samples, and tetracycline alone was detected in most green-food vegetable and traditional vegetable farmland soil samples. Oxytetracycline was detected mainly in vegetable farmlands affiliated with livestock farms and pollution-free vegetable farmland, but not detected in green-food vegetable farmland. Among the four types of farmlands, the total concentration of tetracyclines was highest in vegetable farmland affiliated with livestock farms at 149.9 μg/kg, followed by pollution-free vegetable farmland (132.8 μg/kg), traditional vegetable farmland (37.1 μg/kg), and green-food vegetable farmland (22.6 μg/kg) (Figure 4). The highest individual antibiotic concentrations in various types of farmlands

Table 2. Concentrations of Antibiotics in Soils from Vegetable Farmlands

group of antibiotics	compound	range[a] (μg/kg)	mean ± SD (μg/kg)	detection rate (%)
tetracyclines	oxytetracycline	ND−79.7	9.6 ± 22.9	19
	tetracycline	ND−74.4	44.1 ± 20.4	97
	chlortetracycline	ND−104.6	31.1 ± 42.9	36
	ΣTCs	ND−242.6	84.8 ± 68.9	97
sulfonamides	sulfamerazine	ND−93.5	16.0 ± 24.3	52
	sulfamethazine	ND−74.0	5.5 ± 15.0	29
	sulfadiazine	ND−85.5	13.4 ± 23.0	48
	sulfameter	ND−120.4	51.4 ± 33.2	87
	sulfamethoxazole	ND−54.5	23.5 ± 13.0	94
	sulfadimethoxine	ND−40.4	4.9 ± 10.3	26
	ΣSAs	29.0−321.4	114.8 ± 66.9	100
quinolones	norfloxacin	14.9−150.2	61.9 ± 33.1	100
	ciprofloxacin	5.3−119.8	26.9 ± 26.1	100
	lomefloxacin	ND−13.7	7.4 ± 5.7	97
	enrofloxacin	5.1−1347.6	99.4 ± 262.4	100
	ΣQNs	27.8−1527.4	195.3 ± 293.7	100

[a] ND, not detected; n = 216.

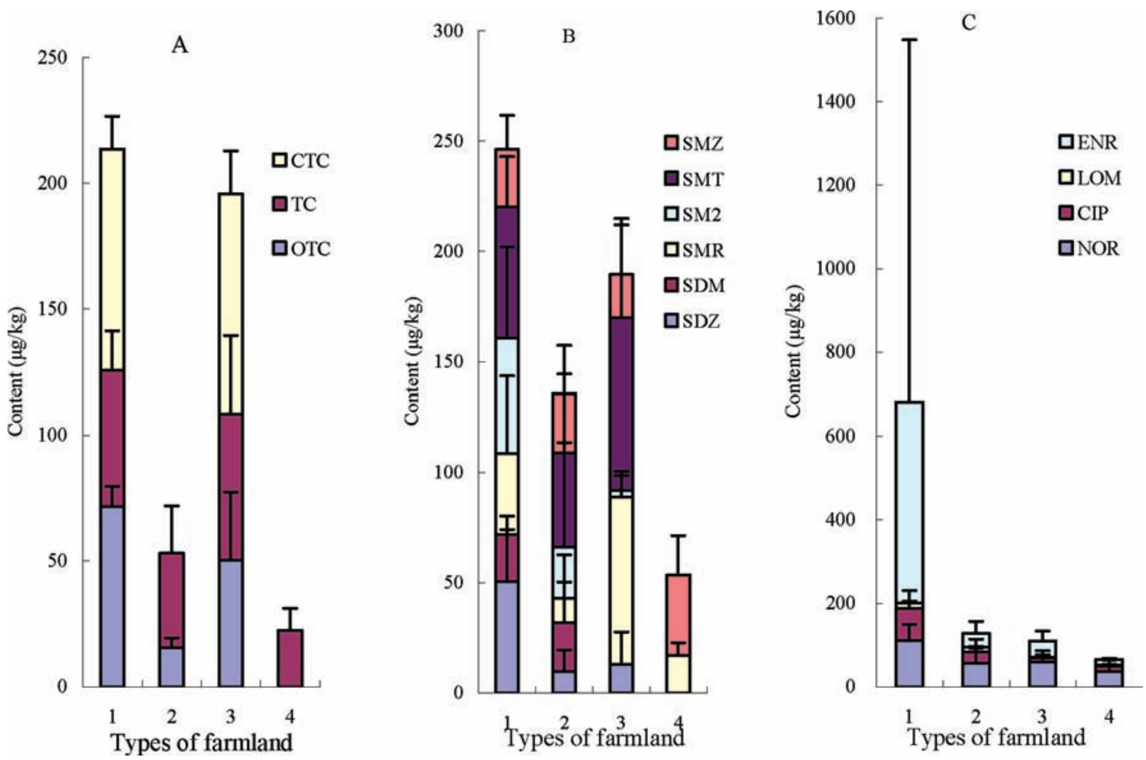

Figure 4. Contents of antibiotics in soil from different types of vegetable farmlands: (A) tetracyclines (TC, tetracycline; OTC, oxytetracycline; CTC, chlortetracycline); (B) sulfonamides (SM_2, sulfamerazine; SDZ, sulfamethazine; SMR, sulfadiazine; SMT, sulfameter; SMZ, sulfamethoxazole; SDM, sulfadimethoxine); (C) quinolones (NOR, norfloxacin; CIP, ciprofloxacin; LOM, lomefloxacin; ENR, enrofloxacin). Bars: 1, affiliated vegetable farmland of livestock farm (n = 26); 2, traditional vegetable farmland (n = 93); 3, pollution-free vegetable farmland (n = 67); 4, green-food vegetable farmland (n = 30).

were 104.6 μg/kg chlortetracycline in the pollution-free vegetable farmland, 100.9 μg/kg chlortetracycline in the vegetable farmland affiliated with livestock farms, 60.2 μg/kg tetracycline in the traditional vegetable farmland, and 24.6 μg/kg tetracycline in the green-food vegetable farmland.

Sulfonamides. Five or six sulfonamides were detected in most vegetable farmlands affiliated with livestock farm soil samples, three or four sulfonamides (sulfamethoxazole, sulfameter, sulfamerazine, and sulfadiazine) were detected in most traditional vegetable farmland soil samples, and two or three sulfonamides were detected in most pollution-free and green-food vegetable farmland soil samples. The highest sum concentrations of sulfonamides were detected in vegetable farmland affiliated with livestock farms (206.9 μg/kg), followed by pollution-free vegetable farmland (117.2 μg/kg), traditional vegetable farmland (91.7 μg/kg), and green-food vegetable farmland (53.6 μg/kg) (Figure 4). The highest individual antibiotic concentrations were 120.4 μg/kg sulfameter in the pollution-free vegetable farmland, 93.5 μg/kg sulfamerazine in the vegetable farmland affiliated with livestock farms, 78.5 μg/kg sulfameter in the traditional vegetable farmland, and 54.5 μg/kg sulfamethoxazole in the green-food vegetable farmland.

Quinolones. All four quinolones were detected in each soil sample, except lomefloxacin in one sample from pollution-free vegetable farmland. The total concentrations of quinolones in vegetable farmland affiliated with livestock farms were highest (682.1 μg/kg), followed by traditional vegetable farmland (127.6 μg/kg), pollution-free vegetable farmland (109.5 μg/kg), and green-food vegetable farmland (65.4 μg/kg) (Figure 4). The highest individual antibiotic concentrations in various types of farmlands were 86.3 μg/kg norfloxacin in pollution-free vegetable farmland, 1347.6 μg/kg enrofloxacin in vegetable farmland affiliated with livestock farms, 114.5 μg/kg norfloxacin in traditional vegetable farmland, and 64.4 μg/kg norfloxacin in green-food vegetable farmland. Enrofloxacin was the dominant compound in vegetable farmland affiliated with livestock farms, whereas norfloxacin was the dominant one in all other types of farmlands (Figure 4).

The antibiotic concentrations in vegetable farmland affiliated with livestock farm soil were generally higher compared with the other types of farmlands. The highest concentrations of oxytetracycline, sulfamethazine, sulfadiazine, norfloxacin, ciprofloxacin, lomefloxacin, and enrofloxacin were detected in this type of farmland. The green-food vegetable farmland had comparably lower antibiotic concentrations, although they were still higher. The highest concentration of sulfamethoxazole was detected in these samples. Antibiotics in the soil from traditional and pollution-free vegetable farmland could come from irrigation water,[7] manure, or antibiotics used as pesticides.[11] Less manure was used on traditional and pollution-free vegetable farmlands than on vegetable farmland affiliated with livestock farms. In addition, the environmental quality requirements for green-food vegetable farmland are stricter than traditional and pollution-free vegetable farmlands. Manure was usually composted before use on the green-food vegetable farmland, causing antibiotics to be effectively degraded.

Occurrence of Antibiotics in Soils Growing Different Vegetables. The composition and content of antibiotics in soils varied considerably for different vegetable species (Figure 5). Of the sulfonamides, sulfameter and sulfamethoxazole were dominant in the soils growing most vegetable species; sulfameter, sulfamethoxazole, and sulfamethazine were dominant in radish soil; and sulfamerazine, sulfameter, and sulfamethoxazole were dominant in tomato soil. Of the three tetracyclines, chlortetracycline and tetracycline were dominant in the soils growing most vegetable species, and tetracycline was dominant in cabbage or tomato soil. Of the quinolones, norfloxacin and enrofloxacin were dominant in the soils growing various vegetable species.

The above results may be related to different antibiotic uptake and bioaccumulation across crop species.[30] Furthermore, there were significant differences in the degradation of organic pollutants in soils growing various plants, which could be related to differences in physiological and biochemical characteristics of the plants, including root exudates and rhizospheric microbial community structure and function. Plant root exudates could stimulate pollutant-degrading bacteria growth in the rhizosphere.

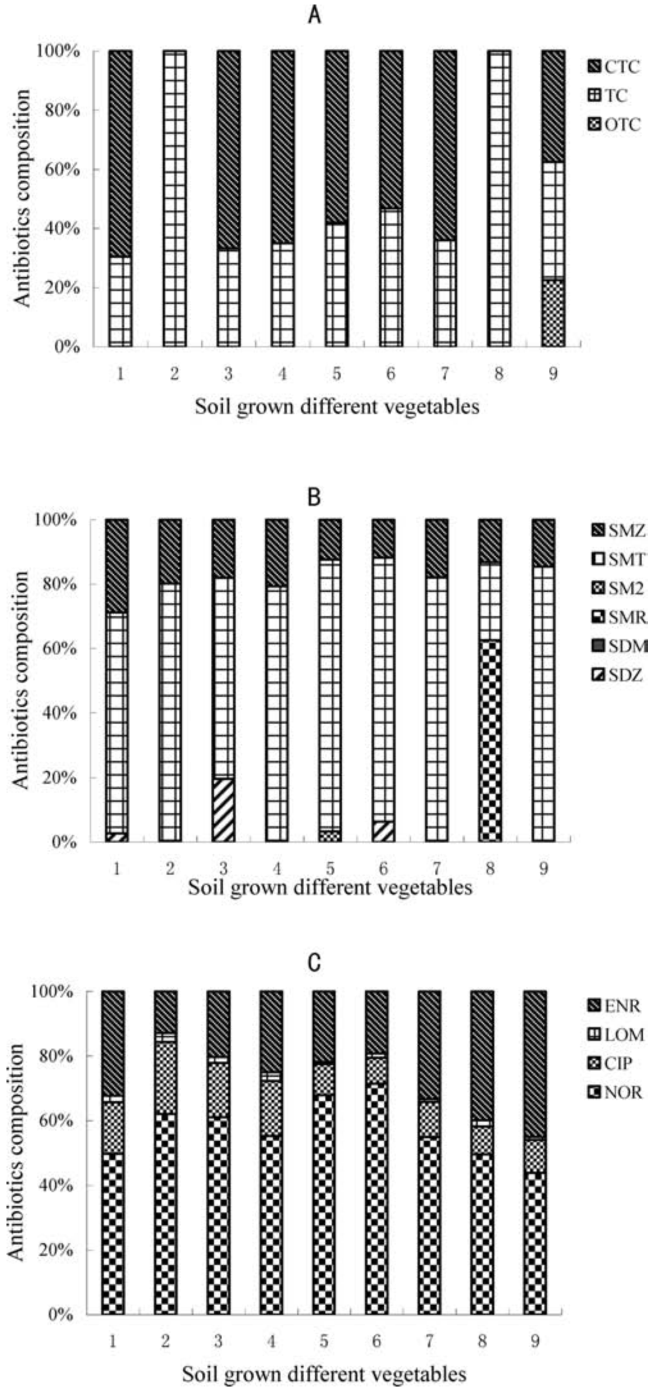

Figure 5. Distribution characteristics of various antibiotics in soils used to grow different vegetables: (A) tetracyclines (TC, tetracycline; OTC, oxytetracycline; CTC, chlortetracycline); (B) sulfonamides (SM_2, sulfamerazine; SDZ, sulfamethazine; SMR, sulfadiazine; SMT, sulfameter; SMZ, sulfamethoxazole; SDM, sulfadimethoxine); (C) quinolones (NOR, norfloxacin; CIP, ciprofloxacin; LOM, lomefloxacin; ENR, enrofloxacin). Bars: 1, lettuce; 2, cabbage; 3, radish; 4, cowpea; 5, Chinese flowering cabbage; 6, broccoli; 7, purple cabbage; 8, tomato; 9, eggplant.

Relationship between Antibiotic Content and Soil Characteristics. All statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS) 13.0 for Windows. The Shapiro−Wilk test (significance level of 0.05) was used to determine whether the data of the following parameters were distributed normally. The results indicated that quinolones, tetracyclines, sand distribution, pH, OM, TN, TP, AN, AP, and AK were non-normal, whereas sulfonamides, clay, silt, CEC, and TK fit the normal curve poorly, indicating Spearman's statistic is applicable to determine the correlation of these parameters.

Spearman's rank correlation coefficients were calculated to determine the correlation between the levels of quinolone, tetracycline, and sulfonamide antibiotics and soil characteristics ($p \leq 0.05$), because Spearman's statistic is valid regardless of variable distribution. The Spearman correlation matrix for the significant correlations was at the 0.05 and 0.01 levels. The highest correlation was for tetracyclines-OM (r = 0.93); higher correlations were found for sulfonamides-OM (r = 0.86), tetracyclines-TN (r = 0.85), sulfonamides-TN (r = 0.79), and quinolones-OM (r = 0.72). Some results such as sulfonamides-OM (r = 0.86) and quinolones-OM (r = 0.72) agreed with published data. The sorption of sulfonamides to soil was affected by organic matter, exchangeable cations, clay surface charge, and pH. Soil texture, CEC, and OM influenced tetracycline levels. Tetracyclines were adsorbed more in acidic and high clay soils. The correlation of quinolones and CEC was not significant, which disagreed with previous work and requires further research.

This investigation demonstrated that antibiotic residues were prevalent in vegetable farmland soil in the Pearl River Delta region of southern China. At least three antibiotics were detected in each sample. Six antibiotics including four quinolones, tetracycline, and sulfamethoxazole were detected in >94% of the samples. Some concentrations of individual sulfonamide, tetracycline, and quinolone compounds exceeded 100 μg/kg, which is the ecological risk trigger value set by the Steering Committee of the Veterinary International Committee on Harmonization based on the eco-toxic effects of antibiotic compounds on a range of organisms. The total concentrations of tetracyclines, sulfonamides, and quinolones in 35, 58, and 69% of the soil samples exceeded 100 μg/kg, and the maximum values reached 242.6, 321.4, and 1527.4 μg/kg, respectively. Some soils had antibiotic concentrations between 10 and 100 μg/kg. Although lower than the trigger value, these levels could affect soil microbial communities through synergistic effects of the various antibiotics and favor antibiotic resistance. Antibiotics pose a potential risk to the soil environment ,and further investigation on the effect of these emerging pollutants on the large-scale soil environment is urgently required.

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