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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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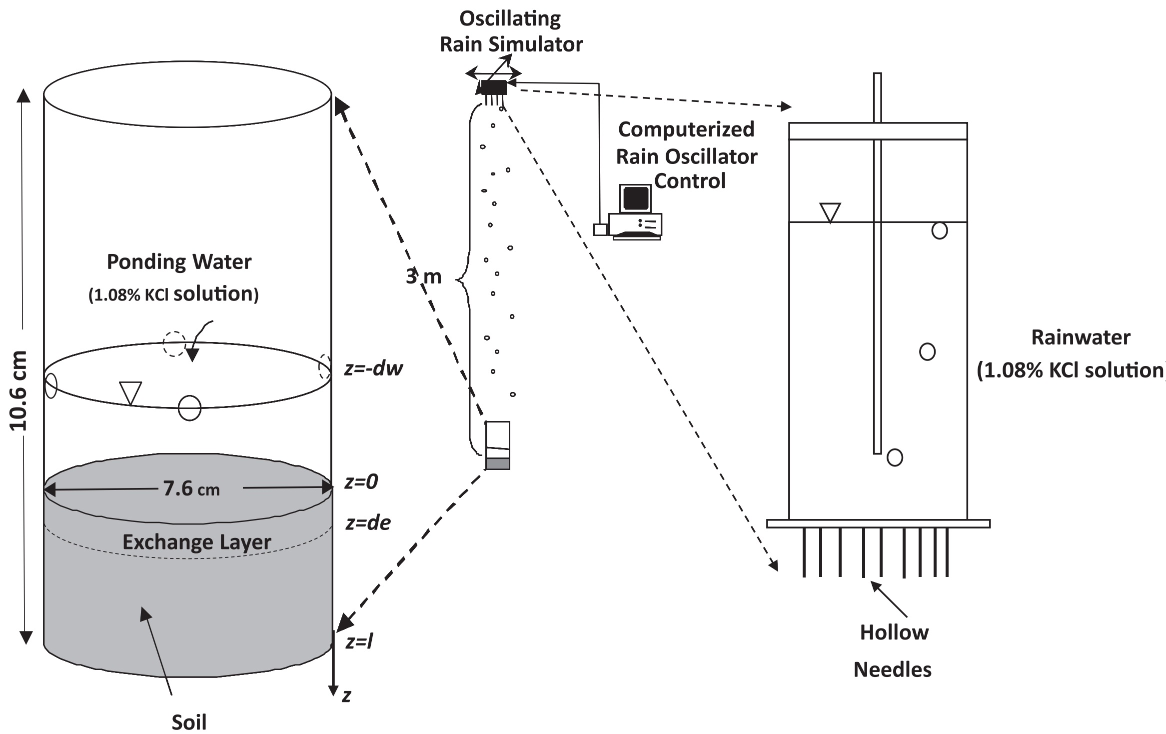

Fig. 1. Experimental set-up, same as Wang et al. (in press), adapted from Gao et al. (2004, 2005). The ponded water (d_w) and exchange layer (d_e) as defined in the Gao model are labeled next to the column.

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 pre-pond 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-

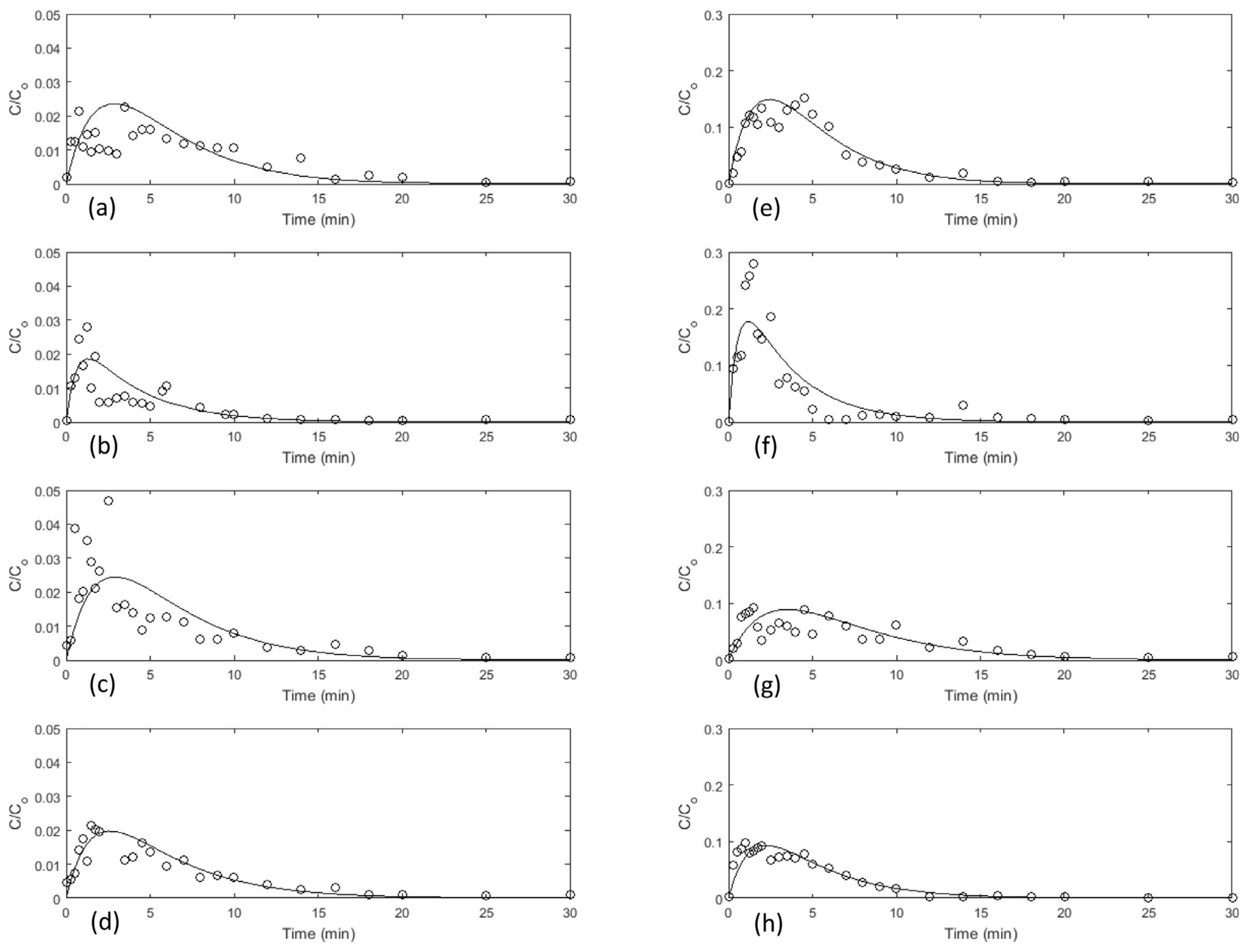

Fig. 2. Gao solute model for relative concentration of *E. coli* from clay and sand soil (left column, (a) to (d) corresponding to run 1 to run 4, respectively, from Wang et al. 2017) and from pure sand soil (right column, (e) to (h) corresponding to run 1 to run 4, respectively; circles = experimental data, lines = Gao model. Note, scales are different between a–d and e–h.

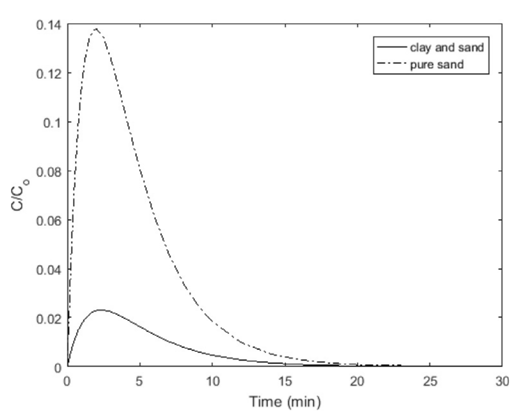

Fig. 3. Comparison of *E. coli* release between sand-clay mixture (solid line) and 100% sand (dashed line) as simulated by the Gao model using average experimental soil properties and applying the same initial *E. coli* concentration. (C_o).

ponded water as the sample at $t = 0$.

A Marriott bottle (Fig. 1) was used to generate constant rainfall intensity without allowing the introduction of bacteria from the ambient air. The rainwater consisted of 1.08% KCl solution to avoid lysing of the *E. coli* cells. The study area was protected with an umbrella until the rainfall was steady. A timer was started when the umbrella was removed. Samples of 0.5 ml were taken from the runoff at varying intervals that transitioned at times 0, 2, 5, 10, and 20 min from the beginning of rainfall. The sampling intervals within each time period were 15 s, 30 s, 1 min, 2 min and 5 min, respectively. Rainfall lasted for a total of 30 min. Wang et al. (2017) showed that the net growth (or net die-off) of *E. coli* during the 30 min rainfall experiment was negligible.

The concentration of bacteria in runoff samples was determined by a dilution and inoculation procedure. Sterilized 1.08% KCl solution was used to dilute each runoff sample. Samples were then plated on *E. coli* media with 4-methylumbelliferyl-β-D-glucuronide (EC-MUG; Neogen Corporation, Lansing, MI) and incubated for 20 h at 37 °C. Colony forming units (CFUs) were manually counted on each plate and converted to the bacteria concentration units (CFU/ml) in the original runoff sample. To verify that there were no unknown sources of *E. coli*, we used a control experimental with no *E. coli* additions to the soil to assure our design was not inadvertently introducing contamination.

A total of four experimental runs (i.e., four replicates) were completed. The rainfall intensity (p) and the ponding water depth (d_w) were measured before and after each experimental run and the averages were used in the Gao model so we could ignore the small variations in these values. The shield layer (a.k.a. mixing layer or exchange layer) depth, d_e, was measured after each experimental run. The exchange layer for the clay-sand mixture was very distinct because the white clay had been removed making it much darker than the underlying soil (following Heilig et al., 2001). The exchange layer for the pure sand was also visually distinguishable because a distinct white layer formed in the soil below the exchange layer (see Wang, 2015 for a figure). Presumably this white layer was also present in the clay-sand experiments, but was masked by the white clay. A subsequent analysis in which we ran experiments with and without *E. coli* suggest that the white precipitate was likely *E. coli* cells, i.e., the layer did not form in experiments that omitted *E. coli* (data not shown). Although we attempted to keep conditions identical between experimental runs, the ponding depth, the rainfall intensity, and the initial *E. coli* concentration varied a from run to run due to inherent variability in our experiment, especially the initial *E. coli* concentration.

3. Gao model

Wang et al. (2017) showed that Gao solute model (Gao et al., 2004) simulates *E. coli* release from soil into overland flow under rainfall impact very well with a diffusion coefficient set to zero. The Gao model performs a mass balance of the material ejected from the exchange layer into the ponded water (Gao et al., 2004). The layers referred to in the Gao model are shown in Fig. 1. Assuming no diffusion of material from the underlying soil into the exchange layer and, therefore, no alteration of the concentration of *E. coli* in the underlying soil, the Gao model for our experimental conditions can be written as:

$$\text{Exchange layer:} \theta d_e \frac{dC_e}{dt} = -e_r C_e \tag{1}$$

$$\text{Ponded water:} d_w \frac{\mathrm{d}C_w}{\mathrm{d}t} = e_r C_e - pC_w \tag{2}$$

$$\text{where } e_r = \frac{ap\theta}{\rho_b} \tag{3}$$

$$\text{Initial conditions:} C_e = C_o, \quad C_w = 0 \tag{4}$$

where C_e and C_w are concentrations of *E. coli* (CFU/ml) in exchange layer pore water and runoff (i.e., ponded water), respectively; C_o is the initial concentration of *E. coli* in soil (CFU/mL); d_e (cm) is the exchange layer depth; t (min) is time; p (cm/min) is the rainfall intensity; d_w (cm) is the ponding water depth; a (g/cm^3) is the soil detachability; θ is the volumetric soil water content (saturated water content in our case); ρ_b (g/cm^3) is the bulk density of the soil. Wang et al. (2017) analytically solved the Gao model for these simple experiments:

$$C_e = C_o \exp\left(-\frac{ap}{\rho_b d_e} t\right) \tag{5}$$

$$C_w = C_o \frac{ap\theta}{\rho_b d_w \left(\frac{p}{d_w} - \frac{ap}{\rho_b d_e}\right)} \left\{ \exp\left[\left(\frac{p}{d_w} - \frac{ap}{\rho_b d_e}\right)t\right] - 1 \right\} \exp\left(-\frac{p}{d_w} t\right) \tag{6}$$

Like Wang et al. (2017), the soil detachability, a, is used as a fitting parameter.

4. Results and discussion

To independently estimate d_e (Eq. 7), as confirmed by our visual observations, we integrated the concentrations of ejected *E. coli* (C_e), over the experimental durations (and dividing by soil bulk density); for the sand-clay columns we used the integral of clay concentrations as an independent check (see Wang et al., 2017). We found that our C_o values were highly variable relative to the total ejected *E. coli*, thus, we used the visually observed exchange layer depths for d_e and fitted C_o by forcing the integral under the model curve equal to the integral under the measured data for each experiment (see Wang et al. 2017 for a more extensive discussion on this aspect of our experiments). There was no systematic patterns for C_o, i.e., consistently higher or lower than the total ejected *E. coli*, so we are inclined to conclude that there is some inherent variability in our experimental design rather than a missing process *per se*.

All the parameter definitions and values are listed in Table 1. The Gao model was able to capture the *E. coli* release from both the sand-clay and sand alone experiments (Fig. 2). Results show that *E. coli* in pure sand columns eroded more readily (y-axes are an order of magnitude higher for 100% sand plots relative to clay-sand plots – Fig. 2). We performed a series of analyses of variance (ANOVA) analyses to determine whether the characteristics of the clay/sand mix and the pure sand were significantly different. Compared to the clay/sand mix, the pure sand had significantly larger peak amount of *E. coli* release (via erosion; $p = .025$), significant larger total amount of *E. coli* release between 0 and 10 min ($p = .014$) and significantly larger total amount of *E. coli* release between 0 and 30 min ($p = .0002$). However, the two soil types have similar soil detachability (a; $p = .125$), rainfall intensity (p; $p = .312$), ponding water depth (d_w; $p = .506$), time to peak ($p = .907$), and integral from 0 to average peak time ($p = .663$). These findings agree with the test showing that the pure sand had a significant larger penetration depth (d_e; $p = .0004$), and was indeed easier to penetrate, which resulted in higher peaks and total eroded amounts of *E. coli*. In addition, pure sand soil had higher soil water content by volume at saturation (θ_s) and lower bulk density of the soil (ρ_b) than clay-sand soil (Table 1). Thus, with similar detachability and exposure to rainfall of similar intensity, more soil water and associated *E. coli* would be ejected from pure sand than the clay/sand mix.

By way of illustration, we ran the Gao model using average soil properties for each of the soil columns (sand-clay mixture vs. 100% sand) and applied the same initial *E. coli* concentrations to both soil conditions, i.e., this was a difficult parameter to control in the experiments. It is obvious that many more E. coli are ejected from the 100% sand soil than the sand-clay mixture (Fig. 3). Thus, clay is not necessary to facilitate *E. coli* release. The differences are most strongly linked to reduced rain-drop penetration depth, d_e, and, to a lesser degree, reduced saturated soil water, θ_s, for the sand-clay mixture compared to the 100% sand.

5. Conclusion

We conclude, based on a combination of empirical and modeling results, that soils with increased clay content will release less bacteria into storm runoff compared to sandy soils, due to the role of clay in decreasing the effectiveness of raindrop impact, i.e., shallower penetration into the soil. With respect to the question that initiated this study, *E. coli* are not dependent on mineral (clay) particles for transfer between soil and overland flow during rainfall, although we still do not know whether they will preferentially attach to clay (compared to sand) if it is present.

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