Quantifying Escherichia coli Release from Soil under High-Intensity Rainfall

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QUANTIFYING *ESCHERICHIA COLI* RELEASE FROM SOIL UNDER HIGH-INTENSITY RAINFALL

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ABSTRACT. *Bacterial loading in surface runoff can only be reasonably assessed or predicted with quantitative knowledge of the release of bacteria from the soil under different rainfall conditions. Most studies of bacterial movement were conducted under rainfall intensities of less than 44 mm h^{-1}. However, in the tropics, intensities higher than 44 mm h^{-1} are frequent. In this study,* Escherichia coli *release from the soil into surface runoff and its distribution in the soil under the impact of heavy rainfall (95 mm h^{-1}) of different durations were investigated. Results of simulated heavy rainfall of different durations on gently sloping grass plots with spray-applied* E. coli *indicated that* E. coli *was released with relative ease, resulting in contaminated runoff. Runoff* E. coli *concentrations ranged from 2.09 log(CFU) mL^{-1} in 5 min simulated rainfall events to 4.45 log(CFU) mL^{-1} in 15 min simulated rainfall events. The first simulated rainfall events after spray applications produced the highest concentration of* E. coli *in the runoff. Runoff loss accounted for 0.001% of the total applied* E. coli *in 5 min rainfall events and 2.1% in 15 min rainfall events. Total solids explained 28% of the variation in the concentrations and 14% of the total loadings.* E. coli *concentration in the surface centimeter of the soil explained 80% to 89% of the variations in runoff concentrations and loadings with regression slope of less than unity. Such quantitative relationships have the potential to predict runoff* E. coli *concentrations under high-intensity rainfall events.*

Keywords. E. coli *concentration,* E. coli *loading, Fecal bacteria, Simulated rainfall, Surface runoff.*

Fecal contamination of soil and surface water is a concern to the public, as livestock can be infected with numerous bacterial, viral, protozoan, and helminthic pathogens that are also infectious to humans (Pell, 1997; Hill, 2003). Runoff from farm sites and manure-applied fields was suspected to be a source of several human disease outbreaks (Smith and Perdek, 2004). Bacterial loading from agricultural waste is identified as the main cause of impairment of rivers in the U.S. (USEPA, 2000). Agricultural practices that potentially contribute to bacterial contamination of surface water include waste deposited by grazing animals in the fields and in the streams, animal waste from concentrated animal feeding operations applied as fertilizer or disposed of on land, and effluent from lagoons (Hooda et al., 2000). Ling et al. (2006a) found the tributary of Serin River that received lagoon effluent and the river downstream of animal farms to have elevated *Escherichia coli* concentrations (0.6 to 4.0 log(CFU) mL^{-1}). To reduce bacterial pollution of rivers, there is a need for further treatment of lagoon wastewater. An alternative could be a soil-based or land-treatment system, which is a natural treatment method with the advantage of microorganism removal through die-off, straining, sedimentation, entrapment, predation, radiation, desiccation, and adsorption (Metcalf and Eddy, 1991). The above would require knowledge of the extent or quantity of bacteria transported to surface water during rainfall events before any recommendation can be made to farm operators.

Bacteria from animal farm wastewater or lagoon effluent are transferred directly to the soil system, whereas those in slurry and solid waste are transferred to the soil system predominantly during rainfall events (Ogden et al., 2001, Joy et al., 1998). Once in the soil, further movement of the bacteria to water bodies depends on the hydrologic conditions (McDowell et al., 2006) through pathways of surface runoff and leaching (Reddy et al., 1981). Ogden et al. (2001) reported that leaching losses accounted for 0.2% to 10% of the total *E. coli* applied to plots and were dependent on natural rainfall. According to Hattori (1970) and Ling et al. (2002a), *E. coli* adsorb onto soil particles and the extent of adsorption depends on clay content. Rain can, thus, transport the bacteria by way of eroded soil particles. Tyrrel and Quinton (2003) postulated that the concentration of microorganisms on the soil surface and the kinetic energy of the rainfall are two of the factors that contribute to the quantity of microorganisms detached from the soil. Therefore, quantitative knowledge of the release of *E. coli* from the soil to the runoff for rainfalls of different intensities and durations is important in assessing or making predictions about the quantity of bacteria transported in the surface runoff pathway. In an attempt to develop a model to continuously simulate *E. coli* density on land and concentration in surface runoff, our previous study (Ling et al., 2006b) indicated the need for a quantitative relationship between *E. coli* concentrations in surface runoff and the concentration in the soil during rainfall-runoff events for predicting the movement of *E. coli*.

A number of rainfall simulation studies with rainfall intensities of 16 to 44 mm h^{-1} have been conducted to investigate

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the transport of *E. coli* from pasture, cowpats, cow manure, poultry litters, and mixture of fecal material with soils (Collins et al., 2005; Muirhead et al., 2005; McDowell et al., 2006; Muirhead et al., 2006; Soupir et al., 2006). In the tropics, rainfall intensities higher than the above range frequently occur. According to the Malaysian Meteorological Department, the classification of rain showers is as follows: slight (<2.0 mm h^{-1}), moderate (2.0 to 10.0 mm h^{-1}), heavy (10.1 to 50.0 mm h^{-1}), and violent (>50.0 mm h^{-1}). Therefore, in this study, *E. coli* release from the soil and distribution in the soil for different rainfall durations were investigated. The objectives of this study were to determine the concentrations and loadings of *E. coli* in surface runoff under violent showers and their relationship to the concentration in the soil, the proportion of the applied *E. coli* lost in surface runoff, and the distribution of *E. coli* in the soil after rainfall events.

MATERIALS AND METHODS

INOCULUM PREPARATION

Fecal bacteria used in this study were wild strains of *E. coli* isolated from fresh pig waste from a commercial farm in Siburan, Sarawak, Malaysia. *E. coli* isolation was performed using spread plate technique on eosin methylene blue (EMB) agar and nutrient agar (Oxoid, Basingstoke, U.K.). A series of biochemical tests including carbohydrate utilization, methyl red, Voges-Proskauer, citrate utilization, and indole was conducted to determine if the isolates were *E. coli*. Analytical profile index (API) 20 E test kit (BioMerieux, Marcy l'Etoile, France) was also used as a final confirmation of the purity of the *E. coli* isolates.

E. coli cultures from nutrient slant agar were grown on lauryl broth (LB) (Fluka, Buchs, Switzerland) in an incubator shaker (New Brunswick Scientific, Edison, N.J.) at 37°C, 200 rpm for 24 h. The 24 h cultures were then grown on LB broth in the same incubator shaker at 37°C, 200 rpm for 18 h. The bacterial suspension was diluted with LB broth to the concentration of 10^8 to 10^9 CFU mL^{-1} as determined by a standard growth curve based on optical density reading using a spectrophotometer (Jenway 6300, Dunmow, U.K.).

SOIL AND CLIMATIC DATA

Soil properties considered relevant to the study included pH, organic matter content, and particle size distribution. Three samples of soil were collected from the study site for analysis. The pH of each soil sample was measured using a pH meter (Waterproof CyberScan pH 300, Eutech Instruments, Singapore) after diluting to 1:2 soil to water ratio. The soil organic matter content of the soils was determined by loss-on-ignition (Nelson and Sommers, 1996), and particle size distribution was performed by the pipette method (Gee and Bauder, 1986). Daily soil temperature, moisture, and solar radiation were monitored continuously by using temperature, solar radiation, and moisture probes (WatchDog model 400, Spectrum Technologies, Plainfield, Ill.) placed at a depth of 2 cm, and the readings were recorded with a data logger.

SIMULATED RAINFALL EXPERIMENT

This rainfall simulation study used nine field plots that included three replicates of plots receiving a tropical-intensity storm for three different rainfall durations. A total of three spray applications of *E. coli* were conducted, and each spray application was followed by two or three simulated rainfall events at 24 h intervals.

The field plots (3 × 3 m) of 3% slope were set up at the University of Malaysia Sarawak Campus, Kota Samarahan. The plots were separated by 20 cm height plywood borders partially driven into the ground to prevent cross-contamination and overland flow entering neighboring plots. At the downslope end of each plot, there was a 1 m width zone that was not spray-applied with *E. coli* and where a plastic drain was installed to direct surface runoff to a bucket. All plots were planted with turf grass (*Axonopus compressus*) five months prior to the experiment. During the experiments, the grass height ranged from 5 to 10 cm. Prior to the first spray application, no *E. coli* was detected in the runoff from simulated rainfall on the plots.

The *E. coli* inocula for spray applications were prepared by diluting concentrated inocula (10^8 to 10^9 CFU mL^{-1}) using 0.1% peptone water. Nine liters of the diluted inocula (10^6 to 10^7 CFU mL^{-1}) were sprayed on each plot using a 16 L capacity, 800 to 896 kPa knapsack sprayer (model B16-2, Teong Hin Plastic Industries Sdn. Bhd., Johore, Malaysia). Before and after each simulated rainfall event, the top 1 cm of the soil was sampled at four random points in each plot and composited for *E. coli* enumeration. The first spray application was followed by two simulated rainfalls, while the second and third spray applications were followed, in each case, by three simulated rainfall events. The first of the simulated rainfalls was meant to represent the worst-case scenario of rain occurring right after spraying of waste or wastewater in the field. At night, the plots were covered with canvas to prevent natural rainfall from washing away the *E. coli*. In the morning, the canvas was removed to expose the plots to natural conditions. The plots were also covered whenever it rained during day time.

Simulated rainfalls of were 5, 10, and 15 min durations and were produced using a Tlaloc 3000 rainfall simulator (Joern's Inc., West Lafayette, Ind.; Franklin et al., 2006). The simulator consists of a TeeJet 1/2 HH-SS 50WSQ nozzle and solenoid, pressure gauge, and pressure regulator. The nozzle of the rainfall simulator was located 3 m from the ground to achieve terminal velocity of 7.0 to 7.4 m s^{-1} in the raindrops (Miller, 1987). A submersible pump (JS Pump RS-400, East Riding Koi Co., East Yorkshire, U.K.) was used to deliver water from the tank to the rainfall simulator. The pressure of the water flow was fixed at 20.7 kPa, and the design rainfall intensity was 95 mm h^{-1}, simulating a heavy downpour typical of the humid tropics. This value corresponds to the rainfall intensity occurring in 1 h for a 6-year return period in the study area. However, the intensities calculated from the water used in the experiment and duration of rainfall yielded a range of 89 to 110 mm h^{-1} (table 1). The area of the plot covered by the simulated rainfall was 2 × 3 m. Tap water used to simulate rainfall events was stored in a 520 L fiberglass tank for two days prior to the experiment. No significant difference between decay of *E. coli* in tap water stored for two days and distilled water was observed in the laboratory. There was a slight difference between the pH of rain water (pH 6.2) and that of stored tap water (pH 6.6). This difference was not likely to have had much impact on the die-off rates, as previous studies showed that *E. coli* die-off rates between pH 6 and pH 7 experimental conditions in a sandy soil were not significantly different (Ling et al., 2005). The runoff produced

Table 1. Spray application dates, total *E. coli* applied, simulated rainfall dates, simulated rainfall durations, amount of water used in simulated rainfall, rainfall intensity, and volume of runoff collected during the rainfall simulation study.

Spray Application Date	*E. coli* Applied (CFU plot^{-1})	Simulated Rainfall Date	Rainfall Duration (min)	Water Used (L)	Rainfall Intensity (mm h^{-1})	Runoff Collected (L)
21 Sept. 2005	9.18×10^{10}	21 Sept. 2005	5	46.3 ±1.2	92.7 ±2.3	6.5 ±1.0
			10	97.7 ±2.5	97.7 ±2.5	29.4 ±4.3
			15	150.7 ±3.2	100.4 ±2.1	61.5 ±4.1
		22 Sept. 2005	5	45.3 ±2.5	90.7 ±5.0	3.7 ±1.8
			10	94.7 ±1.5	94.7 ±1.5	21.6 ±2.6
			15	150.0 ±3.5	100.0 ±2.3	61.1 ±5.1
27 Sept. 2005	1.26×10^{11}	27 Sept. 2005	5	44.3 ±2.9	88.7 ±5.8	10.7 ±2.2
			10	95.3 ±5.0	95.3 ±5.0	40.5 ±2.6
			15	150.3 ±4.2	100.2 ±2.8	78.1 ±2.9
		28 Sept. 2005	5	46.7 ±2.9	93.3 ±5.8	9.2 ±2.1
			10	93.7 ±4.6	93.7 ±4.6	33.0 ±7.5
			15	149.3 ±1.2	99.6 ±0.8	70.2 ±9.2
		29 Sept. 2005	5	43.3 ±2.9	88.7 ±5.8	10.2 ±3.6
			10	94.3 ±2.9	94.3 ±2.9	50.6 ±0.5
			15	152.7 ±2.3	101.8 ±1.5	76.5 ±12.3
25 Oct. 2005	9.00×10^{10}	25 Oct. 2005	5	49.7 ±1.2	99.3 ±2.3	8.2 ±2.6
			10	98.0 ±3.5	98.0 ±3.5	35.2 ±2.9
			15	141.3 ±5.5	94.2 ±3.7	80.9 ±8.6
		26 Oct. 2005	5	50.0 ±3.5	100.0 ±6.9	9.6 ±1.2
			10	100.7 ±2.9	100.7 ±2.9	39.8 ±3.3
			15	149.0 ±3.5	99.3 ±2.3	73.3 ±2.5
		27 Oct. 2005	5	50.3 ±2.3	100.7 ±4.6	9.0 ±2.6
			10	97.0 ±6.9	97.0 ±6.9	39.5 ±2.5
			15	147.0 ±6.0	98.0 ±4.0	76.0 ±4.6

(table 1) by the simulated rainfall events was collected in 50 L plastic containers and sampled for *E. coli* enumeration and total solids analysis.

To study the distribution of *E. coli* in the soil, core samples were collected to a depth of 4 cm using 5 cm diameter aluminum cores at four random points on each plot following the first simulated rainfall events of the first and the second spray applications. The soil cores were then divided into 1 cm slices, and the four samples for each depth were composited for *E. coli* analysis. Following the third spray application, distribution of *E. coli* in the soil of the 10 min duration plots was monitored for three days of consecutive simulated rainfall events by core sampling before and after each of the daily simulated rainfalls. Thereafter, samples were taken daily until *E. coli* was no longer detected.

E. COLI ENUMERATION AND TOTAL SOLIDS ANALYSIS

E. coli in the soil and runoff samples were analyzed using the spread plate technique (APHA, 1998). Each soil sample consisted of 1 g of soil transferred to a test tube, diluted with 0.1% buffered peptone solution and stirred. A plate containing solidified EMB agar was inoculated with 0.1 mL diluted sample and was incubated at 37°C for 24 h. Replicates of three plates were set up at each dilution level for each sample. Since the magnitude of the concentration of *E. coli* in runoff was unknown, we had to use different dilution levels so that colonies between 30 and 300 CFU could be obtained for enumeration. For the runoff sample, 1 mL of the sample was pipetted to a test tube and the dilution and plating proceeded as with the soil samples. Total solids were analyzed according to standard methods (APHA, 1998) by drying a runoff volume of 25 mL to constant weight.

EQUATIONS FOR COMPUTATIONS

Loading of *E. coli* was computed according to equation 1:

$$L_R = V_R\, C_R \tag{1}$$

where L_R is loading (CFU), V_R is the volume of runoff collected (mL), and C_R is the *E. coli* concentration in the runoff (log(CFU) mL^{-1}). Total solids were computed according to equation 2:

$$T_S = V_R\, C_S \tag{2}$$

where T_S is the amount of total solids in runoff (mg), and C_S is the concentration of total solids (mg L^{-1}). Rainfall intensity was computed according to equation 3:

$$I = \frac{V_w}{AT} \tag{3}$$

where I is the rainfall intensity (mm h^{-1}), V_w is the volume of water used (mL), A is the area of the plot rained on (m^2), and T is the duration of simulated rainfall (h). Proportion of the applied *E. coli* lost in runoff was computed according to equation 4:

$$P = \frac{C_R V_R}{C_A V_A} \tag{4}$$

where P is the proportion of *E. coli* lost in runoff (%), C_A is the *E. coli* concentration spray applied (CFU mL^{-1}), and V_A is the volume of *E. coli* spray applied (mL).

STATISTICAL ANALYSIS

E. coli enumeration data were log-transformed for statistical analysis. Data were analyzed using SPSS (SPSS, 2002). Differences in *E. coli* concentrations at different soil depths after the first simulated rainfall event were analyzed using univariate analysis of variance (ANOVA) where depth and rainfall durations were the fixed factors and spray application was treated as the block. To compare the decay of *E. coli* between different soil depths after 10 min rainfalls during the third spray application, univariate ANOVA was used where day and depth were the fixed factors. Differences in *E. coli* concentrations and loadings of surface runoff among simulated rainfall durations for each simulated rainfall day were analyzed using one-way ANOVA. Simple linear regression was performed to investigate the significance of the relationship between *E. coli* concentrations/loadings in runoff and total solids. Similarly, the significance of the relationship between *E. coli* concentrations/loadings in runoff and concentrations at the soil surface was determined by simple linear regression. Slopes of the regression of the runoff concentrations or loadings versus soil surface concentrations were compared among the three rainfall durations according to Zar (1996). In all the statistical analyses, the level of significance was 5% (0.05).

RESULTS

The soil of the experimental plots was of sandy clay loam texture (58% sand and 27% clay) with pH of 4.3 and organic matter of 2.2% (table 2). Daily temperature ranged from 23°C to 35°C, water tension ranged from 0 to 21 kPa, and solar radiation ranged from 0 to 1131 W m^{-2} (table 3). The highest daily temperature and solar radiation were recorded at 1:00 p.m., whereas the highest water tension was recorded at about 4:00 p.m. The lowest daily temperature was recorded at 4:00 a.m.

Mean *E. coli* concentration in the soil surface decreased as a function of time after each spray application and with increasing rainfall duration (fig. 1). Simulated rainfalls resulted in *E. coli* concentration in the soil surface dropping in increasing order of rainfall duration (5 min < 10 min < 15 min), as indicated by the increasing magnitude of the slope of the regression line. After three days, *E. coli* was no longer detected in the 15 min rainfall plots, whereas *E. coli* persisted for four days in the 5 min plots.

After the first simulated rainfall following each spray application, concentration of *E. coli* in the soil decreased with increasing depth (fig. 2) significantly (Tukey's test, $P \leq 0.001$) (Zar, 1996). The concentration drop with soil depth was most apparent for the 5 min duration and least for the 15 min duration, as indicated by the slopes of the respective regression lines. However, there was no significant difference in mean concentrations among the three simulated rainfall durations (P = 0.44).

With repeated simulated rainfalls after the third spray application, concentration of *E. coli* at all soil depths decreased with time (fig. 3). Right after the spray application, *E. coli* concentrations in the top 3 cm were similar, but that of the 3-4 cm was about one order of magnitude lower. However,

Table 2. Properties of the soil at the experimental plots.

Properties	Value
pH	4.32 ±0.05
Clay (%)	26.9 ±1.4
Fine silt (%)	5.2 ±0.5
Coarse silt (%)	9.7 ±1.2
Sand (%)	58.2 ±2.9
Organic matter (%)	2.20 ±0.05

Table 3. Mean (and range) of soil temperature, water tension, and solar radiation during the experimental period following the three spray applications in simulated rainfall plots.[a]

Spray Application	Temperature (°C)	Water Tension (kPa)	Solar Radiation (W m^{-2})
1	28.6 (25.4-34.5)	8.5 (0-15.0)	394 (0-778)
2	28.8 (25.2-35.1)	7.6 (0-14.0)	449 (0-973)
3	29.9 (23.4-34.8)	14.8 (0-20.8)	512 (0-1131)

[a] Values are means of 24 hourly values.

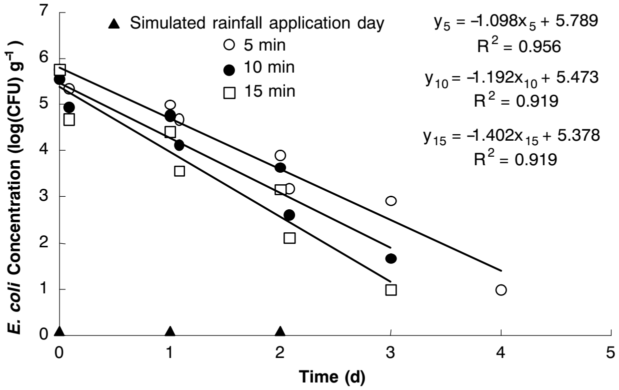

Figure 1. Mean *E. coli* concentrations (three replicates) in the top 1 cm of soil for the second spray application under different simulated rainfall durations.

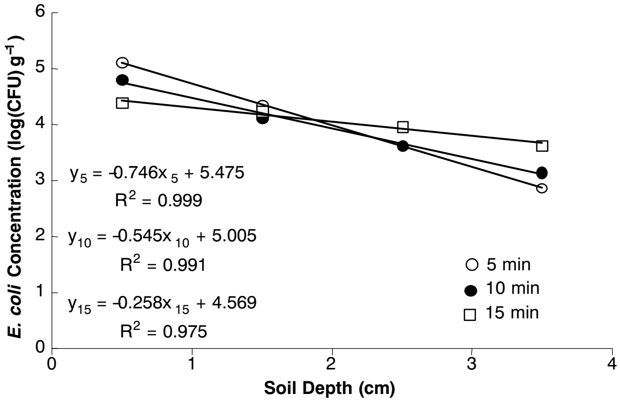

Figure 2. *E. coli* distribution in the soil after the first simulated rainfalls of different durations following the first and second spray applications.

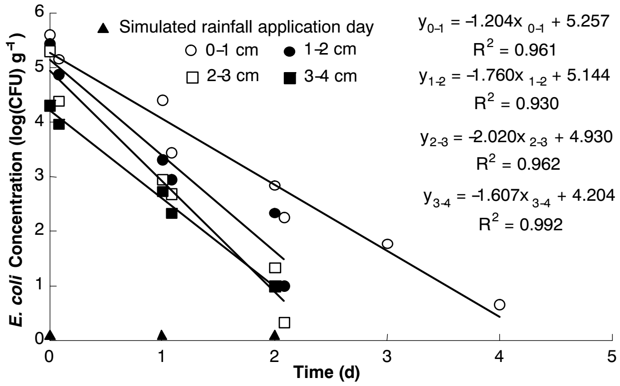

Figure 3. Mean *E. coli* concentrations (three replicates) at different soil depths for the 10 min simulated rainfalls after the third spray application.

Table 4. Mean *E. coli* concentrations, loadings, and proportions lost in surface runoff in three different durations of simulated rainfalls following the three spray applications.

		Runoff Concentration (log(CFU) mL^{-1})[a]			Runoff Loading (log(CFU))[a]			Proportion Lost in Runoff (%)		
Spray	Rainfall	5 min	10 min	15 min	5 min	10 min	15 min	5 min	10 min	15 min
1	1	3.17 a ±0.15	3.84 b ±0.06	4.02 b ±0.08	6.98 a ±0.22	8.31 b ±0.08	8.81 c ±0.06	0.011 ±0.005	0.223 ±0.042	0.701 ±0.100
	2	2.24 a ±0.18	2.64 a ±0.14	3.37 b ±0.33	5.79 a ±0.03	6.97 b ±0.12	8.15 c ±0.29	0.001 ±0.000	0.011 ±0.003	0.177 ±0.098
2	1	3.70 a ±0.19	4.21 b ±0.04	4.45 b ±0.17	7.72 a ±0.26	8.82 b ±0.06	9.34 c ±0.18	0.047 ±0.030	0.526 ±0.074	1.828 ±0.683
	2	3.50 a ±0.21	3.72 a ±0.15	3.68 a ±0.24	7.46 a ±0.29	8.23 b ±0.18	8.53 b ±0.27	0.026 ±0.014	0.144 ±0.061	0.307 ±0.209
	3	2.09 a ±0.20	2.46 a ±0.07	3.27 b ±0.15	6.08 a ±0.14	7.17 b ±0.07	8.09 c ±0.21	0.001 ±0.000	0.012 ±0.002	0.104 ±0.048
3	1	3.75 a ±0.08	4.47 b ±0.07	4.37 b ±0.10	7.65 a ±0.17	9.02 b ±0.11	9.27 b ±0.13	0.053 ±0.018	1.185 ±0.289	2.148 ±0.662
	2	2.87 a ±0.11	3.64 b ±0.14	3.83 b ±0.14	6.85 a ±0.13	8.24 b ±0.18	8.69 c ±0.16	0.008 ±0.002	0.200 ±0.080	0.571 ±0.182
	3	2.16 a ±0.15	2.58 a ±0.21	3.39 b ±0.19	6.10 a ±0.07	7.18 b ±0.23	8.25 c ±0.17	0.001 ±0.000	0.018 ±0.009	0.205 ±0.077

[a] Means within a row followed by the same letter are not significantly different at 5% level.

after the first simulated rainfall event, *E. coli* concentrations were more or less uniformly distributed among the layers. In the 3-4 cm layer, *E. coli* in all the 10 min simulated rainfall plots was not detectable prior to the final simulated rainfall. Persistence of *E. coli* was in the decreasing order of 0-1 cm > 1-2 cm > 2-3 cm > 3-4 cm. Decay coefficients at different depths between simulated rainfalls ranged from 0.74 to 1.52 d^{-1}, and there was no significant difference ($P = 0.068$).

In all the spray applications, the first simulated rainfalls immediately after spray applications produced the highest concentration of *E. coli* in the runoff, in the order of 3 to 4 log(CFU) mL^{-1}. The concentration decreased with successive simulated rainfalls (table 4). For the first and second simulated rainfalls following spray applications of *E. coli*, there was no significant difference in *E. coli* concentrations between the 10 and 15 min duration rainfall events ($0.163 \leq P \leq 0.966$). For the last simulated rainfalls, there was no significant difference between the 5 and 10 min duration rainfall events ($0.054 \leq P \leq 0.163$). Loadings of *E. coli* decreased in the order of 1st rainfall > 2nd rainfall > 3rd rainfall after spray applications of *E. coli* (table 4). Loadings were the highest for the 15 min duration and lowest for the 5 min duration. Multiple comparisons indicated that differences in *E. coli* loading between all pairs of durations of rainfall events were significant ($P \leq 0.032$) except that between the 10 and 15 min durations for the second simulated rainfall of the second spray applications ($P = 0.389$) and for the first simulated rainfall of the third spray applications ($P = 0.144$). The runoff concentrations and loadings were found to be significantly correlated with total solids ($P < 0.0005$ and $P = 0.001$, respectively), where total solids explained 28% of the variations in concentration and 14% of the variations in loading (fig. 4).

The proportion of the total applied *E. coli* lost in surface runoff was dependent on the simulated rainfall duration and the number of rainfall simulations (table 4). The first simulated rainfall after spray application resulted in the highest loss, ranging from 0.01% for the 5 min rainfall to 2.1% for the 15 min rainfall, and the third rainfall resulted in the least lost, ranging from 0.001% for the 5 min rainfall to 0.2% for the 15 min rainfall. As the rainfall duration increased, the percentage of *E. coli* lost increased.

The 5 min rainfall produced the lowest runoff *E. coli* concentrations, and the 15 min rainfall produced the highest runoff *E. coli* concentrations (fig. 5). At high soil *E. coli* concentrations, the 10 and 15 min rainfalls produced similar *E. coli* concentrations in runoff. However, at low soil *E. coli* concentrations, the 15 min rainfalls produced *E. coli* concentrations of about one order of magnitude higher than the 10 min rainfall. *E. coli* concentrations in surface runoff were highly correlated with the concentration in the surface centimeter of the soil. For event mean concentration, the relationships between *E. coli* concentration in the surface runoff and *E. coli* in the surface of the soil for the 5, 10, and 15 min rainfall durations are expressed as:

$$C_{R5} = 0.633C_s \tag{5}$$

$$C_{R10} = 0.771C_s \tag{6}$$

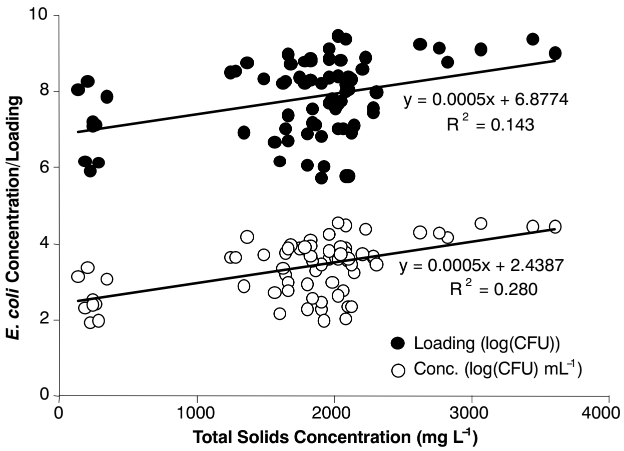

Figure 4. Mean *E. coli* concentrations/loadings as a function of mean total solids concentrations (mg L^{-1}) in runoff for all simulated rainfalls of all spray applications.

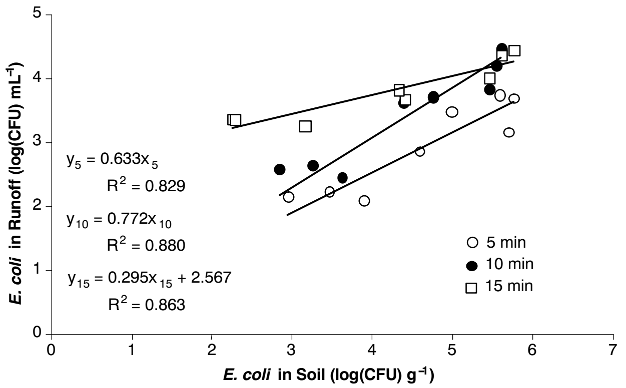

Figure 5. Mean *E. coli* concentrations in runoff as a function of mean *E. coli* concentrations in the top 1 cm of soil for all simulated rainfalls of all spray applications.

$$C_{R15} = 0.295C_s + 2.57 \qquad (7)$$

where C_{Ri} is the rainfall event mean concentration of *E. coli* in surface runoff (log(CFU) mL^{-1}); i = 5, 10, and 15 for the 5, 10, and 15 min rainfall durations, respectively; and C_s is the soil surface *E. coli* concentration (log(CFU) g^{-1} dw). Since regression analysis indicated that the *y*-intercepts of the regression for the 5 and 10 min rainfalls were not significantly different from zero (P = 0.701, P = 0.349), equations 5 and 6 were developed by setting the intercept to zero. However, for the 15 min rainfall events, the constant of the regression was significantly different from zero. Therefore, the intercept is retained, as shown in equation 7. Regression analysis indicated that all three regressions slopes were significantly different from zero ($P \leq 0.001$) and that *E. coli* concentration on the soil surface explained 82.9%, 88.0%, and 86.3% of the variation in *E. coli* in runoff concentration for the 5, 10 and 15 min simulated rainfalls, respectively. The 15 min rainfall regression line showed the lowest slope and the highest intercept. Tukey's test indicated that the regression slopes of the 15 min rainfalls were significantly different from those of the 5 min ($P < 0.05$) and 10 min ($P < 0.005$) rainfalls. However, the regression slopes of the 5 and 10 min rainfalls were not significantly different ($0.2 < P < 0.5$).

Furthermore, the loadings of *E. coli* under different rainfall durations were also highly correlated with the surface concentrations of the soil with coefficients of determination 80.3%, 89.0%, 83.4% for the 5, 10, and 15 min rainfall durations, respectively (fig. 6). Equations for the event mean runoff *E. coli* load for the three durations are expressed as:

$$L_5 = 0.633C_s + 3.903 \qquad (8)$$

$$L_{10} = 0.682C_s + 4.960 \qquad (9)$$

$$L_{15} = 0.306C_s + 7.367 \qquad (10)$$

where L_i is the event mean load of *E. coli* in surface runoff (log(CFU)), and i = 5, 10, and 15 for the 5, 10, and 15 min rainfall durations, respectively. The regression slopes and intercepts were all significantly different from zero ($P \leq 0.003$, $P \leq 0.001$). Similar to the concentration regression lines, the slope for the 15 min rainfall regression line was the lowest. The slope for the 15 min regression line of *E. coli* loading was significantly different from those of the 5 and 10 min rainfalls ($P < 0.05$ and $P < 0.005$, respectively). There was no significant difference between slopes for the 5 and 10 min rainfalls ($P > 0.5$).

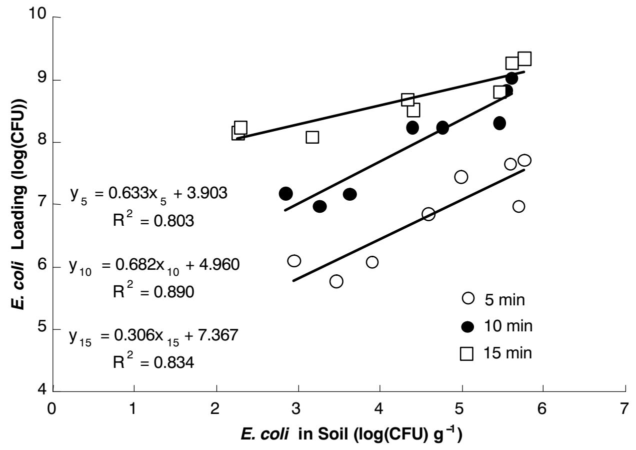

Figure 6. Mean *E. coli* loadings in runoff as a function of mean *E. coli* concentrations in the top 1 cm of soil for all simulated rainfalls of all spray applications.

DISCUSSION

E. coli in the soil surface was no longer detectable after only four days following two or three simulated rainfall events. Factors that contributed to such a drop in population included losses due to surface runoff, decay, and leaching. Runoff was found to contribute only a small percentage of the total *E. coli* applied. The other key loss is most likely due to die-off, as the loss of *E. coli* in the soil surface between consecutive simulated rainfall events was high. This is based on the large decrease in the *E. coli* concentrations in the soil immediately after a simulated rainfall event and immediately before the next simulated rainfall event, and the fact that most of the applied *E. coli* was concentrated in the top 1 cm. This conclusion is further supported by the unpublished results of two previous experiments: (1) an experiment conducted without simulated rain (soil temperature of 26°C to 34°C) to study the die-off of *E. coli* in the same location indicated high die-off rates and persistence of only four days; (2) an experiment on the plot soil under controlled conditions in the laboratory at 100% saturated condition indicated persistence of five days at 30°C and four days at 35°C. Leaching also accounts for a proportion of the loss, as not all the water used in the rainfall simulations was accounted for in runoff. In fact, for the 5 and 10 min duration rainfall events, runoff accounted for less than 50% of the water used for simulated rainfall, indicating substantial infiltration through the soil surface, which would facilitate leaching of *E. coli*. For the 15 min duration rainfall event, although proportionately smaller, the total volume infiltrated was larger than that of the shorter duration events.

Rapid decline of fecal coliform has been reported for swine manure in the soil-runoff mixing zone with persistence of only six to ten days (Gessel et al., 2004). The decay process was mainly attributed to the inability of the *E. coli* to reduce its metabolic rate to meet the low availability of usable organic carbon (Klein and Casida, 1967). In the weakened state of nutrient shortage, the organism is also stressed by other environmental factors (Hill, 2003), such as high soil temperatures of up to 35°C, strong solar radiation, and the acidic soil (pH 4.32) in the present study. According to Ling et al. (2002b), *E. coli* die-off rate in a clay loam in dry, moist, and wet conditions increased as temperature increased from 25 to 35°C. In a study of die-off of *E. coli* in a sandy soil under different pH values, it was reported that the first order die-off rate of *E. coli* at pH 4.4 was much higher than that at pH 6.4 and for temperatures of 20°C and 30°C (Ling et al., 2005).

E. coli concentrations in the soil decreased gradually from the top 1 cm to 3-4 cm after the first simulated rainfalls, indicating continuous downward movement and retention of *E. coli* through the soil depths. Retention occurred due to filtration of *E. coli* in the soil and adsorption of *E. coli* cells to soil particles. Since the size of bacteria ranged from 0.2 to 5 μm compared to the fine to medium pore size of 10 nm to 10 μm, mechanical filtration of bacteria should occur, but it would be incomplete (Matthess et al., 1988). Adsorption has been reported to occur between *E. coli* and soil particles. Previous studies of two strains of *E. coli* indicated that cells adhered rapidly to clay particles and formed cell-clay complexes, which adhere to each other or to other clay particles and form cell-clay aggregates at a much lower rate (Hattori, 1970). *E. coli* was also found to establish equilibrium in the soil-water system, and the percentage of *E. coli* adsorbed depended on the clay content (Ling et al., 2002a). Studies of bacteria movement through a loam soil indicated that only 0.01% to 15% passed through a 5 cm column (Gannon et al., 1991). Therefore, *E. coli* could be both filtered as individual cells or adhered to soil particles while adsorption could occur at all depths.

E. coli in runoff was found to be dependent on the duration of rainfall, amount of runoff, and the time elapsed between rainfall events. For the first simulated rainfalls, the 5 min rainfall produced runoff *E. coli* concentrations that were lower than those of the 10 and 15 min rainfall events. During this short-duration rainfall, infiltration was much larger than runoff. The smaller runoff volume as well as the shorter duration were probably insufficient to allow a more extensive release (desorption) of the *E. coli*. The short-duration rainfall also produced a slower runoff velocity whereby some *E. coli* attached to soil particles could be redeposited along the flow path. Evidence of attachment to particles was shown by the significant relationship between *E. coli* concentrations and total solids in runoff, where total solids explained 28% of the variation in concentrations and 14% of the loadings.

For the two simulated rainfalls after the first spray application, the *E. coli* concentrations and loadings in runoff were found to be lower than those of the corresponding simulated rainfalls after the second and third spray applications. This is most likely related to the lower volumes and velocity of runoff in the two simulated rainfalls, leading to lower releasing and carrying capacity in terms of the *E. coli*. The proportion of *E. coli* lost in runoff for the third spray application were higher than that of the second spray application due to more *E. coli* being applied in the second spray application compared to the third application.

In spite of the short duration rainfall of 5 min, the concentration of *E. coli* in runoff was in the order of 2 to 3 log(CFU) mL^{-1}, which exceeded the standard established for primary contact recreation (USEPA, 1976). This indicates the relative ease with which *E. coli* moved with runoff. Muirhead et al. (2005) also reported low attachment of <25% from totally feces and feces mixed with soil cowpats. The physical filtration of bacteria at the soil surface also increased the chance of losses during runoff (Crane et al., 1983). In stormwater, it was reported that more than 50% of bacteria were not settled or filtered (Schillinger and Gannon, 1985). The significant correlation between *E. coli* concentrations and total solids obtained in the present study was not observed in the study of cowpats (Muirhead et al., 2006), most likely due to the difference between fecal material and turf-grown soil as the source of *E. coli*, where solids in cowpats are basically organic materials.

In the present study, a significant relationship between *E. coli* concentrations in runoff and loadings with that of the source was observed. Such a relationship was also reported by Muirhead et al. (2005, 2006). However, the source materials studied were fresh and aged feces and feces-soil mixture (Muirhead et al., 2005) and fresh feces (Muirhead et al., 2006), and the studies were conducted under a lower rainfall intensity of 25 mm h^{-1}. For the same source concentration of 5 log(CFU) g^{-1}, lower runoff *E. coli* concentrations (1 to 3 log(CFU) mL^{-1}) were recorded by the above authors than those in the present study at 2 to 4 log(CFU) mL^{-1}. The higher intensity rainfall in the present study would have imparted more energy to release more *E. coli* and consequently higher runoff *E. coli* concentration. In terms of regression slope, the slope of the 10 min simulated rainfall is comparable to that of Muirhead et al. (2005) but smaller than that of Muirhead et al. (2006), which was close to unity. This is most likely due to the difference in the source of *E. coli*. The mechanism of release from fecal material in the form of cowpats placed in metal trays is likely to be different from a soil surface with grass growing on it. Fecal material contained mostly organic materials with low density and was therefore more erodible than grass-covered soil (Khaleel et al., 1979).

When *E. coli* concentration at the soil surface was low, the 15 min rainfall produced higher *E. coli* concentration in runoff compared to the 5 and 10 min rainfalls. Furthermore, the concentration and loading regression lines for the 15 min rainfall have the least slope and highest intercept. These observations indicate that at lower concentration, the 15 min rainfall was able to extract more *E. coli* than the 5 and 10 min rainfalls. This is possibly due to greater detachment and entrainment associated with the longer-duration rainfall event and higher-volume of runoff.

CONCLUSIONS AND RECOMMENDATIONS

High-intensity rainfalls of different durations, especially rainfall events occurring right after spray application, released *E. coli* with relative ease, contaminating surface runoff with high *E. coli* concentrations (2 to 4 log(CFU) mL^{-1}). When rainfall duration increased, there was an increase in the *E. coli* concentration in runoff. Runoff loss accounted for only a small percentage of the total spray-applied *E. coli*. Die-off under the tropical intense solar radiation, high temperature, and the acidic nature of the soil most likely eliminated most of the *E. coli*. After the simulated rainfall events, the distribution of *E. coli* in the soil depths indicated decreasing concentrations with depth. Linear regression indicated that the relationships between surface runoff *E. coli* concentrations/loadings and soil surface *E. coli* concentrations were strong, with $R^2 > 80\%$. Total solids explained a small percentage of the variation in *E. coli* concentrations and loadings in surface runoff. Since the worst-case scenario for *E. coli* contamination would be high-intensity rainfall events occurring right after spray application, it is recommended that lagoon effluent be treated by land application instead of direct discharge into rivers, especially during the dry season. Further research is recommended to study the impacts of other factors on the movement of *E. coli* during rainfall events, such as different rainfall intensities, longer-duration rainfalls, different types of waste applied, different types of soil, and different vegetative covers.

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REFERENCES

APHA. 1998. Solids. In *Standard Methods for Examination of Water and Wastewater.* 20th ed. Washington, D.C.: American Public Health Association.

Collins, R., S. Elliott, and R. Adams. 2005. Overland flow delivery of faecal bacteria to a headwater pastoral stream. *J. Appl. Microbiol.* 99(1): 126-132.

Crane, S. R., J. A. Moore, M. E. Grismer, and J. R. Miner. 1983. Bacterial pollution from agricultural sources: A review. *Trans. ASAE* 26(3): 858-866.

Franklin, D. H., M. L. Cabrera, and V. H. Calvert. 2006. Fertilizer source and soil aeration effects on runoff volume and quality. *SSSA J.* 70(1): 84-89.

Gannon, J. T., V. B. Manila, and M. Alexander. 1991. Relationship between cell surface properties and transport of bacteria through soil. *Appl. Environ. Microbiol.* 57(1): 190-193.

Gee, G. W., and J. W. Bauder. 1986. Particle-size analysis: Part I. Physical and mineralogical methods. In *Methods of Soils Analysis*, 383-412. A. Klute, ed. Madison, Wisc.: SSSA.

Gessel, P. D., N. C. Hansen, S. M. Goyal, L. J. Johnston, and J. Webb. 2004. Persistence of zoonotic pathogens in surface soil treated with different rates of liquid pig manure. *Applied Soil Ecol.* 25(3): 237-243.

Hattori, T. 1970. Adhesion between cells of *E. coli* and clay. *J. Gen. Appl. Microbiol.* 16(5): 351-359.

Hill, V. R. 2003. Prospects for pathogens reductions in livestock wastewaters: A review. *Crit. Rev. Environ. Sci. Tech.* 33(2): 187-235.

Hooda, P. S., A. C. Edwards, H. A. Anderson, and A. Miller. 2000. A review of water quality concerns in livestock farming areas. *Sci. Total Environ.* 250: 143-167.

Joy, D. M., H. Lee, C. M. Reaume, H. R. Whiteley, and S. Zelin. 1998. Microbial contamination of subsurface tile drainage water from field applications of liquid manure. *Canadian Agric. Eng.* 40(3): 153-160.

Khaleel, R., G. R. Foster, K. R. Reddy, M. R. Overcash, and P. W. Westerman. 1979. A nonpoint source model for land areas receiving animal waste: III. A conceptual model for sediment and manure transport. *Trans. ASAE* 22(6): 1353-1361.

Klein, D. A., and L. E. Casida Jr. 1967. *Escherichia coli* die-out from normal soil as related to nutrient availability and the indigenous microflora. *Canadian J. Microbiol.* 13(11): 1461-1470.

Ling, T. Y., E. C. Achberger, C. M. Drapcho, and R. L. Bengtson. 2002a. Quantifying adsorption of an indicator bacteria in a soil-water system. *Trans. ASAE* 45(3): 669-674.

Ling, T. Y., K. Apun, and A. L. Mijen. 2002b. Survival of an *Escherichia coli* in a Sarawak soil. *Malaysian J. Soil Sci.* 6: 43-52.

Ling, T. Y., H. J. Jong, and K. Apun. 2005. Die-off rate of *Escherichia coli* as a function of pH and temperature. *J. Physical Sci.* 16(2): 53-63.

Ling, T. Y., H. W. Layang, Y. P. Then, and K. Apun. 2006a. Impacts of pig farming on the water quality of Serin River, Sarawak. *Sains Malaysiana* 35(1): 45-50.

Ling, T. Y., R. L. Bengtson, C. M. Drapcho, E. C. Achberger, G. J. Sabbagh, and J. Jackson. 2006b. Modelling of *Escherichia coli* density on land and concentration in surface runoff. *Malaysian J. Soil Sci.* 10: 25-34.

Matthess, G., A. Pekdeger, and J. Schroeter. 1988. Persistence and transport of bacteria and viruses in groundwater: A conceptual evaluation. *J. Contamin. Hydrol.* 2(2): 171-188.

McDowell, R. W., R. W. Muirhead, and R. M. Monaghan. 2006. Nutrient, sediment, and bacterial losses in overland flow from pasture and cropping soils following cattle dung deposition. *Comm. Soil Sci. Plant Anal.* 37(1-2): 93-108.

Metcalf and Eddy. 1991. Natural treatment systems. In *Wastewater Engineering: Treatment, Disposal* and Reuse, 927-1002. 3rd ed. New York, N.Y.: McGraw-Hill.

Miller, W. P. 1987. A solenoid-operated, variable-intensity rainfall simulator. *SSSA J.* 51(3): 832-834.

Muirhead, R. W., R. P. Collins, and P. J. Bremer. 2005. Erosion and subsequent transport state of *Escherichia coli* from cowpats. *Appl. Environ. Microbiol.* 71(6): 2875-2879.

Muirhead, R. W., R. P. Collins, and P. J. Bremer. 2006. Numbers and transported state of *Escherichia coli* in runoff direct from fresh cowpats under simulated rainfall. *Lett. Appl. Microbiol.* 42(2): 83-87.

Nelson, D. W., and L. E. Sommers. 1996. Total carbon, organic carbon, and organic matter: Part 3. Chemical methods. In *Methods of Soil Analysis*, 1001-100. D. L. Sparks, ed. Madison, Wisc.: SSSA.

Ogden, I. D., D. R. Fenlon, A. J. Vinten, and D Lewis. 2001. The fate of *Escherichia coli* O157:H7 in soil and its potential to contaminate drinking water. *Intl. J. Food Microbiol.* 66(1-2): 111-117.

Pell, A. N. 1997. Manure and microbes: Public and animal health problem? *J. Dairy Sci.* 80(10): 2673-2681.

Reddy, K. R., R. Khaleel, and M. R. Overcash. 1981. Behavior and transport of microbial pathogens and indicator organisms in soils treated with organic wastes. *J. Environ. Qual.* 10(3): 255-266.

Schillinger, J. E., and J. J. Gannon. 1985. Bacterial adsorption and suspended particles in stormwater. *J. Water Pollut. Control Fed.* 57(5): 384-389.

Smith, J. E., Jr., and J. M. Perdek. 2004. Assessment and management of watershed microbial contaminants. *Crit. Rev. Environ. Sci. Tech.* 34(2): 109-139.

SPSS. 2002. SPSS Release 11.5. Chicago, Ill.: SPSS, Inc.

Soupir, M. L., S. Mostaghimi, E. R. Yagow, and C. Hagedorn. 2006. Transport of fecal bacteria from poultry litter and cattle manures applied to pastureland. *Water Air Soil Pollut.* 169: 125-136.

Tyrrel, S. F., and J. N. Quinton. 2003. Overland flow transport of pathogens from agricultural land receiving faecal wastes. *J. Appl. Microbiol.* 94(1): 87-93.

USEPA. 2000. National Water Quality Inventory. Washington, D.C.: U.S. Environmental Protection Agency, Office of Water. Available at: www.epa.gov/305b/2000report/. Accessed 1 August 2007.

USEPA. 1976. Fecal coliform bacteria. In *Quality Criteria for Water*, 42-50. Washington, D.C.: U.S. Environmental Protection Agency.

Zar, J. H. 1996. Multiple comparisons; Comparing simple linear regression equations. In *Biostatistical Analysis*, 212, 353-369. 3rd ed. Englewood Cliffs, N.J.: Prentice Hall.