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Antibiotic Uptake by Plants from Soil Applied with Antibiotic-Treated Animal Manure

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Food contamination through antibiotic uptake by plants has been one of the major concerns regarding animal manure amendment to agricultural land. Antibiotic uptake by plants was tested with three veterinary antibiotics such as chlortetracycline (CTC), tylosin (TYL), and sulfamethazine (SMT) and three plants such as lettuce, tomato, and hairy vetch. Antibiotic-treated swine slurry was applied to a greenhouse soil before transplanting or sowing the plants. The treated antibiotic concentrations to the swine slurry were 22.9 mg CTC L^{-1}, 27.8 mg TYL L^{-1}, and 32.4 mg SMT L^{-1}. Lettuce was harvested on 64 days after transplanting and 94 days for tomato and hairy vetch. Concentrations of CTC were 3.4 ng g^{-1} for lettuce and 0.7 ng g^{-1} for tomato on a fresh weight basis. For TYL, 20.1 ng g^{-1} from lettuce leaves and 3.0 ng g^{-1} from whole hairy vetch were detected. Sulfamethazine showed greatest concentration among the three antibiotics, 63.3 ng g^{-1} in lettuce leaves, 30.2 ng g^{-1} in tomato fruits, and 20.9 ng g^{-1} in hairy vetch. The results imply that antibiotic uptake by plants may be dependent on antibiotic type and plant type.

Key words: Chlortetracycline, Hairy vetch, Lettuce, Sulfamethazine, Tomato, Tylosin

Introduction

Veterinary antibiotics have been routinely administered to even healthy livestock by applying to feed or water in order to increase feed efficiency, to prevent disease, and to promote growth. The mechanisms to increase body weight of livestock by antibiotic treatment include inhibition of subclinical infections, reduction in harmful microbial metabolites, and enhanced nutrient uptake by reducing microbial use (Gaskins et al., 2002). In the Republic of Korea, 998 Mg of veterinary antibiotics were consumed in 2009 (National Veterinary Research & Quarantine Service, 2010).

Antibiotics given to livestock can be excreted via the urine and feces (Boxall et al., 2002; Aga et al., 2003; Vaclavik et al., 2004), and are gradually decomposed by physical, chemical, and biological reactions in animal manure. One of the main routes of antibiotics entering soil environment is via the application of antibiotic-laden animal manure to agricultural land as an organic fertilizer. The decomposition rate of antibiotics in animal manure and soil is determined by some factors including the antibiotic properties and environmental conditions. The antibiotic properties include photosensitivity, biodegradation, water solubility, acid base dissociation constant, and sorption behavior. The major environmental conditions are temperature, pH, bacterial activity, and redox conditions. Seo et al. (2007) determined priority of veterinary antibiotics to environmental risks based on their consumption in Korea and the potential to reach the environment.

Some studies have been conducted to examine the potential risk for the veterinary antibiotics to be taken up by plants from soil amended with antibiotic-laden animal manure. Kumar et al. (2005) reported that corn, green onion, and cabbage absorbed chlortetracycline up to 17 ng g^{-1} fresh weight. Boxall et al. (2006) showed that florfenicol and trimethoprim were taken up by lettuce and enrofloxacin, florfenicol, and trimethoprim were detected in carrot roots. Concentrations of the antibiotics were less than 38 ng g^{-1} fresh weight. Dolliver et al. (2007) showed that sulfamethazine was taken up by corn, lettuce, and potato with concentrations ranging from 0.1 to 1.2 mg kg^{-1} dry weight when the antibiotic applied at rates of 2.8 and

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5.6 kg ha^{-1}. They reported that SMT uptake after 45 days was highest for lettuce, followed by potato and corn.

Although food contamination through antibiotic uptake by plants has been one of the major concerns regarding animal manure amendment to soils, it is largely unknown if plants uptake antibiotics in manure wastes in Korea. The objective of the study was to test whether or not antibiotics are detected from plants grown in soils fertilized with antibiotic-treated swine slurry.

Materials and Methods

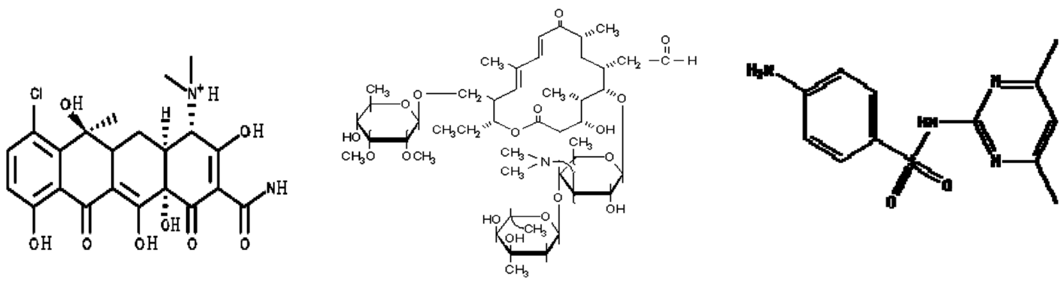
Three antibiotics such as chlortetracycline (CTC), tylosin (TYL), and sulfamethazine (SMT) and three plants such as lettuce (*Lactuca sativa* L.), tomato (*Lycopersicon esculentum* Mill.), and hairy vetch (*Vicia villosa* Roth) were used in the study. The chemical structures of CTC, TYL, and SMT are shown in Fig. 1 Lettuce and tomato were selected to assess food safety of freshly consumed produce and hairy vetch was chosen to search plants to take up the antibiotics as a remediation strategy. Plants absorbing antibiotics can be harvested after they have degraded the contaminants, and then the field can be used to cultivate crops. For instance, Migliore et al. (2000) suggested *Lythrum salicaria* L. for phytoremediation of veterinary drugs. Antibiotic-treated swine slurry was applied to 1.5 by 1.2m confined plots with a Yonggye silty clay loam soil (fine loamy, mixed, mesic family of Typic Dystrochrepts) before transplanting or sowing the plants at a rate of 16,667 L ha^{-1}. Table 1 shows selected chemical properties of the soil. The treatments were control (no antibiotic addition) and antibiotic addition to the slurry with concentrations of 22.9 mg CTC L^{-1}, 27.8 mg TYL L^{-1}, and 32.4 mg SMT L^{-1}, corresponding to between 0.38 and 0.54 kg ha^{-1}. By assuming soil depth of 15 cm and soil bulk density of 1.2 g cm^{-3}, the antibiotic levels ranged from 0.21 to 0.30 mg kg^{-1} soil.

Plants were harvested on 64 days after transplanting for lettuce and 94 days for tomato and hairy vetch. The levels of the antibiotic in the plants were determined by adapting the procedure outlined by Kumar et al. (2005) and Dolliver et al. (2007). Briefly, the antibiotics were extracted from leaves of lettuce, fruit part of tomato, and whole parts of hairy vetch using buffered peptone water, and determined using enzyme-linked immune-sorbent assay (ELISA) method. Agar et al. (2003), Kumar et al. (2004), and Dolliver et al. (2008) reported that the ELISA techniques can be used to determine antibiotics in environmental samples. The fresh plant material was finely ground in a blender and four grams of each sample was mixed with 40 mL of buffered peptone water (pH 7) for one hour. The samples were centrifuged at 2500 ×g and the supernatant was used to analyze the antibiotics. The ELISA analysis was conducted using a commercially available ELISA kit for tetracycline (R-Biopharm AG, Darmstadt, Germany), TYL (Tecna S.r.l., Trieste, Italy), and SMT (R-Biopharm AG, Darmstadt, Germany).

The ELISA kit for tetracycline was used because ELISA kit for CTC was not available and reactivity of the tetracycline kit to CTC was approximately 100% according

Table 1. Selected soil chemical properties of the soil used in the study.

pH (H_2O, 1:5)	Electrical conductivity	Organic matter	Available P_2O_5	Exchangeable cation			NO_3-N
				Ca	K	Mg	
	dS m^{-1}	g kg^{-1}	mg kg^{-1}		cmolc kg^{-1}		mg kg^{-1}
5.6	2.2	12	401	5.3	0.93	1.2	211



CTC TYL SMT

Fig. 1. Chemical structures of chlortetracycline (CTC), tylosin (TYL), and sulfamethazine (SMT).

to the ELISA kit manufacturer. After adding standard solution or sample to wells coated with tetracycline-protein conjugate, antibody solution was added to the wells. Any unbound antibody was removed in a washing step and enzyme conjugate was added. After removing unbound enzyme-labeled antibodies with a washing step, enzyme substrate and chromogen were added. Tetracycline levels were determined by measuring the absorbance at 450 nm after adding stop solution.

The procedure of the ELISA test for TYL and SMT was similar to that of tetracycline. For TYL, after adding standards and samples to wells coated with high affinity capture antibody, the enzyme conjugate was added to compete with TYL in the sample for binding sites on the capture antibody. Any unbound enzyme conjugate was removed by a series of washings and color-developing solution was added. The reaction was stopped by adding stop solution and the absorbance at 450 nm was measured. For SMT, after addition of standard solution or sample to wells coated with immobilized antibody, enzyme conjugate and antibody solutions were added. The unbound enzyme conjugate was removed in a washing step and the same amount of substrate and chromogen were added. After incubation, stop solution was added to stop the reaction and the absorbance at 450 nm was measured to determine SMT concentration in the sample.

Results and Discussion

The concentration of CTC was 3.4 ng g^{-1} for lettuce and 0.7 ng $g^{-1}$for tomato on a fresh weight basis when CTC-treated swine slurry was applied to the soil, while CTC was not detected from hairy vetch (Fig. 2). The result imply that antibiotic uptake by plants may be dependent on plant type. Kumar et al. (2005) reported that the maximum amount of CTC was found in corn, followed by cabbage and green onion. The concentrations of CTC in the three plants ranged from 2 to 17 ng g^{-1} fresh weight by applying CTC-spiked swine manure to a loamy sand soil at levels of 294-794 g CTC kg^{-1} soil. In this study, CTC was treated to a silty clay loam soil at a level of 210 g kg^{-1} soil which was less than those for Kumar et al. (2005).

For TYL, the highest concentration was observed in lettuce, 20.1 ng g^{-1}, followed by hairy vetch, 3.0 ng g^{-1} (Fig. 3). On the other hand, TYL was not detected in tomato fruits. Boxall et al. (2006) reported that 6 ng g^{-1} of trimethoprim and 15 ng g^{-1} of florfenicol were detected in lettuce leaves.

Of the three antibiotics, SMT was detected at greatest concentration; 63.3 ng g^{-1} for lettuce, 30.2 ng g^{-1} for tomato, and 20.9 ng $g^{-1}$for hairy vetch (Fig. 4). The SMT concentrations in the three plants were comparable with the results of Dolliver et al. (2007) who reported 8-100 ng

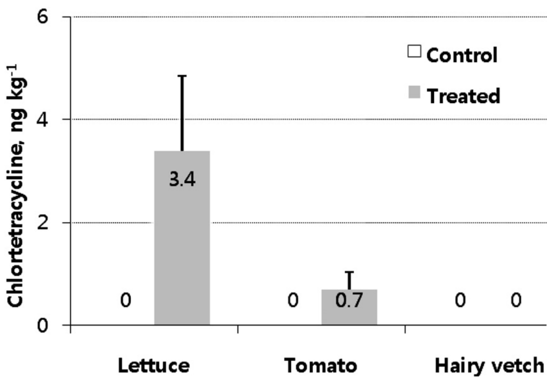


Fig. 2. Chlortetracycline concentrations in lettuce, tomato, and hairy vetch on a fresh weight basis. Error bars indicate +1 standard error.

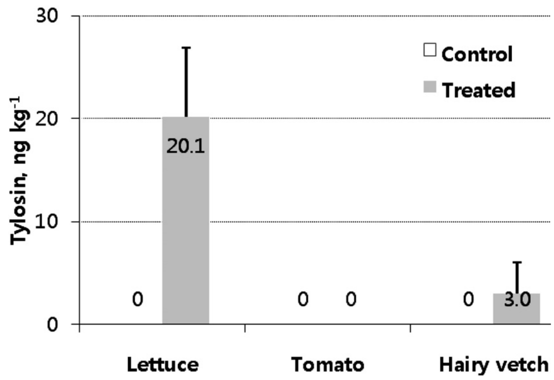


Fig. 3. Tylosin concentrations in lettuce, tomato, and hairy vetch on a fresh weight basis. Error bars indicate +1 standard error.

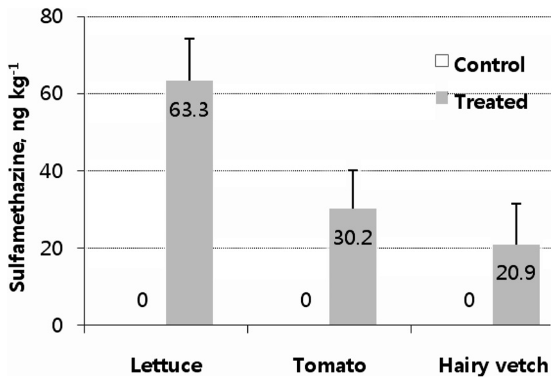


Fig. 4. Sulfamethazine concentrations in lettuce, tomato, and hairy vetch on a fresh weight basis. Error bars indicate +1 standard error.

g^{-1} fresh weight of SMT for lettuce, potato, and corn.

The results imply that antibiotic uptake by plants may be affected by antibiotic type and plant type. Plant root uptake of organic chemicals from soil is generally related to the octanol-water partition coefficient (Kow) of the compound (Burken and Schnoor, 1998), and root uptake is greater for lipophilic compounds than for polar compounds (Briggs et al., 1982). Maximum translocation into shoots is observed at a log Kow of about 1.8 (Boxall et al., 2006). Jjemba (2002) suggested that bioavailability of veterinary antibiotics to plants is greatly dependent on the sorption kinetics of the antibiotics, soil organic matter, and soil acidity. Therefore, further extensive research deserves to be conducted to examine antibiotic uptake influenced by other factors including antibiotic level, soil characteristics, soil structure, type and growth stage of plant, plant part, climate, and agricultural practices.

Consumers may unknowingly obtain some veterinary antibiotics when they eat fresh vegetables and fruits cultivated in soils amended with antibiotic-laden manure wastes. Kumar et al. (2005) discussed some potential adverse effects including allergic or toxic reactions and antibiotic resistance. On the other hand, Phillips et al. (2004) concluded little harm to human from antibiotic resistance because resistant bacteria in animal-derived food are destroyed by adequate cooking. They also showed no correlation between the carriage of resistant enterococci in animal food and human infection with resistant strains. At this time, it is not clear if consuming antibiotics in agricultural products can result in adverse impacts on health.

Conclusion

Three antibiotics were detected in lettuce leaves with concentrations of 3.4 ng g^{-1} for CTC, 20.1 ng g^{-1} for TYL, and 63.3 ng g^{-1} for SMT after applying antibiotic-treated swine slurry to a silty clay loam soil at levels of 0.38-0.54 kg ha^{-1}. Only CTC and SMT were found from tomato at levels of 0.7 ng g^{-1} and 30.2 ng g^{-1}, respectively. For hairy vetch, 3.0 ng g^{-1} of TYL and 20.9 ng g^{-1} of SMT were detected. The results obtained from this study imply that antibiotic uptake by plants may be dependent on antibiotic type and plant type.

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가축분뇨 시용 토양에서 식물체의 축산용 항생물질 흡수양상

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최근 축산용 항생제를 함유한 축산분뇨 퇴액비를 토양에 시용하였을 때 식물체가 항생제를 흡수할 수 있음을 보여주는 연구결과가 유럽과 미국에서 발표되었다. 클로르테트라싸이클린, 타이로신, 설파메타진 등 3종의 항생제를 처리한 돈분 액비를 처리하고, 상추, 토마토, 헤어리베치 등 3종을 기른 다음 식물체의 항생제 함량을 분석하여 항생제를 처리하지 않은 대조구와 비교하였다. 클로르테트라싸이클린 22.9 mg L^{-1}, 타이로신 27.8 mg L^{-1}, 설파메타진 32.4 mg L^{-1} 수준의 항생제를 처리했을 때, 생체중 기준으로 클로르테트라싸이클린은 3.4 ng g^{-1} 이하, 타이로신 20.1 ng g^{-1} 이하, 설파메타진은 20.9−63.3 ng g^{-1}가 검출되었다.