Transport of Bacteria on Sloping Soil Surfaces by Runoff

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Received 3 March 1999; revised 2 August 1999; accepted 18 August 1999

ABSTRACT: Pathogenic bacteria exist at soil surfaces as a result of practices as spreading of liquid manure on agricultural lands or use of treated wastewater for irrigation. Rainfall is a major factor affecting vertical and horizontal movement of bacteria in soil. Surface runoff carries bacteria significant distances downstream causing serious threats to ground and surface waters. This study uses a nalidixic acid-resistant *Escherichia coli* strain as a biotracer monitoring extent of bacterial migration on sloping soil surfaces by runoff action. Two 10 × 10-m plots in two sites having different slopes were sprayed with water containing biotracer. Soil texture at sites was clay loam. Sixteen days after spraying, two heavy rainfalls that caused runoffs were recorded. First rainfall occurred 2 days after spraying plots. Samples were collected from soil and runoff at different distances downstream of the plots. Biotracer was found in soil and runoff samples some 20 m downstream from center point of plot having the milder slope. Biotracer was found in soil and runoff samples further downstream of the second plot with the steeper slope reaching a 35- and 30-m distance respectively. Most soil and runoff samples collected after the second rainfall, occurring 15 days after inoculation, contained no biotracer except small numbers found in soil samples taken from center point of each plot 5 m downstream. Results confirm the important role of runoff in bacterial transport on soil surfaces. They show *E. coli* survives in semiarid areas for a long time and increases potential of contamination. Environ Toxicol 15: 149–153, 2000

Keywords: biotracer; *E. coli*; runoff; soil; bacterial transport

INTRODUCTION

Pathogenic microorganisms found at the soil surfaces may originate from several sources. Practices such the spreading of liquid manure on agricultural lands or the use of treated wastewater for irrigation are potential sources for these pathogens. In a field study conducted in Ontario, Canada, Culley and Phillips (1982) found that manure applications in winter resulted in significantly higher fecal coliform and fecal streptococcus counts in the surface runoff, and fecal streptococcus counts in subsurface discharge when compared with applications during other seasons. Stewart and Reneau (1981) observed migration of coliform bacteria from septic tank drain fields in both vertical and horizontal directions to monitoring wells of 152- and 305-cm depth located within 30 m of the drain fields. The extent of

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Contract grant sponsor: Jordan University of Science and Technology.

Contract grant sponsor: Natural Sciences and Engineering Research Council (NSERC) of Canada.

Contract grant sponsor: Canada Foundation for Innovation's Institutional Innovation Fund for Infrastructure.

migration in both directions varied depending on the position of the monitoring well relative to the drain field. They attributed these differences to variations in water flow.

Some authors reported that the adsorption of microorganisms onto soil is reversible, as reviewed by Abu-Ashour et al. (1994). The term "reversible" implies that adsorbed microorganisms may detach from surfaces of soil particles and desorb in water. They may subsequently be readsorbed. The phenomenon of desorption was suggested by Wellings et al. (1975) who observed that previously virus-free wells near a land application site in Florida, contained viruses after a period of heavy rainfall. They suggested that viruses were initially adsorbed onto the soil particles and hence could not be detected in wells. However, heavy rainfall caused desorption of these viruses to water flowing into the wells where they were detected.

The present study investigated bacterial movement on sloping soil surfaces by the action of runoff. The experiments were conducted in a field located about 15 km east of Irbid in northern Jordan. The area is considered a part of the semi-arid region with an average annual rainfall of 400 mm. However, the rain events in arid and semi-arid regions are normally characterized by high intensity. Such rain events induce runoff, especially on slopes, which increases erosion and the transport of contaminants on the soil surface to areas downstream.

MATERIALS AND METHODS

Microorganism and Culture Conditions

The microorganism used as a biotracer in this study was a nalidixic acid-resistant *Escherichia coli* strain (*E. coli* NAR), kindly provided by G. Palmateer (formerly at Ontario Ministry of Environment Southwestern Laboratory, London, ON, Canada). Laboratory studies by Joy et al. (1992) confirmed the suitability of using this tracer bacterium as an indicator of the soil transport characteristics of naturally occurring bacteria under various testing conditions. This strain has also been used in laboratory (Abu-Ashour et al., 1998) and field studies (Joy et al., 1998; Palmateer et al., 1989; Shadford et al., 1997) elsewhere.

E. coli NAR was grown by adding a loopful of cells from a plate culture to a 125-mL Erlenmeyer flask containing 25-mL tryptic soy broth (TSB, Difco Laboratories, Detroit, MI, USA) and incubated at 20°C for 17–19 h with gyratory shaking at 200 rpm. The cells were harvested by centrifugation at $5000 \times g$ for 20 min. Cells were washed twice with 0.1 M phosphate buffer, pH 7.5, and resuspended in a phosphate buffer. Samples of this cell suspension were used to inoculate 500 mL of TSB and grown as above, for use as the inoculum in field spreading.

Test Sites and Experimental Setup

The experiments were conducted at two sites about 200 m apart and located in a field 15 km east of Irbid in northern Jordan. Both sites had a bare soil surface with an average bulk density of 1350 kg/m^3 and an initial soil water content of 20% on a mass basis. The soil texture at both sites was clay loam. The average slope of the first site was 2% while that of the second site was 6%.

The experiments were carried out in November 1997. At that time of the year, the average day light period was approximately 11 h. During the experimentation period, the skies were mainly partly cloudy. The average minimum and maximum temperatures during the same period were 7 and 14°C, respectively.

At each of the two sites, a 10×10-m plot was inoculated by spraying with the biotracer-containing cell suspension. A 500-mL volume of inoculum was added to 19.5 L of distilled water in a 20-L carboy and shaken vigorously. Prior to inoculation, samples were taken from the liquid cell suspension to determine the biotracer concentration. These samples were serially diluted, spread on tryptic soy agar plates supplemented with 100 $\mu g/mL$ nalidixic acid (TSA-NA), and incubated at 44°C for 24 h before being enumerated for colony forming units (CFU). A total of 2×10^{12} CFU contained in two 20-L carboys (for a total of 40 L) were used for each plot. The rest of the cell suspension was then poured into a plastic watering can, similar to those used to irrigate gardens, and distributed evenly over the surface of test field plots.

Prior to inoculation, samples were also collected from the top 5 cm of soil at three different locations at each site to determine the background concentration of indigenous nalidixic acid-resistant *E. coli* cells prior to inoculation. From each of these samples, 10 g of soil were placed in a graduated cylinder and distilled water was poured into the cylinder until 100-mL volume was reached. The graduated cylinders were shaken vigorously, then 1-mL samples were removed for enumeration of nalidixic acid-resistant *E. coli* cells as described earlier. No background nalidixic acid-resistant *E. coli* cells were detected in any of these samples.

For runoff sample collection, 1-L plastic bottles perforated at the top were inserted into the ground. Care was taken to keep the perforations at the soil surface open to intercept runoff. The bottles were laid at 5-m intervals along the center line of each site. Two ditches along the down slope, each 1 m long and 3 cm deep, were dug near each bottle in a V-shaped pattern as

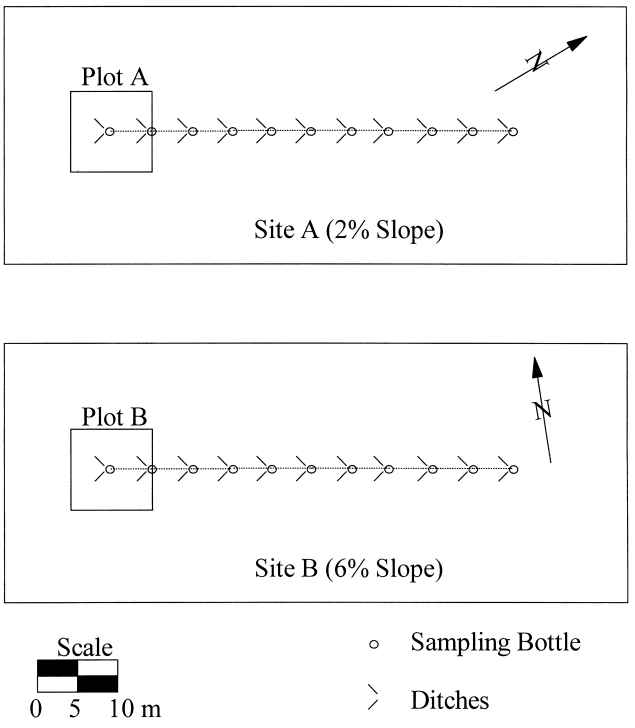

Fig. 1. Experimental site layout.

shown in Figure 1, to intercept runoff and direct it toward the bottles. This arrangement minimized the chance of having some of the bottles remaining empty due to the concentration of runoff in small channels which may have diverted the flow completely away from the bottles. Also, excess runoff would flow over the ditches and the bottles when they became full.

Precipitation was measured using a rain gauge placed at one site. During a period of 16 days after inoculating the plots, two rain events that caused runoff were recorded. The first rain event occurred 2 days after inoculation of the test plots. A total rainfall of 26 mm was recorded in 24 h. The second rain event occurred 15 days after inoculation, and resulted in 21 mm of precipitation in 24 h. After each of the two rain events, samples from the soil surface (top 5 cm) within 1 m of each bottle were collected. Runoff samples were also collected from the water retained in the bottles. All plastic bottles were replaced with new ones after each rain storm. Soil and runoff samples were analyzed for biotracer concentration as described earlier.

Another rain event that recorded less than 2 mm in 24 h occurred 10 days after inoculation of the plots. This rain event did not cause runoff since no water was found in any of the plastic bottles laid along the slopes at both sites, and no samples were collected.

RESULTS AND DISCUSSION

The objective of this study was to determine the effect of surface runoff on the movement of bacteria applied on the soil surface. The experiments were conducted in two sites having different slopes to investigate the effect of surface sloping on bacterial movement.

The biotracer was inoculated over the test plots as described in Materials and Methods. After both major rain events, all the bottles at both sites were found to be filled right to the top with water as well as some soil particles. After the first heavy rain event, the biotracer was found in both soil and runoff samples taken downstream of the plot at Site A, reaching a distance of 20 m from the center of the plot (Table I). Soil and runoff samples taken further downstream did not contain any biotracer cells. Similarly, at site B, after the first heavy rain event, the biotracer was found in soil and runoff samples as far as 35 and 30 m, respectively, downslope from the center of the plot (Table I). Sam-

TABLE I. Biotracer concentrations in soil and runoff samples after first rain event

	Site A (2% Slope)		Site B (6% Slope)	
Distance	Cells in Soil (CFU/g)	Cells in Runoff (CFU/mL)	Cells in Soil (CFU/g)	Cells in Runoff (CFU/mL)
0	4200	220	3300	450
5	5100	340	4500	320
10	330	45	510	170
15	10	60	55	24
20	25	15	10	15
25	0	0	25	10
30	0	0	13	16
35	0	0	15	0
40	0	0	0	0
45	0	0	0	0
50	0	0	0	0

ples collected beyond these distances did not contain any biotracer.

The close correspondence in the extent of migration of the biotracer in the soil and in the bottles at both sites show that the experimental setup was adequate for runoff collection by the bottles. The results confirm that runoff was the main medium that carried the biotracer downstream at both sites. After spraying, the biotracer cells initially interacted with some of the soil particles via adsorption. Runoff likely caused detachment of some of the adsorbed biotracer cells. The detached cells were then carried downstream by the water flow where they were found in soil and runoff samples. The other likely mechanism for the transport of these cells is by advection in the sorbed phase. Runoff caused erosion of some soil particles to which biotracer cells were attached. Some soil particles were observed in the sampling bottles which may indicate the occurrence of erosion. The amount of soil found in the bottles was not measured, hence the relative contribution by erosion could not be adequately assessed. A combination of these two mechanisms may be responsible for finding the biotracer cells downstream of the plots at both sites. The results also show that bacteria migrated to a greater extent in the steeper site (site B) than the site with a milder slope (site A). This result is expected. Likely, the higher water velocity at the steeper slope resulted in higher shear force which caused more erosion of soil particles. It may also cause greater detachment of biotracer cells from the soil surface.

The presence of small numbers of biotracer cells downslope of the plots may indicate that adsorption of the cells onto soil was strong and far from being instantaneously reversible. This may also be the reason for finding the greatest number of cells in the plots where the biotracer was initially applied. If the attachment of biotracer cells was reversible and weak, then one would have expected to find higher biotracer concentrations in the soil and runoff samples with peak values further downslope of the plots. Such trend was not found in the experimental results.

The second rain event which occurred 15 days after inoculation of the test plots with the biotracer also led to runoff. The results summarized in Table II show that small concentrations of biotracer cells were recovered from soil samples taken from the center point of each plot about 5 m downstream of that point. No biotracer was found in other soil samples. All samples taken from the bottles at both sites were free of biotracer cells.

Several reasons may be responsible for the large reduction in biotracer concentration in the soil samples taken after the second rain event. First, the biotracers may have died off. As reviewed by Abu-Ashour et al. (1994), sun light may adversely affect bacterial survival in soil. The biotracer cells on the soil surface were exposed to direct sunlight during the experimentation period which may have affected their survival. Die-off may also have occurred through competition with indigenous microorganisms. Second, biotracer cells may have moved vertically deeper into the soil, as infiltrating rain water may have carried some of the cells to layers below the 5-cm sampling depth.

It was remarkable that some biotracer cells remained sufficiently viable for at least 16 days in a semi-arid environment to allow their transport and enumeration. This capability to survive increases the potential of contamination.

In conclusion, the present study shows that runoff is an important mechanism by which microorganisms applied at the soil surface may be transported downstream to areas where they may pose a serious threat

TABLE II. Biotracer concentrations in soil and runoff samples after second rain event

	Site A (2% Slope)		Site B (6% Slope)	
Distance	Cells in Soil (CFU/g)	Cells in Runoff (CFU/mL)	Cells in Soil (CFU/g)	Cells in Runoff (CFU/mL)
0	100	0	70	0
5	60	0	90	0
10	0	0	0	0
15	0	0	0	0
20	0	0	0	0
25	0	0	0	0
30	0	0	0	0
35	0	0	0	0
40	0	0	0	0
45	0	0	0	0
50	0	0	0	0

to the quality of our water resources. Pathogenic bacteria and viruses applied at the soil surface will cause more danger if they have the ability to survive for long periods of time.

REFERENCES

Abu-Ashour, J.; Joy, D. M.; Lee, H.; Whiteley, H. R.; Zelin, S. Water Air Soil Pollut 1994, 75, 141–158.

Abu-Ashour, J.; Joy, D. M.; Whiteley, H. R.; Zelin, S. Trans Am Soc Agric Eng 1998, 41, 1043–1050.

Culley, J. L. B.; Phillips, P. A. J Environ Qual 1982, 11, 155–158.

Joy, D. M.; Abu-Ashour, J.; Botari, J. L.; Etches, C.; Lee, H.; Sopher, C.; Whiteley, H. R.; Zelin, S. Microbial Transport in Soils With and Without Macropores; Proceedings of the Technology Transfer Conference; Sponsored by the Ontario Ministry of Environment and Energy: Toronto, Canada, 1992.

Joy, D. M.; Lee, H.; Reaume, C. M.; Whiteley, H. R.; Zelin, S. Can Agric Eng 1998, 40, 153–160.

Palmateer, G. A.; McLean, D. E.; Walsh, M. J.; Kutes, W. I.; Janzen, E. M.; Hocking, D. E. Toxicol Assess 1989, 4, 377–397.

Shadford, C. B.; Joy, D. M.; Lee, H.; Whiteley, H. R.; Zelin, S. J Contamin Hydrol 1997, 28, 227–246.

Stewart, L. W.; Reneau, R. B. J Environ Qual 1981, 10, 528–531.

Wellings, F. M.; Lewis, A. L.; Mountain, C. W.; Pierce, L. V. Appl Microbiol 1975, 29, 751–757.