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Adhesion of *Escherichia coli* and *Salmonella enterica* to Soil in Runoff as Influenced by Polyacrylamide

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Abstract

Polyacrylamide (PAM) is used in agriculture to reduce soil erosion and has been reported to reduce turbidity, nutrients, and pollutants in surface runoff water. The objective of this work was to determine the effect of PAM on the concentration of enteric bacteria in surface runoff by comparing four enteric bacteria representing phenotypically different motility and hydrophobicity from three soils. Results demonstrated that bacterial surface runoff was differentially influenced by the PAM treatment. Polyacrylamide treatment increased surface runoff for adhered and planktonic cells from a clay soil; significantly decreased surface runoff of adhered bacteria, while no difference was observed for planktonic bacteria from the sandy loam; and significantly decreased the surface runoff of planktonic cells, while no difference was observed for adhered bacteria from the clay loam. Comparing strains from a final water sample collected after 48 h showed a greater loss of *Escherichia coli* while *Salmonella enterica* serovar Poona was almost not detected. Thus, (i) the PAM efficiency in reducing the concentration of enteric bacteria in surface runoff was influenced by soil type and (ii) variation in the loss of enteric bacteria highlights the importance of strain-specific properties that may not be captured with general fecal indicator bacteria.

APPLICATION of slurry to agricultural land may lead to enteric pathogens entering water used for drinking, irrigation, and recreation (Warnick et al., 2001; Van Donkersgoed et al., 2009; Haley et al., 2009; Jenkins et al., 2009;). Curriero et al. (2001) found that heavy rainfall and disease outbreaks correlated well over the period from 1948 to 1994 in the United States. Human exposure to recreational waters containing pathogens is a recognized risk for infection, the probability increasing with the extent of bodily contact and pathogen loading. The importance of agricultural pollution was recently highlighted by the USEPA, who ranked agriculture as the most probable source of threatened or impaired rivers and streams (USEPA, 2013).

The public health objective to control agricultural pollution has led to implementation of best management strategies including no-till, riparian buffer zones, cover crops, and nutrient management plans. Establishment of vegetated filter strips, managed wetlands, and riparian buffer zones increase particle settling and filter surface runoff before entering vulnerable surface and coastal waters. An alternative and potentially synergistic approach to reduce runoff involves the application of polymers such as the anionic polyacrylamide (PAM) to soil, which has been shown to reduce soil erosion by up to 84% (Lentz and Sojka, 1994, 2009; Zhang and Miller, 1996; Sepaskhah and Shahabizad, 2010; Tümsavaş and Kara, 2011). In addition, PAM has reduced the environmental impact of agricultural activities by reducing particulate phosphorus (Goodson et al., 2006), total phosphorus, fecal coliform bacteria (Sojka and Entry, 2000), biochemical oxygen demand, and nitrate (Lentz and Sojka, 1994, 2000; Aase et al., 1998; Goodson et al., 2006) in runoff water. Polyacrylamide has been used as a soil-stabilizing compound since 1995 and is recommended by the NRCS for agricultural purposes. It is a high-weight linear polymer containing >150,000 acrylamide monomers and is negatively charged.

Polyacrylamide application may reduce bacterial load in surface runoff and agricultural effluent water associated with

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Abbreviations: BPB, Butterfield's phosphate buffer; CFU, colony forming units; M9, M9 minimal salts, 5X buffer; OD, optical density PAM, polyacrylamide; PCR, polymerase chain reaction; RIF, Rifampicin; TDS, total dissolved solids; TSA, Tryptic soy agar; TSB, Tryptic soy broth; TSS, total suspended solids.

irrigation as a result of sequestering of bacteria to soil aggregates or fractions. Relatively few have studied the effect of PAM application on bacterial load in surface runoff. Sojka and Entry (2000) found that a PAM treatment reduced the total bacterial number in surface runoff compared with nontreated silt loam soils. Entry and Sojka (2000) found that PAM+$Al(SO_4)_3$ and PAM+CaO reduced total coliform bacteria, fecal coliform bacteria, and fecal streptococci by 10- to 1000-fold from animal waste in water flowing 1 and 27 m downstream from the inlet at a silt-loam field site.

Previous studies have focused on coliform bacteria, total bacteria, total bacterial biomass, fecal coliform bacteria, and fecal streptococci. However, recent studies and reviews have detailed the lack of correlation between indicator microorganisms and the presence of pathogenic bacteria in surface water (Duris et al., 2009; Pachepsky et al., 2011; Shelton et al., 2011) Therefore, the aim of this study was to evaluate how PAM treatment influenced surface runoff water carriage of four enteric bacteria with different hydrophobicity and motility in three soils: clay, clay-loam, and sandy-loam soil.

Materials and Methods

Soils

Surface soil (A horizon) was collected from major irrigated, cool-season, vegetable production farms in Salinas, CA. The soils used include a Valpac soil (a fine-loamy, mixed, superactive, thermic Fluvaquentic Haploxerolls; clay), a Yorkville soil (fine-loamy, mixed, thermic Typic Argixerolls; sandy loam), and Riz soil (fine, smectitic, thermic Typic Natrixeralfs; clay loam) (Source: http://casoilresource.lawr.ucdavis.edu/gmap/). For details on soil properties see Table 1. Soils were collected 1 mo before experiment onset and were stored at 4°C in 18-L sealed plastic containers.

Bacterial Strains and Inoculum Preparation

Strains of *Escherichia coli* (TVS354), *Salmonella enterica* serovar Newport (PTVS73), *S. enterica* serovar Poona (PTVS124) and *E. coli* O157:H7 (PTVS46) were used in this study. All strains were rifampicin (RIF) resistant (80 mg L^{-1}), which facilitated detection, recovery, and enumeration with negligible interference from soil background bacteria. Strains were made RIF-resistant under pressure selection in our laboratory.

Bacterial strains were cultured overnight at 37°C on Tryptic soy agar (TSA, BD Diagnostics), supplemented with 80 mg L^{-1} of RIF (Sigma-Aldrich). One colony was transferred to 5 mL Tryptic soy broth (TSB, BD Diagnostics) with 80 mg L^{-1} RIF for 18 h at 37°C. A total of 100 µL of cell suspension (stationary phase) was transferred to fresh 5 mL TSB RIF. Cells were harvested after 6 h at the end of the exponential phase. The bacterial suspension was centrifuged at 1500 × g for 10 min. The pellet was washed twice in Butterfield's phosphate buffer (BPB) (Whatman, Inc.) and resuspended in BPB. Cultures were stored at 10°C for 18 h followed by adjustment of to an optical density (OD) of 0.7 at 620 nm (OD$_{620}$), which corresponds to approximately 10^8 colony forming units (CFU) mL^{-1}. Cultures were serially diluted and plated to determine the best estimate of cell concentration in the bacterial suspension.

Hydrophobicity and Motility of Selected Strains

The hydrophobicity of the strains was measured using an assay based on the bacterial adherence to hydrocarbons using octane as the organic liquid (Rosenberg et al., 1980). The cells were grown as described above and resuspended in a buffer containing 22.2 g $K_2HPO_4\cdot3H_2O$, 7.26 g KH_2PO_4, 1.8 g urea, and 0.2 g $MgSO_4\cdot7H_2O$ in 1 L of deionized water adjusted to pH 7.1. One mL of a washed-cell suspension with an OD$_{620}$ of 0.7 and 0.5 mL of octane were vortexed for 60 s in a 2-mL test tube. The difference in OD$_{620}$ was measured after 30 min. Hydrophobicity was determined as $[100 \times (OD_A - OD_B)/OD_A]$, where OD$_A$ and OD$_B$ represent the optical density of the aqueous phase before and after the hydrophobicity test, respectively.

Motility was tested in soft agar as described previously (Fredslund et al., 2008). A 5-µL aliquot of 10^8 CFU mL^{-1} culture was carefully placed in the center of a 10% TSA plate with 0.3% agar. The plates were incubated at 29°C overnight in a humid environment to achieve a measurable swarm diameter within the agar dish. Swarm radii were measured with a caliper and evaluations were made in triplicate.

Model Runoff Experiment

Before the surface runoff experiment, the recently collected soil was sieved through an 8-mm sieve and allowed to air dry at 25°C. The runoff trays were packed with dry soil at a density of 1.3 g cm^{-3} in boxes (40 cm long, 4 cm deep, and 11 cm wide). One day before the onset of the surface runoff, 500 mL of M9, minimal salts, 5X buffer (M9; 33.9 g L^{-1} $Na_2HPO_4\cdot7H_2O$, 15 g L^{-1} KH_2PO_4, 5 g L^{-1} NH_4Cl, 2.5 g L^{-1} NaCl; Difco) adjusted to an electric conductivity of 1.3 dS m^{-1} was added to the soil resulting in gravimetric water content of 20%. All surface runoff experiments were conducted in these microcosms inclined to a 5% slope and were repeated in triplicate. Surface runoff was collected immediately in the collection chamber that was separated from the soil by a small plastic barrier equaling the height of the soil. The bacterial strains were grown as previously described and a cocktail consisting of 0.25 mL of each strain was diluted into 9 mL M9 solution and pipetted evenly on the soil surface 2 h before the onset of the surface runoff to allow adhesion (Huysman and Verstraete, 1993). The total amount of

Table 1. Chemical and physical properties of the clay loam, clay, and sandy loam soil.

Soil	pH	Sand	Silt	Clay	SOM†	CEC‡	Fe	Al
		——————————— % ———————————				cmol kg^{-1}	——— mg kg^{-1} ———	
Clay loam	6.83	40	25	35	2.37	33.4	23,400	22,100
Clay	7.39	34	20	46	3.48	43.3	38,200	29,100
Sandy loam	6.34	74	13	13	1.46	13.5	15,700	12,900

† SOM, soil organic matter.

‡ CEC, cation exchange capacity.

each strain was 10^8 CFU per microcosm, resulting in an average concentration in the soil of 5×10^4 CFU g^{-1} of dry soil. The M9 solution was introduced to the side of the microcosm distal to the collection reservoir using a Rainin peristaltic pump (Rainin Instrument LLC) onto the top of the soil at a flow rate of 31 mL min^{-1} to generate surface runoff that equaled an irrigation intensity of approximately 40 mm h^{-1}. Polyacrylamide was thoroughly mixed into the M9 solution to a concentration of 10 µg PAM mL^{-1} according to the PAM NRCS guideline, whereas in the control experiment, a M9 solution without PAM was used. From each surface runoff experiment, ten independent water samples of 50-mL aliquots were collected for analysis. Surface runoff first occurred on soil saturation, and the ten samples were collected during approximately 20 min.

A subsample of 10 mL was used for a buoyant-density separation procedure separating sediment-adhered cells from planktonic cells. Below the soil suspension, 1 mL of Histodenz (Sigma Aldrich) at a density of 1.3 was added followed by centrifugation for 20 min at $3000 \times g$ in a swing-out rotor centrifuge (Thermo CL3R). The two layers were separated by decanting the fractions into a sterile 15-mL Falcon test tube (BD Biosciences), and the pellet was resuspended in 5 mL of BPB. Cell concentrations were determined in both supernatant and soil pellet by preparing 10-fold dilutions and plating 100 µL on TSA RIF.

The remaining 40 mL were centrifuged in a swing-out rotor centrifuge for 10 min at $910 \times g$ to determine the solid-matter content. The settling time for the fraction >0.45 µm was determined as described by Gimbert et al. (2005). The supernatant was collected, and both fractions were dried at 60°C for at least 48 h (Throop et al., 2012) to determine total suspended solids (TSS) (>0.45 µm) and total dissolved solids (TDS) (<0.45 µm). Due to low recovery of TSS, these were dismissed due to uncertainty of the measured weight.

Distribution of Bacterial Strains in Surface Runoff

A second surface runoff assessment from each microcosm was conducted 48 h later, where one water sample was collected and 32 randomly collected colonies from the adhered and planktonic fraction from each surface runoff event was isolated. Collected colonies were streaked on TSA RIF and incubated overnight at 37°C. Two to three colonies were picked and resuspended in BPB to yield an OD$_{620}$ of 0.3 to 0.5. The cell suspension was subsequently centrifuged at $13,000 \times g$ for 1 min and suspended in 200 µL of Tris EDTA 1X buffer (Sigma Aldrich). Cell lysates were obtained by heating cell suspensions for 10 min at 95°C. The DNA from isolated colonies were analyzed through repetitive element polymerase chain reaction (PCR) (Versalovic et al., 1991) to identify their unique band patterns and to determine the distribution of bacterial strains in the surface runoff.

Each PCR contained 5 µL of 5X colorless GoTaq buffer (Promega) supplemented with 2.5 µL 25 mM MgCl (Promega), 0.5 µL 10 mM dNTP (Promega), 2.5 µL 10 µM REP1R-I (IIIICGICGICATCIGGC), 2.5 µL 10 µM REP2-I (ICGICTTATCIGGCCTAC) (Versalovic et al., 1991), 2.5 µL 10% DSMO (Sigma-Aldrich), 1.25 unit GoTaq (Promega), 7.75 µL H$_2$O, and 1.5 µL DNA sample. In each PCR well, 15 µL mineral oil was placed on top to avoid evaporation.

Polymerase chain reaction amplifications were done in a thermocycler (Applied Biosystems) with an initial denaturation (4 min, 94°C) followed by 30 cycles of denaturation (94°C, 1 min), annealing (40°C, 1.5 min), and extension (65°C, 8 min), followed by a single final extension (65°C, 16 min). The DNA fragments were separated in a 1.2% agarose gel for 3 h at 120 V. Isolates showing indistinguishable band patterns were grouped manually and compared with the band patterns of matched inoculum-source pure colonies from the culture collection.

Bacterial Adhesion

A soil suspension containing 1 g of soil and 9 mL of M9 solution, as described previously, was mixed in a 15-mL Falcon test tube. Seventy-four microliters of a PAM (SoilFloc E300, Hydrosorb, Inc.) stock solution (0.37%) was added to achieve a final concentration of 0.09 µg PAM g fresh soil^{-1}. The soil buffer suspensions were mixed for 36 h at 150 rpm at 10°C. A total of 50 µL of inoculum (OD$_{620}$ 0.7) was added to the soil suspension resulting in a concentration of 10^6 to 10^7 CFU mL^{-1} in soil suspension. Samples were analyzed after 5 h at 150 rpm at 10°C to allow adhesion between strain and soil. Adhered and unadhered cells were determined by the buoyant-density separation procedure as described above. The percentage of adhered cells was calculated based on the bacterial concentration from both fractions. Further, the effect of PAM on bacterial flocculation was tested in the range 0 to 10 mg PAM L^{-1}, and comparable levels of enumeration among treatments were observed (data not shown).

Statistics

Enumerated colonies were expressed as CFU per g of fresh soil or CFU mL^{-1} with calculated standard deviations. Data were compared with either t test or ANOVA in SigmaPlot 12.5 (Systat Software, Inc., 2013). If normality testing failed, the data were then analyzed with the Mann–Whitney Rank Sum test. Statistical significant differences among treatments was established when $P < 0.05$.

Results and Discussion

Model Runoff Experiment

Irrigation was applied to the unplanted soil boxes in the present experiment at a rate that equaled 0.7 mm min^{-1}. Soil erosion was greatest from the clay and clay-loam soil with a decreasing trend over time, whereas, erosion from the sandy-loam soil was both lower and constant (Fig. 1). The effect of PAM on adhered and planktonic cells and TDS varied with soil type. In the clay soil, PAM significantly decreased TDS in the surface runoff water compared with the control ($P = 0.002$). However, PAM significantly increased the bacterial concentration from the applied bacterial cocktail in the surface runoff water for both adhered ($P = 0.048$) and planktonic cells ($P = 0.014$). The surface runoff from the PAM treatment in the clay loam soil showed a significant decrease in TDS ($P = 0.001$), planktonic cells ($P < 0.001$), and adhered cells ($P = 0.008$). For the sandy-loam soil, the PAM treatment did not have a significant effect on the amount of TDS in the surface runoff water ($P = 0.07$), while there was a significant decrease in the adhered cells with
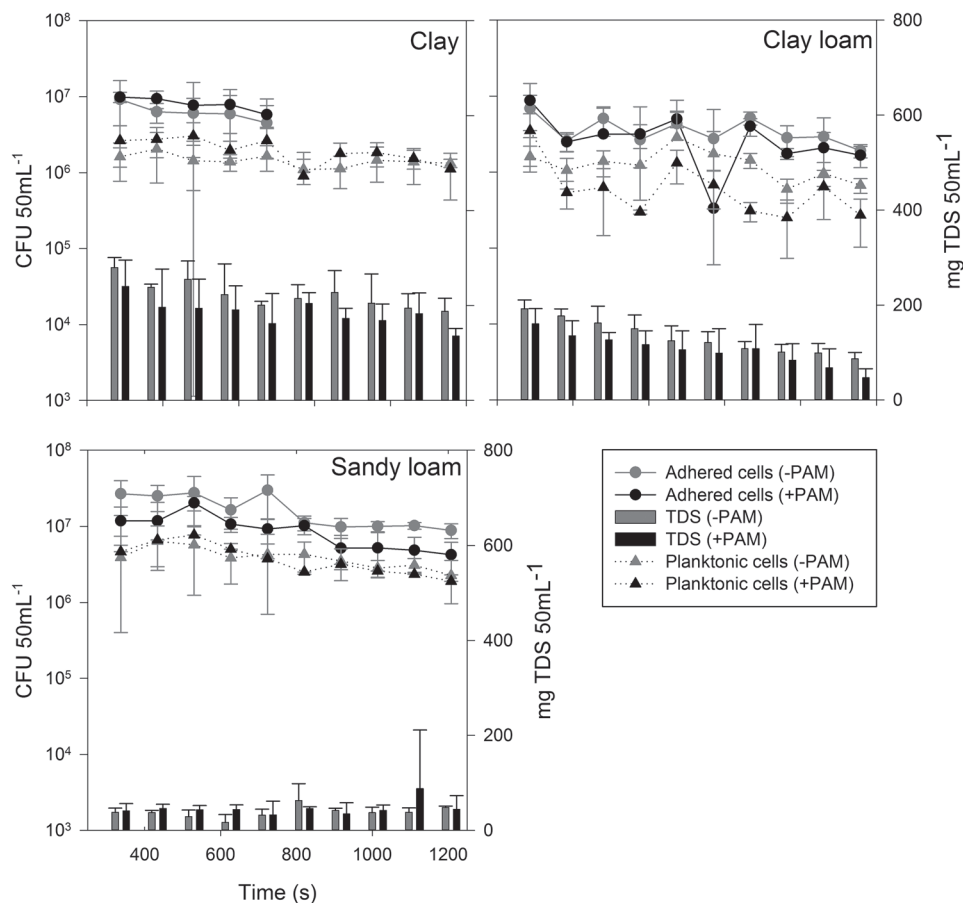
Fig. 1. Runoff data for the three soils based on triplicate experiments. Bars represent total dissolved solids (TDS) (<0.45 μm), comparing a control (gray) with a polyacrylamide (PAM) treatment (black). The number of planktonic cells is given as colony forming units (CFU) 50 mL^{-1} (triangles) and the number of adhered cells are given as CFU 50 mL^{-1} (circles). Black represents PAM treatments and gray represents controls. Error bars represent standard deviations (n = 3).

the PAM treatment in the surface runoff water ($P < 0.001$) and no difference for planktonic cells ($P = 0.824$).

Previous research has generally concluded that PAM reduces soil erosion (Petersen et al., 2007; Lentz and Sojka, 2009; Murphy et al., 2010; Li et al., 2011). Fewer studies have compared the effect of PAM on different soil types even though Miller et al. (1998) reported that the PAM efficiency as a soil conditioner depends on soil texture. This was supported by Sepaskhah and Shahabizad (2010) who compared sandy-loam, loam, and silty-clay-loam soils in a surface runoff experiment and found a reduced effect of PAM from the sandy loam. They explained this finding by lack of surface sealing in sandy soils and since PAM reduce surface seals; it would not make a difference in this type of soil. An additional study by Tümsavaş and Kara (2011) found that effectiveness of PAM was greater in clay and clay loam compared with a sandy-clay loam. They explained this observation by the smaller specific surface area of the sand particles, where a small contact area between sand particles would decrease the likelihood of PAM bridging. This is in accordance with the present experiment, as the PAM treatment did not significantly decrease erosion from the sandy-loam soil.

The recovery of bacteria in the PAM-treated surface runoff was ~40% for the clay-loam soil, ~100% for the clay soil, ~100% for the sandy-loam soil, and ~50, ~80, and ~100% for the control soils, respectively. The recovery of enteric bacteria was therefore influenced by both soil type and PAM treatment. From the

clay soil there was an observed increase in the runoff of enteric bacteria with the PAM treatment, whereas a decrease, or no difference, was observed for the sandy-loam and clay-loam soils, respectively. These observations contradict a study by Entry et al. (2003) comparing runoff from three soil types (heavy clay, loam, and light sandy loam) concluding that PAM treatments reduced the loss of total coliform and fecal coliform. Previous studies conclude that PAM decreases bacterial concentration in surface runoff water indiscriminate to the type of microorganisms (Entry et al., 2000, 2003; Sojka and Entry, 2000). Common among these studies was a focus on fecal indicator organisms compared with our study with four pure bacterial cultures. Further, the studies by Entry and Sojka (2000) and Sojka and Entry (2000) were conducted at the same research site. The soil properties at this site differ from the soils used in the present experiment by having a high silt content of 60 to 75%. Spackman et al. (2003) found no difference of total coliform in the surface water regardless of PAM treatment from a silty loam.

The distribution between adhered and planktonic bacteria in surface runoff was influenced by the soil type. From the clay and clay-loam soil, approximately 80% of cells in runoff were adhered, whereas the opposite was seen for the sandy loam where 20% were associated with particles. Characklis et al. (2005) found that 20 to 35% of *E. coli* was associated with rapidly settling particles in storm water. In a laboratory controlled experiment, Muirhead et al. (2006b) compared the

transport of adhered and planktonic *E. coli* in a saturated soil and found that planktonic cells were more easily transported across the soil and thereby to the receiving waters. Furthermore, when *E. coli* was adhered to soil particles (>45 μm) before the runoff experiment, a significant reduction was seen compared with inoculation of unadhered cells. Soupir et al. (2010) found that the percentage of *E. coli* and enterococci adhered to soil particles in surface runoff ranged from 28 to 49% and that this proportion increased in time when comparing surface runoff at 10 and 30 min. As bacterial suspensions were applied to the soil surface 2 h before the surface runoff, this could facilitate a higher proportion of adhesion in this experiment. This initial adhesion between soil and bacteria may also explain why the PAM treatment did not alter the distribution between adhered and planktonic bacteria.

Bacterial Strains in Surface Runoff

Genotyping of colonies collected from the surface runoff after 48 h were used to determine the distribution of the four bacterial strains with repetitive element PCR as a fingerprinting technique (Table 2). This comparison does not provide information on the temporal variation between strains in the surface runoff but solely a snapshot at 48 h. When comparing the four bacteria, *S.* Poona was least abundant, whereas *E. coli* was most abundant in the surface runoff from the three soils regardless of the PAM treatment. Adhesion and detachment are important processes that influence the potential loss of bacteria through surface runoff, where there is an increased risk of transport for planktonic bacteria (Muirhead et al., 2006a). Comparing the effect of PAM on three soil types and four bacterial strains did not show a clear pattern, and the PAM treatment did not change the distribution between the four bacteria in the surface runoff sample at 48 h.

Bacterial Adhesion

The adhesion experiment showed that the presence of PAM decreased bacterial adhesion compared with the controls (Fig. 2). The effect of the PAM treatment on bacterial adhesion differed between soil types and decreased in the following order: sandy loam > clay > clay loam. The effect of the PAM treatment on species level was highest for the sandy-loam soil, where only the adhesion of *S.* Poona was not significantly different. Compared with the other strains, regardless of the PAM treatment, *S.* Poona showed the strongest adhesion. A decrease in bacterial adhesion due to the PAM treatment was significant for the following combinations: Clay and *S.* Newport, Clay loam and *E. coli* O157:H7, Sandy loam and *E. coli*, Sandy loam and *E. coli* O157:H7, and Sandy loam and *S.* Newport. Bacteria with a strong adhesion are therefore less likely to be influenced by a PAM treatment. However, for all treatment combinations, there was an observed decrease in adhesion in the presence of PAM. This may be explained by physical reduction in the surface area due to agglomeration of soil particles by the PAM treatment (Lu et al., 2002). In addition, the lack of divalent cations in the M9 solution would have reduced cation bridging between the repulsive negative charges of the bacterial surface and the PAM molecule. However, by using this solution, the natural variation in divalent cation from the three soils would have a more profound effect on the adhesion. A preliminary adhesion experiment for *E. coli* showed larger adhesion in 0.005 M $CaCl_2$ solution compared with a 0.01 M NaCl solution; though, in both solutions, there was observed decreased adhesion with the PAM treatment. The greatest decrease in adhesion was seen in the sandy loam, most likely explained by fewer sorption sites compared with the clay and clay loam. Limited sorption sites would increase competition between PAM molecules and bacterial cells, as both contain carboxyl groups. When comparing the clay and clay-loam soil, a similar effect of the PAM treatment was seen.

Table 2. Distribution percentage of the four bacteria in the surface runoff collected at 48 h.

Soil type	Planktonic or adhered	PAM† treatment	*E. coli*‡	*E. coli* O157:H7§	*S.* Newport¶	*S.* Poona#
			%—————————————————			
Clay	Planktonic	+PAM (*n* = 32)††	31.3	40.6	25.0	3.1
		−PAM (*n* = 32)	40.6	34.4	25.0	0.0
	Adhered	+PAM (*n* = 32)	50.0	31.3	18.8	0.0
		−PAM (*n* = 32)	31.3	50.0	12.5	6.3
Clay loam	Planktonic	+PAM (*n* = 32)	65.6	12.5	21.9	0.0
		−PAM (*n* = 32)	50.0	18.8	31.3	0.0
	Adhered	+PAM	ND‡‡	ND.	ND	ND
		−PAM	ND	ND	ND	ND
Sandy loam	Planktonic	+PAM (*n* = 32)	78.1	9.4	9.4	3.1
		−PAM (*n* = 32)	56.3	21.9	12.5	9.4
	Adhered	+PAM (*n* = 32)	84.4	9.4	6.3	0.0
		−PAM (*n* = 21)	71.4	14.3	9.5	4.8

† PAM, polyacrylamide; +PAM, with PAM treatment; −PAM, no PAM treatment.

‡ *Escherichia coli* (TVS354).

§ *Escherichia coli* O157:H7 (PTVS46).

¶ *Salmonella enterica* serovar Newport (PTVS73).

Salmonella enterica serovar Poona (PTVS124).

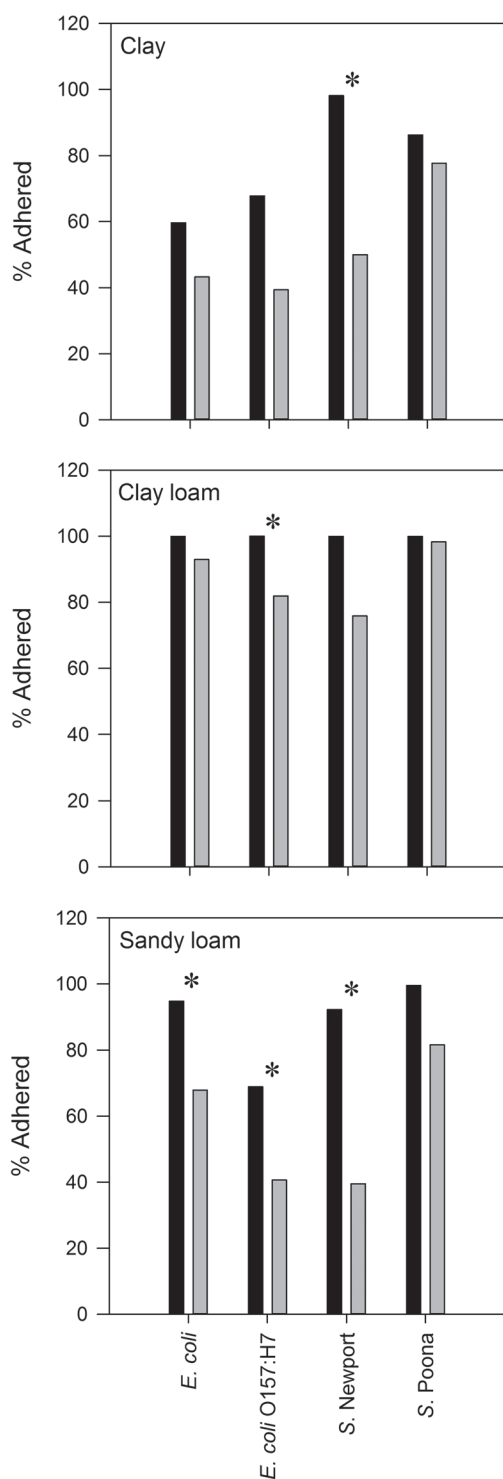†† Thirty-two colonies were isolated from each treatment.

‡‡ ND, not detected.

Fig. 2. Percentage of bacterial strains adhered to the clay, clay loam, and sandy loam soil comparing a control (black bars) with a polyacrylamide (PAM)-treated soil (gray bars). Values are based on triplicate adhesion experiments, where the percentage of adhered cells is calculated as the fraction of the sum of adhered and planktonic cells. An asterisk indicates a significant difference between the control and PAM-treated soil ($P < 0.05$).

Similar soil properties such as texture, soil organic matter, and cation exchange capacity (CEC) (Table 1) could explain this observation. Huysman and Verstraete (1993) attributed a higher bacterial adhesion in a clay-loam soil to greater CEC.

Table 3. Hydrophobicity and motility for *Escherichia coli*,† *Salmonella* Newport,‡ *S.* Poona,§ and *E. coli* O157:H7.¶ Mean value and standard deviations are based on triplicate measurements.

Bacteria strain	Motility	Hydrophobicity
	cm	%
PTVS142 *S.* Poona	1.6 ± 0.66	0.9 ± 0.6
PTVS73 *S.* Newport	3.4 ± 1.4	1.4 ± 1.2
TVS354 *E. coli*	2.9 ± 0.17	15.1 ± 7.3
PTVS46 *E. coli* O157:H7	0.5 ± 0	6.5 ± 2.0

† *Escherichia coli* (TVS354).

‡ *Salmonella enterica* serovar Newport (PTVS73).

§ *Salmonella enterica* serovar Poona (PTVS124).

¶ *Escherichia coli* O157:H7 (PTVS46).

Motility and Hydrophobicity

Motility and hydrophobicity phenotype of the four single bacteria used in the runoff experiments was tested (Table 3). We found that the motility of the *E. coli* O157:H7 isolate was limited as compared with the other three strains. *Salmonella* Poona was the most hydrophilic strain and showed the highest adhesion affinity in the adhesion experiment (Fig. 2). This contradicts some of the existing literature, where a positive correlation between adhesion and hydrophobicity generally is accepted (Stenstrom, 1989; Huysman and Verstraete, 1993)

Conclusions

This study examined runoff from a sandy-loam, clay, and clay-loam soil and how the water transport of four enteric bacteria with different surface properties was influenced by a PAM treatment. We found that the PAM efficiency in reducing the concentration of enteric bacteria in surface runoff was influenced by soil type. This implies that the runoff of enteric bacteria differs from common inorganic pollutants that, in general, are reduced in surface runoff by PAM treatments according to previous literature. Therefore, before application of PAM to agricultural soil, one should evaluate soil type and potential pollutants the treatment is targeting.

In addition, comparison between the four bacteria in the surface runoff collected at 48 h showed a low concentration of *S.* Poona whereas *E. coli* was recovered in the greatest proportion. This difference in the loss of enteric bacteria highlights the importance of strain-specific properties that may not be captured with general fecal indicator bacteria. Hydrophobicity and motility did not explain observed differences. Therefore, further investigation is needed to pinpoint what surface properties influence the loss of enteric bacteria in surface runoff.

Acknowledgments

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