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The effect of composting on the persistence of four ionophores in dairy manure and poultry litter

Osman A. Arikan[a,b,*], Walter Mulbry[a], Clifford Rice[a]

[a] USDA-ARS, Beltsville Agricultural Research Center, Sustainable Agricultural Systems Laboratory, Beltsville, MD 20705, USA
[b] Istanbul Technical University, Department of Environmental Engineering, Istanbul 34469, Turkey

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ABSTRACT

Manure composting is a well-described approach for stabilization of nutrients and reduction of pathogens and odors. Although composting studies have shown that thermophilic temperatures and aerobic conditions can increase removal rates of selected antibiotics, comparable information is lacking for many other compounds in untreated or composted manure. The objective of this study was to determine the relative effectiveness of composting conditions to reduce concentrations of four widely used ionophore feed supplements in dairy manure and poultry litter. Replicate aliquots of fresh poultry litter and dairy manure were amended with monensin, lasalocid, salinomycin, or amprolium to 10 mg kg^{-1} DW. Non-amended and amended dairy manure and poultry litter aliquots were incubated at 22, 45, 55, or 65 °C under moist, aerobic conditions. Residue concentrations were determined from aliquots removed after 1, 2, 4, 6, 8, and 12 weeks. Results suggest that the effectiveness of composting for contaminant reduction is compound and matrix specific. Composting temperatures were not any more effective than ambient temperature in increasing the rate or extent of monensin removal in either poultry litter or dairy manure. Composting was effective for lasalocid removal in poultry litter, but is likely to be too slow to be useful in practice (8–12 weeks at 65 °C for >90% residue removal). Composting was effective for amprolium removal from poultry litter and salinomycin in dairy manure but both required 4–6 weeks for >90% removal. However, composting did not increase the removal rates or salinomycin in poultry litter or the removal rates of lasalocid or amprolium in dairy manure.

1. Introduction

Ionophores are commonly used in livestock production as coccidiostats as well as for growth promotion (Duffield and Bagg, 2000; Callaway et al., 2003; Sassman and Lee, 2007; Clarke et al., 2014). Ionophores are toxic to both prokaryotic and eukaryotic cells because these compounds interfere with the formation of ion gradients across cell membranes (reviewed in Hansen et al., 2009a). Ionophores are toxic to human cells and are therefore not used except for veterinary purposes. According to a Food and Drug Administration (FDA) report, >4500 tons of ionophores were sold for use in livestock in the United States in 2012 (FDA, 2014).

Since ionophores aren't used in humans, land application of ionophore-containing manure does not pose a potential threat to public health by influencing the development of antibiotic resistance in human pathogens. Many ruminal bacteria are ionophore-resistant but there is little evidence that ionophore resistance can be spread from one bacterium to another (Russell and Houlihan, 2003). Ionophore use is unlikely to lead to resistance in people as this resistance tends to develop more slowly, and may well be a reversible change (O'Neill, 2015). However, these compounds inhibit the activity of many bacteria and eukaryotic cells and can affect soil invertebrates and aquatic organisms (Hansen et al., 2009b; Žižek et al., 2011). Development of resistance against ionophores and cross-resistance in bacteria to more than one ionophore have also been observed (VKM, 2015). Results for raw vegetables indicate that coccidiostats can be incorporated into vegetables from the soil although detected concentrations are relatively low (Peteghema et al., 2012). Therefore, application of manure with ionophores is a considerable concern.

Ionophores have been primarily studied in manure (Donoho, 1984; Schlüsener et al., 2003; Sassman and Lee, 2007; Storteboom et al., 2007; Watanabe et al., 2008; Varel et al., 2012) poultry litter (Webb and Fontenot, 1975; Furtula et al., 2009; Ramaswamy et al.; 2010; Biswas et al., 2012; Bak et al., 2013; Žižek et al., 2015) and soil (Sassman and Lee, 2007; Bak et al.,

* Corresponding author at: Istanbul Technical University, Department of Environmental Engineering, Istanbul 34469 Turkey.
E-mail address: arikan@itu.edu.tr (O.A. Arikan).

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2013; Žižek et al., 2015). Although reported half-lives for these compounds in soils are very short (typically 2–4 days) (Sassman and Lee, 2007; Žižek et al., 2015) ionophores have been detected in surface water (Cha et al., 2005; Kim and Carlson, 2006; Hao et al., 2006, 2008; Song et al., 2007; Kurwadkar et al., 2013; Bak et al., 2013), groundwater (Watanabe et al., 2008) and sediment (Kim and Carlson, 2006). Hansen et al. (2009a) suggest that ionophores constitute an environmental risk because predicted environmental concentrations of ionophores and their measured concentrations in sediments could be above predicted no-effect levels.

Treatment of ionophore-containing manure prior to land application is one possible means of reducing the amount of these compounds that is released into the environment. Manure composting is a treatment process that is widely used for stabilization of nutrients and reduction of pathogens and odors (US Composting Council, 2000). The major factors that affect composting are oxygen, moisture, temperature, carbon and nitrogen (Epstein, 2011). Mean temperatures between 45 and 65 °C can be achieved during composting by applying different intensities of composting practices (Arikan et al., 2009a; Dolliver et al., 2008). Previous laboratory and field-scale composting studies (Arikan et al., 2009a,b; Dolliver et al., 2008) have shown that thermophilic temperatures (50–60 °C) and aerobic conditions rapidly reduce chlortetracycline levels in dairy manure and turkey litter. However, there is limited information on the effect of composting on ionophores, especially among the range of temperatures expected within minimally managed compost piles.

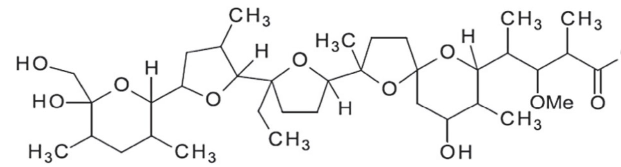
The specific objective of this study was to determine the relative effectiveness of composting temperatures to reduce the concentrations of four widely used ionophores (monensin, lasalocid, salinomycin, amprolium) (Mellon et al., 2001). Laboratory-scale incubations were conducted using both dairy manure and poultry litter to determine the influence of the composting matrix on removal rates. Replicate aliquots of fresh poultry litter and dairy manure were amended with monensin, lasalocid, salinomycin, or amprolium to 10 mg kg^{-1} DW. Non-amended and amended dairy manure and poultry litter aliquots were incubated for 12 weeks under moist, aerobic conditions at 22 °C or at three elevated temperatures (45, 55, or 65 °C) that represent temperatures achievable at different levels of composting intensity.

Based on our previous results with tetracyclines and from results from other studies with monensin, we predicted that temperature would influence ionophore removal rates with higher temperatures leading to higher removal rates and extent of removal. In addition, we predicted that removal rates would vary between ionophores and between poultry litter and dairy manure. We also predicted that the influence of temperature on removal rates would be similar in both matrices. The results show that ionophore removal rates were influenced by the specific ionophore, temperature, and matrix. However, in some instances, the influence of temperature on ionophore removal varied between dairy manure and poultry litter samples.

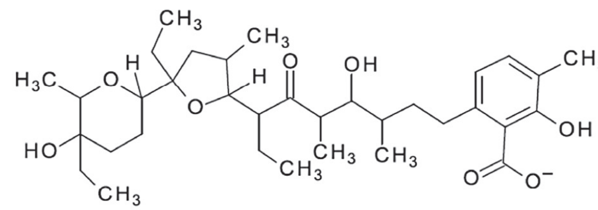
2. Materials and methods

2.1. Chemicals

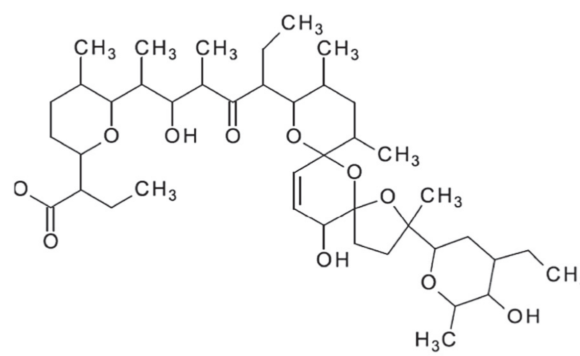
Monensin sodium salt, lasalocid A sodium salt, salinomycin mono-sodium salt hydrate and amprolium hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). Structures of these compounds are shown in Fig. 1. HPLC grade acetonitrile and methanol were purchased from Fisher Scientific (Pittsburg, PA). All reagents used in this study were analytical grade. Water used for extractions was purified using reverse osmosis and activated carbon. Stock solutions of the ionophore standards for extraction and analysis were prepared monthly by dissolving each compound in methanol at a concentration of 100 mg L^{-1} and stored at −20 °C in the dark.



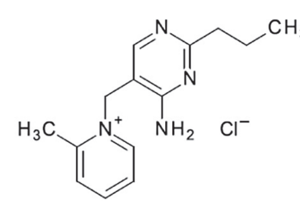
Monensin (M_w= 671 g mol^{-1}, pK_a = 6.7, log K_{ow} = 2.8-4.2)



Lasalocid (M_w= 591 g mol^{-1}, pK_a = 4.4, log K_{ow} = 1.4-2.8)



Salinomycin (M_w= 751 g mol^{-1}, pK_a = 4.5, 6.4, log K_{ow} = 5.2)



Amprolium hydrochloride (M_w= 278 g mol^{-1}, log K_{ow} = -2.5)

Fig. 1. Structures (adapted from Clarke et al., 2014) and selected physicochemical properties (adapted from Hansen et al., 2009b; Ding, 2011) of ionophores used in this study.

2.2. Dairy manure and poultry litter

Fresh dairy manure was collected from the USDA's Beltsville Dairy Research Unit (Beltsville, Maryland). Manure was air-dried in a fume hood for 48 h to a moisture content of 6%. The dried manure was ground using a hammer mill to pass a 4.7 mm sieve and stored at 4 °C in a covered container prior to use.

Poultry litter was obtained from a covered storage pile at the University of Maryland Eastern Shore's broiler production facility (Princess Anne, Maryland). The moisture content of the litter as received was 16%. The litter was sieved to remove feathers and large debris. Material that passed a 4.7 mm sieve was collected and stored in a covered container at 4 °C prior to use.

The collected dairy manure and poultry litter samples were analyzed for ionophores using the method below and found to contain some of the studied ionophores (shown in Table 3).