Final Report for the project "Potential Lettuce Uptake of Antimicrobials from Animal Waste Composts"

Abstract

Animal waste composts are widely used as amendments and fertilizers in the lettuce industry. The only regulatory scrutiny for composts at present is microbial hazard, which is primarily focused on *E. coli* testing in accordance with the Arizona and California leafy greens marketing agreement. The livestock industries use substantial amounts of antimicrobials much of which passes into the animal waste. A number of studies have shown plant uptake of antimicrobials when the growing medium contains animal wastes. However, little attention has been given to the risks of antimicrobials in composts. The objective of this study was to evaluate potential uptake of antimicrobials and hormones by lettuce and spinach produced with animal waste composts. We evaluated several antimicrobials commonly used in animal feeding operations. When compost was applied at 128,000 kg/ha several of the antimicrobials tested for were detected in the compost, soils amended with the compost, and the roots and shoot of lettuce and spinach. Overall, the concentrations of antimicrobials detected in the edible plant portion were very low and well below therapeutic levels. When the rate of compost was reduced to 64,000 kg/ha levels of all antimicrobials were below detection limits in the edible leaves. Because organic production fields use compost as the principal source of N and P fertilizer, and rates applied over time exceed rates used in our evaluations, further testing of organic production systems is warranted.

Introduction

Animal waste compost are widely used in the lettuce industry provided they meet certain microbial testing standards. For both conventional and organic production systems composts are used for their positive effects on soil tilth. For organic production compost are often the primary source of N and P fertilizers and applied at higher rates than conventional production systems. However, little attention has been given to the risks of antimicrobials and hormones in composts.

The USEPA estimated that in 1998, 13.7 million kg of antimicrobials were used in the United States. It has been estimated that approximately 80% of all antimicrobials marketed are used in the livestock industries, with 4.7 million kg specifically associated with cattle feeding (Mellon et al., 2001). Antibiotics are commonly used at therapeutic levels to treat disease and at sub-therapeutic levels to increase feed efficiency and improve growth rates (Cohen, 1998). The Union of Concerned Scientists has estimated that 70% of the antibiotics are used for non-therapeutic purposes (Mellon et al., 2001). Major products used in the cattle industries include chlortetracycline, oxytetracycline, tylosin, sulfametazine, monoensin, and lasalocid (Kumar et al., 2005). A more recent survey found that antimicrobial use in the dairy industries remained unchanged from 2002 through 2007; with sulfonamide and tetracycline the most commonly used products (APHIS, 2008). Many antimicrobials are poorly absorbed in the digestive tracks of animals and are often present in animal waste in significant quantities (Elmund et al., 1971; Feinman and Matheson, 1978).

Another existing concern with antimicrobials is the identification of growing resistance in microbial populations (Schwartz et al., 2006; Gilchrist et al., 2007). Resistance has been found in bacteria isolated from the innards of animals treated with antimicrobials, in the corresponding manure (Berger et al., 1986), and in agricultural soils receiving manure (Esiobu et al., 2002). There is concern that non-pathogenic bacteria can serve as a platform for gene transfer to pathogenic organisms as a result of promiscuous exchange of genetic material among microbes (Kümmerer, 2004; Bazquero et al., 2008) and have a profound effect on human health. Enteric bacterial in soil collected from a dairy showed multidrug resistance (Burgos et al., 2005). Antimicrobial resistant bacteria have been found in surface water (Schwartz et al., 2003, Schwartz et al., 2006), sediments (Samuelson et al., 1992; Andersen and Sandaa, 1994), and ground water (McKeon et al., 1995).

An estimated 376,000 livestock operations contain confined animals in the U. S. producing 130 billion pounds of manure, and most of this is land-applied (USEPA, 2000). Land applications are usually guided by the nutrient requirement of feed and food crops produced on this land, without regard to the pharmaceutical contaminants. It has been estimated antibiotic loading from manure can approach the kilogram per hectare level (Winckler and Grafe, 2001

Antimicrobials in animal wastes added to land might be degraded, sorbed to soil constituents, transported to ground and surface waters through leaching and runoff, or sometimes even transferred to terrestrial plants (Kumar et al., 2005). Plant uptake is a potential route of human exposure since manures and compost are applied to food and feed crops. Uptake of sulfamethazine has been shown in wheat, corn, lettuce, and potato produced on manure-amended soils (Dolliver et al., 2007; Grote et al., 2007). More recently, we have found uptake of several macrolide antibiotics by food crops grown in greenhouse sand culture and in fields irrigated with treated effluent (Jones-Lepp et al., 2010). However, the potential for lettuce uptake from animal waste used in the region has not been evaluated. The objective of was to conduct a preliminary study of the potential for lettuce to take up antimicrobials and hormones from composts used in the region.

Materials and Methods

All these preliminary studies were conducted in the greenhouse. In all experiments, surface soil was collected in the field, sieved, and 1.6 kg was weighed into 15 cm diameter pots.
The compost was collected from stock piles currently being used in the Yuma area.
Rates of composts were equivalent to 0, 4000, 8000, 16,000, 32,000, 64,000 kg/ha, and 128,000 kg/ha on an area basis. We used high rates to simulate repeated applications. N and P fertilizers was applied to all pots so that these nutrients would not be limiting.

Although our primary interest was lettuce, we used spinach as a test plant as well because of its known propensity to accumulate contaminants. Lettuce and spinach seedlings (one-leaf stage), pre-germinated in trays, was transplanted into the pre-watered pots. Above ground lettuce tissue and spinach were harvested. Soils were sampled with a probe and roots were harvested by washing away the soil. All samples were frozen and then freeze dried as space became available on the freeze drier. We selected some antimicrobials currently used in animal feeding operations. The compounds we tested for and their principal applications are briefly summarized in Table 1.

After sample clean-up, the selected antimicrobials in the composts, soil, and plants were determined by GC/MS at the USDA ARS Aridlands Research Center. Because of the costs of these analysis we started analyzing samples at the high rates and continued through lower rates until we got no detection for a majority of the samples.

Results

Although we applied N and P to all pots for maximum production, lettuce and spinach responded positively to compost rate (Table 20. Perhaps the compost release N more slowly and it was less subject to N leaching loss and/or the composts provided benefits beyond fertility.

With the exceptions of Invermectin, Sulfamethoxazole, and Tylosin, all products analyzed for were found in the compost. We do not know if these three products were not found because they were not used by the animal feeding operation or because they degraded in the compost. Most products found in the compost could be detected in the soil that received 128,000 kg compost per hectare. One exception was Oxytetracycline that we did not detect in soil but found a small amount in lettuce shoots. Some products seemed to show a tendency to bioaccumulate in plant shoots including Diclofenac, Chlortetracycline, Ivermectin, and Ofloxacin. Nevertheless, the concentrations of products detected in the edible plant portion are very low and well below therapeutic levels.

Interestingly, when, an equivalent rate 64,000 kg compost were applied, most of products tested for could not be detected in the soil or plant parts. The exception were small levels of Monesin.

We used high rates of composts in this greenhouse study in an attempt to capture potential effects from repeated applications. Organic production fields, where compost represents the principal source of N and P fertilizers, total rates applied over a period of five years might exceed the rates we applied. However, over time we might expect microbial degradation in the soil to have a greater impact than that we may have experienced in the short term greenhouse experiment we conducted. Overall, these results suggest that plant accumulation of antimicrobials from animal waste derived compost are small and risk to human health minimal. However, it would be prudent to do some testing on commercial organic production fields were total rates of compost applied over time approach and exceed the highest rates we applied in these studies.

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Table 1. Antimicrobials tested for in greenhouse study with compost amended. Lettuce and spinach

Product	Type
Chlortetracycline	Tetracycline antibiotic
Diclofenac	Nonsteroidal anti-inflammatory drug
Invermectin	Broad spectrum antiparasitic
Monesin	Polyether antibiotic isolated from *Streptomyces cinnamonensis*
Ofloxacin	Synthetic antibiotic
Oxytetracycline	Broad spectrum tetracycline antibiotic
Sulfamethoxazole	General antibiotic
Tetracycline	General antibiotic
Tylosin	Macrolide antibiotic

Table 2. Shoot and root growth of lettuce and spinach to compost rate.

Compost Rate (kg/ha)	Lettuce Shoots (g/pot)	Lettuce Roots (g/pot)	Spinach Shoots (g/pot)	Spinach Roots (g/pot)
0	83.1	42.2	10.5	8.1
4000	86.4	28.7	12.1	12.2
8000	90.2	33.7	12.5	7.4
16,000	80.0	33.5	11.9	5.7
32,000	101.3	33.1	20.0	11.7
64,000	122.7	46.7	20.2	15.1
128,000	106.2	28.2	20.2	16.1
	L**Q**	NS	L**Q*	L**

Table 3. Levels of selected antimicrobials found in compost, soil amended with compost and roots and shoots of lettuce and spinach.

Product	Compost	Lettuce			Spinach		
		Soil	Roots	Shoots	Soil	Roots	Shoots
	ng/g						
	128,000 kg/ha Compost Applied.						
Chlorotetracycline	26.8	3.6	49.3	46.2	3.8	54.2	60.8
Diclofenac	107	1.3	375	45.6	2.6	587	79.5
Invermectin	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Monesin	596	4.3	61.9	64.9	12.5	65.4	58.4
Ofloxacin	79.9	20.1	43.8	64.6	17.1	159	41
Oxytetracycline	20.9	<DL	<DL	14.5	<DL	<DL	<DL
Sulfamethoxazole	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Tetracycline	7	<DL	<DL	14.8	<DL	40.2	<DL
Tylosin	<DL	<DL	<DL	<DL	<DL	<DL	<DL
	64,000 kg/ha Compost Applied						
Chlorotetracycline	26.8	<DL	<DL	<DL	<DL	<DL	<DL
Diclofenac	107	<DL	<DL	<DL	<DL	<DL	<DL
Invermectin	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Monesin	596	<DL	3.5	1.7	<DL	5.3	<DL
Ofloxacin	79.9	<DL	<DL	<DL	<DL	<DL	<DL
Oxytetracycline	20.9	<DL	<DL	<DL	<DL	<DL	<DL
Sulfamethoxazole	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Tetracycline	7	<DL	<DL	<DL	<DL	<DL	<DL
Tylosin	<DL	<DL	<DL	<DL	<DL	<DL	<DL