JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Antibiotic Uptake by Vegetable Crops from Manure-Applied Soils

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Supporting Information

ABSTRACT: This study quantified the uptake of five antibiotics (chlortetracycline, monensin, sulfamethazine, tylosin, and virginiamycin) by 11 vegetable crops in two different soils that were fertilized with raw versus composted turkey and hog manures or inorganic fertilizer. Almost all vegetables showed some uptake of antibiotics from manure treatments. However, statistical testing showed that except for a few isolated treatments the concentrations of all antibiotics in vegetable tissues were generally less than the limits of quantification. Further testing of the significant treatments showed that antibiotic concentrations in vegetables from many of these treatments were not significantly different than the corresponding concentrations from the fertilizer treatment (matrix effect). All five antibiotic concentrations in the studied vegetables were <10 μg kg^{-1}. On the basis of the standards for maximum residue levels in animal tissues and suggested maximum daily intake based on body weight, this concentration would not pose any health risk unless one is allergic to that particular antibiotic.

KEYWORDS: *Organic agriculture, manure, animal agriculture, composting, antibiotics*

INTRODUCTION

Since their discovery, antibiotics have been instrumental in treating infectious diseases that were previously known to cause the death of humans and animals. However, their widespread use as a feed additive in livestock production has raised concerns about (1) the development of antibiotic resistance bacteria in the environment and (2) the appearance of antibiotics in food and water supplies. The main pathway for these impacts is when manure containing antibiotics is land-applied.[1]

There is limited literature on antibiotic uptake by plants from manure-applied soils. Most of the earlier research was directed toward identifying the stimulation or toxicity of antibiotics to plants.[2−4] However, many of the studies in the literature were done in cultures, agar, or soils that were artificially spiked with antibiotics at very high concentrations. The earliest published research on antibiotic uptake by vegetable crops was reported by Bewick who used tylosin and terramycin (oxytetracycline) fermentation wastes as sources of fertilizer for tomatoes (*Solanum lycopersicum*).[5] The author reported that there was no antibiotic detected in any of the tomatoes when these wastes were mixed with compost containing peat. However, two recent greenhouse studies showed that onions (*Allium cepa*), cabbage (*Brassica oleracea*, var. *capitata*), and corn (*Zea mays*) take up chlortetracycline[6] and corn, lettuce (*Lactuca sativa*), and potato (*Solanum tuberosum*) take up sulfamethazine[7] from soils (loamy sand and sandy loam) mixed with antibiotic containing raw (noncomposted) hog manure. The chlortetracycline treatments included manure where antibiotics had passed through the animal gut as well as manure that was artificially spiked. The chlortetracycline uptake amount increased from 0 to 17 μg kg^{-1} of fresh weight with an increase in antibiotic concentration in the soil from 0 to 1600 μg pot^{-1}.[6] In this study, there was no uptake of tylosin by corn, cabbage, and onions, possibly because of its larger molecular size.[6] Dolliver et al. showed a significant difference in sulfamethazine uptake by lettuce and onions between the control (soil amended with manure with no antibiotics) and the sulfamethazine treatments (soil amended with manure containing sulfamethazine).[7] However, there was no significant difference in sulfamethazine uptake between 50 (5 mg pot^{-1}) and 100 mg L^{-1} (10 mg pot^{-1}) treatments for lettuce. Although there was some sulfamethazine uptake by corn, there was no statistical difference between the treatments because of the large variability in its concentration in plant samples. Both studies were conducted for 6 weeks, and antibiotics were measured only in the leaf tissue.

A study performed in the United Kingdom reported the uptake of 10 veterinary medicines by lettuce and carrots (*Daucus carota*) from soils that had been artificially spiked with these drugs.[8] The seven antibacterial drugs included amoxicillin, enrofloxacin, florfenicol, oxytetracycline, sulfadiazine, trimetho-

Received: May 17, 2013
Revised: September 22, 2013
Accepted: September 24, 2013
Published: September 24, 2013

prim, and tylosin. The nominal antibiotic concentration in this study was 1.5 mg of a given antibiotic in each container of sand with low organic matter content. The results showed that after 5 weeks of plant growth, florfenicol and trimethoprim were present in lettuce at concentrations of 15 and 6 μg kg^{-1}, respectively. Similarly, enrofloxacin, florfenicol, and trimethoprim were present in carrot roots at concentrations of 2.8, 5, and 5.3 μg kg^{-1}, respectively. The authors also reported that enrofloxacin (8.5 μg kg^{-1}) and florfenicol (38 μg kg^{-1}) were higher in carrot peel than the carrot pith. However, oxytetracycline, tylosin, and sulfadiazine concentrations in lettuce and carrot were below detection limits.

Recently, Herklotz et al. used Chinese cabbage (*Brassica rapa* var. *pekinensis*) and Wisconsin Fast Plants (*Brassica rapa*) to test potential uptake and accumulation of carbamazepine, salbutamol, sulfamethoxazole, and trimethoprim at a concentration of 232.5 μg L^{-1} under hydroponic conditions.[9] All four chemicals were detected in the root and leaves of cabbage. Although all four chemicals were detected in the leaf, stem, and root of the Wisconsin Fast Plant, only carbamazepine and salbutamol were detected in the seedpods.

In a greenhouse study, Jones-Lepp et al. tested the uptake of azithromycin (macrolide), clindamycin (lincosamides), and roxithromycin (macrolide) by lettuce, spinach (*Spinacia oleracea*), and carrots grown in washed silica sand and irrigated with antibiotic-spiked Colorado River water at concentrations of 0 (control), 0.1, 1, 10, 100, and 1000 ng L^{-1}.[10] None of these antibiotics were detected in the leafy part of these vegetables. Only a trace amount of clindamycin (lettuce, spinach, and carrot) and roxithromycin (lettuce and carrot) was present in the root portion of these vegetables at the spiked antibiotic concentration of 1000 ng L^{-1}.

With the current emphasis on healthy food and sustainability, there is increased demand for organic foods, where manure is the primary source of nutrients. At this time, there are no guidelines on the presence of antibiotics in the manure used for certified organic farming. The goal of this study was to determine the extent of antibiotic uptake by vegetable crops from soils that have been fertilized with antibiotic-laden turkey and hog manures, both raw and after composting, under field conditions. In this article, we refer to raw manure as manure that is just taken out of a turkey barn or from a hog lagoon and has not gone through composting or an extensive curing process. The specific vegetable crops tested in this study were spinach, lettuce, cabbage, carrot, radish (*Raphanus sativus*), onion, garlic (*Allium sativum*), tomato, green bell pepper (*Capsicum annuum*), sweet corn, and potato.

■ MATERIALS AND METHODS

Field studies were conducted at two locations, the Southern Research and Outreach Center (SROC) in Waseca, Minnesota, and the Central Lakes College Agricultural Station in Staples, Minnesota. The soil at the Waseca site is a Webster clay loam (fine loamy, mixed, mesic, Typic Haplaquoll), whereas the soil at the Staples site is a Verndale sandy loam (coarse-loamy, mixed, frigid Udic Agriboroll). The five antibiotics studied in this experiment were chlortetracycline ($C_{22}H_{23}ClN_2O_8$; a tetracycline), monensin ($C_{36}H_{62}O_{11}$; an ionophore), sulfamethazine ($C_{12}H_{14}N_4O_2S$; a sulphonamide), tylosin ($C_{46}H_{77}NO_{17}$; a macrolide), and virginiamycin ($C_{28}H_{35}N_3O_7$ for M_1 and $C_{43}H_{49}N_7O_{10}$ for S_1; a polypeptide). The properties of these antibiotics have been reported by several investigators[11−19] and some of those properties are summarized in review articles by Tolls[20] and Thiele-Bruhn.[21] These antibiotics were selected for our study because of their common use for the treatment of infection as well as for the growth of and feed efficiency for many food animals.[1] For example, chlortetracycline is used for the treatment of infection in swine and sheep and for growth promotion in cattle, swine, and poultry. Comparatively, monensin is used for growth promotion in cattle only. Sulfamethazine is mostly used in swine for the treatment of infection. Tylosin is used for the treatment of infection in cattle, swine, and poultry and for growth promotion in swine and poultry. Virginiamycin is used for the treatment of infection in poultry and for growth promotion in both swine and poultry.[1]

To simulate realistic conditions as much as possible, we used concentrated antibiotic animal products that are mixed with animal feed to supply antibiotics to livestock. The products used were CTC-50, Tylan-40, Rumensin-80, and Stafac-20, and sulfamethazine powder. The commercial products Tylan, Rumensin, and Stafac contain tylosin, monensin, and virginiamycin, respectively. Table 1 lists the amount of antibiotic product (River County Cooperative, Hastings, MN) or powder (INVESCO LLC, Mankato, MN) that was mixed with manures.

Table 1. Antibiotic Feed or Powder Mixed with Liquid Hog and Turkey Manures before Composting

antibiotic feed or antibiotic powder	liquid hog manure (mg L^{-1})	solid turkey manure (mg kg^{-1})
CTC-50[a]	30.0−36.0	63.2
Tylan-40[b]	37.7−45.2	78.9
Rumensin-80[c]	18.9−22.6	39.5
sulfamethazine powder	3.32−3.98	6.9
Stafac-20[d]		157.5

[a]CTC-50 contains 50 g of chlortetracycline per pound of product. [b]Tylan-40 contains 40 g of tylosin per pound of product. [c]Rumensin-80 contains 80 g of monensin per pound of product. [d]Stafac-20 contains 20 g of virginiamycin per pound of product.

Eleven vegetables representing leafy greens, root crops, and fruit were grown in soils amended with four manure treatments and a control (inorganic fertilizer but no manure). The four manure treatments were (1) raw (solid) turkey manure, (2) composted turkey manure, (3) raw (liquid) hog manure, and (4) composted (solid) hog manure. Raw hog manure was taken from the gestation barn at the SROC hog facility at Waseca, Minnesota, whereas the raw turkey manure was obtained from a conventional farm where antibiotics were being used per industry standard. Limited antibiotics had been used at the hog facility at SROC. The composting of both manures was done at Waseca. Details on the antibiotic mixing with manure, the composting process, including compost properties (Table S1) and composting temperatures (Figure S1 and S2), manure and fertilizer rates, plot design, and vegetable planting are given in the Supporting Information.

Vegetable Harvest and Processing. Vegetables were hand-harvested at peak maturity from the middle of each plot, and a sub sample of the produce was processed within a day. Processing involved the careful washing of the vegetables with deionized water, wiping the excess water off of the vegetables with paper towels, chopping the vegetables in coarse chunks, taking a subsample of the chopped vegetable for the water content, and then taking another subsample (20 g) of chopped vegetables and liquefying it with 40 mL of buffered peptone water (BPW, pH 7.0) in a blender. BPW (10 g of proteose peptone, 5 g of sodium chloride, 3.5 g of disodium phosphate anhydrous, and 1.5 g of monopotassium phosphate in 1 L of deionized water) was selected mainly as a mild extractant of antibiotics from vegetables and because of its buffering capacity to prevent the degradation of antibiotics in the extract.[7] BPW was prepared in the laboratory, and all reagents were purchased from Becton, Dickinson and Company (Sparks, MD). The blended mixture was then mixed in a rotating mixer (20 rpm) for 30 min in a cold room (4 °C) and then centrifuged at 4435*g* for 15 min. The supernatant of the centrifuged mixture was then decanted and filtered through a 0.45 μm nonsorptive filter (Milex-HV, Millipore Corp., Carrightwohill, Co. Cork, Ireland),

dx.doi.org/10.1021/jf404045m | J. Agric. Food Chem. 2013, 61, 9992−10001

frozen (−20 °C), and stored for future analysis. Peels of carrots, radishes, and potatoes were analyzed separately to determine if there was any difference in the antibiotic concentrations between the peel and the pith. Radish was peeled with a knife, and the peel was about 1 to 2 mm thick. Carrots and potatoes were peeled with a potato peeler, and the peel thickness was around 1 mm. The moisture contents of the vegetables expressed on wet basis were measured by oven drying the subsample of chopped vegetable at 60 °C.

Soil Sample Collection and Processing. Surface soil (0−15 cm) was collected just before planting (22 April at Staples and 29 May at Waseca) and after vegetable harvest to evaluate the fate of the manure-applied antibiotics in soils. Because the timing of the vegetable harvest was different for different vegetables, the timing of soil sampling at harvest also varied. Soil samples from the fertilizer plots served as the control.

The processing of the soil samples was similar to the vegetable-extraction procedures. The antibiotic-extraction procedure from the soil samples involved mixing 5 g of moist soil with 20 mL of BPW in centrifuge tubes, mixing the suspension for 30 min in a rotating mixer (20 rpm) in a cold room (4 °C), and then centrifuging the suspension at 4435*g* for 15 min. Similar to plant analysis, BPW was used as a mild extractant of antibiotics from the soil sample. The supernatant was decanted and saved. To the remaining soil an additional 20 mL of BPW was added, and the mixture was shaken and then centrifuged again as outlined above. The supernatant was decanted and added to the earlier saved supernatant. The combined supernatant was then filtered through a 0.45 μm nonsorptive filter, and the filtrate was frozen until the time of antibiotic analysis. The water content of the soil samples expressed on an oven-dried basis was measured by oven drying the soil at 105 °C.

Antibiotic Analysis. Antibiotic analysis was done using commercially available enzyme-linked immunosorbent assay (ELISA) kits for all five antibiotics used in this study. The commercial vendors for these kits are chlortetracycline (r-Biopharm AG, Darmstadt, Germany), monensin (Immuno-Diagnostic Reagents, Vista, CA), sulfamethazine (Neogen Corporation, Lexington, KY), tylosin (International Diagnostic Systems Corporation, St. Joseph, MI), and virginiamycin (EuroProxima B.V. Arnhem, The Netherlands). These kits were used primarily because they can measure small quantities of antibiotics in samples, which was the case for vegetable samples.

Several of these kits have been tested in the investigators' laboratory for various matrices and cross-reactivity.[6,7,22,23] For example, Kumar et al. showed that the ELISA kits for chlortetracycline and tylosin used in this study were highly sensitive and selective and had detection limits of 0.10 and 0.05 μg L^{-1}, respectively.[22] Furthermore, the tetracycline ELISA kit was highly specific to tetracycline and chlortetracycline and not to other forms of tetracycline (oxytetracyline, demeclocyline, and doxycycline). Analysis of a liquid swine manure sample by liquid chromatography−mass spectrometry (LC−MS) showed lower concentration of chlortetracycline than the ELISA test concentrations. However, the tylosin concentrations were comparable to those obtained by LC−MS. For a series of matrices varying from nanowater, lake waters, runoff samples, and soil saturation extracts, the recovery of both tylosin and tetracycline was near 100%. Subsequently, Kumar et al. used these ELISA kits to quantify the uptake of chlortetracycline and tylosin in corn, onion, and cabbage leaves.[6]

Dolliver et al. showed that the ELISA kit for monensin was highly sensitive, with limits of detection and quantification of 1.5 and 3.0 μg L^{-1}, respectively.[23] Furthermore, these authors did not observe any cross reactivity with similar poly ether ionophore (salinomycin), tetracyclines (chlortetracycline and doxycycline), macrolides (erythromycin and tylosin), a sulfonamide (sulfamethazine), and a polypeptide (virginiamycin). Monensin concentrations in water, cattle manure, and turkey manure samples were generally higher with the ELISA test than with LC−MS. The authors used the monensin ELISA kit in their study on antibiotic degradation during manure composting.[24]

The sulfamethazine ELISA kit was tested by Dolliver et al. in a plant-uptake study of sulfamethazine from manure-amended soil.[7] The authors were able to confirm the sulfamethazine peak in a plant sample using HPLC (a Beckman Coulter System Gold 128 Solvent Module equipped with a UV−vis diode array detector), but they were uncertain of the HPLC-measured concentrations because of the noise and interference despite an extensive cleanup procedure. The kit manufacturer had also reported a cross reactivity of less than 5.7% for other sulfa chemicals (sulfamerazine, sulfadiazine, sulfachloropyridazine, sulfamethoxypyridazine, sulfamethoxydiazinium, sulfamethoxydine, sulfamethoxazole, and sulfadimethoxine). In the above testing, pure antibiotic were purchased from Sigma Chemicals Co. (St. Louis, MO).

Development of the virginamycin ELISA kit was based on research by Situ and Eliott for banned antibiotics growth promoters mixed in with animal feed in Europe.[25] These authors showed that the viginiamycin ELISA kit reactivity to a polypeptide (bacitracin), a coccidiostat (halofuginone), quinoxalines (carbodox and olaquindox), macrolides (spiramycin and tylosin) was minimal. In further testing, the authors found correct identification of the test kit results using LC−MS/MS.[26]

The procedures for antibiotic analysis followed ELISA kit manufacture's instructions and measured the optical density (OD) of the sample extracts after incubation. The details of these procedures have been outlined by Kumar et al.[6,22] and Dolliver et al.[7,23] Optical density was converted to percent inhibition, which was then regressed against standard antibiotic concentrations to develop a standard curve (eq 1). A standard curve was developed each time the ELISA test was run.

$$\%\ \text{inhibition} = 100 - \left(\frac{\text{OD}_{\text{sample or standard}}}{\text{OD}_{0\mu\text{g L}^{-1}}}\right) \times 100 \quad (1)$$

Assessment of Matrix Effects in Plant Sample Analysis. A matrix effect can occur when a substance or substances in the matrix interfere with the ELISA assay, thus producing inaccurate results. In general, the more complex the matrix, the more likely a matrix effect will be encountered during ELISA analysis. In this study, the matrix effect for various ELISA assays was assessed for samples extracted from vegetables. This assessment was made with the pepper extract. Pepper extract was selected because it had a dark-green color. Because the ELISA test measures optical density, we assumed this may represent the largest matrix interference. The procedure involved blending 20 g of pepper from the inorganic fertilizer (control) treatment with 20 mL of buffered peptone water in a blender, transferring the mixture to a 50 mL centrifuge tube, and then shaking the mixture for 30 min on a rotary mixer (20 rpm) in a cold room (4 °C). After shaking, the mixture was centrifuged at 4435*g* for 15 min and then filtered through a 0.45 μm nonsorptive filter. To evaluate the matrix effect from plant materials, a set of antibiotic and pepper extract mixtures was prepared by mixing 0.3 mL of pepper extract with 0.3 mL of antibiotic solution of varying concentrations. Antibiotic concentrations ranged from 1.35 to 12.15 μg L^{-1} for chlortetracycline, 1 to 25 μg L^{-1} for monensin, 0.5 to 5 μg L^{-1} for sulfamethazine, 0.1 to 10 μg L^{-1} for tylosin, and 1.56 to 12.5 μg L^{-1} for virginiamycin. These mixtures were then analyzed for various antibiotic concentrations using different antibiotic ELISA kits. The antibiotics used in testing the matrix effects were taken from the stock solution or antibiotic salt that came with the ELISA kits.

Statistical Analysis. Statistical testing of antibiotic uptake by vegetables was done by first comparing antibiotic concentrations in plant tissues against the limit of quantification (LOQ) values. If any of the treatments were significantly different, then the antibiotic concentration in the plant tissue of that treatment was compared with the corresponding concentration in the fertilizer treatment. The first comparison assessed if the differences in tissue concentrations were over and above the variability in the ELISA technique, whereas the second comparison assessed if the differences in tissue concentrations were over and above the matrix effects. Statistical analyses (regression relationship, *t* test for significance of slope and intercept values, and *t* test for average values) were performed using Microsoft Excel.[27] In the statistical testing of antibiotic uptake, a two-tailed *t* test was used. Analyses were performed separately for each vegetable within a given manure treatment.

RESULTS

ELISA Analytical Procedure. The coefficient of determination (R^2 values) of the standard ELISA curves relating the percent inhibition versus log of antibiotic concentration for five antibiotics tested in this study are summarized in Table 2.

Table 2. Limit of Detection (LOD), Limit of Quantification (LOQ), and R^2 Values for ELISA Standard Curves Relating Optical Density vs Log Antibiotic Concentration for Five Antibiotics Tested in This Study

antibiotics	R^2	LOD (μg L^{-1})	LOQ (μg L^{-1})
chlortetracycline	0.93−0.97	0.10	0.13
monensin	>0.99	0.58	1.0
sulfamethazine	0.97−0.99	0.10	0.27
tylosin	0.97−1	0.02	0.14
virginiamycin	0.97−0.98	0.09	0.12

Average percent inhibition ranged from 21% for the 0.15 μg L^{-1} standard to 87% for the 12.15 μg L^{-1} standard in the case of chlortetracycline, 22% for the 1 μg L^{-1} standard to 72% for the 25 μg L^{-1} standard in the case of monensin, 13% for the 0.05 μg L^{-1} standard to 83% for the 5 μg L^{-1} standard in the case of sulfamethazine, 44% for the 0.1 μg L^{-1} standard to 89% for the 100 μg L^{-1} standard in case of tylosin, and 39% for the 0.39 μg L^{-1} standard to 87% for the 12.5 μg L^{-1} standard in the case of viginiamycin. For all five antibiotics, there was a high coefficient of determination.

For various antibiotics, the limit of detection (LOD) and the LOQ varied from 0.02 to 0.58 μg L^{-1} and 0.12 to 1.0 μg L^{-1}, respectively (Table 2). Because different vegetables had different water contents and also a different proportion of buffered peptone water was added to extract the antibiotics, LOQ, expressed as micrograms of antibiotic per kilogram of vegetable, varied by vegetable type as well as between the peel and the pith of a given vegetable. For various vegetables, the LOQ values of chlortetracycline, monensin, sulfamethazine, tylosin, and virginiamycin varied from 0.13 to 0.39 μg kg^{-1}, 0.95 to 2.96 μg kg^{-1}, 0.25 to 0.79 μg kg^{-1}, 0.13 to 0.73 μg kg^{-1}, and 0.11 to 0.35 μg kg^{-1}, respectively. For comparisons of antibiotic uptake by various crops, we used the LOQ value of a given antibiotic at the time a given vegetable was tested.

Antibiotic Degradation during Composting. The degradation of all antibiotics during composting followed the first-order rate reaction (eq 2) for both hog and turkey manures.

$$C_t = C_0 \, e^{-kt} \quad (2)$$

where C_t is the concentration of a given antibiotic at a given time (t), C_0 is the initial concentration of the same antibiotic, and k is the rate of its degradation (d^{-1}). Except for chlortetracycline, antibiotic degradation was slower during the composting of turkey manure than the composting of hog manure (Table 3). Tylosin had the shortest half-life (5 days) during hog manure composting followed by chlortetracycline (46 days), sulfamethazine (74 days), and monensin (128 days). Comparatively, during turkey manure composting, the half-life ($t_{1/2}$) trends were tylosin (16 days) < chlortetracycline (17 days) < virginiamycin (25 days) < monensin (257 days) < sulfamethazine (462 days). These values are similar or slightly higher than the half-life values measured by Dolliver et al.[24] for turkey manure composting over 35 days. Dolliver et al. reported a half-life of ≤1 day for chlortetracycline, 11−22 days for monensin, and 19−23 days for tylosin.[24] In their study, there was also very little degradation of sulfamethazine. Table 4 lists the amount of antibiotics applied with raw and composted manures to the vegetable plots.

Table 3. First-Order Degradation Rate Constant ($-k$) and Half-Life ($t_{1/2}$) of Monensin, Sulfamethazine, Tylosin, Virginiamycin, and Chlortetracycline during Composting of Hog and Turkey Manures

	hog manure		turkey manure	
antibiotic	μ, d^{-1}	$t_{1/2}$, d	μ, d^{-1}	$t_{1/2}$, d
chlortetracycline	−0.0149	46.2	−0.0421	16.5
monensin	−0.0054	128.4	−0.0027	256.7
sulfamethazine	−0.0094	73.7	−0.0015	462.1
tylosin	−0.1456	4.8	−0.0448	15.5
virginiamycin	N/A	N/A	−0.0274	25.3

Table 4. Amount of Antibiotics Applied to Soil with Raw and Composted Hog and Turkey Manures

	g ha^{-1}			
antibiotics	raw hog	composted hog	raw turkey	composted turkey
chlortetracycline	11.5	7.2	0.1	0.3
monensin	303.0	226.7	10.3	22.9
sulfamethazine	238.2	64.2	36.1	50.1
tylosin	16.0	0.0	2.4	0.3
virginiamycin	N.A.[a]	N.A.	0.2	1.7

[a] N.A., not added.

Water Content of Vegetables and Soil at Harvest. As expected, the water content of the vegetables and the soil at the time of harvest varied with the type of vegetable. For a given vegetable, it also varied with the type of tissue sampled. Generally, the water content (wet basis) of a given vegetable was slightly higher from Waseca than from Staples.[28] Except for garlic bulb and garlic scapes at Staples and potato tuber and corn kernels at both sites, the water content of all vegetables were greater than 80%. The water contents of garlic bulbs and scapes at Staples were 62 and 77%, respectively. The water content of potato tubers at Waseca and Staples was 66 and 78%, respectively. The water content of corn kernels averaged around 74% for the two sites. The water content (oven dry basis) of the soil samples at the time of harvest ranged from 23 to 39% for the Waseca site and from 6 to 14% for the Staples site. The higher water contents at the Waseca site are due to higher clay and organic matter contents of Webster clay loam (clay = 39%, organic matter = 5%) than the Verndale sandy loam (clay = 8%, organic matter = 1.2%) soil at Staples.

Antibiotic Concentrations in Soil at Planting and Harvest. Concentrations of various antibiotics in soil just before planting and at the time of harvest are shown in Table 5. Each number in this table is an average of 33 (11 crops and three replications) measurements. Because different vegetables were harvested at different times, the antibiotic concentration in soil at the time of harvest represents an average over all harvest times. Except for sulfamethazine, one case of monensin (raw hog manure treatment at planting), one case of tylosin (raw hog manure after harvest), and two cases of virginiamycin (raw and composted turkey manure at planting), the soil antibiotic concentrations were below the limits of quantification

Table 5. Average Antibiotic Concentration in Soil before Planting and after Harvest for Various Nutrient Source Treatments at Both Waseca and Staples[a]

treatment	fertilizer (μg kg^{-1})		raw hog (μg kg^{-1})		composted hog (μg kg^{-1})		raw turkey (μg kg^{-1})		composted turkey (μg kg^{-1})	
Chlortetracycline (LOQ: 1.2 μg kg^{-1})										
veg.	Waseca	Staples	Waseca	Staples	Waseca	Staples	Waseca	Staples	Waseca	Staples
before planting	0.1 (±0.0)	0.1 (±0.0)	0.9 (±1.3)	0.7 (±1.0)	0.3 (±0.1)	0.1 (±0.1)	0.2 (±0.1)	0.1 (±0.0)	0.2 (±0.0)	0.1 (±0.1)
after harvest	0.2 (±0.1)	0.1 (±0.0)	0.6 (±0.9)	0.1 (±0.0)	0.6 (±0.3)	0.1 (±0.1)	0.5 (±0.4)	0.1 (±0.0)	0.4 (±0.6)	0.10 (±0.1)
Monensin (LOQ: 9.0 μg kg^{-1})										
veg.	Waseca	Staples	Waseca	Staples	Waseca	Staples	Waseca	Staples	Waseca	Staples
before planting	1.9 (±0.1)	1.1 (±0.1)	18.0 (±25.2)	27.1 (±15.2)	3.2 (±0.3)	1.7 (±0.2)	2.2 (±0.5)	2.8 (±1.1)	3.0 (±0.3)	7.0 (±7.6)
after harvest	2.8 (±1.0)	3.3 (±0.4)	3.3 (±0.7)	3.3 (±0.4)	3.7 (±0.35)	3.7 (±0.6)	3.4 (±1.2)	3.5 (±0.4)	3.2 (±0.9)	3.6 (±0.3)
Sulfamethazine (LOQ: 2.4 μg kg^{-1})										
veg.	Waseca	Staples	Waseca	Staples	Waseca	Staples	Waseca	Staples	Waseca	Staples
before planting	0.8 (±0.0)	1.1 (±0.78)	56.2 (±15.8)	72.6 (±14.7)	19.2 (±4.0)	14.7 (±7.5)	9.2 (±6.5)	11.6 (±8.5)	10.4 (±2.7)	27.4 (±20.7)
after harvest	0.7 (±0.3)	0.5 (±0.09)	21.0 (±13.0)	23.0 (±5.3)	12.0 (±3.6)	5.9 (±1.7)	2.4 (±1.2)	1.4 (±0.9)	5.8 (±1.3)	2.8 (±2.0)
Tylosin (LOQ: 1.2 μg kg^{-1})										
veg.	Waseca	Staples	Waseca	Staples	Waseca	Staples	Waseca	Staples	Waseca	Staples
before planting	0.0 (±0.0)	0.0 (±0.0)	0.0 (±0.0)	0.0 (±0.0)	N.A.	N.A.	0.0 (±0.0)	0.00 (±0.0)	N.A.	N.A.
after harvest	0.2 (±0.2)	0.5 (±0.2)	0.9 (±0.4)	2.4 (±2.6)	N.A.	N.A.	0.5 (±1.2)	0.8 (±1.9)	N.A.	N.A.
Virginiamycin (LOQ: 1.1 μg kg^{-1})										
veg.	Waseca	Staples	Waseca	Staples	Waseca	Staples	Waseca	Staples	Waseca	Staples
before planting	0.7 (±0.2)	0.5 (±0.6)	N.A.	N.A.	N.A.	N.A.	0.8 (±0.4)	3.1 (±0.4)	0.6 (±0.3)	2.8 (±0.9)
after harvest	0.5 (±0.1)	0.2 (±0.1)	N.A.	N.A.	N.A.	N.A.	0.5 (±0.1)	0.3 (±0.2)	0.6 (±0.3)	0.3 (±0.4)

[a]For the samples before planting, soil samples were collected on 05/29/2009 at Waseca and 04/22/2009 at Staples. The antibiotic concentration in the soil at harvest represents an average of all harvest dates for various crops. N.A., not added.

(LOQ) at both planting and at harvest for all antibiotics. Because the plant uptake was very minimal (discussed later), these results suggest that for the most part the antibiotics present in composted and raw manures at the time of their application either degraded or were strongly adsorbed to the soil.

Monensin. Except for raw hog manure, the monensin concentrations in soil at planting were below the LOQ (9.0 μg kg^{-1}). High monensin concentrations in soil at planting for the raw hog manure treatment appear to be due to its higher amount in manure at the time of its application (Table 4). The monensin application in the raw hog manure plot was 303 g ha^{-1}, as compared to 10 to 227 g ha^{-1} for the raw turkey, composted hog, and composted turkey manure plots. The monensin concentrations in soils after raw hog manure application were 18.0 ± 25.2 μg kg^{-1} at the Waseca site and 27.1 ± 15.2 μg kg^{-1} at the Staples site. Higher monensin concentrations in the soil at the Staples site compared to the Waseca site are likely because raw hog manure to all plots at Waseca was applied at one time in the fall. Comparatively, raw hog manure applications at the Staples site occurred in the fall in some plots and then the next spring (closer to the time of planting) in the remaining plots. Delayed (spring) application of raw hog manure at Staples occurred because of poor weather conditions, including soil freezing, in the fall. Even in the spring, raw hog manure applications at Staples occurred over several dates because of limited transport capacity to haul raw hog manure from Waseca to Staples. The monensin concentration in the soil at the time of harvest represents the residual concentration, and this concentration was below LOQ for all treatments (Table 5).

Sulfamethazine. The sulfamethazine concentration in the soil at planting was higher than the LOQ (2.4 μg kg^{-1}) for all treatments (Table 5). At planting, sulfamethazine concentrations in the soil for the raw hog manure treatment were the highest at both sites (56.2 ± 15.8 μg kg^{-1} in the Waseca plots and 72.6 ± 14.7 μg kg^{-1} in the Staples plots), which is likely due to a higher sulfamethazine amount present in raw hog manure at the time of its application (238 g ha^{-1}) (Table 4). Except for the raw turkey manure treatment at both sites, residual (at harvest) sulfamethazine concentrations in soil for other treatments were still higher than the LOQ. This observation is consistent with the slow degradation of sulfamethazine even during composting in this and an earlier study.[24] The highest residual sulfamethazine concentrations detected in the soil at the Waseca (21.0 ± 13.0 μg kg^{-1}) and the Staples (23.0 ± 5.3 μg kg^{-1}) plots were also observed for the raw hog manure treatment.

Chlortetracycline, Tylosin, and Virginiamycin. Concentrations for all three antibiotics in the soil at both sites (Table 5) were mostly below the LOQ (i.e., 1.2 μg kg^{-1} for chlortetracycline, 1.2 μg kg^{-1} for tylosin, and 1.1 μg kg^{-1} for virginiamycin). These lower concentrations in the soil were partially due to their lesser amounts applied with manure (<11.5 g ha^{-1} for chlortetracycline, <16.0 g ha^{-1} for tylosin, and <1.7 g ha^{-1} for virginiamycin; Table 4) and partially due to their rapid degradation, as evident during composting both in this study (Table 3) and in a study by Dolliver et al.[24] An exception to the above observation was the residual tylosin concentration in the raw hog manure treatment at Waseca that was higher than LOQ.

Assessment of Matrix Effects in Plant Sample Analysis. Except for considerably higher standard deviation at higher antibiotic concentrations, the measured and added antibiotic concentrations in the pepper extract from the fertilizer treatment were comparable. Plots of measured versus added antibiotic concentration showed that except for chlortetracycline the matrix effect from pepper extract was statistically absent (slope and intercept were not different than 1 and 0, respectively) for the remaining four antibiotics tested in this study. The coefficient of determination (R^2) for the measured versus added antibiotic concentration plots varied from 0.97 to 1.0 for all five antibiotics tested. The slope of the best fit line for chlortetracycline was statistically different than 1.0 (P = 0.05), indicating some matrix effect. Because the chlortetracycline concentrations in vegetables were mostly below the LOQ, the matrix effect in the ELISA measurement of chlortetracycline in vegetables should not affect the interpretation of the results.

Antibiotic Concentration in Vegetables. Figures 1−5 show the concentration of various antibiotics in different vegetables and vegetable parts. In the case of tylosin, we analyzed only one or two samples of some vegetables. This was mainly because our earlier studies had shown that tylosin degradation was very rapid[24] and we had not detected any uptake of tylosin by plants.[6] In this study, we also did not detect a presence of tylosin in the composted manure samples and thus we did not make any further measurements of tylosin uptake by plants either from the composted hog or composted turkey manure treatments. The detection of antibiotics in the fertilizer treatment over and above LOQ reflects a matrix effect, possibly from other natural chemicals present in the vegetables. The following text describes the uptake of each individual antibiotic by various vegetables.

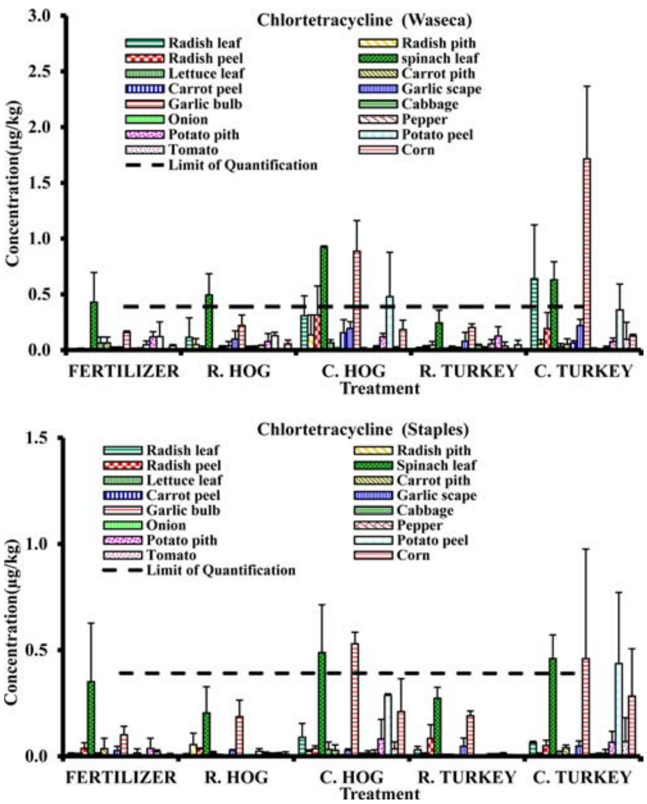

Figure 1. Concentration of chlortetracycline in plant tissue of different vegetable crops for various nutrient-source treatments at Waseca and Staples, Minnesota. Error bars indicate standard deviation of the mean. Prefixes R and C refer to raw and composted hog or turkey manure, respectively. The broken line is the maximum LOQ measured during vegetable analysis.

Chlortetracycline. The highest LOQ value for chlortetracycline during all vegetable testing was 0.4 μg kg^{-1}. At the Waseca site, spinach from the composted hog (P = 0.000) and the composted turkey (P = 0.01) manure treatments was the only vegetable in which the chlortetracycline concentration was higher than the LOQ (Figure 1a). Subsequent testing showed that chlortetracycline concentrations in spinach from both of these treatments were not statistically different (P = 0.05) than the corresponding concentrations from the fertilizer plots. At the Staples site (Figure 1b), spinach (P = 0.033), garlic bulb (P = 0.029), and potato peel (P = 0.003) from the composted hog manure were the only vegetables in which the chlortetracycline concentration was statistically higher than LOQ. Additional testing showed that there was no significant difference (P > 0.05) in the chlortetracycline concentrations in spinach and in potato peel from the composted hog manure treatment versus the fertilizer treatments. However, the chlortetracycline concentration in garlic bulb from the composted hog manure treatment was significantly higher (P = 0.000) than the fertilizer treatment. For both sites, chlortetracycline concentrations in soils at planting and harvest (Table 5) did not exceed their corresponding LOQ (1.2 μg kg^{-1} for chlortetracycline).

Monensin. The highest LOQ value for monensin in all vegetable testing was 3.0 μg kg^{-1}. Except for garlic bulbs (P = 0.002) from the raw turkey manure plots at Waseca, there was no difference in the monensin concentration of the vegetables and the corresponding LOQ value for any of two sites (Figure 2). Subsequent comparison of the monensin concentrations in garlic bulb from the raw turkey manure treatment and the fertilizer treatment did not show any statistical difference (P > 0.05). The absence of monensin in most vegetables is likely due to its degradation, as indicated by its low concentrations in soil

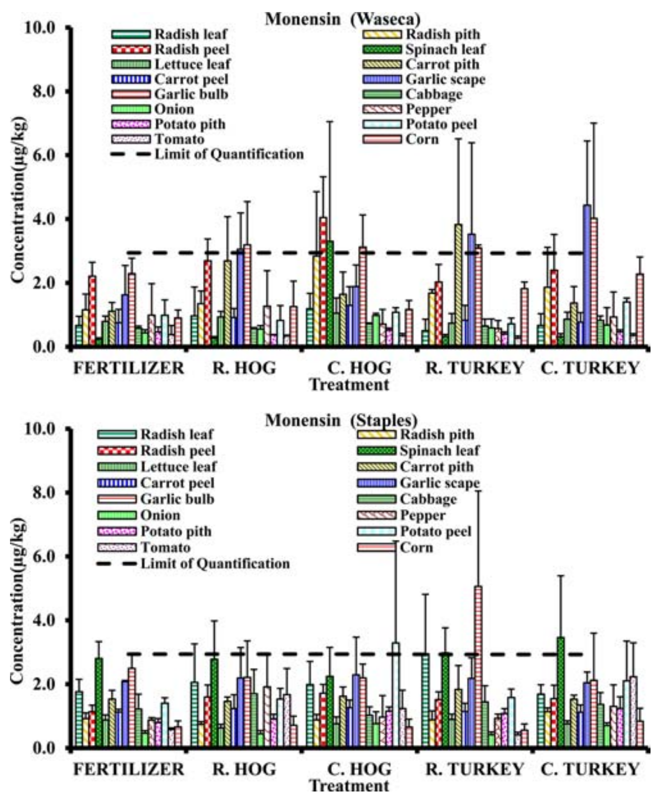

Figure 2. Concentration of monensin in plant tissue of different vegetable crops for various nutrient-source treatments at Waseca and Staples, Minnesota. Error bars indicate standard deviation of the mean. Prefixes R and C refer to raw and composted hog or turkey manure, respectively. The broken line is the maximum LOQ measured during vegetable analysis.

at harvest (2.8 ± 1.0 to 3.7 ± 0.3 μg kg^{-1}; LOQ = 9.0 μg kg^{-1}) (Table 5).

Sulfamethazine. The highest LOQ for sulfamethazine in vegetable testing was 0.8 μg kg^{-1}. Statistically, there was no difference in the sulfamethazine concentration in various vegetables and the corresponding LOQ value at both sites (Figure 3). The lack of significant differences is due to the large

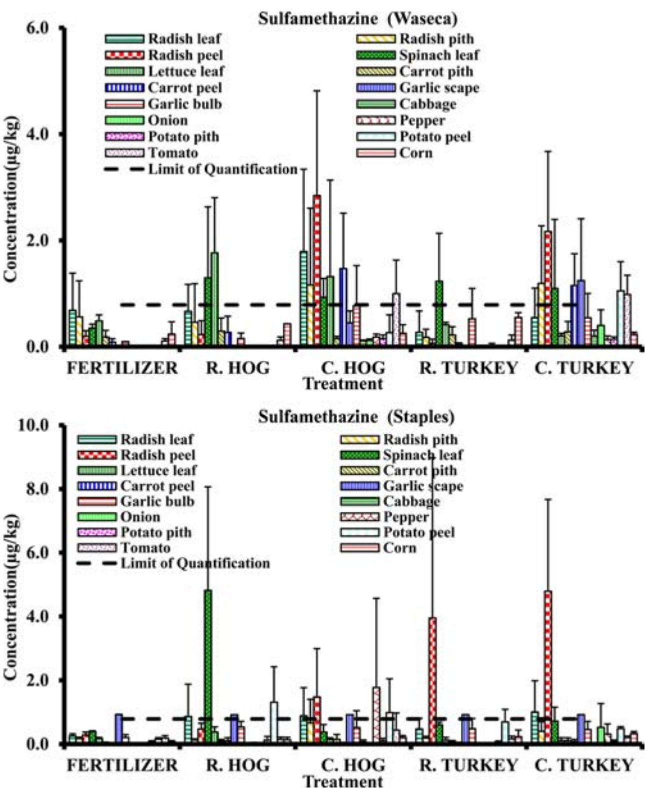

Figure 3. Concentration of sulfamethazine in plant tissue of different vegetable crops for various nutrient-source treatments at Waseca and Staples, Minnesota. Error bars indicate standard deviation of the mean. Prefixes R and C refer to raw and composted hog or turkey manure, respectively. The broken line is the maximum LOQ measured during vegetable analysis.

variability in the measured sulfamethazine concentrations in various vegetable crops. The differences in the sulfamethazine concentration relative to LOQ were greater at the Waseca sites than at the Staple sites. These differences between the sites are likely related to the higher clay content at the Waseca site, which possibly led to an increased presence of sulfamethazine in small pores and also on the soil particles. Although there was a large decrease in the sulfamethazine concentration in the soils from planting to harvest, there was still a significant presence of sulfamethazine in the soil at harvest in the manure treatments (1.4 ± 0.9 to 23.0 ± 5.3 μg kg^{-1}; LOQ = 2.4 μg kg^{-1}) (Table 5). However, this significant presence of sulfamethazine did not result in a significant uptake by the vegetables crops tested in this study.

Tylosin. The highest LOQ value for tylosin during all vegetable testing corresponded to 0.7 μg kg^{-1}. At the Waseca site, tylosin concentrations in all plant tissues (Figure 4a) were statistically ($P > 0.05$) less than the LOQ values. At the Staples site, there were only two vegetables in which the tylosin concentration was higher than the LOQ values (Figure 4b). These vegetables were carrot (peel) ($P = 0.000$) from the raw

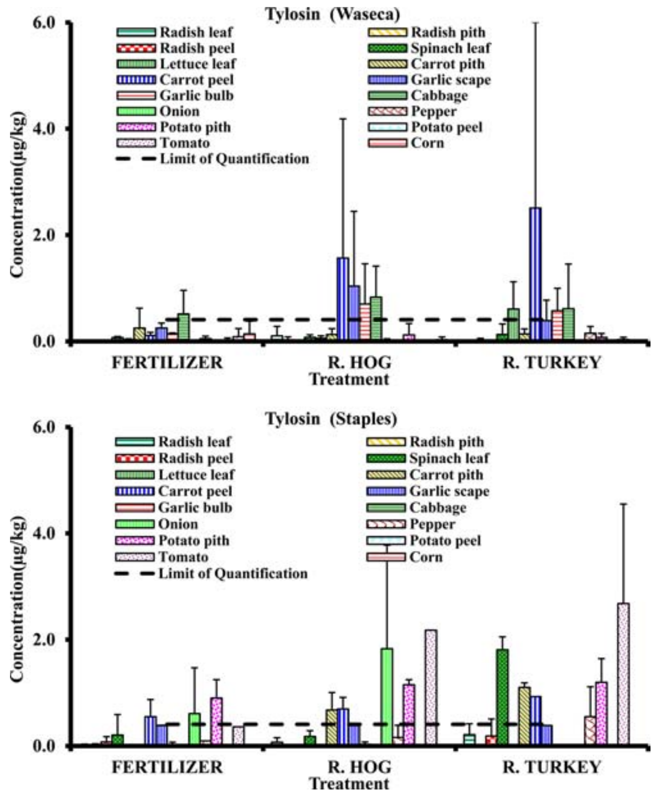

Figure 4. Concentration of tylosin in plant tissue of different vegetable crops for various nutrient-source treatments at Waseca and Staples, Minnesota. Error bars indicate standard deviation of the mean. Prefix R refers to raw hog or turkey manure. The broken line is the maximum LOQ measured during vegetable analysis.

turkey manure treatment and potato pith ($P = 0.004$) from the raw hog manure treatment. Subsequent comparisons between the manure treatments and the fertilizer treatment showed that the tylosin concentration in carrot peel from the raw turkey manure plots and potato pith from the raw hog manure plots were not statistically different ($P > 0.05$) than the corresponding concentrations from the fertilizer treatment. For both sites, the tylosin concentrations in the soils at planting and harvest (Table 5) did not exceed their corresponding LOQ (1.3 μg kg^{-1} for tylosin).

Virginiamycin. The highest LOQ value of virgniamycin in vegetables was 0.4 μg kg^{-1}. At the Waseca site, garlic scapes from both the raw ($P = 0.040$) and composted turkey ($P = 0.029$) manure treatments and corn from the composted turkey manure ($P = 0.007$) treatment were the only vegetables in which the virginiamycin concentration was higher than the LOQ (Figure 5a). Further testing showed that garlic scapes from both the raw ($P = 0.044$) and composted ($P = 0.030$) turkey manure plots were the only vegetables where the virgniamycin concentrations were significantly higher than the corresponding concentrations from the fertilizer treatment. At the Staples site, there was no difference ($P > 0.05$) in the concentration of virginamycin in the vegetables and the corresponding LOQ values (Figure 5b). Except at planting for the Staples sites, virginiamycin concentrations in the soils both at planting and harvest (Table 5) did not exceed their LOQ (1.1 μg kg^{-1}).

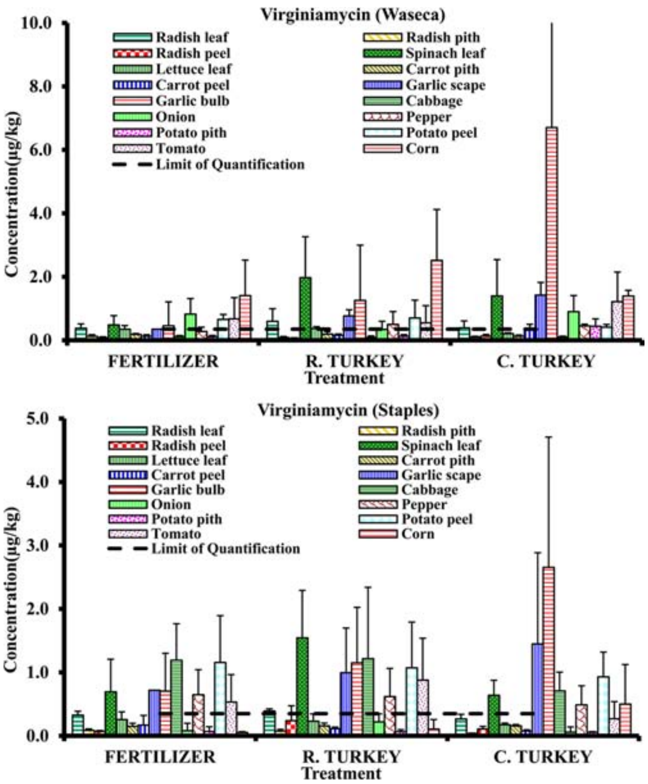

Figure 5. Concentration of virginiamycin in plant tissue of different vegetable crops for various nutrient-source treatments at Waseca and Staples, Minnesota. Error bars indicate standard deviation of the mean. Prefixes R and C refer to raw and composted hog or turkey manure, respectively. The broken line is the maximum LOQ measured during vegetable analysis.

■ DISCUSSION

Antibiotic uptake by 11 different vegetable crops showed that 90 and 83% of the plant tissues had chlortetracycline and monensin concentrations less than the LOQ values, respectively. Comparatively, 76, 76, and 44% of the plant tissues had tylosin, sulfamethazine, and virginiamycin concentrations less than LOQ, respectively. In spite of some vegetable tissue showing an antibiotic concentration above LOQ, these concentrations were generally very low and often not statistically different than the matrix effect measured in vegetables from the fertilizer treatment. Overall, the highest concentration of an antibiotic in any vegetable in our field study was <10 μg kg^{-1}. By considering that there is some matrix effect in ELISA test measurements, the real concentrations are likely even lower. The vegetable antibiotic concentrations measured in this study are similar to or smaller than the concentration reported by others in the literature. Kumar et al. reported a maximum chlortetracycline concentration of 17 μg kg^{-1} of fresh weight in onion, cabbage, and corn leaves grown in loamy sand (10% clay and 2% organic matter) after 6 weeks of growth in a greenhouse study.[6] Dolliver et al. measured a maximum sulfamethazine concentration of 100 μg kg^{-1} of fresh weight in corn, lettuce, and potato leaves grown in sandy loam (10% clay and 4.7% organic matter) after 6 weeks of a greenhouse study.[7] Boxall et al. reported a maximum concentration of 38 μg kg^{-1} of fresh weight for enrofloxacin, florfenicol, and trimethoprim in lettuce and carrots grown in sand (3.6% clay and 0.7% organic matter) after 103 and 152 days, respectively.[8] In the above three studies and the present study, the rate of antibiotic mixing with soil was nearly similar. The slightly lower concentration of antibiotics in the plant tissue in this study could be partially because (1) the soils used in this study had higher clay and organic matter content (Webster clay loam: 34% clay and 5.8% organic matter; Verndale sandy loam: 6.1% clay and 1.8% organic matter) and thus potentially greater binding of antibiotics with soil and organic particles and less availability for plant, (2) this study was conducted under field conditions over a longer time period and thus likely had greater antibiotic degradation, and (3) the rooting volume in the field is much greater than in a pot and thus the roots may have explored deeper parts of the soil that was not exposed to antibiotics. Herklotz et al. reported higher concentrations of carbamazepine (99 μg kg^{-1}), salbutamol (114.7 μg kg^{-1}), sulfamethoxazole (138.3 μg kg^{-1}), and trimethoprim (91.3 μg kg^{-1}) in Chinese cabbage, which is likely due to the feeding of plants under hydroponic conditions with a constant supply of these pharmaceuticals at 232.5 μg L^{-1}.[9] The absence of azithromycin (macrolide), clindamycin (lincosamides), and roxithromycin (macrolide) in lettuce, spinach, and carrots at concentrations <1000 ng L^{-1} in the Jones-Lepp et al. study[10] is likely a reflection of the low concentration of these antibiotics in the spiked Colorado River irrigation water and is possibly due to rapid degradation of macrolides as shown by Dolliver et al.[24] as well as the present study.

Several organizations have established acceptable daily intake (ADI) values for some of the veterinary pharmaceuticals. These include the United Nations' Food and Agriculture Organization in collaboration with World Health Organization (FAO-WHO), Australian Health authority, and the Drinking Water Authority in Germany.[29] The ADI values are the levels that can be ingested daily over a lifetime without any health risks.[7] For regulatory purposes, maximum residue levels (MRLs) in animal tissue have also been established, and these values are less than 1.2 mg per kilogram of raw weight of animal tissue. The minimum ADI value given is 10 μg kg^{-1} of body weight per day (Table 6). Assuming the body weights of an average adult vary from 50 to 75 kg, the total permissible intake will be equivalent to 500−750 μg of pharmaceutical per day. Because the maximum concentration of any antibiotic in vegetable produce in this study was less than 10 μg kg^{-1} fresh weight, our results suggest that one would need to consume 50−75 kg of produce every day to reach the recommended ADI values. These simple calculations suggest that antibiotic concentrations in various

Table 6. Antibiotic Daily Intake and Maximum Residue Limit of Five Antibiotics Used in This Study

antibiotic	ADI (μg kg^{-1} body weight per day)	maximum residue limit (MRL) in animal tissue (μg kg^{-1})
Chlortetracycline	30[a]	100−1200[a]
Monensin	10[a]	2−100[a]
Sulfamethazine		100[b]
Tylosin	30[a]	100−300[a]
Virginiamycin		100−400[c]

[a]Food and Agriculture Organization of the United Nations MRL for veterinary drugs. http://www.fao.org/ag/agn/jecfa-vetdrugs/search.html (accessed 1 May, 2013). [b]MRL. http://www.dsm.com/le/nl_NL/delvotest/downloads/LesRisquesDeResidus_extrait2_En.pdf (accessed 1 May, 2013). [c]MRL. http://www.legislation.gov.hk/blis_ind.nsf/CURALLENGDOC/4D1FA97EA098B39C48256A7F001792E0?OpenDocument (accessed 1 May, 2013).

dx.doi.org/10.1021/jf404045m | J. Agric. Food Chem. 2013, 61, 9992−10001

vegetable crops measured in this study do not pose a major health concern for most adults unless one is allergic to that particular pharmaceutical. Because most antibiotics degrade over time, a practical guide for organic producers who use conventional manure as a source of plant nutrients will be (1) to apply composted manure rather than fresh manure and/or (2) to let the manure sit in the soil for as long as possible before planting vegetables in the spring. This extra time will help degrade the antibiotics and thus lower their concentration for plant uptake.

■ ASSOCIATED CONTENT

Supporting Information

Details on the composting of hog and turkey manures, mixing of antibiotics in manure, plot design for the field study, field manure and fertilizer application rates, vegetables varieties and planting procedures, and a bioassay to test whether sulfamethazine was biologically active in vegetable extracts. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors gratefully acknowledge the funding for this project from the North Central Region Sustainable Agriculture Research and Education (NCR-SARE) program. Earlier work on antibiotic uptake by vegetables by Dr. Kuldip Kumar and subsequently by Dr. Holly Dolliver and Dr. Kumar form the basis for this project. The PIs are grateful to Drs. Kumar and Dolliver for laying the foundation for this project. There were several individuals who helped the PIs in their field and laboratory work. We are thankful to Josh Worely for his help in vegetable harvest and in laboratory processing of vegetable produce; Curt Miller, Scott Coy, Sue Schoenfeld, Kraig Deling, and Gabriel Malima for their help with the composting of manure, field set up, and maintenance of vegetable plots at the Southern Research and Outreach Center at Waseca; and Bob Schafer, Michelle Johnson, Matt McNearney, Anina Christensen, Brendan Ward, and Anubha Garg for their help with plot set up and other field work at the Central Lakes Irrigation Center at Staples. S.G. also acknowledges helpful discussions with Mr. Tom Halbach on manure composting.

■ ABBREVIATIONS USED

ADI, acceptable daily intake; BMD, bacitracin methylene disalicylate; BPW, buffered peptone water; CT, composted turkey manure; CTC, chlortetracycline; ELISA, enzyme-linked immunosorbent assay; Fer, fertilizer; HPLC, high-performance liquid chromatography; LOD, limit of detection; LOQ, limit of quantification; OD, optical density; RH, raw hog manure; SROC, Southern Research and Outreach Center; RT, raw turkey manure; TKN, total Kjeldahl nitrogen

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