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Sulfamethazine Uptake by Plants from Manure-Amended Soil

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ABSTRACT

Animal manure is applied to agricultural land as a means to provide crop nutrients. However, animal manure often contains antibiotics as a result of extensive therapeutic and subtherapeutic use in livestock production. The objective of this study was to evaluate plant uptake of a sulfonamide-class antibiotic, sulfamethazine, in corn (*Zea mays* L.), lettuce (*Lactuca sativa* L.), and potato (*Solanum tuberosum* L.) grown in a manure-amended soil. The treatments were 0, 50, and 100 μg sulfamethazine mL^{-1} manure applied at a rate of 56 000 L ha^{-1}. Results from the 45-d greenhouse experiment showed that sulfamethazine was taken up by all three crops, with concentrations in plant tissue ranging from 0.1 to 1.2 mg kg^{-1} dry weight. Sulfamethazine concentrations in plant tissue increased with corresponding increase of sulfamethazine in manure. Highest plant tissue concentrations were found in corn and lettuce, followed by potato. Total accumulation of sulfamethazine in plant tissue after 45 d of growth was less than 0.1% of the amount applied to soil in manure. These results raise potential human health concerns of consuming low levels of antibiotics from produce grown on manure-amended soils.

Antibiotics are extensively used in animal agriculture as growth promoters or to reduce the risk of disease outbreak in large-scale animal confinement operations. Until recently, the major concerns with agricultural antibiotic usage have been their presence in animal-based food products, the development and spread of antibiotic resistant bacteria, and the transport to aquatic environments from lands amended with antibiotic-laden manure. There is a growing concern that antibiotics may be taken up by food crops and make there way into food supply systems (Kumar et al., 2005a; Boxall et al., 2006).

Antibiotic concentrations in manure range from trace levels to greater than 200 mg kg^{-1} or L^{-1}, with typical concentrations in the 1 to 10 mg kg^{-1} or L^{-1} range (Kumar et al., 2005b). An estimated 132 million metric tons (dry weight) of manure is produced annually from cattle, swine, and poultry production in the USA (USDA-ERS, 2005), which is applied to approximately 9.2 million hectares of land (USDA-NASS, 2005). Once manure is applied to agricultural land, crops are exposed to antibiotics because they can persist in soils from a few to several hundred days depending on the antibiotic compound, sorption interactions with soil, and environmental conditions (Tolls, 2001; Kumar et al., 2005b; Thiele-Bruhn, 2003).

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The majority of research on the impact of antibiotic exposure to plants has focused on evaluating phytotoxicity (Batchelder, 1981, 1982; Bradel et al., 2000; Jjemba, 2002). Migliore et al. (1995, 1996) found that sulfadimethoxine concentrations of 300 mg L^{-1} in agar and soil-based laboratory systems significantly reduced root, stalk, and leaf growth in millet (*Panicum miliaceum*), pea (*Pisum sativum* L.), corn, and barley (*Hordeum vulgare*). These authors identified bioaccumulation as the mechanism causing the phytotoxic response. In their studies, as high as 1000 and 2000 mg sulfadimethoxine kg^{-1} dry weight was reported in foliage and root materials, respectively, for 10- to 20-d agar-based laboratory trials. Bioaccumulation in soil-based experiments was an order of magnitude lower than in agar-based experiments (Migliore et al., 1996).

A bioaccumulation-focused study by Kumar et al. (2005a) evaluated plant uptake of chlortetracycline and tylosin in cabbage (*Brassica oleracea* L.), corn, and green onion (*Allium cepa* L.) from manure-amended soil with antibiotic concentrations ranging from 25 to 125 mg kg^{-1} manure. These authors found chlortetracycline uptake between 0.002 and 0.017 mg kg^{-1} fresh weight; however, tylosin was not taken up by these food crops. The authors speculated that the large size of the tylosin molecule possibly prohibited mass flow or active uptake. Similarly, a study by Boxall et al. (2006) evaluated plant uptake of seven antibacterials in lettuce and carrot (*Daucus carota*) tissues from a sandy soil spiked at a concentration of 1 mg antibiotic kg^{-1} soil. Florfenicol and trimethoprim were detected in lettuce leaves, whereas enrofloxacin, florfenicol, and trimethoprim were detected in carrot. Concentrations ranged from approximately 3 to 38 μg kg^{-1} fresh weight.

The major concern surrounding antibiotic uptake by plants is contamination of the food supply and associated health risks. Although health implications of antibiotic residues in plant-based products are largely unknown, several potential adverse impacts include allergic/toxic reactions, chronic toxic effects as a result of prolonged low-level exposure, the development and spread of antibiotic-resistant bacteria, and disruption of digestive system functioning (Kumar et al., 2005a; Doyle, 2006).

Acceptable daily intake (ADI) values have been established for veterinary pharmaceuticals (JEFCA, 2006). The ADI value indicates the level of a chemical that can be ingested daily over a lifetime without health risk. For most antibiotic compounds, ADI values are less than 50 μg kg^{-1} body weight per day (JECFA, 2006). For regulatory purposes, maximum residue levels (MRLs) have been established for food products. In general,

Abbreviations: ADI, acceptable daily intake; ELISA, enzyme-linked immunosorbent assay; HPLC, high-performance liquid chromatography; MRL, maximum residue limit.

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MRLs for antibiotics in animal tissues are below 1 mg kg^{-1} fresh weight (JEFCA, 2006). Although MRLs are set for various animal-based food products, limits have not been established for plant-based products.

The objective of this research was to evaluate plant uptake of sulfamethazine by food crops. Sulfamethazine (Fig. 1), a sulfonamide-class antibiotic, is extensively used in animal agriculture for therapeutic and subtherapeutic purposes. An estimated 400 tons of sulfamethazine is used annually as a feed additive for cattle and swine production in the USA (Mellon et al., 2001). Relative to other antibiotic compounds, sulfamethazine has a low molecular weight and is not strongly sorbed to soil particles, which may facilitate uptake in plant tissues.

MATERIALS AND METHODS

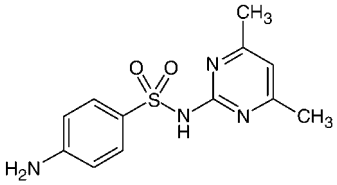
Experimental Design

Sulfamethazine uptake by plants was tested in a greenhouse study involving three crops and three antibiotic treatments. The crops were corn, lettuce, and potato, and the antibiotic treatments were control (no antibiotic addition), 50 mg sulfamethazine L^{-1} manure, and 100 mg sulfamethazine L^{-1} manure. The experiment was established as a randomized, complete-block design with two replications. To each 4-L plastic container (diameter, 21 cm; length, 19 cm), 4 kg of an agronomic soil mixture (33% topsoil, 33% sand, 28% peat, and 6% compost) was added. The topsoil in the mixture was Waukegan silt loam (Typic Hapludolls) taken from the St. Paul campus of the University of Minnesota. Textural analysis of the resulting mixture was 71% sand, 18% silt, and 10% clay. Organic matter content and pH of the soil mixture were 4.7% and 7.0, respectively. Swine manure was obtained from a local farmer and showed no presence of sulfamethazine. For antibiotic treatments, a known amount of pure sulfamethazine (Sigma Aldrich, St. Louis, MO) was dissolved in a minimal (<5 mL) amount of water and mixed with swine manure. Manure was applied at 100 mL pot^{-1}, equivalent to a typical manure application rate of 56 000 L ha^{-1}. Manure was mixed with soil to an approximate depth of 15 cm to simulate manure incorporation or injection. These experimental parameters (sandy soil, high antibiotic concentration, and raw manure application) were selected to simulate a worse-case scenario.

Corn and lettuce were planted from seeds (6 corn seeds pot^{-1} and ~50 lettuce seeds pot^{-1}), and potato was planted from seed tubers (4 tubers pot^{-1}). Crops were grown in a greenhouse with 24-h temperature maintained between 18 and 23°C. Artificial lighting was maintained for 10 h during the daytime. On average, plants were watered three times weekly. Approximately one half of potato tubers emerged, whereas a majority of the lettuce emerged. Corn emergence was generally low (<2 plants pot^{-1}) across all treatments. Additional corn seeds were planted in every corn pot during the third week of the experiment. During the sixth week of the experiment, two corn plants (V1 stage) were transplanted into one 50-mg and one 100-mg sulfamethazine L^{-1} manure replication.

After 45 d, plants were harvested approximately 2 cm above the soil surface, washed with nanopure water, and dried with adsorbent papers. After finely chopping the fresh plant materials from each pot, total fresh weight, moisture content, and total dry weight was determined (Table 1). Along with plant tissues, one intact potato seed tuber (approximately 60 g), including roots, was collected from a 100 mg sulfamethazine L^{-1} manure potato treatment, washed with nanopure water, and dried with adsorbent papers. Along with roots, three parts from the potato seed tuber were obtained using a small stainless steel knife: skin, inner material (approximately 5 mm from skin), and center material (approximately 15 mm from skin). Depending on the available quantity, between 1 and 3 g fresh weight of root and tuber material was used for analysis. Along with plant materials, soil samples from a control, 50 mg sulfamethazine L^{-1} manure, and 100 mg sulfamethazine L^{-1} manure treatment were also analyzed for sulfamethazine.

Molecular weight:	278.33†
Solubility:	1.9 g L^{-1}†
K_{ow}:	0.89‡
K_d:	0.6-3.1‡



† Source: O'Neil et al., 2001
‡ Source: Tolls, 2001

Fig. 1. Chemical structure and characteristics of sulfamethazine (O'Neil et al., 2001).

Antibiotic Analysis

Sample Preparation

Sulfamethazine uptake in plant tissues was primarily evaluated using enzyme-linked immunosorbent assay (ELISA) analysis, with secondary confirmation by high-performance liquid chromatography (HPLC). For ELISA analysis, two different mild extractions were performed using 5 g of fresh plant material from each pot. The first extraction was performed using 20 mL of buffered peptone water, and the second extraction was performed using 20 mL of a salt-based extractant suggested by the ELISA kit manufacturer (0.96 g sodium phosphate, 0.17 g potassium phosphate, 9 g sodium chloride, and 0.5 mL tween 80 suspended in 1000 mL of nanopure water). Due to limited sample mass, root and tuber materials were extracted with 20 mL of the salt-based extractant only. Soil samples (1 g) were extracted using 30 mL of buffered peptone water. Samples were shaken on a rotary shaker for 12 h at 4°C, vortexed for 2 min, and centrifuged for 15 min, and the supernatant was collected for antibiotic analysis.

Unlike ELISA analysis, HPLC analysis is generally not sensitive to harsh extractants. For HPLC analysis, plant sample extraction was modified from the procedure outlined by

Table 1. Average plant fresh weight, moisture content, and dry weight ± SD by treatment.

Parameter	Avg. fresh weight	Moisture content	Avg. dry weight
	g	g g^{-1}	g
Corn			
Control	42.3 ± 5.6	0.87 ± 0.04	5.9 ± 0.8
50 mg L^{-1}	7.0 ± 1.9	0.93 ± 0.01	0.5 ± 0.2
100 mg L^{-1}	22.9 ± 28.7	0.91 ± 0.07	3.1 ± 4.2
Lettuce			
Control	50.2 ± 6.6	0.92 ± 0.02	4.2 ± 0.4
50 mg L^{-1}	50.7 ± 5.3	0.93 ± 0.01	3.8 ± 1.0
100 mg L^{-1}	70.1 ± 0.5	0.94 ± 0.01	4.2 ± 0.3
Potato			
Control	99.8 ± 41.4	0.91 ± 0.01	9.2 ± 4.4
50 mg L^{-1}	138.7 ± 1.3	0.92 ± 0.00	10.9 ± 0.1
100 mg L^{-1}	156.5 ± 27.8	0.92 ± 0.00	12.8 ± 2.5

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Migliore et al. (1996). First, 500 mg of dried and crushed plant material was extracted with 8 mL methanol/HCl (95:5), manually shaken for 5 min, and centrifuged for 15 min, and the supernatant was collected. The remaining pellet was extracted with 5 mL of acetone using the same procedure as that of the methanol/HCl. Supernatants were pooled and dried under a stream of N_2. The residue was resuspended in 1 mL methanol/nanopure water (50:50) and defatted with 1 mL hexane three times. Hexane was removed each time after liquid–liquid partition. The remaining liquid was dried under a stream of N_2 to 1.5 mL for solid phase extraction with an OASIS HLB 500 mg (Waters Corporation, Milford, MA) cartridge. The cartridge was preconditioned with 10 mL of methanol and 10 mL of nanopure water. The sample was passed through the cartridge at a flow rate of 5 mL min^{-1} and eluted with 2 mL of methanol.

Enzyme-Linked Immunosorbent Assay Antibiotic Analysis

Analysis was conducted using a commercially available sulfamethazine ELISA kit (Abraxis LLC, Warminster, PA). The kit consisted of microtitre plates precoated with sheep antibodies to rabbit immunoglobulin G. After addition of standards (0–10 μg L^{-1}) and samples (50 μl), enzyme conjugate (25 μl) and rabbit antisulfamethazine antibody (25 μl) solutions were added. Rabbit antisulfamethazine antibodies were bound by the precoated immobilized rabbit antibodies. At the same time, free sulfamethazine in the standards or samples and enzyme conjugate competed for rabbit antisulfamethazine antibody binding sites. After 1 h incubation at 4°C, the solutions were discarded, and the nonbound enzyme conjugate was removed by a series of washings. A chromogen substrate (100 μl) was added, which was transformed into a colored product by bound enzyme. This reaction was stopped after 30 min by the addition of sulfuric acid (100 μl). Color intensity (optical density) was measured photometrically at 450 nm.

For quantification, the mean optical density for each of the standards was subtracted from the mean optical density of the zero standard and divided by the mean optical density of the zero standard to obtain a maximal absorbance percentage. Linear regression analysis was used to develop a relationship between percent maximal absorbance and sulfamethazine concentration. This relationship was used to determine sulfamethazine concentration in the plant materials and soil samples. The standard curve was log-linear in the range of 0 to 10 μg L^{-1}, with a $R^2 > 0.99$.

High-Performance Liquid Chromatography Antibiotic Analysis

Sulfamethazine presence in selected plant samples was confirmed using HPLC. A Beckman Coulter System Gold 128 Solvent Module equipped with a UV-VIS diode array detector was used for HPLC analysis. An Adsorbosphere OPA HR, 150 × 4.6 mm, 5 μm column (Alltech Associates, Inc., Deerfield, IL) was used for analyte separation. Mobile phase A was 0.4% formic acid in nanopure water, and mobile phase B was HPLC grade pure methanol. Mobile phase (A:B) was run at a linear gradient of 90:10 to 60:40 from 0 to 20 min with a flow rate of 1 mL min^{-1}. Sample injection volume was 20 μl.

Statistical Analysis

Data were evaluated using Statistical Analysis Systems software (SAS Institute, 2004). Summary statistics were obtained using the PROC UNIVARIATE procedure. The PROC TTEST procedure was used to compare sample means. Treatment differences were evaluated using the PROC GLM procedure. Statistical significance for all procedures was tested at a probability level of 0.05. Unless stated, analysis and results are reported on a dry weight basis due to differences in plant moisture content among crops, treatments, and replications (Table 1).

RESULTS

Sulfamethazine Uptake

Sulfamethazine was taken up by all three food crops from manure-amended soil containing sulfamethazine (Fig. 2). As a result of large within-treatment variation, statistical analysis showed no significant difference ($p \gg 0.05$) between the performance of the buffered peptone water and salt-based extractant for all treatments (data not shown); therefore, treatment results (Fig. 2 and 3) are averages over both extractants. A

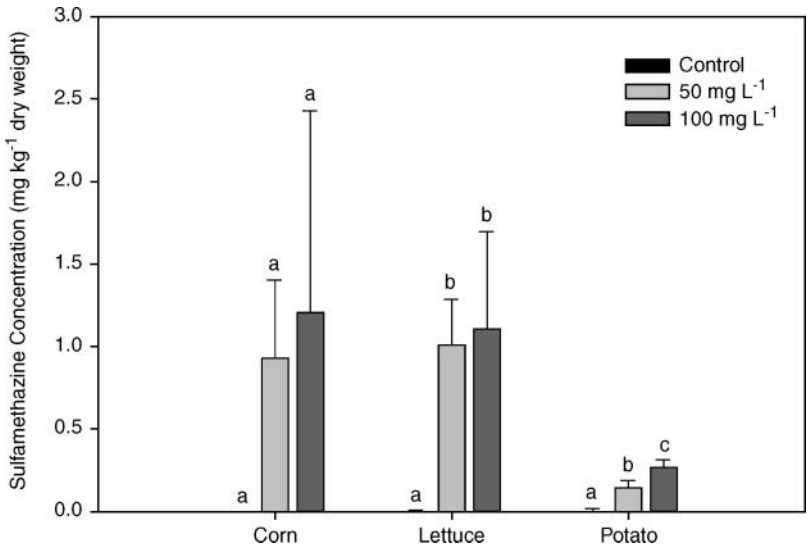

Fig. 2. Sulfamethazine concentration in plant tissue by crop and antibiotic treatment. Error bars indicate SD of the mean. Within crop type, different letters designate statistical significance at the 0.05 probability level.

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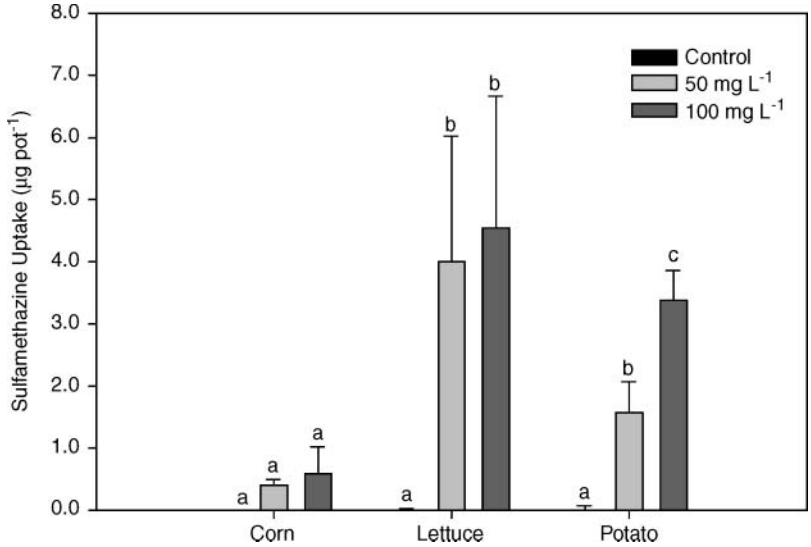

Fig. 3. Sulfamethazine uptake by crop and antibiotic treatment. Error bars indicate SD of the mean. Within crop type, different letters designate statistical significance at the 0.05 probability level.

small matrix effect (<2 μg sulfamethazine kg^{-1} fresh weight) was detected in the control plant samples, which was normalized to zero using the average value for each food crop. This matrix effect adjustment was also made to plant sample results for the two sulfamethazine treatments by subtracting the control values for each crop.

Sulfamethazine concentration in plant tissue was higher in manure-amended soils than in the control treatments; however, these differences were only statistically significant for lettuce and potato. Sulfamethazine concentration in plant tissue increased with increasing concentration in manure (Fig. 2); however, this trend was only statistically significant for potato. Increasing sulfamethazine concentration in manure from 50 mg L^{-1} to 100 mg L^{-1} increased sulfamethazine concentration in lettuce, corn, and potato tissue by 9, 30, and 88%, respectively.

The statistical nonsignificant differences in sulfamethazine concentrations between treatments for the corn crop were a result of high variability in emergence and growth (Table 1). However, our results clearly show uptake of sulfamethazine by corn plants from pots that were amended with sulfamethazine-containing manure. Although initial reseeding of corn plants took place early in the experiment (week 3), transplanting was done near the end of the experiment (week 6). Sulfamethazine concentration in transplanted plants varied from 150 to 1000% over and above (>1 mg kg^{-1}) that of the nontransplanted plants (<0.6 mg kg^{-1}). This may be due to low plant biomass, fine root structure, intense nutrient requirements, or high exudation of soluble organic carbon compounds from young plants. It has been hypothesized that soluble carbon may increase availability and ultimately increase uptake of antibiotic compounds (Tolls, 2001).

Total uptake of sulfamethazine after 45 d was highest for lettuce, followed by potato and corn, and increased with increasing concentration of sulfamethazine in manure (Fig. 3). Without assessing the relationship between concentration and biomass over time, it is difficult to evaluate or compare antibiotic uptake ability between crops. Increasing sulfamethazine concentration in manure from 50 mg L^{-1} to 100 mg L^{-1} increased total sulfamethazine uptake in lettuce, corn, and potato by 14, 45, and 117%, respectively. Similar to concentration results, this trend was only statistically significant for potato. Despite limited growth and low biomass, total uptake for transplanted corn was between 25 and 150% of nontransplanted plants, thus suggesting a high rate of antibiotic uptake during early growth stages. Overall, the recovery of sulfamethazine in plant tissues was less than 0.1% of the sulfamethazine applied in manure for all crops and treatments.

Along with plant tissues, sulfamethazine was detected in the planted potato seed tuber extracted from one of the 100 mg sulfamethazine L^{-1} manure treatments (Fig. 4). For the potato seed tuber, the highest concentration of sulfamethazine was observed in roots and skin materials (>1 mg kg^{-1}). Sulfamethazine may have been adsorbed to the outside of root and skin materials due to contact with soil particle.

At the conclusion of the experiment, soil samples were collected for sulfamethazine analysis to assess potential degradation during the experiment. The water-extractable concentration of sulfamethazine in the soil samples was 2.4 and 3.6 mg kg^{-1} for the 50 and 100 mg sulfamethazine L^{-1} manure treatments, respectively. Assuming that the top 50% of the soil in the pot was equally mixed with the manure/antibiotic, these concentrations suggest that a majority of sulfamethazine (>70%) remained in the soil.

High-Performance Liquid Chromatography Antibiotic Analysis

The HPLC analysis confirmed sulfamethazine presence in selected plant samples. Average retention time for sulfamethazine was 16.3 ± 0.1 min. A considerable

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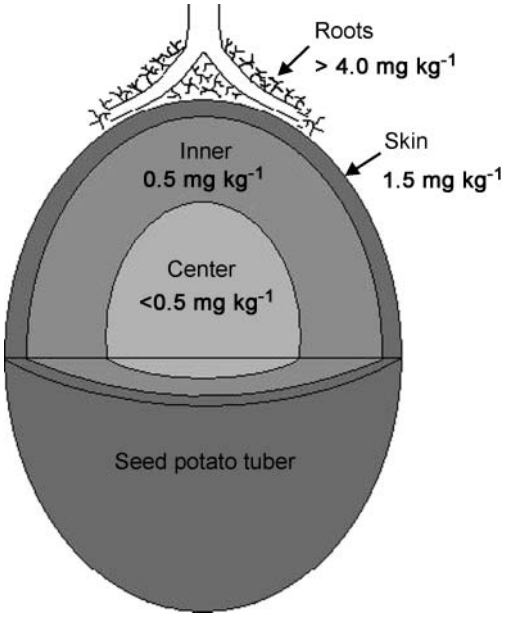

Fig. 4. Sulfamethazine concentration in the potato seed tuber.

amount of background noise and interference was present in plant samples despite extensive clean-up procedures (Fig. 5A). To validate the sulfamethazine peak for the sample represented in Fig. 5A (lettuce, 100 mg sulfamethazine L^{-1} manure treatment), the sample was spiked with pure sulfamethazine (Fig. 5B). Although background noise and interference limit accurate quantification, sulfamethazine concentration in the sample represented in Fig. 5A is greater than 3 mg kg^{-1} dry weight, which is more than 100% greater than ELISA results for the same sample. This suggests that the mild water-based extraction for the ELISA analysis was not sufficient to extract all of the sulfamethazine present in the plant samples.

DISCUSSION

Antibiotic analysis using the ELISA methodology offers a rapid, low-cost assessment of antibiotic presence and concentration in plant materials. However, comparisons of ELISA and HPLC results suggest that mild extractants commonly used in ELISA analysis may not be sufficient to fully extract antibiotics from plant materials. On the other hand, harsh extractants (solvents, acids, bases, etc.) may not be compatible with ELISA analysis. Typically, HPLC analysis is more costly and time consuming and requires optimization and extensive sample processing to minimize background noise and enhance signal.

Sulfamethazine concentrations in plant tissue from this study (0.008–0.100 mg kg^{-1} fresh weight) were higher than chlortetracycline results (0.002–0.017 mg kg^{-1} fresh weight) reported by Kumar et al. (2005a) and were higher than enrofloxacin, florfenicol, and trimethoprim results (0.003–0.038 mg kg^{-1} fresh weight) reported by Boxall et al. (2006). However, concentrations of a similar sulfonamide class compound, sulfadimethoxine, reported by Migliore et al. (1996) in barley were considerably greater (11–19 mg kg^{-1} dry weight) than concentrations of sulfamethazine reported in this study (0.1–1.2 mg kg^{-1} dry weight), which is likely a result of high initial sulfadimethoxine concentration in soil (110 mg sulfadimethoxine kg^{-1} soil) for the Migliore et al. (1996) study. There is limited understanding of the interactions of antibiotic concentrations in manure/soil, antibiotic chemical characteristics, specific crop, plant growth stage, and plant physiology on plant uptake of antibiotics, which makes it difficult to compare and evaluate results from different studies.

An interesting aspect of this research was the detection of sulfamethazine in the existing potato tuber. During potato growth, nutrients and water are provided to the plant by the roots or the seed tuber itself; therefore, active nutrient or water uptake by the seed tuber is not necessary for plant growth and is likely not a mechanism for sulfamethazine accumulation in the seed tuber. We speculate that diffusion through the peel resulted in sulfamethazine accumulation in the potato seed tuber and the decreasing trend in concentration from the skin to the center of the potato. A recent study by Boxall et al. (2006) also found higher antibiotic accumulation in carrot peels than in the whole carrots. These results suggest antibiotic accumulation may be of particular concern for edible root and tuber crops that are directly exposed to soil containing antibiotics (i.e., root and tuber crops).

Although some plant-based materials are consumed raw, limited information is available on the effect of cooking on antibiotic concentrations in plant-based (or animal) products. A study by Rose et al. (1995) found that sulfamethazine was stable for 6 h in boiling water but not in hot oil ($t_{1/2}$ = 120 min at 180°C and 5 min at 260°C). Sulfamethazine spiked into raw swine meat was also found to be stable during a variety of common cooking processes (casseroling, roasting, grilling, pressure cooking, microwaving, and frying). Conversely, oxytetracycline was not stable in water, oil, or cooking processes (Rose et al., 1996).

Although antibiotics are not regulated in plant-based products, the maximum residue level for sulfamethazine in animal-based products has been established at 0.1 mg kg^{-1} fresh weight (JEFCA, 2006). In this study, fresh weight concentrations were typically below this level; average fresh weight concentration across all crops for antibiotic treatments was less than 0.05 mg kg^{-1}. However, in a few instances, fresh weight concentrations were near this level. Dry weight concentrations of sulfamethazine for antibiotic treatments exceeded the 0.1 mg kg^{-1} level. Unlike animal-based products, fresh and dried plant materials are used in processed food products, making it important to account for fresh and dry weight concentrations.

Do these sulfamethazine residues in plant tissues pose a human heath risk? The established ADI value for sulfamethazine is 5 mg kg^{-1} body weight (JEFCA, 2006). It is estimated that a typical adult consumes approximately 0.6 kg of fresh and processed cereal, pulse, and vegetable crops on a daily basis (WHO, 2003). Assuming a strictly plant-based diet and using the maximum fresh

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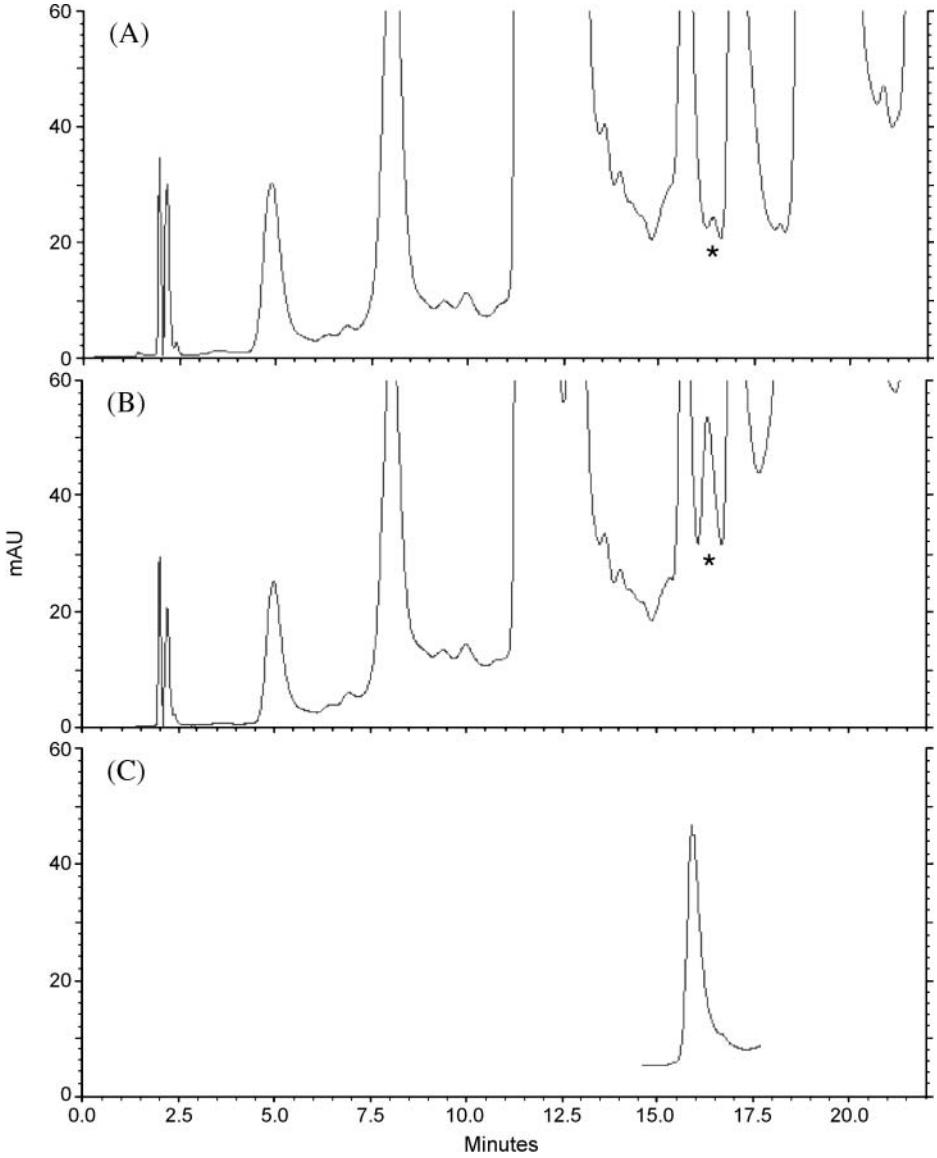

Fig. 5. High-performance liquid chromatography chromatogram (A) plant sample, (B) spiked plant sample, and (C) sulfamethazine standard. Asterisk (*) indicates sulfamethazine peak.

weight concentration for sulfamethazine observed in plant tissue in this study (0.1 mg kg^{-1}), daily intake is considerably below the ADI value. However, these ADI values do not account for issues such as development and spread of antibiotic resistance, which is a major problem globally.

Although antibiotics in plant tissues do not seem to be a major health risk, antibiotic uptake by plants may be of particular concern to organic crop producers. Because synthetic fertilizers are not permitted for use in organic farming, manure is often an important source of crop nutrients. According to the United States Department of Agriculture National Organic Program regulations (§205.203), producers must manage animal materials (i.e., manure) in a manner that "…does not contribute to contamination of crops, soils, or water by…residues of prohibited substances" (USDA-NOP, 2006), which includes antibiotic compounds. To our knowledge, there is no current plan or standardized methodology for monitoring antibiotics in animal manure, which is often obtained from nonorganic farms where antibiotics are commonly used.

CONCLUSIONS

In this study, uptake of sulfamethazine was demonstrated in corn, lettuce, and potato plants. Using ELISA analysis, concentration of sulfamethazine in plant tissues ranged from 0.1 to 1.2 mg kg^{-1} dry weight. Sulfamethazine concentration in plant tissue increased with increasing concentration in manure-amended soil. Overall, less than 0.1% of applied sulfamethazine was accumulated in plant materials, with greater than 70% remaining in the soil. More research is needed (i) to evaluate the effects of crop type, crop physiology, and growth stage on antibiotic uptake; (ii) to determine

whether specific antibiotic physical or chemical characteristics affect plant uptake ability; (iii) to evaluate the fate of antibiotics in plant materials; (iv) to assess human health implications of antibiotic exposure from plants; and (v) to validate experimental data with organic contaminant plant uptake models (e.g., Paterson et al., 1990).

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