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Short communication

Release of *Escherichia coli* under raindrop impact: The role of clay

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ABSTRACT

A recent paper by Wang et al. (2017) showed that the release of *Escherichia coli* (*E. coli*) from soil into overland flow under raindrop impact and the release of clay follow identical temporal patterns. This raised the question: what is the role of clay, if any, in *E. coli* transfer from soil to overland flow, e.g., does clay facilitate *E. coli* transfer? Using simulated rainfall experiments over soil columns with and without clay in the matrix, we found there was significantly more *E. coli* released from the non-clay soil because raindrops penetrated more deeply than into the soil with clay.

1. Introduction

Non-point source (NPS) pathogen pollution contributes to human health risks in drinking and recreational waters, as well as to contamination of agricultural crops. More than 40% of US rivers and streams were found to be impaired for at least one designated use and pathogens are one of the leading causes (USEPA, 2009). Livestock manure is a primary source of pathogens, which is increasingly problematic with the rising number of concentrated animal feed lot operations: 350 million tons manure per year are produced by more than 200 thousand animal feeding operations (James and Joyce, 2004; USEPA, 2001). Other sources include septic systems and wildlife (e.g., Falbo et al., 2013). It is critical to understand the mechanisms that transport pathogens from sources to water resources in order to develop strategies for reducing NPS pathogen transport. One of the main categories of pathogens is bacteria (thers are viruses, protozoa and helminth worms) (James and Joyce, 2004). Because bacteria and clay (mineral) particles are similar in size, i.e., characterized as colloids −1 nm to 10 µm (Chrysikopoulos and Sim, 1996; Vasiliadou and Chrysikopoulos, 2011) it is difficult to determine if bacterial transport is facilitated by mineral particles or if they are (or can be) transported independently.

Two separate studies have investigated mineral-colloid-associated bacteria transport using soil columns. Vasiliadou and Chrysikopoulos (2011) found that the kaolinite colloids inhibited the transport of *Pseudomonas putida* (*P. putida*), a rod-shaped bacteria of similar size as Escherichia *coli* (*E. coli*), because *P. putida* were attached to kaolinite and kaolinite stayed attached to the solid matrix. Working with both *Salmonella typhimurium* and *E. coli* O157: H7, Chen (2012) found that mineral colloids either facilitated or retarded the transport of these bacteria in soil, depending on whether the mineral colloids were mobile or immobile. Mobile and immobile here refers to suspended in aqueous phase and attached to solid matrix, respectively.

An additional two experiments have specifically investigated mineral-colloid-associated transport of *E. coli* in overland flow. Muirhead et al. (2006) looked at the interaction between *E. coli* and soil particles in overland flow across saturated soil and found that *E. coli* were mainly attached to mineral particles smaller than 2 µm and, once mobilized, *E. coli* remained in suspension. Using lab experiments, Wang et al. (2017) studied mineral colloid and bacteria co-release from soil into overland flow under raindrop impact (splash erosion). They found that the temporal patterns of release of mineral colloid and bacteria were identical (Wang et al., 2017). But they were unable to specifically identify the role of clay in the *E. coli* release process (Wang et al., 2017). Because the temporal patterns were so similar, they speculated that there were *E. coli*-clay micro-aggregates such that *E. coli* transfer might be dependent on the bacteria attaching to mobile mineral particles, i.e., clay (Wang et al., 2017). Note, Wang et al.'s (2017) previous research and that presented here did not consider shear-stresses associated with overland flow, i.e., the primary transfer mechanism is assumed to be due to raindrop impact.

The goal of this study is to compare the release of *E. coli* from soil into overland flow when clay is and is not part of the soil matrix.

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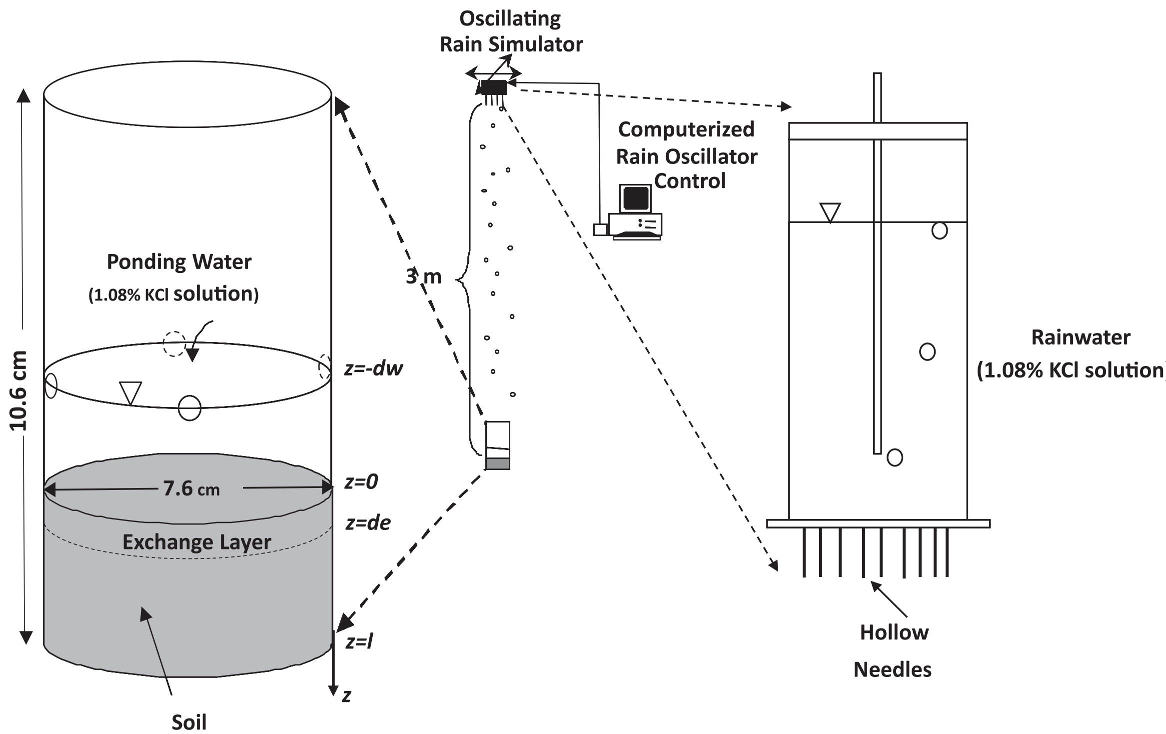

Fig. 1. Experimental set-up, same as Wang et al. (in press), adapted from Gao et al. (2004, 2005). The ponded water (d_w) and exchange layer (d_e) as defined in the Gao model are labeled next to the column.

Table 1
Summary of parameters and the ways they were determined (parameter values for clay-sand mixture were copied from Wang et al. (2016)).

Notation	Definition (Unit)	Value							
		Clay-sand mixture				Pure sand			
		run 1	run 2	run 3	run 4	run 1	run 2	run 3	run 4
a	Soil detachability [c] (g/ml)	0.350	0.800	0.450	0.450	1.500	5.000	0.900	1.400
C_o	Initial concentration of *E. coli* in soil [c] ($\times 10^6$ CFU/ml)	7.05	13.4	3.20	3.17	1.42	2.73	1.41	9.56
d_e	Exchange layer (shield layer) depth [b] (cm)	0.175	0.085	0.180	0.126	0.656	0.501	0.532	0.413
d_w	Ponding water depth [a] (cm)	0.800	0.900	0.950	0.950	0.700	0.800	0.950	0.950
p	Rainfall intensity [a] (cm/min)	0.276	0.260	0.260	0.240	0.276	0.260	0.260	0.280
θ	Soil water content by volume at saturation [a]	0.288	0.288	0.288	0.288	0.416	0.416	0.416	0.416
ρ_b	Bulk density of the soil [a] (g/cm^3)	1.543	1.543	1.543	1.543	1.475	1.475	1.475	1.475
R^2		0.53	0.63	0.42	0.83	0.89	0.82	0.58	0.86

[a] Directly measured, see Section 2 for details.
[b] Calculated from directly measured values, explained in Section 3.
[c] Curve fitted, elaborated in Section 3.

Wang et al. (2017) demonstrated that *E. coli* and clay release from soil under raindrop impact can be modeled equally well as a "non-settling particle" via the Hairsine–Rose model (Hairsine and Rose, 1991; Heilig et al., 2001) or as a "non-diffusing solute" via the Gao model (Gao et al., 2004); of course, bacteria are particles (not solutes), but the Gao model is easier to apply to bacterial experiments because we do not need to make assumptions about the number of bacteria that initiate a colony forming unit (CFU), which is needed in order apply use the Hairsine–Rose model (see Wang et al., 2017 for a full explanation). So, here we will use the Gao model to infer mechanistic differences between *E. coli* release from soil under raindrop impact.

2. Experimental design

We used the same analytical procedures and experimental set-up as Wang et al. (2017) (Fig. 1). These methods are briefly described below. The only experimental difference from theirs is that here we used a second soil column composed of pure sand (250–300 µm sand) in addition to the 9:1 sand-clay mixture (250–300 µm sand, kaolinite clay).

E. coli ATCC 25,922, a common nonpathogenic surrogate of pathogenic *E. coli* O157: H7 (Muirhead et al., 2006; Salleh-Mack and Roberts, 2007; Sauer and Moraru, 2009), was grown in Tryptic Soy Broth (TSB) for 18 h at 37 °C. Two milliliters of this culture were then mixed with 80 mL of 1.08% potassium chloride (KCl) solution (ionic strength = 0.145 M) and added to 250 g of soil; 1.08% KCl prevented lysing the *E. coli* cells and dispersing the clay, while avoiding uncontrollable aggregation.

The pre-saturated soil was packed into 7.6 cm-diameter plexiglass columns using a shaking table. A 0.5 ml sample was taken from the solution that was ponded on the surface of the column during this procedure to determine the initial *E. coli* concentration, before the ponded solution was poured off. The columns were then placed under a rainfall simulator and *E. coli*-free KCl solution was gently added to prepond the soil columns; we did this so a steady-state runoff assumption would be valid at t = 0. A 0.5 ml sample was extracted from this pre-