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Antibiotic Uptake by Plants from Soil Fertilized with Animal Manure

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ABSTRACT

Antibiotics are commonly added to animal feed as supplements to promote growth of food animals. However, absorption of antibiotics in the animal gut is not complete and as a result substantial amounts of antibiotics are excreted in urine and feces that end up in manure. Manure is used worldwide not only as a source of plant nutrients but also as a source of organic matter to improve soil quality especially in organic and sustainable agriculture. Greenhouse studies were conducted to determine whether or not plants grown in manure-applied soil absorb antibiotics present in manure. The test crops were corn (*Zea mays* L.), green onion (*Allium cepa* L.), and cabbage (*Brassica oleracea* L. Capitata group). All three crops absorbed chlortetracycline but not tylosin. The concentrations of chlortetracycline in plant tissues were small (2–17 ng g^{-1} fresh weight), but these concentrations increased with increasing amount of antibiotics present in the manure. This study points out the potential human health risks associated with consumption of fresh vegetables grown in soil amended with antibiotic laden manures. The risks may be higher for people who are allergic to antibiotics and there is also the possibility of enhanced antimicrobial resistance as a result of human consumption of these vegetables.

Since their discovery in the early 1900s, antibiotics have been instrumental in treating infectious diseases that were previously known to kill humans and animals. Although most antibiotics are used for the treatment of infections in humans and animals, a significant quantity of these is also used as a feed supplement to promote growth of food animals (Halling-Sørensen et al., 1998; Phillips et al., 2004; Kumar et al., 2005). At least four mechanisms have been suggested as explanations for antibiotic mediated growth enhancement (Gaskins et al., 2002): (i) inhibition of subclinical infections, (ii) reduction in growth-depressing microbial metabolites, (iii) reduction in microbial use of nutrients, and (iv) enhanced uptake of nutrients through the thinner intestinal wall of antibiotic-fed animals.

The antibiotic dose varies from 3 to 220 g Mg^{-1} of feed depending on the type and size of the animal and the type of antibiotic (McEwen and Fedorka-Cray, 2002; Kumar et al., 2005). It is claimed that these low quantities of antibiotics encourage the selection of antibiotic resistant bacteria in the environment (Hirsch et al., 1999; Khachatourians, 1998; Boxall et al., 2003). Most antibiotics fed to animals are poorly absorbed in the animal gut and as a result a substantial amount of these are excreted in urine and feces, which in turn end up in manure. As much as 90% of some antibiotics may be excreted as the parent compound (Phillips et al., 2004; Kumar et al., 2005).

Land application of manure is a common practice in many parts of the world including the United States. Manure is land-applied because of its value in supplying nutrients to crops as well as a means of disposing unwanted waste. Although it is strongly recommended that manure application rates be based on the nutrient status of the soil and crop needs, this recommendation is not always followed and thus the manure applications have frequently been higher than the recommended rate.

In 1997, the USDA estimated a livestock population of more than 8 billion animals (more than 95% being chickens and turkeys) producing up to 1.32 billion Mg of manure in the United States (USDA, 1997). These numbers would suggest that the presence and persistence of antibiotics in this large quantity of manure presents a significant environmental problem, both in terms of toxicity of these antibiotics to soil microflora and fauna as well as potentially increasing antibiotic resistance in the environment. Baguer et al. (2000) claim that land application of antibiotic-laced manure appears to be the dominating pathway for the release of antibiotics in the terrestrial environment.

The most common antibiotics present in swine, beef, and turkey manures are tetracyclines (oxytetracycline and chlortetracycline), tylosin, sulfamethazine, amprolium, monensin, virginiamycin, penicillin, and nicarbazine (Webb and Fontenot, 1975; De Liguoro et al., 2003; Kumar et al., 2004, 2005). The concentration of these antibiotics varies from traces to as high as 216 mg L^{-1} of manure slurry (Kumar et al., 2005). Manure samples obtained from four swine producers in Minnesota contained as high as 7.73 mg L^{-1} chlortetracycline and 4.03 mg L^{-1} tylosin (Kumar et al., 2004). A manure application rate of approximately 50 000 L per hectare (equivalent to 168 kg ha^{-1} N application) can thus result in land application of up to 387 g of chlortetracycline and 202 g of tylosin per hectare. Studies by Boehm (1996) and Migliore et al. (1995) have shown that these antibiotics generally remain stable during manure storage and end up in agricultural fields on manure applications.

Livestock manure is traditionally a key ingredient in organic and sustainable farming systems. Here, manure is applied either as raw manure (fresh or dried) or composted (Kuepper, 2003) as a source of fertilizer. Although there are clear restrictions on the use of raw manure in organic farming in the United States (National Organic Program Regulations) due to the concern of bacterial contamination (*Salmonella* and *E. coli*), there are no guidelines on the presence of contaminants such as hormones and antibiotics in manure (Kumar et al., 2005).

Until recently, research on antibiotic use has been

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mainly directed toward their beneficial and adverse effects on the end user, humans and animals. However, there have been relatively few studies on the effect of these antibiotics in the environment including uptake by plants from manure-amended soils. Consumers may unknowingly be ingesting some of these antibiotics when they eat vegetables grown on manure-applied lands. The objective of this study was to evaluate whether or not plants take up antibiotics from manure–soil mixtures, and if so to what extent? The study was conducted as a greenhouse pot trial.

MATERIALS AND METHODS

Experiment 1: Plant Uptake of Antibiotics from Soil Spiked with Antibiotics

In this experiment, green onion (four plants per pot) and cabbage (two plants per pot) were transplanted in three 1-L-capacity pots each containing Hubbard loamy sand soil (sandy, mixed, frigid Entic Hapludolls from Becker, MN). After 1 wk, 50 mL each of chlortetracycline and tylosin antibiotics (20 μg L^{-1} concentration) were added to each pot. These pots were watered with 100 mL of nanopure water twice a week. Two onion plants from each pot and one cabbage plant from each pot were harvested at 3 and 6 wk after transplanting. Plants were harvested 2.5 cm above the surface of potting mix, washed with deionized water, and dried using blotting paper. The fresh plant material was finely ground in a food processor. Five grams of each sample was suspended in 50 mL of buffered peptone water (pH 7) and vortexed for 5 min. After vortexing, the samples were centrifuged at 2000 $\times$ *g*, and the supernatant was removed and analyzed for antibiotics on the same day (Kumar et al., 2004).

Experiment 2: Plant Uptake of Antibiotics from Manure-Applied Soils

The manure used in this experiment was taken from pigs that were part of an experiment on antibiotic feeding. The feeding trial was conducted at the Southern Research and Outreach Center at Waseca, MN. Briefly, in this feeding trial, 32 pigs (18 d old) housed in 16 pens (each pen housed two pigs) were fed with or without antibiotics for 1 mo. The two treatments were: (i) control, and (ii) with antibiotics (100 g chlortetracycline, 100 g sulfamethazine, and 50 g penicillin per ton feed). During the last week pigs were transferred to metabolic crates and manure from these pigs was collected. These two manures, refered to hereafter as control-manure (C-M) and antibiotics manure (Ab-M), were used in greenhouse antibiotic uptake study.

The treatments in the uptake study were (i) the above two manures (C-M and Ab-M); (ii) the same two manures that were artificially spiked with an additional 100 mg of chlortetracycline and tylosin each per kg of dry manure (C-M + antibiotics and Ab-M + antibiotics); and (iii) the three crops, corn, green onions, and cabbage. The experiment was laid out as a factorial treatment arrangement (2 $\times$ 2 $\times$ 3) with three replications in a randomized complete block design.

The manure was mixed with 2 kg of a Hubbard loamy sand soil and potted up into a 2-L pot. Manure was applied at rates equivalent to 200 kg N ha^{-1}. The amount of fresh manure applied per pot was 21.5 g and 23.8 g for C-M and Ab-M, respectively. Corn was planted as two seeds per pot, green onions as four seedlings per pot, and cabbage one seedling per pot. The pots were watered with 100 mL deionized water every day and the temperature in the greenhouse was maintained at 25°C during the day and 20°C at night. After 6 wk, the tops were harvested about 2.5 cm above the soil surface, washed with deionized water, and then blotted dry of any extra water and weighed. Samples were prepared and analyzed as outlined in Experiment 1 (Kumar et al., 2004).

Table 1. Concentration of antibiotics in plant tissues after 3 and 6 wk.

Antibiotic	Green onions 3 wk	Green onions 6 wk	Cabbage 3 wk	Cabbage 6 wk
	ng g^{-1} fresh weight			
Tylosin	ND†	ND	ND	ND
Chlortetracycline	14.4 ± 2.3	12.8 ± 1.7	11.4 ± 2.1	10.0 ± 1.8

† Not detected.

Antibiotic Analysis in Plant Tops

The buffered peptone water extracts were analyzed for tylosin and chlortetracycline antibiotics using ELISA method (Kumar et al., 2004). Antibiotic concentration in plant tops is reported on a fresh weight basis. We did not analyze the plant samples for sulfamethazine or penicillin because we do not have methods developed for trace analysis of these antibiotics in our laboratory.

RESULTS AND DISCUSSION

Experiment I

Both green onions and cabbage plants took up chlortetracycline from the potting mix but not tylosin (Table 1). Concentration of chlortetracycline in both plants decreased with time probably due to increase in plant biomass and decreased availability of chlortetracycline over time.

Experiment 2

Manure obtained from pigs fed with antibiotics contained chlortetracycline, but manure from the control pigs did not contain any chlortetracycline (Table 2). In this experiment also, chlortetracycline was taken up by all three plants (green onions, cabbage, and corn) both in the presence of naturally present antibiotics as well as when manures were spiked with additional quantities of antibiotics (Table 3). However, tylosin was not detected in any of the plant tops for any of the antibiotic treatments.

The amount of chlortetracycline absorbed by different plants increased with increasing antibiotic present in manure–soil mixture in a pot (Fig. 1). This suggests that if increasing amounts of chlortetracycline are present in manure–soil, plants will absorb this antibiotic to

Table 2. Amount of antibiotics present per pot in various treatments.

Treatment†	Chlortetracycline	Tylosin
	μg pot^{-1}	
C-M	0	0
C-M + antibiotics	1000	1000
Ab-M	587	0
Ab + antibiotics	1587	1000

† See "Experiment 2" section of Materials and Methods for treatment descriptions.

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Table 3. Mean amount of chlortetracycline recovered in plant tops in various treatments.†

	Recovery			
	C-M	Ab-M	C-M + antibiotics	Ab-M + antibiotics
	%			
Green onions	0	0.34	0.30	0.21
Cabbage	0	0.64	0.80	0.62
Corn	0	1.04	1.02	0.80

† See "Experiment 2" section of Materials and Methods for treatment descriptions.

a greater extent. The maximum amount of chlortetracycline in plant tops per pot was found in corn followed by cabbage and green onions. The recovery of chlortetracycline antibiotic in plant tops was variable with 0.21% recovered from Ab-M + antibiotics treatment in the case of green onions to a maximum of 1.04% recovered in corn tops from Ab-M treatment (Table 3).

It was interesting to note that plants absorbed chlortetracycline but not tylosin. These differences cannot be explained on the basis of differences in adsorption coefficients ($K_{d,solid}$) of these antibiotics for soils because the $K_{d,solid}$ values of tylosin for Hubbard loamy sand are much lower than those of chlortetracycline (Tolls, 2001; Kumar et al., 2005), which means that tylosin will be more readily available to plants than chlortetracycline. Another explanation for this may be that tylosin is a larger molecule, with almost double the mass of a chlortetracycline molecule and thus could not be taken up easily by plants both in mass flow (in transpiration stream) or as active uptake. In general, macrolides class antibiotics, to which tylosin belongs, are less soluble in water (0.45–15 mg L^{-1}) compared to tetracycline class antibiotics (230–52000 mg L^{-1}) and have higher log K_{ow} values (octanol–water partition coefficient) as compared to tetracycline class antibiotics (Table 4).

CONCLUSIONS

The adverse impacts of ingesting antibiotics present in plants by humans are not known at this stage. A few adverse impacts of consuming antibiotics in fresh vegetables (sweet corn) and fruits are speculated in the following discussion:

- Allergic or toxic reactions: Some of these antibiotics when ingested by humans, especially children, may cause serious allergies or may be toxic (Patterson et al., 1995). Furthermore, there may be some interaction effects from simultaneous ingestion of two different antibiotics. It has been shown that some of the macrolide antibiotics present in animal feed have interacted with other antibiotics like monensin and have resulted in toxicity of monensin and the death of affected cattle (Basaraba et al., 1999).
- Antibiotic resistance: Antibiotics present in plant materials ingested by humans may provide resistance to human pathogens thus resulting in illnesses that may be difficult to cure with presently available antibiotics. This can be a serious threat, as has been proved in many animal studies. For example, it has been shown that resistance of gut bacteria to antibiotics increased with increasing concentrations of penicillin in contaminated waste milk fed to dairy calves as compared to dairy calves fed with noncontaminated milk (Selim and Cullor, 1997; Langford et al., 2003). Recently, it has also been shown that presence of small amounts of tetracycline can act as a catalyst in triggering the horizontal gene transfer between different bacteria (Shoemaker et al., 2001). This increasing resistance may be of concern both for human and animal health if antibiotics are present in food crops.

Our knowledge regarding the implications of manure antibiotics on the terrestrial environment and impacts on human health is limited. There is an urgent need to study (i) the fate of different antibiotics present in manure, (ii) which antibiotics and their degradation products may be taken up by plants grown on manure amended soils, (iii) whether or not antibiotics present in food degrade when cooked, and (iv) whether or not antibiotics or their degradation products are still bioactive to impart antibiotic resistance to gut bacteria or cause adverse immunological reactions in humans.

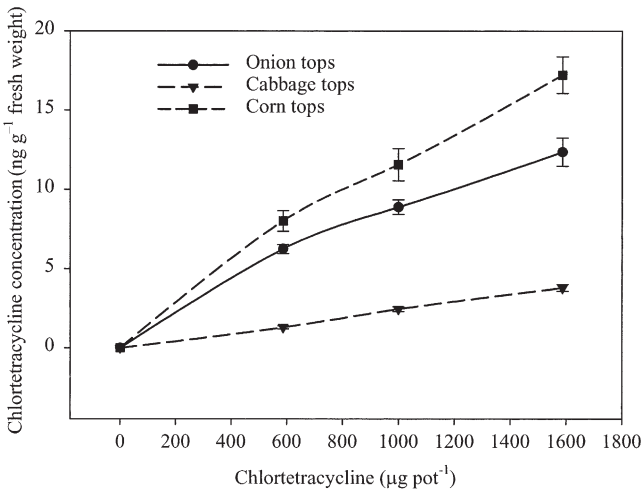

Fig. 1. Concentration of chlortetracycline in plant tops in relation to the amount of chlortetracycline present in the soil–manure mixture in a pot.

Table 4. Characteristics of chlortetracycline and tylosin antibiotics.

Antibiotic (class)	Mass	Water solubility†	log K_{ow}†‡
		mg L^{-1}	
Chlortetracycline (tetracyclines)	479	230 to 52 000	−1.3 to 0.05
Tylosin (macrolides)	916	0.45 to 15	1.6 to 3.1

† Range provided for the whole class of antibiotics.
‡ Octanol–water partition coefficient.

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