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Transport and attenuation of *Salmonella enterica*, fecal indicator bacteria and a poultry litter marker gene are correlated in soil columns

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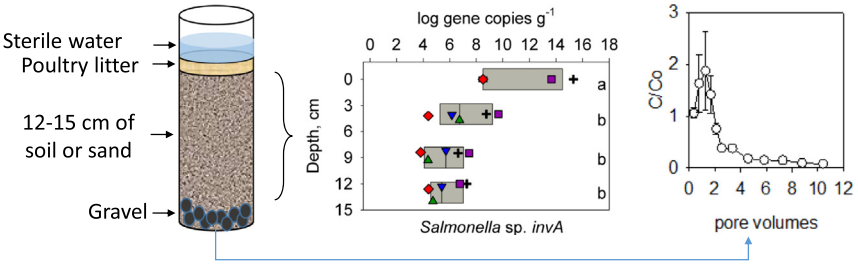
HIGHLIGHTS

- Pathogens and FIB are released from poultry litter during rainfall and irrigation.
- *S. enterica* in soil leachate correlates with a poultry litter marker gene and FIB.
- Microorganisms decline 2 to 9 log gene copies (g soil)$^{-1}$ within 12 in. of soil.

GRAPHICAL ABSTRACT



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ABSTRACT

Millions of tons of fecal-contaminated poultry litter are applied to U.S. agricultural fields annually. Precipitation and irrigation facilitate transport of fecal-derived pathogens and fecal indicator bacteria (FIB) to groundwater. The goal of this study was to compare transport of pathogens, FIB, and a microbial source tracking marker gene for poultry litter (LA35) in a simulated soil-to-groundwater system. Nine laboratory soil columns containing four different soil types were used to evaluate microbial transport to groundwater via infiltration. Quantitative polymerase chain reaction was used to monitor *Salmonella enterica* Typhimurium, *Escherichia coli*, *Enterococcus* spp., *Brevibacterium* sp. LA35 and *Bacteroidales* leached from soil columns inoculated with poultry litter. *S. enterica* was correlated with LA35 poultry litter marker gene and FIB concentrations in column soils containing organic matter, but not in acid washed sands. In contrast, *S. enterica* was found to correlate with LA35 and FIB in the leachate from columns containing sand, but not with leachate from organic soil columns. The majority of recovered DNA was found in leachate of predominately sandy soil columns, and in the soil of loamy columns. At least 90% of the DNA retained in soils for each microbial target was found in the top 3 cm of the column. These studies suggest that poultry litter associated pathogens and FIB are rapidly released from litter, but are influenced by complex attenuation mechanisms during infiltration, including soil type. This study advances our understanding of the potential for subsurface transport of poultry litter associated pathogens and FIB, and support the use of the LA35 marker gene for evaluating poultry litter impacts on groundwater.

1. Introduction

An estimated 896,000 acres of US farmland in 2006 were fertilized with poultry litter (USDA, 2009). In total, 1.5 billion broiler chickens

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and 100 million turkeys were sold in the US in 2012 (USDA, 2014). Assuming 1.14 kg of litter produced per bird harvested (Tabler et al., 2009), approximately 1.7 billion kg of poultry litter (broiler chickens and turkey) was available for use as a fertilizer in 2012. Microorganisms, nutrients, sediments and metals can be released from the poultry litter applied as fertilizer during rainfall runoff (Brooks et al., 2009; Weidhaas et al., 2014) which then contaminates streams (Weidhaas and Lipscomb, 2013; Weidhaas et al., 2010) or infiltrates to groundwater potentially emerging in springs (Gooddy, 2002; Weidhaas et al., 2011).

Poultry litter harbors pathogens and fecal indicator bacteria such as *Campylobacter* sp. (Keramas et al., 2004), *Clostridium perfringens* (Brooks et al., 2009), *enterococci*, *Escherichia coli*, *Salmonella* sp., *Staphylococcus* sp., (Weidhaas et al., 2014) and viruses (Guan et al., 2009). These pathogens in land applied animal manure can migrate to groundwater during surface water or rainwater infiltration (Pachepsky et al., 2006; Weidhaas et al., 2014; Weidhaas et al., 2011). However complex fate and transport mechanisms affect pathogen migration during infiltration through the vadose zone. First, after surface application, microorganisms must survive exposure to environmental stressors (e.g., desiccation, UV light, nutrient scarcity) before the first rainfall or irrigation event. After microorganisms desorb from manure or litter during rainfall, rainwater volume must be sufficient to transport microorganisms through the macropores of the vadose zone to the saturated zone. If rainfall volume is insufficient or infiltration rates are too limited, microbes may be retained in micropores until sufficient infiltration water carries the microorganisms to groundwater. Factors shown to affect the transport of microorganisms through soils with infiltrating water include: soil grain size (Fontes et al., 1991) and soil particle surface properties (Sen, 2011), cell size and microbe surface properties (Gannon et al., 1991; Schinner et al., 2010), and soil water content. Finally, when present in the soil micropores or macropores, microorganisms may replicate or die (Wilkins et al., 2014), form biofilms or remain planktonic (Or et al., 2007).

Once microorganisms enter the saturated zone, their transport follows the general laws governing macropore flow and the interaction between soil and microbial surfaces of variable charges. Mechanisms shown to attenuate microbial transport in soils include: 1) macropore flow characteristics due to soil structure and porosity, 2) filtration effects in soil micropores, 3) adhesion or adsorption of microbial cells to soil minerals and organic particles (Unc and Goss, 2004). Despite these mechanisms to retard microbial movement in soils, microorganisms have been shown to be transported long distances (e.g., 456 m (Gerba et al., 1975)) depending on the soil type. However the abundance of microorganisms has been shown to be severely attenuated during transport with groundwater (e.g., 88% removal (Pang et al., 2004)).

Several studies have evaluated pathogen transport during surface runoff from poultry litter amended fields (Brooks et al., 2009; Jenkins et al., 2006; Sistani et al., 2010; Sistani et al., 2009; Weidhaas et al., 2014), however very few studies have evaluated infiltration of microorganisms from poultry litter into soil pore water and groundwater (Gooddy, 2002; McMurry et al., 1998). Previous infiltration studies with poultry litter have shown $2 \cdot 10^3$ to $3 \cdot 10^5$ fecal coliforms$\cdot(\text{mL})^{-1}$ of fecal coliforms in leachate from 42.5 cm deep, intact soil blocks with $1 \cdot 10^1$ to $2 \cdot 10^4$ fecal coliforms$\cdot(\text{g soil})^{-1}$ (McMurry et al., 1998). In these field studies the fecal coliforms leached as a pulse, but had variable areal abundance in the leachate from the bottom of the soil blocks, despite homogeneous application of poultry litter across the surface. In contrast, fecal coliforms, fecal enterococci, *Salmonella* spp., *E. coli* and *Campylobacter* spp. were not found in the 0.45 m deep chalk soils below turkey litter manure (Gooddy, 2002). To date, none of the previously published studies have systematically evaluated the mechanisms of microbial attenuation during transport of leachate from poultry litter applied as fertilizer.

Monitoring for pathogens in environmental samples has traditionally been dependent on indicator organisms such as fecal indicator bacteria (FIB). However, FIB such as *E. coli* and *Enterococcus* spp. are not specific to fecal sources such as poultry litter, rather they are associated with most warm blooded animal feces. Therefore various microorganisms been proposed to be used for monitoring environmental samples for impacts from chicken feces (Kobayashi et al., 2013; Lu et al., 2007) or poultry litter (Ryu et al., 2013; Weidhaas et al., 2010). Herein we propose to use the *Brevibacterium* sp. LA35 (hereafter LA35 marker gene), which has been shown to be highly concentrated in soiled poultry litter and rarely found in the feces of non-target animals (Ryu et al., 2013; Weidhaas et al., 2010).

Infiltration of pathogens originating from poultry litter applied as fertilizer to groundwater poses a potential risk to human health through exposure to these pathogens in water. The objective of this study was to evaluate the infiltration and attenuation of pathogens, FIB and a poultry litter marker gene originating from poultry litter. Specifically, studies were undertaken to determine if there was a correlation in the release, transport and attenuation of microorganisms originating from poultry litter through soil columns during simulated infiltration studies. Understanding of the attenuation of microorganisms in various soils will allow for better design of water quality monitoring programs and identification of the most appropriate microorganisms to quantify during sampling campaigns. By knowing which indicator organisms are transported similarly to pathogens in leachate or from groundwater seepage, one can more appropriately select monitoring methods, and better inform fate and transport models.

2. Materials and methods

2.1. Soil handling and characterization

Four soil types were used in nine column studies (Table 1). Sakrete natural play sand (Bonsal American, Inc. USA) was acid washed to remove organic material prior to its use. Briefly, the sand was sifted through 0.25 mm sieve (ASTM #60) and then boiled in a 2-L conical flask containing 1 M hydrochloric acid (Fischer Scientific, Pittsburgh, PA) for 2 h. The sand was then rinsed with de-ionized water until the rinse water had a pH of 5.6 (Orion ROSS pH electrode, Thermo Scientific, Waltham, MA). The sand was then dried in an oven at 105 °C overnight. The oven-dried sand was again re-rinsed in de-ionized water the following day and dried again overnight at 105 °C. The second soil type, a loamy sand was collected from the West Virginia University (WVU) Evansdale campus. A silt loam soil was collected from the WVU Organic Farm, while a loam soil was collected from the WVU downtown campus. All soils, excluding the sand were heated at 105 °C overnight to remove moisture, then sterilized by autoclave prior to packing into the soil columns. DNA was extracted from autoclaved soils (ea. 0.25 g) prior to setting up the soil column experiments to determine if any target microorganisms naturally present in the soils remained detectable by quantitative polymerase chain reaction (qPCR) after autoclaving. None of the target organisms were quantifiable by qPCR in the autoclaved soils (data not shown).

Soils were characterized by sieve analysis and by the pipette method as follows. First the sand and loamy sand were sieved through a 2 mm (ASTM #10) sieve, then sand and loam sand grains that passed a 0.074 mm (ASTM #200) sieve were considered clay and silt, the remainder was considered sand. Second, the silt loam and loam soils were sieved through a 0.595 mm sieve (ASTM #30), and percent clay in the silt loam and loam soils was determined by the pipette method (Gee and Bauder, 1986; Kilmer and Alexander, 1949). Organic content for all soils (Table 1) was determined by weight loss via ignition at 360 °C (Ball, 1964).

2.2. Column construction

All materials used for the column construction (PVC column and tubing) were bleached with a 10% bleach solution prior to use. A total