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Transport of manure-borne testosterone in soils affected by artificial rainfall events

Yong Qi [a, *], Tian C. Zhang [b]

[a] 136 PKI, Civil Engineering Dept., University of Nebraska-Lincoln, Omaha, NE, 68182, USA
[b] 205D PKI, Civil Engineering Dept., University of Nebraska-Lincoln, Omaha, NE, 68182, USA

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ABSTRACT

Information is very limited on fate and transport of steroidal hormones in soils. In this study, the rainfall simulation tests were conducted with a soil slab reactor to investigate the transport of manure-borne testosterone in a silty-clay loam soil under six controllable operation conditions (i.e., three rainfall intensities and two tillage practices). The properties [e.g., rainwater volume, particle size distribution (PSD)] of the slurry samples collected in runoff and leachate at different time intervals were measured; their correlation with the distribution of testosterone among runoff, leachate and soil matrix was analyzed. The results indicated that more than 88% of the testosterone was held by the applied manure and/or soil matrix even under the rainfall intensity of 100-year return frequency. The runoff facilitated testosterone transport through both dissolved and particle-associated phases, with the corresponding mass ratio being ~7 to 3. Soil particles collected through runoff were mainly silt-sized aggregates (STA) and clays, indicating the necessity of using partially-dispersed soil particles as testing materials to conduct batch tests (e.g., sorption/desorption). No testosterone was detected at the soil depth >20 cm or in the leachate samples, indicating that transport of testosterone through the soil is very slow when there is no preferential flow. Tillage practice could impede the transport of testosterone in runoff. For the first time, results and the methodologies of this study allow one to quantify the hormone distribution among runoff, leachate and soil matrix at the same time and to obtain a comprehensive picture of the F/T of manure-borne testosterone in soil-water environments.

1. Introduction

In the U.S., there are about 238,000 animal feeding operations, many of them are concentrated animal feeding operations (CAFOs). These CAFOs generate more than 500 million tons of animal waste annually (USEPA, 2001), and the majority of the manure from CAFOs is applied to agricultural land, leading to the risk of natural and synthetic hormones in CAFO wastes entering the environment (Kolpin et al., 2002; Bhandari et al., 2009; Webster et al., 2012). These hormones have been of an increasing concern due to their ability to alter the sexual behavior and endocrine systems of animals and aquatic species even at a very low concentration (as low as 1 ng/L) (Teles et al., 2004; D'Alessio et al., 2014). Hormones and their metabolites have been found in cattle waste with significant concentrations, in runoff from CAFOs, and in ~40% of the 139 streams sampled across 30 states in the U.S (Kolpin et al., 2002). Being persistent in the soil (Finlay-Moore et al., 2000; Schiffer et al., 2001) and lipophilic with most of their log K_{ow} being 2.6–4.0 (Lai et al., 2002; Schiffer et al., 2004; Khanal et al., 2006), most steroid hormones are expected to be sorbed on soils/organic matter, and thus, are unlikely to be very mobile. However, this expectation cannot explain the frequent detection of hormones in ground and surface water. For example, testosterone was detected in the soil sampled from 45-m deep below a dairy-farm wastewater lagoon (Shai et al., 2008).

Column studies have been conducted to investigate the non-equilibrium sorption, vertical transport and degradation of hormones in soils. The results of column studies identified a significant mass loss of hormones due to sorption and degradation; most hormones were retained in the first several inches of the soil column, indicating a low potential for the hormone leaching through the soils (Das et al., 2004). Schiffer et al. (2004) studied the transport of trenbolone and melengestrol acetate in a soil column. They found that dissolved organic carbon (DOC)-hormone associates

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* Corresponding author.
E-mail address: yqi01@unomaha.edu (Y. Qi).

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might lead the hormone to be more or less mobile, depending on the DOC affinity to the soil. It was reported that 17 β-estradiol and testosterone placed on the soil surface contaminated groundwater via preferential flow as demonstrated in a column study (Sangsupan et al., 2006). However, in these soil column studies, water was pumped directly to the surface of the soil columns; therefore, the interaction between water and soil surface was not as intense as that in the real world rainfall events. In addition, in order to obtain the breakthrough point quickly, the hydraulic loading rates were unreasonably high (e.g., the hydraulic loading of one test was more than two times of the average annual precipitation in the Mid-west of the U.S.) to make the water penetrate through the columns less than 12 min; the preferential flow might exist along the edge of the column. These drawbacks may lead to a misunderstanding of the transport of hormone through the soils. In addition, these columns could not generate information on surface runoff.

Field study is a direct way to understand the fate and transport (F/T) of manure-borne steroidal hormones in soils. Van Donk et al. (2013) conducted a four-year field study to investigate the potential contamination of groundwater from manure-borne hormones. They monitored the leaching of steroidal hormones at the bottom of 73.2-cm deep percolation lysimeters installed in plots treated with beef cattle manure, and detected the steroid hormones in only 5% of the leachate samples. Mansell et al. (2011) detected steroid hormones in the runoff in both filtered (dissolved) and particle-associated phases, which indicated the potential mechanism of particle- or colloid-facilitated transport of hormones through runoff. However, field studies often involve complicated situations (e.g., uncontrollable rainfall events, involvement of a large number of test plots, difficulty in sampling runoff and leachate simultaneously and in detection of micro-pollutants, unpredictable contaminant sources). Dutta et al. (2010) conducted the study on the transport of free and conjugated estrogen in surface runoff from poultry litter-amended soil. They found that the organic matters played very important roles during the transport of estrogen through runoff; no-tillage practice resulted in a lower export of estrogens with surface runoff. Due to the difference in soil types (e.g., texture, composition) and climate conditions, the results of previous field studies often are location-based or need special interpretation (e.g., only applied to specific type of soils and rainfall events), and thus, have not be able to provide a comprehensive picture about the F/T of hormones in soil environments. Therefore, it is imperative to develop a new methodology for conducting rainfall simulation tests in a consistent way and under controllable conditions so that the contribution of runoff, leachate, and soil matrix to the distribution and F/T of hormones can be determined simultaneously.

In nature, soils contain a wide range of particles with different particle size distributions (PSDs) and different mobility during rainfall events. It was reported that over 80% of the total soil particles found in storm water runoff were less than 20 μm (Randall, 1982; Furumai et al., 2002). Previous studies indicated that the sorption and desorption of pesticide and hormones associated with soil particles were highly particle-size dependent (Wang and Keller, 2008; Qi et al., 2014). Furthermore, total organic carbon (TOC) in the soil was also reported to be a critical factor that would affect the F/T of hormones in soils (Sarmah et al., 2008; Gineys et al., 2012). Sarmah et al. (2008) studied the sorption of 17β-estradiol (E2) and 17α-ethynylestradiol (EE2) on six selected soils with batch reactors. They found that the sorption capacity of the soil was attributed to both surface area and soil organic carbon content. Therefore, the properties (e.g., soil particle size, TOC content) of runoff and leachate and their contribution to the distribution and F/T of manure-borne hormones should be investigated.

The goal of this study was to use a lab-scale soil slab reactor to investigate the transport of hormones in soil environments via rainfall simulation tests. The specific objectives were to: 1) investigate the properties (e.g., PSD, TOC) of soil-water samples taken away by runoff and leachate; 2) determine the manure-borne hormone distribution among runoff, leachate and soil matrix under six different conditions (i.e., rainfall intensity, tillage practices); and 3) evaluate the correlation between the properties of the soil-water samples and the hormones' F/T in soils.

2. Materials and methods

2.1. Manure and hormone spiking

Testosterone was selected as the representative hormone because 1) it can transform to other metabolites, and it is a prototype for other synthetic androgenic hormones (Kanayama and Pope, 2012); 2) little is known about the F/T of androgenic hormones (Casey et al., 2004); and 3) it was used previously (Qi et al., 2014; Qi and Zhang, 2015; Ma et al., 2015). Stockpiled female manure was sampled from the feedlot (CAFO) owned by the Haskell Agricultural Laboratory (HAL, Concord, NE, USA) at the University of Nebraska–Lincoln (UNL). The testosterone concentration in the female and male mixed manure was ~5.8 ng/g (Van Donk et al., 2013), but no testosterone was in the female manure sampled in this study. Once in the laboratory, the manure was packed in aluminum foil, sterilized with an autoclave (Mol. # 10985, Market Forge Co. New York City, USA) at the temperature of 121 °C for 30 min to eliminate the possible testosterone biodegradation by the microorganisms (Berns et al., 2008), and then dried at 40 °C for 24 h. This sterilized and dried manure (3.5 g) was then mixed with 3.5 mL of the 10 μg/L testosterone stock solution (including 0.3 μg/L of ^{14}C-labeled and 9.7 μg/L of unlabeled testosterone) to make testosterone-spiked slurry of 10 ng/g testosterone. Then, the slurry of the testosterone-spiked manure was put in a 50-mL glass centrifuge tube (#0553841A, Fisher Scientific, Inc. USA) and rotated (top to bottom, 360°/5 s) for 24 h and dried in the oven at 40 °C. Then the testosterone-spiked manure was mashed to powder (in order to spread on the soil surface evenly) with the ceramic masher and ready for applying to the soil slab reactor.

2.2. Soil, soil slab reactor and manure application

The HAL soil was selected because it could be obtained together with manure samples from the CAFO at HAL and was used in the previous studies (Qi et al., 2014; Ma et al., 2015). The top 0–15, 15–30 cm (0–6, 6–12 inch) of the field soils were collected separately. The measured bulk density of the intact field soil was 1.32 g/mL (Table S1 in Supplementary Materials). The soil field slope was approximately 8%, and the soil had the permeability in the range of 15–50 mm/h and the available water holding capacity of 0.17–0.22 mm/mm. The HAL soil (Nora silty clay loam, fine-silty, mixed, mesic Udic Haplustolls) contains 0.95% total organic carbon (TOC). The total Fe, Cu, Ca, Mg are 21, 4.7, 2131, 318 mg/kg, respectively. The cation exchange capacity (CEC) of the HAL soil is 13.9 meq/100 g. No residual testosterone was detected in the soil samples (Ma et al., 2015).

As shown in Fig. 1, the soil slab (36 cm × 36 cm) was placed into a container. Two rectangle glass boxes (each L × W × H = 20.32 cm × 7.62 cm × 7.62 cm, see Fig. S2 of Supplementary Materials) (no sorption to hormones) were inserted into the soil for creating two test areas. Two PVC pipes on the soil surface with a 7.62 cm (3-inch) wide slot linking the glass boxes to collect runoff from each of the two test areas. To collect leachate, another two rectangle glass trays (each