Journal of Integrative Agriculture 2016, 15(7): 1625–1637

Available online at www.sciencedirect.com

ScienceDirect

RESEARCH ARTICLE

Wetting patterns and bacterial distributions in different soils from a surface point source applying effluents with varying *Escherichia coli* concentrations

WEN Jie, LI Jiu-sheng, LI Yan-feng

State Key Laboratory of Simulation and Regulation of Water Cycle in River Basin, China Institute of Water Resources and Hydropower Research, Beijing 100048, P.R.China

Abstract

Understanding bacterial transportation in unsaturated soil is helpful for reducing and avoiding pathogenic contamination that may be induced by irrigation with reclaimed waste water and for developing better irrigation management practices. Experiments were conducted to study the transport of a typical bacterium, *Escherichia coli* (*E. coli*), in a sandy and a sandy loam soil under different application rates and input concentrations. A 30° wedge-shaped plexiglass container was used to represent one twelfth of the complete cylinder in the experiments. The apparent cylindrical application rate varied from 1.05 to 5.76 L h^{-1} and the input concentration of *E. coli* from magnitude of 10^2 to 10^7 colony-forming unit (CFU) mL^{-1}. For a given volume of water applied, an increase in application rate resulted in an increase in the wetted radius and a decrease in the wetted depth. In the sandy loam soil, the water spread out in a circular-arc shaped saturated zone on the surface, and the ultimate saturated entry radius increased with the application rate. An increasing application rate of water suspended bacteria allowed a more rapid transport of bacteria, thus accelerating *E. coli* transport rate and resulting in a larger distributed volume of *E. coli* for both soil types. For the sandy soil, more than 70% of the *E. coli* that was detected within the entire wetted volume concentrated in the range of 10 cm from the point source, and the concentration of *E. coli* decreased greatly as the distance from the point source increased. More than 98% of the *E. coli* was detected in a range of 5 cm around the saturated wetted zone for the sandy loam soil. For both soil types tested, an extremely high concentration of *E. coli* was observed in the proximity of the point source, and the peak value increased with an increased input concentration. In principle, using an emitter with relative lower application rate would be effective to restrict *E. coli* transport. To reduce bacterial concentration in the sewage effluent during wastewater treatment is important to decrease the risk of soil contamination caused by irrigation with sewage effluent.

Keywords: application rate, drip irrigation, *Escherichia coli*, input concentration

Received 6 September, 2015 Accepted 20 November, 2015
Correspondence LI Jiu-sheng, Tel: +86-10-68786545, E-mail: lijs@iwhr.com; jiushengli@126.com

doi: 10.1016/S2095-3119(15)61249-7

1. Introduction

Water scarcity has already affected every continent. Around 1.2 billion people, or almost one-fifth of the world's population, live in areas of physical scarcity, and 500 million people are approaching this situation. Increased water

shortages and new environmental policies and regulations have stimulated significant development in reuse programs (UNDP 2006). Reuse of wastewater for agricultural irrigation is practiced not only in dry and water deficient areas, but in water abundant regions as well (USEPA 2004). Because of the increase of reclaimed water irrigation, its health and environmental impacts cause wide concerns.

The structure of soil and microbial community might be changed after irrigating by the sewage. Liu and Haynes (2011) studied the influence of land application of dairy factory effluent on catabolic capability of the soil microbial community and found that effluent irrigation substantially changed soil chemical properties, while the metabolic quotient and decomposition functions of the soil microorganisms and the structure and diversity of the bacterial community have remained unaffected by dairy factory effluent irrigation. Tian *et al*. (2015) worked on responses of microbial activity, abundance, and community in soil after three years of heavy fertilization with manure-based compost and inorganic nitrogen and found that the size and activity of the microbial community increased due to regular inputs of soluble organic matter, and the most abundant taxa were found to be correlated with the moisture content, pH, organic C, total N, and available P.

When used for irrigation, the toxic chemicals and microbes in wastewaters or recycled water usually pose threats to human health and environment. Bacteria including pathogenic *Escherichia coli* (*E. coli*), *Salmonella enterica*, *Shigella*, *Staphylococcus*, and fungi in irrigation water may stay in soil or on the surface of crops, transmit to people and cause disease (Crook and Rao 1996). *E. coli* are one of the most common pathogenic bacteria that cause disease in human. Due to its simple detection and high numbers in agricultural wastes, *E. coli* are consistently used as an indicator microorganism for the risk assessment of microbial contamination (Foppen and Schijven 2006).

Bacteria transport is mostly passive (Unc and Goss 2004). Their transport and fate are affected by cell properties, characteristics of the porous medium and transporting solution (Beven and Germann 1982; Abu-Ashour *et al*. 1994; Powelson and Mills 1998; Becker *et al*. 2004; Mosaddeghi *et al*. 2009, 2010). Governing soil characteristics include texture, especially the clay content, structure, pore space, and pore size distribution. The physical and chemical composition, like organic matter content, solution ionic strength, pH and metal oxide coatings also impact the behaviors of *E. coli* in soil (Fontes *et al*. 1991; Abu-Ashour *et al*. 1994; Wan and Wilson 1994; Naclerio 2009; Safadoust *et al*. 2012).

Irrigation methods influenced bacterial transportation. Oliveira *et al*. (2012) have proved that *E. coli* O157:H7 survived in soil and lettuce leaves while applying surface and sprinkler irrigation with contaminated water. Drip irrigation has the priority in reducing the risk of bacterial contamination compared to furrow irrigation (Fonseca *et al*. 2011), since no surface runoff of sewage effluent occurred during irrigation.

Soil columns are routinely used to evaluate bacterial transport in experimental settings. Flow conditions, including water content, the path taken by water and water fluxes, affect the fate of the bacteria (Powelson and Mills 2001). Smith *et al*. (1985) and Tan *et al*. (1994) reported that the breakthrough bacteria increased with the increase of water flow velocity. Tan *et al*. (1994) also found that bacterial transport was enhanced at higher cell concentrations. While Jiang *et al*. (2007) pointed out that percent adsorption (P_a) of *E. coli* to silica sands was not related to the input *E. coli* concentration, and the main controlling mechanisms for bacterial retention in soil are attachment and detachment at particle surfaces (Ginn *et al*. 2002; Banks *et al*. 2003). Smith *et al*. (1985) reported that the activity and penetration of *E. coli* strains were influenced by pore size. Their result is consistent with the hypothesis that small pore throats restrict microbial activity and penetration. Hassan *et al*. (2005) studied the influences of irrigation rates (mL d^{-1}) and dosing frequencies (doses d^{-1}) on coliforms' transport when applying subsurface drip irrigation systems, and gave the result that the number of coliforms in leachates increased significantly at the highest rate of application (2.071 mL d^{-1}).

Most researches regarding the *E. coli* transport in soil focused on breakthrough experiments in which the transport and fate of the *E. coli* were investigated by detecting extraction and leaching of soil solution. Few researches were conducted on the transport of *E. coli* in an unsaturated soil that is frequently a common situation for the promising efficient irrigation method of drip irrigation while applying sewage effluent. The objectives of the study were to quantify the effects of the technical parameters of surface drip irrigation on water and *E. coli* distribution in different soils through laboratory experiments and to give recommendations for management of surface drip irrigation systems applying sewage effluent.

2. Materials and methods

2.1. Experimental setup for *E. coli* suspension transport

A 30° wedge-shaped plexiglass container, which was 60 cm high and 40 cm radius (Fig. 1), was used to conduct the experiments. It was assumed that each container represented one twelfth of the complete cylinder. Lv (2000) investigated the influence of the angle of the wedge-shaped container on water and solute movement, and found that there was no significant difference between the 15° wedge-

shaped container and a 90° one. Such devices had been successfully used for investigating the transport of water and nitrogen in different soils (Li *et al*. 2004). Prior to each experiment, the container was sterilized by an ultraviolet germicidal lamp for 1 h.

2.2. Soil properties

A sandy and a sandy loam soil were used in the experiments. Soil was air-dried and homogenized by passing through a 2-mm sieve. The air-dried soil was packed in the container with 5 cm increments to obtain a constant bulk density of 1.43 and 1.4 g cm^{-3} for the sandy soil and the sandy loam soil, respectively. Particle size analysis yielded an average value of 95.46% sand, 4.52% silt, and 0.02% clay for the sandy soil and 33.88% sand, 52.38% silt, and 13.74% clay for the sandy loam soil. The organic matter content for the sandy and sandy loam soil was 1.32 and 8.59 g kg^{-1}, respectively. In this research, both soils were not sterilized in order to simulate the actual soil environments.

2.3. Source of *E. coli*

The *E. coli* (DH*5α*-3C-GFP, a gift from Dr. Cong Haolong, Institute of Microbiology, Chinese Academy of Sciences, Beijing), which possess penicillin resistant gene, was propagated and maintained in Luria Broth (Helbling and Vanbriesen 2008) liquid medium (Thermo Fisher Scientific Inc., USA) supplemented with penicillin (50 μg mL^{-1}) at 37°C for 16 h. *E. coli* liquid was then suspended in sterile deionized water. A plate count approach (Goldman and Green 2009) was used to determinate *E. coli* concentrations. The supernate was ten-fold serially diluted in order to obtain at least one plate with a countable number of bacteria. The serially dilutions were cultivated in Luria Broth solid medium supplemented with penicillin (50 μg mL^{-1}), at 37°C for 24 h. The results were reported as colony-forming units (CFU) per milliliter (mL). The property of the colony, which randomly selected from the plates, was determined by Gram staining (Holt *et al*. 1994) and this procedure confirmed that the bacteria counted by the plate approach were Gram-negative.

The *E. coli* suspension with designed concentration was added to the soil through a no. 7 needle connected to a peristaltic pump (Tianli Industrial Equipment Co., Ltd., China) with a flow rate ranging from 0.06 to 30 mL min^{-1} through a sterile flexible hose. The outlet needle was located on the soil surface at the corner of the soil container (Fig. 1). To maintain zero evaporation, the soil was covered with a polyethylene sheet. In each experiment, the positions of the moving wetting front on the soil surface and in the vertical plane were recorded visually at several times. After a predetermined volume of solution had been applied, the vertical

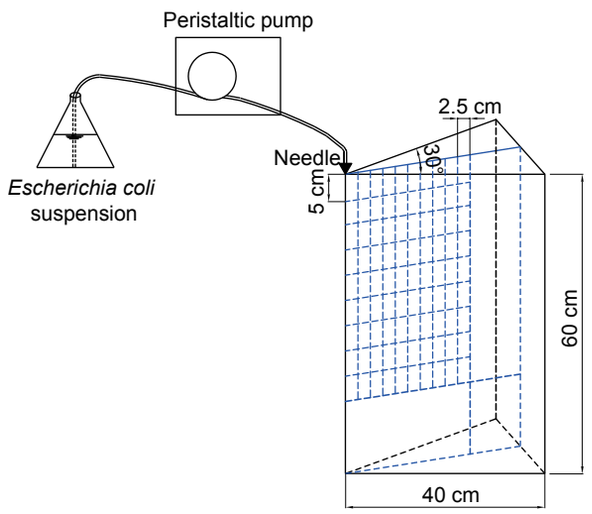

Fig. 1 Schematic descriptions of the experimental device and soil sampling positions.

plane of the container was placed on a horizontal surface to avoid redistribution of water and solute in the soil. The container was then opened and immediately sampled. The sampling layout was radial intervals of 2.5 cm and a vertical interval of 5 cm (Fig. 1). All experiments were conducted in a room with an approximately constant temperature of 20°C.

2.4. Isolation and characterization of *E. coli*

Soil sample for *E. coli* test was extracted with a sterile test tube (inside diameter 2 cm). For each sample, 10 g of soil was added to 100 mL sterile deionized water, and mixed evenly in a shaker for 15 min. Then the solution was homogenized for 2 min to obtain the supernate. The plate count approach was used to determinate *E. coli* concentrations in the supernate. The serially dilutions were also cultivated in Luria Broth (Helbling and Vanbriesen 2008) solid medium supplemented with penicillin (50 μg mL^{-1}), at 37°C for 24 h. Similarly, the Gram-negative property of the counted bacteria was confirmed by the Gram staining.

For each experiment by applying sandy soil, three soil samples were taken outside of the wetted volume to establish the initial conditions of water content and initial bacteria concentrations in the soil. In order to further confirm the detected Gram-negative bacteria were from the input *E. coli* suspension, the polymerase chain reaction (PCR) experiment was conducted using a PCR thermocycle instrument (DL-2000; Shine Gene Molecular Biotech, Shanghai, China). The soil samples showed a similar primer amplification fragment to the added *E. coli* sample (data not shown). This confirmed that the bacteria detected in the soil were the species of *E. coli* that had been added during the ex-

periments. The total *E. coli* and the proportion of penicillin resistant *E. coli* were then determined as described above.

For experiments using sandy loam soil, similarly, three soil samples were also collected outside of the wetted volume to establish the initial conditions of water and bacteria. As the sandy loam soil was collected from 0–30 cm depth of a field just after the harvest of lettuce, the soil activity was stronger and probably more bacteria existed. Two extra experiments were conducted to detect the distribution of background penicillin resistant bacteria in the sandy loam soil and to confirm that the *E. coli* detected in the wetted soil volume were the added indicator *E. coli*.

2.5. Application rate and input *E. coli* concentration

Two variables, application rate and input *E. coli* concentration, which may affect the water flow and *E. coli*'s transport, were considered. The experiments using sandy soil were referred to as group A, and those using sandy loam soil were referred to as group B. In total, 17 experiments were conducted. Table 1 summarizes the apparent application rates, initial water contents, and input *E. coli* concentrations of all the experiments. The variables are presented on the basis of completely cylindrical system in the article based on the verified assumptions that the 30° wedge-shaped container can represent one twelfth of the complete cylinder, and the shape of test device has negligible impact on flow patterns (Lv 2000). In order to compare with actual drip irrigation, the apparent application rates were reported by multiplying the actual variables by 12 in the article. Different application rates were obtained by regulating the peristaltic pump speed. For all the experiments, no dripper (needle) blockage caused by *E. coli* was detected possibly due to the relatively short application duration.

Table 1 Summary of the apparent application rate (q), initial volumetric water content (θ_0) and input *Escherichia coli* concentration (C_0) for each of the 17 experiments

Exp. no.[1)]	q (L h^{-1})[2)]	θ_0 (cm^3 cm^{-3})[2)]	C_0 (CFU mL^{-1})
A1	1.05	0.034	6.7×10^5
A2	1.76	0.033	6.8×10^5
A3	2.88	0.031	7.3×10^5
A4	3.60	0.031	4.7×10^5
A5	5.76	0.034	5.4×10^5
A6	1.75	0.036	9.5×10^2
A7	1.75	0.036	4.6×10^4
A8	1.74	0.034	2.1×10^6
B0	1.76	0.155	0
B1	1.05	0.159	3.6×10^7
B2	1.79	0.158	3.6×10^7
B3	3.51	0.156	9.0×10^7
B4	5.76	0.149	9.2×10^7
B5	1.76	0.147	8.1×10^4
B6	1.76	0.156	4.1×10^5
B7	1.76	0.150	3.6×10^7
B+	1.78	0.159	6.3×10^7

[1)] Exp. no. means experiment numner.
[2)] Actual value multiplied by 12. The same as below.

For group A, the application rates of irrigation varied from 1.05 to 5.76 L h^{-1} that almost covered the current range of emitter discharges for commercial products (experiment numbers (Exp. nos.) A1–A5). The concentrations magnitude of *E. coli* was 10^2 to 10^6 CFU mL^{-1} for sandy soil experiment (Exp. nos. A6–A8). For group B, the application rates of irrigation also varied from 1.05 to 5.76 L h^{-1} (Exp. nos. B1–B4). In order to avoid the possible background bacterial influence and stronger attachment of the sandy loam soil, a greater *E. coli* concentration magnitude of 10^4 to 10^7 CFU mL^{-1} was used for the sandy loam soil (Exp. nos. B5–B7). An approximately similar apparent volume of 7.2 L was used for all experiments.

In order to detect the distribution of background penicillin resistant bacteria in the sandy loam soil, an extra experiment without indicator *E. coli* addition (Exp. no. B0) was conducted. Another extra experiment (Exp. no. B+) was conducted to confirm the transport of indicator *E. coli* under condition of without background penicillin resistant bacteria. The sandy loam soil that was disinfected by an autoclave sterilizer was used for this experiment.

3. Results

3.1. Wetting front movement

Wetting patterns were characterized by the radial and vertical distance of the wetting front from the point source. Figs. 2 and 3 illustrate the surface wetted radius and the vertical wetted depth as a function of time for different application rates in the sandy soil (Exp. nos. A1–A5) and the sandy loam soil (Exp. nos. B1–B4), respectively. For a larger application rate, the wetting front moved faster both in the radial and vertical directions. For a given volume applied, the final wetted radius increased with the application rate while the wetted depth decreased with the rate. This influence is more obvious for sandy loam soil. For example, the surface wetted radii are approximately 27.1, 29.3, 30.0 and 30.4 cm while the wetted depths are 26.0, 25.3, 20.3, and 16.3 cm for 1.05, 1.79, 3.51 and 5.76 L h^{-1} application rates, respectively. This suggest that increasing application rate allows more water to distribute in the horizontal direction, while decreasing the rate allows more water to distribute in the vertical direction.

Power equations shown in eqs. 1 and 2 were fitted to estimate the movement of the wetting front:

$$R_h = bt^d \quad (1)$$

$$R_v = b_1 t^{d_1} \quad (2)$$

Where, R_h is the surface wetted radius (cm); R_v is the vertical wetted depth (cm); t is the elapsed time (min); b and b_1 are regression coefficients; and d and d_1 are regression power value. The fitted parameters of the equations describing the movement of the wetting front in the radial and vertical directions are summarized in Table 2. The average power value (d and d_1) is 0.29 and 0.37 for the sandy soil, and 0.33 and 0.41 for the sandy loam soil, respectively. The average fitted parameter of surface wetted radius is 0.31, which is similar to the findings of Li *et al*. (2004). The b values shows an increasing trend with application rate, especially for the sandy loam soil, suggesting that the surface wetted radius is controlled by both elapsed time and application rate.

It was also found that the water spread out in a circular-arc shaped saturated zone on the sandy loam soil surface. This saturated zone through which the water entry into the soil was initially small, but its radius became larger as time increased and then reached almost a constant value for a unique application rate (Fig. 4). The greater the application rate was, the faster the constant surface-saturated wetted radius reached. Li *et al*. (2003) reported the similar result and given the function of application rate and the ultimate radius of the saturated zone on the surface.

3.2. *E. coli* distribution in the sandy soil

For the experiments of group A, the amounts of initial bacteria measured from the three samples collected outside of the wetted sandy soil volume in cultivation environment with penicillin and without penicillin are compared in Table 3. For the sandy soil, in the LB solid medium plate without penicillin, the total bacteria were up to 1.4×10^2 to 8.6×10^3 CFU mL^{-1}. However, in the cultivation environment with penicillin added, only samples from Exp. no. A1 had bacteria been detected, and the value was less than 1% of the total bacterium. This means the resistance screening was effective to eliminate the interference from the background bacteria in the sandy soil on the distribution of *E. coli* detected in the wetted soil volume since the cells injected during the experiments were penicillin resistant.

The detected *E. coli* within the total wetted volume were summed up, the ratio of *E. coli* in each 10 cm layer was estimated for each of the 8 experiments conducted with sandy soil. It was found that more than 70% of the detected *E. coli* was located in the top 10 cm layer (Table 4). This result is similar to the findings of Jiang *et al*. (2005) who reported that bacterial deposition was concentrated in the top 10 cm soil

Table 2 Estimation of parameters for wetted distance of sandy and sandy loam soil in horizontal ($R_h=bt^d$, cm) and vertical direction ($R_v=b_1t^{d_1}$, cm) and corresponding determination coefficient R^2 value

Exp. no.	q (L h^{-1})	b	b_1	d	d_1	R^2	
						Horizontal	Vertical
A1	1.05	3.26	3.52	0.32	0.39	0.993	0.998
A2	1.76	4.74	5.29	0.26	0.34	0.992	0.970
A3	2.88	5.08	5.09	0.28	0.37	0.998	0.995
A4	3.60	4.63	5.17	0.31	0.38	0.996	0.997
A5	5.76	6.24	5.97	0.28	0.38	0.998	0.994
B1	1.05	4.04	2.33	0.31	0.40	0.991	0.986
B2	1.79	4.34	2.81	0.34	0.41	0.990	0.997
B3	3.51	6.19	2.74	0.32	0.41	0.996	0.986
B4	5.76	7.44	2.59	0.35	0.42	0.920	0.983

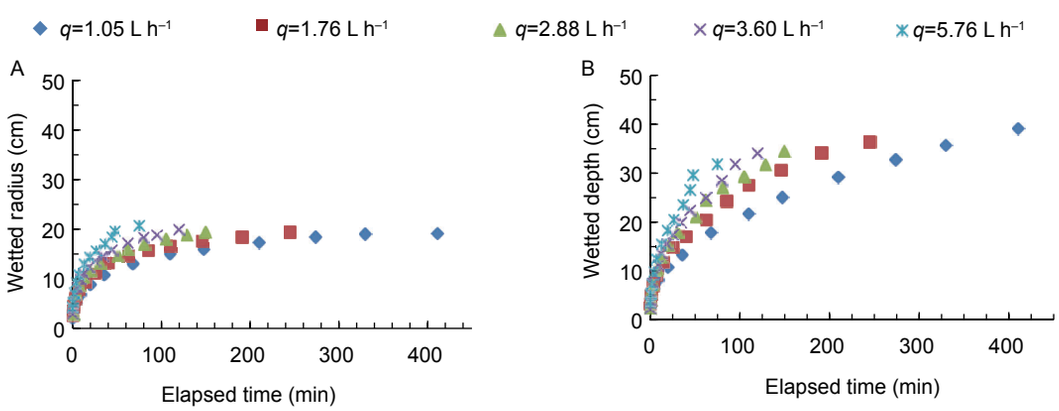

Fig. 2 Surface wetted radius (A) and vertical wetted depth (B) as a function of elapsed time for apparent application rates (q) ranging from 1.05 to 5.76 L h^{-1} for the sandy soil.

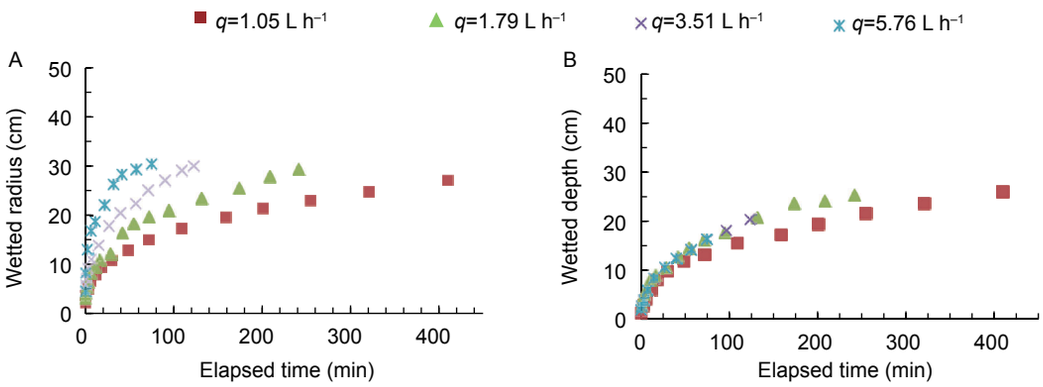

Fig. 3 Surface wetted radius (A) and vertical wetted depth (B) as a function of elapsed time for apparent application rates ranging from 1.05 to 5.76 L h^{-1} for the sandy loam soil.

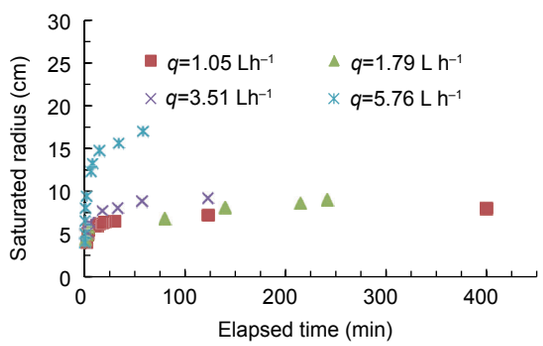

Fig. 4 The saturated wetted radius on the surface as a function of elapsed time for apparent application rates ranging from 1.05 to 5.76 L h^{-1} for the sandy loam soil.

and then decreased abruptly with depth. Mosaddeghi *et al*. (2010) also found that *E. coli* concentrations were greater at the first sampling depth (i.e., 20 cm). The concentration decreased greatly as the distance from the point source increased. Fig. 5 illustrates the bubble diagram of the *E. coli* concentrations in the sandy soil for different apparent application rates ranging from 1.05 to 5.76 L h^{-1}. The r and z represent the radial distance from the point source and the depth from the surface, respectively. A bigger bubble represents a higher *E. coli* concentration. There existed an extremely high *E. coli* concentration (Table 4) in the proximity of the point source (about 0–5 cm from the source) and the concentration decreased greatly as the distance from the

Table 3 Background bacteria under cultivation environment with and without penicillin for the sandy and the sandy loam soil, and the ratio between the indicator *E. coli* within the top 5 cm layer and the *E. coli* detected within the entire wetted volume

Exp.no.	Background bacteria in soil (CFU mL^{-1})		*E. coli* ratio in 5 cm surface layer (%)[1)]
	No penicillin added	Penicillin added	
A1	3.8×10^3	12	
A2	1.4×10^2	0	
A3	1.8×10^3	0	
A4	1.0×10^3	0	
A5	8.6×10^3	0	
A6	9.4×10^2	0	
A7	6.8×10^2	0	
A8	1.8×10^2	0	
B1	1.4×10^2	6	98
B2	2.4×10^3	3	99
B3	6.7×10^2	2	99
B4	7.7×10^4	66	99
B5	5.4×10^3	0	
B6	7.5×10^4	3	
B7	7.8×10^2	0	

[1)] The *E. coli* ratio in 5 cm surface layer for the sandy soil (Exp. nos. A1–A8) was not calculated as more detailed distribution of the *E. coil* was provided in Table 4. Besides, no meaningful values of the *E. coli* ratio in 5 cm surface layer for the sandy loam soil (Exp. nos. B5–B7) was obtained due to the significant effects of the background bacteria on the *E. coil* distribution when the input *E.* coli concentration was lower.

Table 4 *E. coli* distribution characteristics and ratio in the different vertical layers for the sandy soil

Exp. no.	*E. coli* concentration in a given soil layer (CFU mL^{-1})											
	0–10 cm			10–20 cm			20–30 cm			30–40 cm		
	Max	Sum	Ratio (%)[1)]	Max	Sum	Ratio (%)[1)]	Max	Sum	Ratio (%)[1)]	Max	Sum	Ratio (%)[1)]
A1	2.4×10^5	4.1×10^5	73	1.5×10^4	1.1×10^5	19	5.7×10^3	3.8×10^4	7	1.0×10^3	3.0×10^3	1
A2	1.8×10^5	3.5×10^5	91	5.6×10^3	3.0×10^4	8	7.5×10^2	3.6×10^3	1	40	1.3×10^2	0
A3	9.9×10^4	1.9×10^5	76	6.5×10^3	4.4×10^4	17	5.9×10^3	1.6×10^4	6	1.8×10^2	5.7×10^2	0
A4	6.7×10^4	3.0×10^5	72	1.0×10^4	8.3×10^4	20	7.2×10^3	3.2×10^4	8	93	1.5×10^2	0
A5	5.8×10^4	3.3×10^5	74	1.6×10^4	8.5×10^4	19	6.9×10^3	3.0×10^4	7	0	0	0
A6	8.8×10^2	2.3×10^3	83	1.3×10^2	3.4×10^2	16	0	0	0	0	0	0
A7	3.6×10^4	7.1×10^4	72	2.2×10^3	1.7×10^4	19	1.5×10^3	9.7×10^3	10	0	0	0
A8	2.7×10^6	7.0×10^6	89	2.1×10^5	8.8×10^5	11	3.1×10^4	6.0×10^4	0	27	73	0

[1)] It means the ratio between the *E. coli* detected in a given 10 cm layer and the *E. coli* detected within the entire wetted volume.

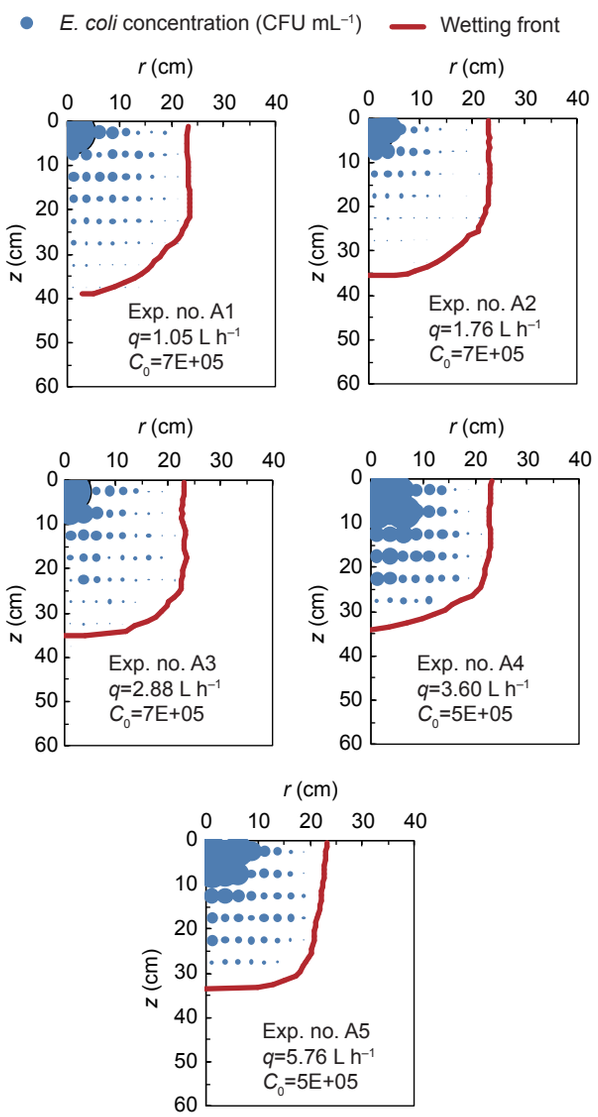

Fig. 5 Distribution of the *E. coli* concentration and wetting front in the sandy soil when 7.2 L solutions with an *E. coli* concentration magnitude around 10^5 CFU mL^{-1} were applied at apparent application rates ranging from 1.05 to 5.76 L h^{-1}. The *r* and *z* represent the radial and vertical distance from the point source, respectively. The same as below.

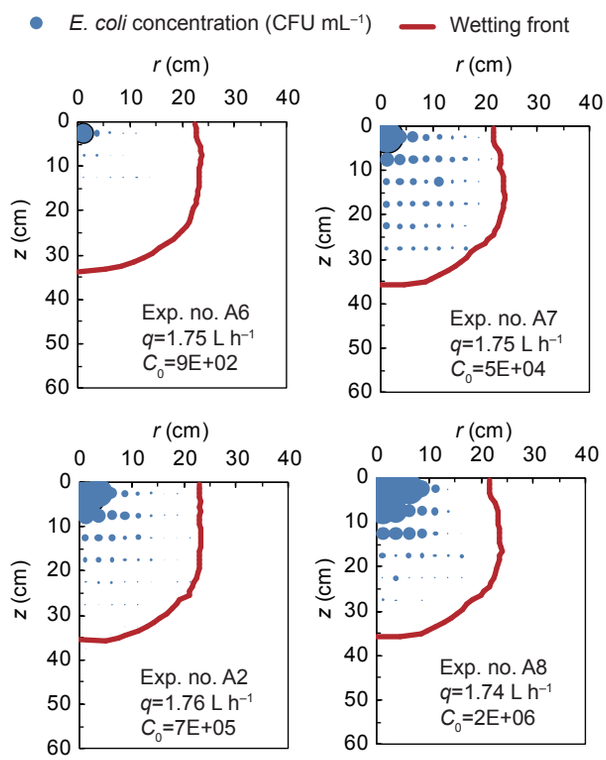

Fig. 6 Distribution of *E. coli* concentration and wetting front in the sandy soil when 7.2 L solutions with *E. coli* concentration magnitudes ranging from 10^2 to 10^6 CFU mL^{-1} applied at an approximate apparent application rate of 1.75 L h^{-1}.

point source increased. Generally, a greater application rate resulted in a larger distributed volume of *E. coli*. For example, an obviously larger distributed volume of *E. coli* was observed for the Exp. nos. A4 and A5 with greater application rates of 3.6 and 5.76 L h^{-1}.

Fig. 6 compares the distributions of *E. coli* in the sandy soil when input concentration magnitude was 10^2, 10^4, 10^5 and 10^6 CFU mL^{-1} at an approximately similar apparent rate of 1.75 L h^{-1}. The *E. coli* concentration in the soil was highly dependent on input concentration. For example, the

peak value was 8.8×10^2 CFU mL^{-1} when the input *E. coli* concentration was 9.5×10^2 CFU mL^{-1}, while it reached 2.7×10^6 CFU mL^{-1} when the input concentration was 2.1×10^6 CFU mL^{-1} (Table 4).

The input concentration also influenced the transport distance of *E. coli*. For example, the maximum transport depth of the *E. coli* was less than 20 cm for a low input concentrations of 9.5×10^2 CFU mL^{-1} (Exp. no. A6), while the depth exceeded 30 cm when the input concentration reached 2.1×10^6 CFU mL^{-1} (Exp. no. A8).

3.3. *E. coli* distribution in the sandy loam soil

The initial bacteria in the sandy loam soil under cultivation environment with and without penicillin are also indicated in Table 3. The total bacterium ranged from 1.4×10^2 to 7.7×10^4 CFU mL^{-1}, while the penicillin-resistant bacteria were less than 1% of the total bacteria. Again, the result confirmed that the resultant screening was effective in controlling the influence of initial bacteria in the sandy loam soil.

The experiment B0 which was conducted without indicator *E. coli* addition is compared with the experiments B1–B7 with *E. coli* additions in Fig. 7. In Fig. 7, the bacterial distributions below 5 cm depth for the experiments B1–B7 are illustrated. The bacteria were distributed randomly in the soil for both experiments B0 and B1–B7, and an approximately similar magnitude of peak bacterial value (10^2–10^3 CFU mL^{-1}) was detected for all experiments.

Experiment B+, which aimed at confirming the transport of the indicator bacterial when the disinfected sandy loam soil was used, showed that more than 99.9% of the detected *E. coli* was located in the 5 cm layer around the saturated wetted zone, and the largest concentration is labeled in Fig. 8. These results suggested that the bacteria detected below 5 cm depth were not the intendedly added *E. coli*.

Fig. 9 illustrates the distributions of *E. coli* in the sandy loam soil for apparent application rates ranging from 1.05 to 5.76 L h^{-1} with an input *E. coli* concentration magnitude of 10^7 CFU mL^{-1}. An extremely high *E. coli* concentration area was observed around the saturated zone, and *E. coli* that were concentrated in the zone accounted for more than 98% of the total bacteria detected in the entire soil volume (Table 3). The maximum concentrations were 9.0×10^5, 1.0×10^6, 6.0×10^5 and 9.0×10^5 CFU mL^{-1} for different apparent application rates ranging from 1.05 to 5.76 L h^{-1}. A greater application rate that produced a larger saturated zone on the soil surface (Fig. 4) resulted in an increased distribution volume of *E. coli* in the soil. Also, a greater application rate also drove the peak position to move farther from the point source. A maximum distance of 17 cm between the peak *E. coli* concentration and the emitter was observed for the largest application rate of 5.76 L h^{-1}. It is worth pointing out that, as a result of strong attachment, *E. coli* distribution in sandy loam soil was almost irrelevant with the wetting front.

In order to eliminate soil background bacteria's influence, Fig. 10 compares the distributions of *E. coli* in the 5 cm surface layer with input concentrations ranging from 10^4 to 10^7 CFU mL^{-1} at an approximately similar apparent rate of 1.75 L h^{-1}. One noticeable result was that the *E. coli* concentration in the first layer was highly dependent on input concentration. For example, the value closest to the point source was 1.09×10^3 CFU mL^{-1} when the input *E. coli* concentration was 8.1×10^4 CFU mL^{-1}, while it reached 8.2×10^6 CFU mL^{-1} for the input concentration of 3.6×10^7 CFU mL^{-1}.

The result shows similar trend comparing with the concentration influence experiment in the sandy soil. *E. coli* concentration in the 5 cm surface layer was also highly dependent on input concentration. For example, the value closest to the point source was 1.09×10^3 CFU mL^{-1} when the input *E. coli* concentration was 8.1×10^4 CFU mL^{-1}, while it reached 8.2×10^6 CFU mL^{-1} when the input concentration is 3.6×10^7 CFU mL^{-1}.

3.4. Effects of soil types on *E. coli* distribution

In order to compare the effect of soil types on *E. coli* distribution, the relative concentration was defined as the ratio of *E. coli* detected in the soil to the input concentration of *E. coli* in this study. Fig. 11 compares the relative concentrations in the top surface layer of the sandy and the sandy loam soil at 2.5, 5, 7.5 and 10 cm radial distances from the point source. The reason to choose these positions was that the *E. coli* distribution around the emitter was much larger than other positions. A greater relative *E. coli* concentration in the sandy soil was observed than that in the sandy loam soil. At 2.5 cm from the point source, for example, the relative *E. coli* concentration ranged from 0.1 to 1.4 for the sandy soil, while a considerably smaller value of 0.017 to 0.024 for the sandy loam soil was found. The relative concentration decreased greatly as the distance from the emitter increased for both soils due to attachment.

4. Discussion

Experiments were conducted using a sandy and a sandy loam soil to study the influence of irrigation strategies on water and *E. coli* distributions from a surface point source. The results showed that the increase in the surface wetted radius and in the vertical wetted depth with the increase of volume applied can be represented by a power function with power values of about 0.3 and 0.35, respectively. Increasing application rate allows more water to distribute in the

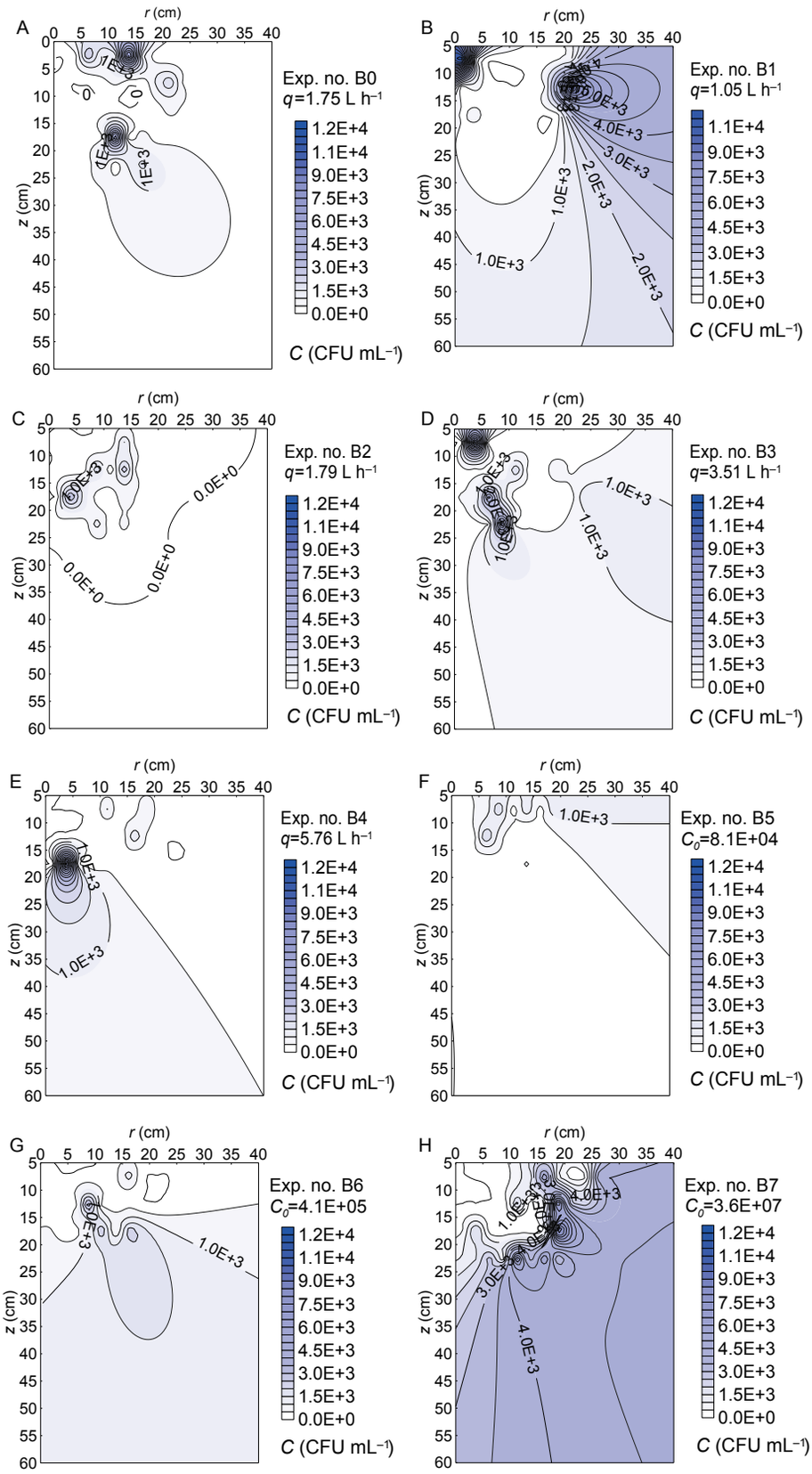

Fig. 7 Distribution of penicillin-resistant bacteria concentration (CFU mL^{-1}) in the sandy loam soil without indicator *E. coli* addition (Exp. no. B0) and with indicator *E. coli* addition (Exp. nos. B1–B7).

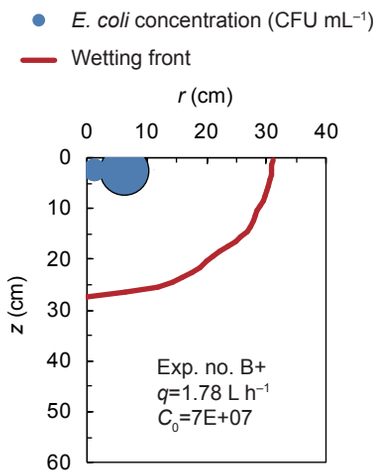

Fig. 8 Distribution of *E. coli* concentration and wetting front in the sandy loam soil which was autoclave-sterilized when 7.2 L solutions with *E. coli* concentration magnitudes 10^7 CFU mL^{-1} were applied at an apparent application rate of 1.78 L h^{-1}.

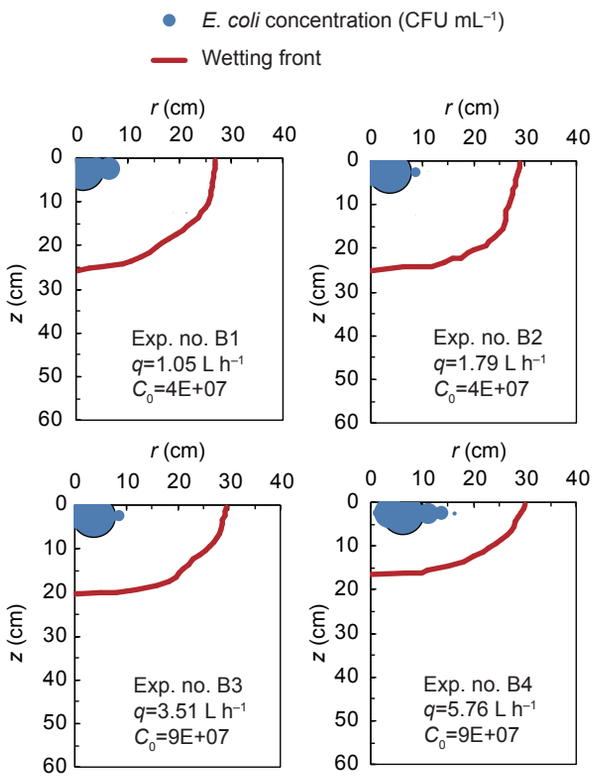

Fig. 9 Distribution of the *E. coli* concentration and wetting front in the sandy loam soil when 7.2 L solutions with an approximate input *E. coli* concentration magnitudes of 10^7 CFU mL^{-1} were applied at apparent application rates ranging from 1.05 to 5.76 L h^{-1}.

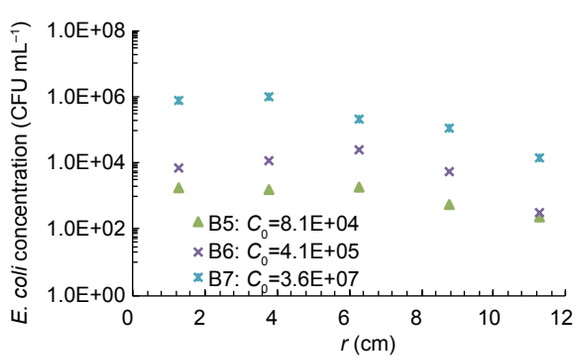

Fig. 10 Distributions of the *E. coli* concentration in 5 cm surface layer of the sandy loam soil with input *E. coli* concentrations ranging from 10^4 to 10^7 CFU mL^{-1}.

horizontal direction, while decreasing the rate allows more water to distribute in the vertical direction. The phenomenon is more obvious in the sandy loam soil experiments. The reason is that a higher application rate produced a larger saturated zone on the sandy loam soil surface, which increases the water transport in the radial direction. The findings is similar to the results of Li *et al*. (2004).

The transport of *E. coli* in two different soils was investigated under unsaturated condition of surface drip irrigation. For both sandy and sandy loam soil types, *E. coli* were mainly concentrated in the top layer and decreased greatly with the increase of distance from the point source. Differing from saturated breakthrough experiment which had greater water flux and no significant air-water interface, more bacteria concentrated above the wetting front under unsaturated condition. These results were agreed with the findings of Powelson and Mills (2001) who reported that the transport of hydrophilic bacteria was drastically reduced under unsaturated conditions.

The mechanism of pollutant migration in medium mainly includes convection, molecular diffusion and mechanical dispersion (Wang 2008). For both soil types, a greater application rate resulted in a larger distributed volume of *E. coli*. The possible reason is that the increasing application rate enhances convection, thus promoting the *E. coli* transportation. Several studies have reported that increasing flow velocity resulted from increasing application rate of water suspended bacteria allowed a more rapid transport of bacteria to the depth where these macrospores are continuous, thus accelerating *E. coli* transport rate (Smith *et al*. 1985; Garbrecht *et al*. 2009).

For the sandy loam soil, a substantially shorter moving distance of *E. coli* was observed than that in the sandy soil. Different behaviors of *E. coli* are possibly due to the smaller particle size in the sandy loam soil, which creates smaller pores and greater staining within the medium and restricts the mechanical dispersion. These results were also supported by the breakthrough experiments conducted by Garbrecht *et al*. (2009). They demonstrated a significant

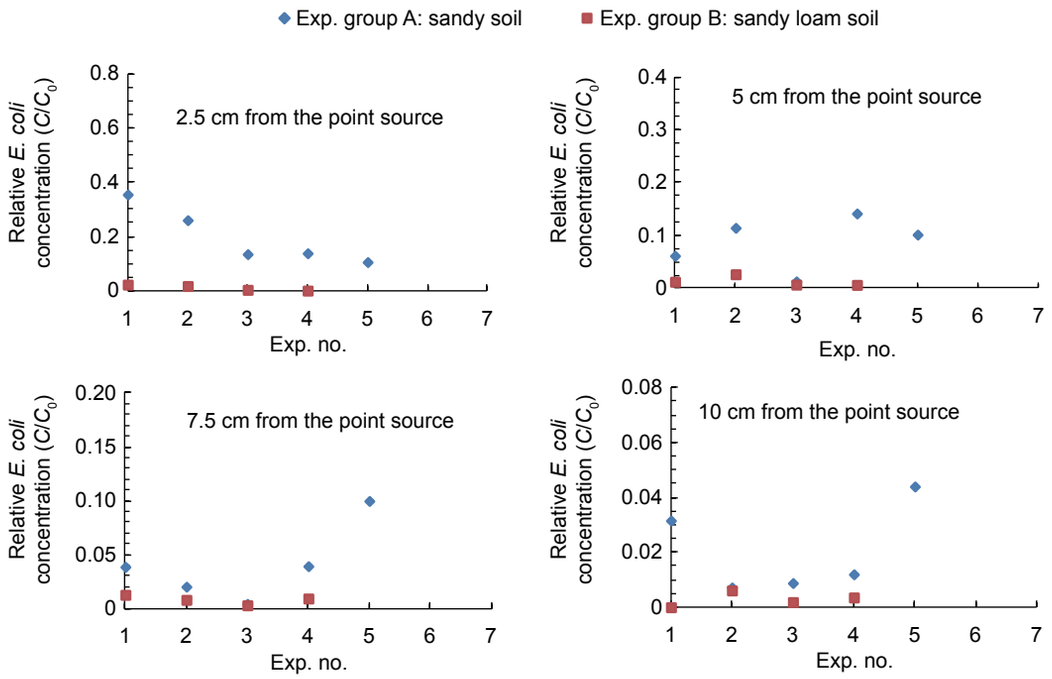

Fig. 11 The relative *E. coli* concentrations at different radial distances from the point source in the 5 cm surface soil layer for the sandy soil and the sandy loam soil with varying application rates.

influence of soil texture on transport of *E. coli* and reported that the final effluent concentrations were approximately 80–100% of the influent concentration in coarse sand, while in loamy sand, the final effluent concentrations were only approximately 12% of the influent concentration. Li and Li (2003, 2006) studied the transport of *E. coli* through saturated sandy and sandy loam soils. They reported that the decreased outflow of *E. coli* in sandy soil was mainly due to retention, while in sandy loam was due to absorption. The greater clay and organic matter content in the sandy loam soil might be the possible reason of the strong attachment since *E. coli* cells are significantly adsorbed to the clay fraction of the soil (Naclerio *et al*. 2009) and organic matter flocks may obstruct soil pores, thus resulting in a reduced water flow through macropores under unsaturated condition (Mosaddeghi *et al*. 2010).

5. Conclusion

The research demonstrated that soil texture, especially the smaller particles played a significant role on migration of *E. coli* cells. A substantially smaller distributed volume of *E. coli* in the sandy loam soil was observed than that in the sandy soil. An increasing application rate of water suspended bacteria accelerated *E. coli* transport rate, resulting in a larger distributed volume of *E. coli* for both soil types. In the proximity of the point source, an extremely high concentration of *E. coli* was observed and the value increased with an increased input concentration.

The results obtained from this study will be useful for the management of surface drip irrigation with effluent. In principle, using an emitter with relative lower application rate would be effective to restrict *E. coli* transport. What is more, to reduce bacterial concentration in the sewage effluent during wastewater treatment is important to decrease the risk of soil contamination caused by irrigation with sewage effluent.

Acknowledgements

This study was financially supported by the National Natural Science Foundation of China (51339007). The authors thank Dr. Cong Haolong (assistant research fellow, Chinese Academy of Sciences, China) for giving *E. coli* DH5α-3C-GFP, and appreciate his help during the PCR detection and preparation of the manuscript. We also thank Dr. Zhang Jianjun (assistant research fellow, Chinese Academy of Agricultural Sciences, China) for suggestions in the experiment preparation and sample taking.

References

Abu-Ashour J, Joy D M, Lee H, Whiteley H R, Zelin S. 1994. Transport of microorganisms through soil. *Water*, *Air*, *and*

Soil Pollution, **75**, 141–158.

Banks M K, Yu W, Govindaraju R S. 2003. Bacterial adsorption and transport in saturated soil columns. *Journal of Environmental Science and Health* (Part A, Toxic /Hazardous Substance & Environmental Engineering), **38**, 2749–2758.

Becker M W, Collins S A, Metge D W, Harvey R W, Shapiro A M. 2004. Effect of cell physicochemical characteristics and motility on bacterial transport in groundwater. *Journal of Contaminant Hydrology*, **69**, 195–213.

Beven K, Germann P. 1982. Macropores and water flow in soils. *Water Resources Research*, **18**, 1311–1325.

Crook J, Rao Y S. 1996. Water reclamation and reuse criteria in the U.S. *Water Science & Technology*, **33**, 451–462.

Fonseca J M, Fallon S D, Sanchez C A, Nolte K D. 2011. *Escherichia coli* survival in lettuce fields following its introduction through different irrigation systems. *Journal of Applied Microbiology*, **110**, 893–902.

Fontes D E, Mills A L, Hornberger G M, Herman J S. 1991. Physical and chemical factors influencing transport of microorganisms through porous media. *Applied and Environmental Microbiology*, **57**, 2473–2481.

Foppen J W A, Schijven J F. 2006. Evaluation of data from the literature on the transport and survival of *Escherichia coli* and thermotolerant coliforms in aquifers under saturated conditions. *Water Research*, **40**, 401–426.

Garbrecht K, Fox G A, Guzman J A, Alexander D. 2009. *E. coli* transport through soil columns: Implications for bioretention cell removal efficiency. *Transactions of the ASABE*, **52**, 481–486.

Ginn T R, Wood B D, Nelson K E, Scheibe T D, Murphy E M, Clement T P. 2002. Processes in microbial transport in the natural subsurface. *Advances in Water Resources*, **25**, 1017–1042.

Goldman E, Green L H. 2009. *Practical Handbook of Microbiology*. 2nd ed. CRC Press, Taylor and Francis Group, USA. pp. 17–18.

Hassan G, Reneau Jr R, Hagedorn C, Saluta M. 2005. Modeling water flow behavior where highly treated effluent is applied to soil at varying rates and dosing frequencies. *Soil Science*, **170**, 692–706.

Helbling D E, Vanbriesen J M. 2008. Continuous monitoring of residual chlorine concentrations in response to controlled microbial intrusions in a laboratory-scale distribution system. *Water Research*, **42**, 3162–3172.

Holt J G, Krieg N R, Sneath P H A, Staley J T, Williams S T. 1994. *Bergey's Manual of Determinative Bacteriology*. *Genus Klebsiella*. Lippincolt Williams and Wilkins, USA.

Jiang G, Noonan M J, Buchan G D, Smith N. 2005. Transport and deposition of *Bacillus subtilis* through an intact soil column. *Soil Research*, **43**, 695–703.

Jiang G, Noonan M J, Buchan G D, Smith N. 2007. Transport of *Escherichia coli* through variably saturated sand columns and modeling approaches. *Journal of Contaminant Hydrology*, **93**, 2–20.

Li G, Li B. 2003. Transport of *Escherichia coli* through saturated sandy soil: Experiment and modelling. *Acta Pedologica Sinica*, **40**, 783–786. (in Chinese)

Li G, Li B. 2006. Non-equilibrium transport of *Escherichia coli* through saturated sandy loam and its simulation with CDE model. *Acta Pedologica Sinica*, **43**, 197–202. (in Chinese)

Li J, Zhang J, Rao M. 2004. Wetting patterns and nitrogen distributions as affected by fertigation strategies from a surface point source. *Agricultural Water Management*, **67**, 89–104.

Li J, Zhang J, Ren L. 2003. Water and nitrogen distribution as affected by fertigation of ammonium nitrate from a point source. *Irrigation Science*, **22**, 19–30.

Liu Y Y, Haynes R J. 2011. Influence of land application of dairy factory effluent on soil nutrient status and the size, activity, composition and catabolic capability of the soil microbial community. *Applied Soil Ecology*, **48,** 133–141.

Lv D. 2000. Test research and simulating model of soil water flow and salt transfer. MSc thesis, Xi'an University of Science and Technology, China. (in Chinese)

Mosaddeghi M R, Mahboubi A A, Zandsalimi S, Unc A. 2009. Influence of organic waste type and soil structure on the bacterial filtration rates in unsaturated intact soil columns. *Journal of Environmental Management*, **90**, 730–739.

Mosaddeghi M R, Safari Sinegani A A, Farhangi M B, Mahboubi A A, Unc A. 2010. Saturated and unsaturated transport of cow manure-borne *Escherichia coli* through *in situ* clay loam lysimeters. *Agriculture*, *Ecosystems and Environment*, **137**, 163–171.

Naclerio G, Nerone V, Bucci A, Allocca V, Celico F. 2009. Role of organic matter and clay fraction on migration of *Escherichia coli* cells through pyroclastic soils, southern Italy. *Colloids Surf* (B Biointerfaces), **72**, 57–61.

Oliveira M, Viñas I, Usall J, Anguera M, Abadias M. 2012. Presence and survival of *Escherichia coli* O157: H7 on lettuce leaves and in soil treated with contaminated compost and irrigation water. *International Journal of Food Microbiology*, **156**, 133–140.

Powelson D K, Mills A L. 1998. Water saturation and surfactant effects on bacterial transport in sand columns. *Soil Science*, **163**, 694–704.

Powelson D K, Mills A L. 2001. Transport of *Escherichia coli* in sand columns with constant and changing water contents. *Journal of Environmental Quality*, **30**, 238–245.

Safadoust A, Mahboubi A A, Mosaddeghi M R, Gharabaghi B, Unc A, Voroney P, Heydari A. 2012. Effect of regenerated soil structure on unsaturated transport of *Escherichia coli* and bromide. *Journal of Hydrology*, **430–431**, 80–90.

Smith M S, Thomas G W, White R E, Ritonga D. 1985. Transport of *Escherichia coli* through intact and disturbed soil columns. *Journal of Environmental Quality*, **14**, 87–91.

Tan Y, Gannon J T, Baveye P, Alexander M. 1994. Transport of bacteria in an aquifer sand: Experiments and model simulations. *Water Resources Research*, **30**, 3243–3252.

Tian W, Wang L, Li Y, Zhuang K, Li G, Zhang J, Xiao X, Xi Y. 2015. Responses of microbial activity, abundance,

and community in wheat soil after three years of heavy fertilization with manure-based compost and inorganic nitrogen. *Agriculture*, *Ecosystems and Environment*, **213**, 219–227.

UNDP. 2006. *Human Development Report*. Published for the United Nations Development Programme, Uzbekistan. pp. 131–169.

Unc A, Goss M J. 2004. Transport of bacteria from manure and protection of water resources. *Applied Soil Ecology*, **25**, 1–18.

USEPA. 2004. Guidelines for Water Reuse. Report No. EPA/625/R-04/108. U.S. Environmental Protection Agency, Washington, D. C., USA.

Wang H. 2008. *Dynamics of Fluid Flow and Contaminant Transport in Porous Media*. Higher Education Press, China. pp. 36–37. (in Chinese)

Wan J, Wilson J L. 1994. Colloid transport in unsaturated porous media. *Water Resource Research*, **30**, 857–864.

(Managing editor SUN Lu-juan)