

Microbial transport in soil caused by surface and subsurface drip irrigation with treated wastewater

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A b s t r a c t. The objective of this work was to compare and simulate transport and fate of microorganisms from wastewater for surface and subsurface irrigation methods. Adsorption isotherms and water-content dependent survival were measured for fecal coliforms, somatic coliphages and F-specific RNA phages in batch experiments with treated wastewater and clay loam soil. Column experiments with surface and subsurface trickle irrigation were carried out for the same soil. Results of the column experiments were simulated with a combination of Richards equation for water transport and advective-dispersive model with the first-order nonlinear adsorption and moisture-dependent first-order die-off. Simulations showed that die-off rates in column experiments were much higher than in batch experiments for all three organisms. Somatic coliphages were the most persistent organisms probably because of lower adsorption and die-off. The subsurface irrigation appeared to be efficient in decreasing the number of pathogens in irrigated water and preventing their appearance on soil surface that could lead to produce contamination.

K e y w o r d s: bacteriophage, fecal coliforms, irrigation, soil, wastewater

INTRODUCTION

Availability of water for irrigation is one of the most important factors in arid and semiarid regions, and treated domestic wastewater becomes an important source. More than $365 \times 10^6 \text{ m}^3$ per year of wastewater is used for irrigation in the US (Rose and Gerba, 1991). More than 70% of Israel sewage is treated and reused in agriculture irrigation. Even after treatment, wastewater may contain a great variety of microorganisms that are pathogenic for humans. Therefore the potential transmission of diseases is a principal concern associated with the

agricultural use of treated wastewater (Crook, 1998). Various irrigation techniques can be used to supply treated wastewater to crops (Oron *et al.*, 1991), and different pathways may be available to microorganisms to travel because the transport of water in soils depends on the irrigation method (Huysman and Verstraete, 1993).

The objective of this work was to compare and simulate transport and fate of microorganisms from wastewater for surface and subsurface irrigation methods. We selected fecal coliforms as a common indicator of bacterial contamination, and somatic coliphages and F-specific RNA phages that were proposed as indicators of viral contamination (Morinigo *et al.*, 1992; Havelaar *et al.*, 1993). Batch data on adsorption and survival of the organisms in soil were obtained and used in a model to simulate transport of the organisms in soil columns irrigated with wastewater.

MATERIALS AND METHODS

Experimental

Wastewater

Treated wastewater was from the treatment system in Sde-Boker (Israel). The treatment system consists of two connected ponds lined by compressed clay and plastic foil. The effluent is applied for irrigation. The total area of the system is 3650 m^2 , the average pond depth is 150 cm, the sludge holding and the stabilization pond volumes are 3500 and 4250 m^3 , respectively, the influent and the effluent daily volumes are about 400 and 350 m^3 , the retention time is 8 to 12 days. The chemical composition of the wastewater used

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in experiments is given in Table 1. Electrical conductivity of the influent and the effluent was 2.1 DS m^{-1} and 1.7 DS m^{-1} , respectively. The influent pH value was 8.1 and did not change during the treatment. Concentrations of fecal coliforms, somatic coliphages and F-specific RNA phages were $5.1 \cdot 10^3 \text{ CFU cm}^{-3}$, $1.3 \cdot 10^3 \text{ PFU cm}^{-3}$, and $6.1 \cdot 10^2 \text{ PFU cm}^{-3}$, respectively.

Table 1. Chemical composition of the wastewater ($\mu\text{g cm}^{-3}$)

Flow	BOD		COD		TSS	Inorganic cations				
	Total	Filtered	Total	Filtered		Cl ⁻	SO ₄ ²⁻	NH ₄ ⁺	NO ₃ ⁻	PO ₄ ²⁻
Inflow	438	42	2500	140	460	330	32.4	36.4	-	10.0
Outflow	54	24	190	140	120	289	56.0	9.9	-	8.8

BOD – Biochemical Oxygen Demand, COD – Chemical Oxygen Demand, TSS – Total Suspended Solids.

Soil

The loess clay loam soil was sampled at a commercial field near the city of Arad (Israel). The field had been irrigated for several years with treated wastewater. Clay, silt, and sand contents were 29, 45, and 26%, respectively, the organic matter content was 0.8%.

Microorganism contents

Somatic coliphages and F-specific RNA phages were determined using standard procedures (ISO 1995; ISO 1996) with *E. coli* CN (ATCC 700078) and *E. coli* HS (pFamp)R respectively as host strains. The double layer agar technique was used for both types of bacteriophage.

Fecal coliforms were quantified using the membrane filtration technique after various dilutions in peptone water (1 g l^{-1} peptone, pH 7.2) as described in the Difco manual (Difco, 1984). Wastewater or soil suspension was filtered through a $0.45 \mu\text{m}$ membrane. The filters were placed on mFC agar and incubated for 24 h at 44.5°C (Britton and Greeson, 1987). Colonies were counted for each dilution after incubation.

Adsorption isotherms

The wastewater used in this experiments contained $5.4 \cdot 10^3 \text{ CFU cm}^{-3}$ fecal coliforms, $1.2 \cdot 10^3 \text{ PFU cm}^{-3}$ somatic coliphages, and $5.4 \cdot 10^2 \text{ PFU cm}^{-3}$ F-specific RNA phages. Three, 10 and 40 g of air-dry soil were placed in 200 ml sterile glass vials, and 100 ml of wastewater or wastewater diluted with autoclaved wastewater was added. The suspension was agitated with a magnetic stirrer for 30 min and then allowed to settle for 30 min at room temperature. Thirty minutes each for stirring and settling was found to be sufficient to achieve adsorption equilibrium and to allow the soil to settle (data not shown). Contents of fecal coliforms, somatic coliphages and F-specific RNA phages were determined in the suspension after 30 min stirring and in

supernatant after 30 min settling. The adsorbed amount of microorganisms was calculated as the difference between the total number of microorganisms present in the suspension and that in the supernatant. The concentration in solution was equal to that in supernatant. A control with 40 g soil and 100 ml autoclaved wastewater was included to determine the initial concentration of the microorganisms in soil.

Survival of microorganisms

Survival in soil was assessed with wastewater containing $3.5 \cdot 10^3 \text{ CFU cm}^{-3}$ fecal coliforms, $1.5 \cdot 10^3 \text{ PFU cm}^{-3}$ somatic coliphages, and 10^3 PFU cm^{-3} F-specific RNA phages. Ten g of air-dry soil was placed in one hundred sterile plastic vials. Wastewater was added to reach moisture content of 15% (20 vials), 25% (60 vials) or 35% (20 vials). Contents of microorganisms were measured daily for ten days. To assess survival rates in wastewater, 500 ml of wastewater was agitated with a magnetic stirrer for 30 min at 20°C . Then 10 ml aliquots were transferred to three hundred capped sterile plastic vials. Ten vials were incubated at $22 \pm 2^\circ\text{C}$. Contents of microorganisms were measured daily for ten days. Data for other temperatures are published elsewhere (Gantzer *et al.*, 2001).

Column experiments

Eight PVC columns, 50 cm high, 18 cm *in dia*, were filled with air-dry loess clay loam soil. The average bulk density value was 1.51 g cm^{-3} . The columns were slowly saturated with autoclaved wastewater. Then they were leached with the same water until constant flow regime was achieved. The saturated hydraulic conductivity was $36 \pm 4 \text{ cm day}^{-1}$. The columns were subsequently allowed to drain for 10 days.

Three of the eight columns were randomly selected to estimate soil hydraulic properties. The first of those columns was destroyed immediately after cessation of drainage. The second and the third columns were irrigated with autoclaved wastewater for 13 h with the application rate of 50 ml h^{-1} . The second and the third columns were disassembled after 1 and 3 days after cessation of irrigation, respectively, and soil moisture content was measured at 10 cm depth increments.

The five remaining columns were used for experiments with microorganisms. One column was used to determine initial microorganism and soil moisture content. Two

columns were irrigated from the surface and two were irrigated with the subsurface emitter buried at the depth of 25 cm in the middle of the columns. A total of 1000 ml of wastewater was applied at the rate of 50 ml h⁻¹ to each of the columns. Initial concentrations of fecal coliforms, somatic coliphages and F-specific RNA phages were 6.65 10³ CFU cm⁻³, 6.5 10² PFU cm⁻³, and 2.05 10² PFU cm⁻³, respectively.

One column from each pair was disassembled after 24 h and another was disassembled after 72 h after the beginning of irrigation to determine the microorganism contents.

Column experiments were carried out at 22±2°C. Soil samples were taken every 10 cm for determination of soil moisture content and every 5 cm for determination of microorganism distributions in soil profile.

Microbial transport modeling

Governing equations

The Darcian water flow in variably saturated soil was considered. The governing flow equation in a rigid porous medium is given by a form of Richards's equations:

$$\frac{\partial \theta}{\partial t} = \frac{\partial}{\partial x} \left[K(h) \frac{\partial}{\partial x} (h-x) \right] + F_\theta \quad (1)$$

where: θ is the volumetric water content (cm³cm⁻³), h is the capillary pressure (cm), $K(h)$ is the hydraulic conductivity (cm day⁻¹), x is the vertical coordinate measured downward (cm), t is the time (day), F_θ is the volumetric rate of water influx from subsurface drip emitter (cm³cm⁻³day⁻¹). Van Genuchten - Mualem (van Genuchten, 1980) equations for soil hydraulic properties were used:

$$\theta(h) = \theta_s - \frac{\theta_s - \theta_r}{[1+(\alpha h)^n]^m}$$

$$K(h) = K_s S_e^{0.5} \left[1 - \left(1 - S_e^{\frac{1}{m}} \right)^m \right] \quad (2)$$

Here S_e is the relative saturation, $S_e = (\theta - \theta_r) / (\theta_s - \theta_r)$, θ_s and θ_r are saturated and residual water content (cm³cm⁻³), K_s is the saturated hydraulic conductivity (cm day⁻¹), α , m and n are parameters, $m=1-1/n$.

Transport of bacteria and viruses in the unsaturated soil can be simulated as a result of a combination of advection and dispersion affected by growth, die-off, attachment and detachment of microorganisms (Peterson and Ward, 1989; Tan *et al.*, 1992; Schafer *et al.*, 1998; Corapcioglu and Choi, 1996; Yates and Ouyang, 1992). The model was chosen in the following form (Shelton *et al.*, 2003):

$$\frac{\partial}{\partial t} (\theta_a c + \rho s) = \frac{\partial}{\partial x} \left(\theta_a D \frac{\partial c}{\partial x} - J_w c \right) - \mu_l \theta_a c - \mu_s \rho s + F_\theta c_w \quad (3)$$

Here c is the volume-averaged microorganism concentration in the soil solution, CFU or PFU cm⁻³, θ_a is the porosity available for microorganism transport in soil (cm³cm⁻³), ρ is soil bulk density (g cm⁻³), s is the adsorbed amount of microorganisms, CFU or PFU g⁻¹, D is the dispersion coefficient (cm² day⁻¹), J_w is the volumetric water flux density (cm day⁻¹), μ_l and μ_s (day⁻¹), are first-order die-off coefficients to simulate removal of microorganisms from solution and solid phase due to deposition and entrainment (Hornberger *et al.*, 1992). Values of μ_l and μ_s include also growth rate constants with negative signs if re-growth occurs, c_w is the microorganisms concentration in the irrigation water, CFU or PFU cm⁻³.

The change of the adsorbed amount of microorganisms was simulated as:

$$\frac{\partial (\rho s)}{\partial t} = k_1 \Omega_s \theta_a c^N - k_2 \rho s - \mu_s \rho s \quad (4)$$

where: k_1 and k_2 are microbial adsorption and desorption rate constants (day⁻¹), N is the exponent in Freundlich isotherm equation:

$$s = K_F c^N,$$

Ω_s is the relative retention capacity of soil defined as (Corapcioglu and Choi, 1996):

$$\Omega_s = 1 - s / s_{\max},$$

where: s_{\max} is the maximum retention capacity of soil (cells g⁻¹), the rate constant k_2 is related to k_1 and μ_s as:

$$k_2 = k_1 \Omega_s \theta_a / K_F - \mu_s,$$

where: K_F is the Freundlich isotherm coefficient.

The microorganisms in irrigation water were subject to die-off:

$$\frac{\partial c_w}{\partial t} = -\mu_w c_w \quad (5)$$

where: μ_w is the die-off rate constant.

Equations of the model were solved numerically using the 2DSOIL code (Timlin and Pachepsky, 1997) that used the finite-element method to solve equations of water and solute transport. A microbial transport routine was added. The numerical solution of the microorganism transport equations was obtained by employing the two-point backward difference approximation for time derivatives in Eq. (3) and the Runge-Kutta method for Eq. (4). Equations

(3) and (4) were solved sequentially for each time step (Kim and Corapcioglu, 1996), and iterations continued while the maximum relative change in concentration of interest was larger than 10^{-6} .

Parameter estimation

Parameters of water transport model included van Genuchten parameters α, n, θ_s and θ_r , and the saturated hydraulic conductivity K_s . The latter was estimated from the values of the steady state flux in columns. Van Genuchten parameters α, n , and θ_r were estimated from water content distributions in column profiles 24 and 72 h after the irrigation ended. The Marquardt algorithm (van Genuchten, 1981) was used to minimize the mean-root-square error of calculated water content. The initial estimates of $\alpha = 0.0261 \text{ cm}^{-1}$, $n = 1.754$, and $\theta_r = 0.208 \text{ cm}^3 \text{ cm}^{-3}$ were derived from data of Mualem (1976), θ_s was $0.43 \text{ cm}^3 \text{ cm}^{-3}$.

The parameters of the microbial transport model include the dispersion coefficient D , the porosity available for microorganisms transport in soil, θ_a , soil bulk density ρ , the maximum retention capacity of soil s_{\max} , the microbial deposition rate constant k_1 , parameters of the Freundlich isotherm K_F and N , first-order die-off coefficients μ_l and μ_s , and the die-off rate constant for the irrigation water μ_w . The dispersion coefficient reflects Brownian diffusion, motility, and mechanical dispersion (Gerba and Bitton, 1984):

$$D = D_B + D_{disp}, \quad (6)$$

where: D_B is Brownian diffusion and motility coefficient, and D_{disp} is the mechanical dispersion coefficient. The value of D_B was calculated as (Corapcioglu and Haridas, 1984):

$$D_B = \tau D_b, \quad (7)$$

where: D_b is the diffusion coefficient of microorganisms in free solution and τ is the tortuosity factor. Values D_b of $10^{-9} \text{ cm}^2 \text{ day}^{-1}$ and $5 \cdot 10^{-10} \text{ cm}^2 \text{ day}^{-1}$ were taken from Characklis (1990) for bacteria and viruses, respectively. The tortuosity factor was estimated as (Pachepsky, 1990):

$$\tau = \begin{cases} 0.71 - 0.133 \log_{10} |h|, & h < -2Pa \\ 0.67, & h \geq -2Pa \end{cases} \quad (8)$$

The dispersion coefficient was calculated as $D_{disp} = \lambda J \theta$ where λ is the dispersivity. The value of $\lambda = 10 \text{ cm}$ was estimated from the soil clay content (Pachepsky, 1990). Parameters θ_a and k_1 were estimated from data from columns with surface irrigation for each of the three microorganisms separately. The Marquardt algorithm was used to optimize the values of these parameters. Freundlich isotherm parameters were estimated from adsorption experiments as shown below. The die-off rate coefficients μ_l and μ_s were separately estimated for each of the three organisms as:

$$\mu_s = \mu_l = b_s f_\theta, \quad (9)$$

where: b_s is the specific inactivation rate in soil, f_θ is the moisture correction factor:

$$f_\theta = 1 - \frac{\theta_s - \theta}{\theta_*}. \quad (10)$$

The linear equation was used because the range of water contents in the experiment was relatively narrow (see below).

No temperature corrections were applied because temperature was the same during both batch and laboratory experiments. The values of b_s and θ_* and h were first estimated from data from survival experiments; then the values of b_s and θ_* were re-estimated as shown below.

The subsurface irrigation data were not used in parameter estimation and served as a testing data set.

RESULTS

Adsorption isotherms

The soil did not contain fecal coliforms and bacteriophages of interest before the experiment. The results of the adsorption experiments are shown in Fig. 1, parameters of the Freundlich isotherm equation are in Table 2. The Freundlich equation provided a good fit to the data. Slopes on the isotherms did not differ from one another significantly at the 0.05 significance level. Ranges of the soil partition coefficient K_d were similar for the two bacteriophages, and range limits were about three times less for bacteriophages than for the fecal coliforms.

Microorganism survival data

The survival characteristics of each type of microorganism in soil as a function of moisture are given in Table 3. At a temperature of 22°C , the moisture content of

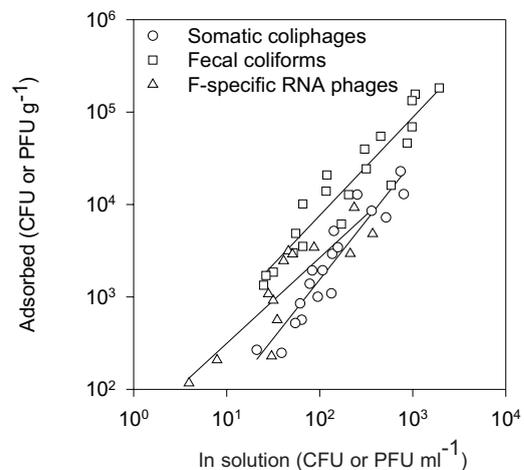


Fig. 1. Adsorption isotherms of three microorganisms on clay loam soil in this work.

Table 2. Parameters of the Freundlich isotherm equation for concentrations in PFU or CFU cm⁻³ and adsorbed amounts in PFU or CFU (g soil)⁻¹

Microorganisms	Number of samples	N	K _F	Determination coefficient (R ²)	K _d (cm ³ g ⁻¹)	
					Max	Min
Fecal coliforms	20	1.066±0.086	55.3±26.7	0.884	174.6	27.5
Somatic coliphages	20	1.253±0.128	4.33±2.55	0.922	50.7	6.4
F-specific RNA phages	13	0.897±0.156	40.7±23.3	0.792	68.0	7.5

Table 3. Survival of fecal coliforms, somatic coliphages, and F-specific RNA phages in soil

Microorganisms	Gravimetric water content (%)	Number of data points	Average die-off rate, k (day ⁻¹)	t ₉₀	R ²
Fecal coliforms	15	6	0.043	23.4	0.757
	25	6	0.083	12.0	0.903
	35	6	0.152	6.6	0.922
Somatic coliphages	15	6	0.027	37.7	0.624
	25	6	0.040	25.2	0.723
	35	6	0.048	20.7	0.960
F-specific RNA phages	15	7	0.141	7.1	0.980
	25	7	0.121	8.3	0.922
	35	8	0.154	6.5	0.828

the soil had a significant effect on the survival of fecal coliforms and somatic coliphages (P-value < 0.05). The values of t₉₀, defined as time of population decimation, generally decreased for somatic coliphages and fecal coliforms as moisture increased. At all moisture contents, the die-off rate of somatic coliphages was significantly higher (1.5 to 4 times higher) than that of fecal coliforms (P-value < 0.05). For the F-specific RNA phages moisture content had no significant effect (P-value > 0.1), and values were lower than those for the other two types of microorganism. Of the three microorganisms, somatic coliphages were the most persistent microorganisms in soil.

The values of the die-off rates *k* were found from the zero-intercept linear regressions:

$$\ln \frac{N}{N_0} = -kt, \tag{11}$$

where: *N*₀ and *N* are the initial count of microorganisms and the count for the time *t*, respectively. Values of the determination coefficients of regressions in Table 3 show that the regressions have reproduced the shape of the ‘N-on-t’ dependencies.

Column experiment data

Figure 2a shows the distribution of water contents after one and three days after irrigation in the columns used to estimate soil hydraulic properties. A gradual redistribution of water content occurred within the top 40 cm while the

40-50 cm layer was left unaffected. Figures 2b and 2c show the distribution of water contents in the columns where the microorganisms were introduced with the wastewater. The pattern of redistribution of surface-applied water in Fig. 2b is quite typical. In subsurface irrigated columns, the maximum contents were observed in the vicinity of the emitters

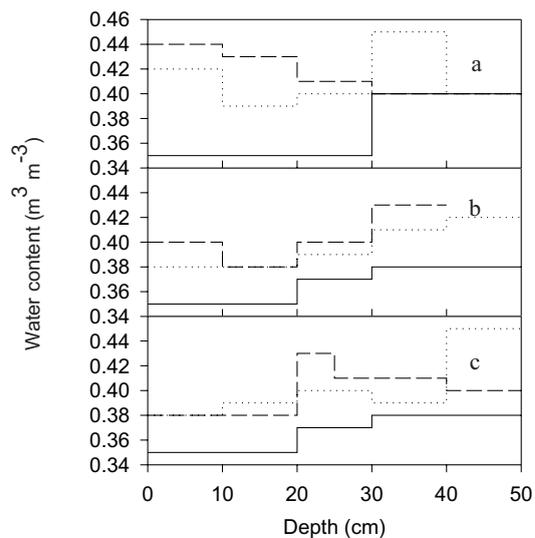


Fig. 2. Profile distributions of water contents; a - columns used to estimate soil hydraulic properties, b - surface irrigation, c - subsurface irrigation; — initial distribution, - - - - - 24 h after the end of irrigation, ······ 72 h after the end of irrigation.

after 24 h of the end of irrigation (Fig. 2c). After 72 h the moisture distribution became more uniform and maximum water content was observed at the bottom of the columns.

Measured distributions of microorganisms in the soil columns are shown in Figs 3-5. A substantial die-off is observed during the second and third days of experiment with surface irrigation (Table 4). The die-off of fecal coliforms and somatic coliphages was similar, the F-specific RNA phages disappeared four times faster than fecal coliforms and somatic phages. Somatic coliphages were present whereas fecal coliforms and F-specific RNA phages were absent at depths of 10-20 cm three days after surface irrigation. The F-specific RNA phages and fecal coliforms were detected only close to the emitter 24 h after the subsurface irrigation. Seventy two hours after the subsurface irrigation, F-specific RNA phages were no longer detectable. Somatic coliphages and fecal coliforms persisted in soil after 72 h.

Simulation results

Simulated and measured water soil water contents compared reasonably well (Fig. 6), the mean simulation error and the root-mean-square error were $0.002 \text{ cm}^3 \text{ cm}^{-3}$ and $0.008 \text{ cm}^3 \text{ cm}^{-3}$, respectively. Optimized parameters of

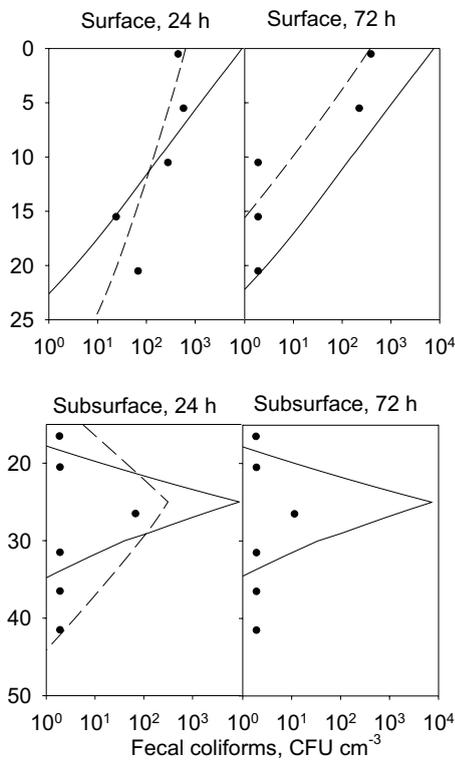


Fig. 3. Measured (symbols) and simulated (lines) fecal coliform contents in soil after surface and subsurface irrigation; solid line - simulations with die-off rates from batch experiments, dashed line - simulations with optimized die-off rates from surface irrigation experiments.

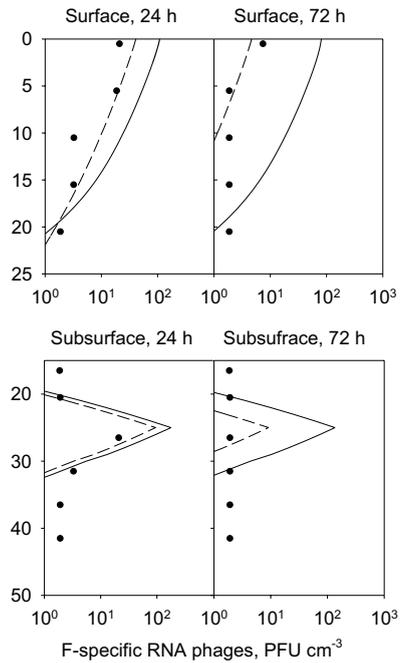


Fig. 4. Measured (symbols) and simulated (lines) contents of F-specific RNA phages in soil after surface and subsurface irrigation; solid line - simulations with die-off rates from batch experiments, dashed line - simulations with optimized die-off rates from surface irrigation experiments.

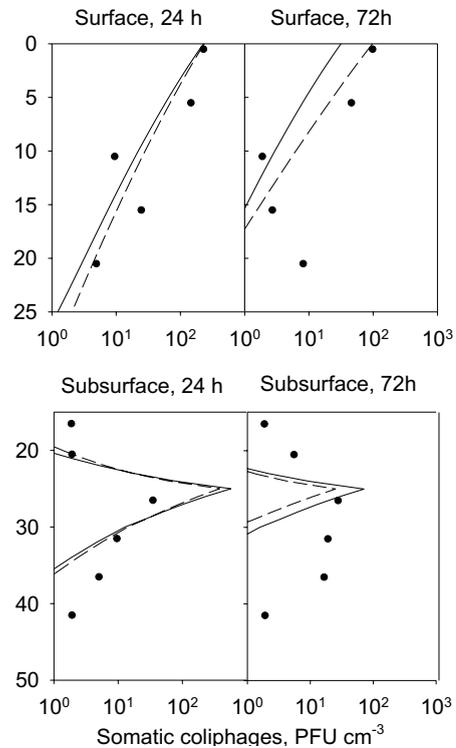


Fig. 5. Measured (symbols) and simulated (lines) somatic coliphage contents in soil after surface and subsurface irrigation; solid line - simulations with die-off rates from batch experiments, dashed line - simulations with optimized die-off rates from surface irrigation experiments.

Table 4. Proportion of microorganisms from irrigation water that remained in soil

Microorganisms	24 h after surface irrigation	72 h after surface irrigation	24 h after subsurface irrigation	72 h after subsurface irrigation
Fecal coliforms	0.236	0.088	0.017	0.004
Somatic coliphages	0.285	0.099	0.054	0.078
F-specific RNA phages	0.078	0.025	0.065	0.0166

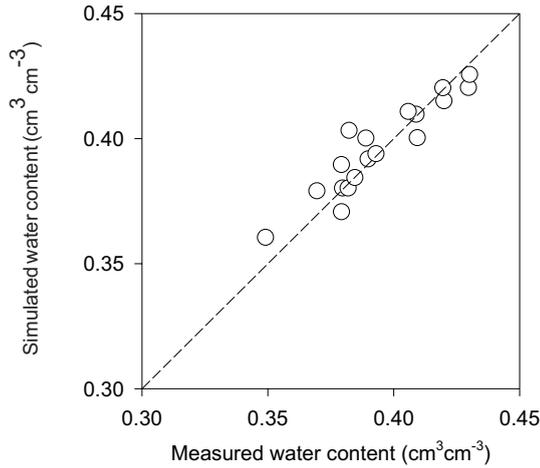


Fig. 6. Comparison of measured and simulated water contents in soil columns; - - - one-to-one line.

soil water retention Eq. (1) were $\alpha = 0.0276 \text{ cm}^{-1}$, $n = 1.19$, $\theta_r = 0.01 \text{ cm}^3 \text{ cm}^{-3}$.

Microbial transport simulations were run first with the parameters of Eq. (11) found from laboratory die-off experiments in Table 3. Values of porosity available for microorganisms transport in soil, θ_a , in Eq. (3) and microbial adsorption rate constant, k_1 in Eq. (4) were optimized to have the smallest value of mean-root-square errors with the surface irrigation data. Figures 3-5 show that the results were mostly unsatisfactory. Varying the available porosity and deposition rate changed the position of simulated microbial distributions in the columns but did not provide right shapes of those distributions. Die-off rates were too low with fecal coliforms, and simulated fecal coliform contents were substantially higher than measured. The same was true for F-specific RNA phages. Simulated

contents of somatic coliphages were too high in 24 h and too low in 72 h after surface irrigation compared with measured values.

The parameters of the die-off rate Eq. (11) were then optimized along with values of θ_a and k_1 with data from columns with surface irrigation. The results are shown in Fig. 3. Optimized parameters provided better fit of data from surface irrigation (Figs 3-5). More important, those parameters provided much better fit in the testing data set from subsurface-irrigated columns (Figs 3-5). This was reflected in the values of all the model performance statistics (Table 6).

DISCUSSION

All three organisms demonstrated substantial adsorption in this work. Values of the bacteriophage soil partition coefficient K_d were significantly lower than values in the range of $10^4 - 10^5$ often found in virus adsorption studies on clay minerals (ie Chattopadhyay and Puls, 1999), but larger than values in the range of 0.1-10 found in experiments with some coarse-textured soils (ie Dowd *et al.*, 1998).

The observed rates of microorganism die-off in soil after irrigation were comparable with data from other studies on irrigation with municipal sewage lagoon effluent. Percentages of surviving fecal coliforms were 28 and 7 after 24 and 72 h periods after irrigation (Table 4). These values corresponded to the values of daily die-off rates $k = 1.15$ and 0.81 day^{-1} , respectively. Bell and Bole (1978) reported 10% fecal coliform survival after 48 h period ($k = 1.15 \text{ day}^{-1}$) in loamy sand soil irrigated with effluent from the municipal lagoon. Dazzo *et al.* (1973) found similar rates of 1.07 day^{-1} in fine sandy soil irrigated with manure slurry. Such values are less than FC die-off rate values observed after the manure application ie 0.29 day^{-1} (Crane *et al.*, 1980), possibly because of the nutrition availability.

Table 5. Parameters of microbial transport model

Microorganisms	Die-off parameters from batch experiments		Die-off parameters optimized from the column experiment data		Available porosity	Adsorption rate
	$b_s (\text{day}^{-1})$	$\theta_* (\text{cm}^3 \text{cm}^{-3})$	$b_s (\text{day}^{-1})$	$\theta_* (\text{cm}^3 \text{cm}^{-3})$	$\theta_a (\text{cm}^3 \text{cm}^{-3})$	$k_1 (\text{day}^{-1})$
Fecal coliforms	0.124	0.285	9.089	0.072	0.182	3.16
Somatic coliphages	0.047	-0.584	2.990	-0.082	0.146	21.06
F-specific RNA phages	0.124	1.555	1.135	1.532	0.168	44.41

Table 6. Model performance statistics in terms of to logarithms of microorganism contents in soil

Micro-organisms	With survival parameters from batch experiments				With survival parameters optimized with surface irrigation data			
	RMSE*		MAD**		RMSE		MAD	
	Surface	Subsurface	Surface	Subsurface	Surface	Subsurface	Surface	Subsurface
Fecal coliforms	1.0254	0.947	1.912	1.635	0.3784	1.011	0.708	1.651
Somatic coliphages	0.478	0.564	0.910	1.216	0.404	0.483	0.910	1.042
F-specific RNA phages	0.779	0.349	1.387	0.667	0.247	0.280	0.498	0.404

*Root-mean-square error, **Maximum absolute difference.

Compared with the surface irrigation, the subsurface irrigation caused much faster die-off of the three organisms studied in this work (Table 4). This was possibly related to higher soil water content in the water application zone. Increasing die-off with the increase of moisture content was observed in batch laboratory experiments (Table 2), and the same presumably happened in the column experiments. Somatic coliphages were the most persistent and mobile organisms. That suggested that somatic coliphages could be used as primary indicators of fecal contamination in the environment of this study.

Parameter estimation from the surface irrigation data provided similar accuracy levels in simulations of surface and subsurface irrigation. The parameter values were sufficiently robust.

Simulations were useful to show that batch survival data were not applicable to transient soil conditions. Much faster die-off occurred in column soil. Bitton *et al.* (1983) found a close agreement between laboratory and field experiments with regard to survival of microorganisms in groundwater. Possible reason for the survival rate differences in this work could be the presence of air-water interfaces in soil columns that have been shown to affect virus survival during transport (Wan *et al.*, 1994). Inactivation by entrainment during transport could be another factor of enhanced die-off. Finally, predation in aerobic columns might be the factor absent in soil pastes.

Fecal coliforms and F-specific phages had the experimental vertical distributions steeper than the simulated ones. One of the reasons for that could be overestimation of dispersivity values. Shelton *et al.* (2003) observed a very low longitudinal dispersion of fecal coliforms in soil monoliths, and hypothesized pore water velocities obviously did not have substantial variability in the pore space available for bacteria transport. Our estimates of the dispersivity were based on data on transport of chloride-ion (Pachepsky *et al.*, 1990). This tracer could experience much larger variation of pore velocities compared with large

bacterial cells. Unfortunately we did not have enough data for the estimation of dispersivity along with other parameters of the microorganism transport models.

The estimated adsorption rates (Table 5) were lower than values reported in studies with coarse soils (Dowd *et al.*, 1998; Powelson and Mills, 1998). The available water content values were in the range from 0.14 to 0.18 cm³cm⁻³. That left about 0.25 cm³cm⁻³ of soil water unavailable for microorganisms. We used equations from Rawls *et al.* (1982) and our data on soil texture to estimate soil matric potential P that corresponds to this water content. The values of P were obtained in the range from 1000 to 10000 cm. The Laplace capillary law equation $P=2\sigma/r$ was then inverted to obtain the pore radius. The values of pore radii were in the range from 0.0003 to 0.003 mm and corresponded to the size of microorganisms in this study.

CONCLUSION

Subsurface irrigation appeared to be efficient in decreasing the number of pathogens in irrigated water and preventing their appearance on soil surface that could lead to produce contamination. This implied that this type of irrigation was safer than surface drip irrigation as previously suggested.

REFERENCES

- Bell R.G., and Bole J.B., 1978.** Elimination of fecal coliform bacteria from soil irrigated with municipal sewage lagoon effluent. *J. Environ. Qual.*, 7, 193-196.
- Bitton G., Farrah S.R., Ruskin R.H., Butner J., and Chou Y.J., 1983.** Survival of pathogenic and indicator organisms in groundwater. *Ground Water*, 213, 405-410.
- Britton L.J., Greeson P.E., (Eds), 1989.** Methods for collection and analysis of aquatic biological and microbiological samples: U.S. Geological Survey Techniques of Water Resources Investigations, 5, A4, 37-40.

- Characklis W.G., 1990.** Biofilm processes. In: Biofilms (Eds W.G. Characklis and K.C. Marshall). Wiley-Interscience, New York, 195-231.
- Chattopadhyay S. and Puls R.W., 1999.** Adsorption of bacteriophages on clay minerals. *Environ. Sci. Technol.*, 33, 3609-3614.
- Corapcioglu M.Y. and Haridas A., 1984.** Transport and fate of microorganisms in porous media: a theoretical investigation. *J. Hydrology*, 72, 149-169.
- Corapcioglu M.Y. and Choi H., 1996.** Modeling colloid transport in unsaturated porous media and validation with laboratory column data. *Water Resour. Res.*, 32, 3437-3449.
- Crane S.R., Westerman P.W., and Overcash M.R., 1980.** Die-off of fecal indicator organisms following land application of poultry manure. *J. Environ. Qual.*, 9, 531-537.
- Crook J., 1998.** Water reclamation and reuse criteria. In: *Wastewater Reclamation and Reuse* (Ed. Asano T.). Technomic Publishing, Lancaster, 627-703.
- Dazzo F., Smith P., and Hubbell D., 1973.** Vertical dispersal of fecal coliforms in Scranton fine sand. *Proc. Soil Crop Sci. Soc., Florida*, 32, 99-102.
- Difco, 1984. *Dehydrated Culture Media and Reagents for Microbiology*. 10th Ed. Difco Laboratories, Detroit.
- Dowd S.E., Pillai S.D., Wang S., and Corapcioglu M.Y., 1998.** Delineating the specific influence of virus isoelectric point and size on virus adsorption and transport through sandy soils. *Appl. Environ. Microbiol.*, 64, 405-410.
- Gantzer C.J., Gillerman L., Kouznetsov M., and Oron G., 2001.** Adsorption and survival of fecal coliforms, somatic coliphages, and F-specific RNA phages in soil irrigated with wastewater. *Water Science and Technology*, 43, 117-124.
- Gerba C.P. and Bitton G., 1984.** Microbial pollutants: Their survival and transport pattern to groundwater. In: *Groundwater Pollution Microbiology* (Eds G. Bitton and C.P. Gerba), J. Wiley and Sons, NY, 65-88.
- Havelaar A.H., van Olphen M., and Drost Y., 1993.** F-specific RNA bacteriophages are adequate model organisms for enteric viruses in fresh water. *Appl. Environ. Microbiol.*, 59, 2956-2962.
- Hornberger G.M., Mills A.L., and Herman J.S., 1992.** Bacterial transport in porous media – evaluation of a model using laboratory observations. *Water Resour. Res.*, 28, 915-923.
- Huysman F. and Verstraete W., 1993.** Water-facilitated transport of bacteria in unsaturated soil columns: Influence of inoculation and irrigation methods. *Soil Biol. Biochem.*, 25, 91-97.
- ISO, 1995. *Water quality - detection and enumeration of bacteriophages. Part 1: Enumeration of F-specific RNA bacteriophages. ISO/CD 10705-1.* Int. Organization for Standardization, Geneva.
- ISO, 1996. *Water quality - detection and enumeration of bacteriophages. Part 2: Enumeration of somatic coliphages. ISO/CD 10705-1.* Int. Organization for Standardization, Geneva.
- Kim S. and Corapcioglu M.Y., 1996.** A kinetic approach to modeling bacteria-facilitated groundwater contaminant transport. *Water Resour. Res.*, 32, 321-331.
- Morinigo M.A., Wheeler D., Berry C., Jones C., Munoz M.A., Cornax R., and Borrego J.J., 1992.** Evaluation of different bacteriophage groups as fecal indicators in contaminated natural waters in Southern England. *Water Res.*, 26, 267-271.
- Mualem Y., 1976.** A catalogue of hydraulic properties of unsaturated soils. Research Report 000002165417, Technion, Israel Institute of Technology, Haifa, 19.
- Oron G., DeMalach Y., Hoffman Z., Keren Y., Hartman H., and Plazner N., 1991.** Wastewater disposal by subsurface trickle irrigation. *Water Sci. Tech.*, 23, 2149-2158.
- Pachepsky Y., 1990.** *Mathematical Models of Physical Chemistry in Soil Science* (in Russian). Nauka, Moscow.
- Peterson T.C. and Ward R.C., 1989.** Development of a bacterial transport model for coarse soils. *Water Resour. Bull.*, 25, 349-357.
- Powelson D.K. and Mills A.L., 1998.** Water saturation and surfactant effects on bacterial transport in sand columns. *Soil Sci.*, 163, 694-704.
- Rawls W.J., Brakensiek D.L., and Saxton K.E., 1982.** Estimation of soil water properties. *Transactions of the ASAE*, 25, 1316-1320.
- Rose J.B. and Gerba C.P., 1991.** Assessing potential health risks from viruses and parasites in reclaimed water in Arizona and Florida, USA. *Water Sci. Tech.*, 23, 2091-2098.
- Schafer A., Ustohal P., Harms H., Stuffer F., Dracos T., and Zehnder A.J.B., 1998.** Transport of bacteria in unsaturated porous media. *J. Contam. Hydrol.*, 33, 149-169.
- Shelton D.D., Pachepsky Y.A., Sadeghi A.M., Stout W.L., Karns J.S., and Gburek W.J., 2003.** Release rates of manure-borne coliform bacteria from data on leaching through stony soil. *Vadose Zone J.*, 2, 34-39.
- Tan Y., Bond W.J., and Griffin D.M., 1992.** Transport of bacteria during unsteady unsaturated soil water flow. *Soil Sci. Soc. Am. J.*, 56, 1331-1340.
- Timlin D.J. and Pachepsky Y.A., 1997.** A modular soil and root process simulator. *Ecological modeling*, 94, 67-80.
- Van Genuchten M.T., 1980.** A closed-form equation for predicting the hydraulic conductivity of unsaturated soils. *Soil Sci. Soc. Am. J.*, 44, 892-898.
- Van Genuchten M.T., 1981.** Non-equilibrium transport parameters from miscible displacement experiments. Research Rep. No. 119. U.S. Salinity Laboratory, USDA-SEA-ARS, Riverside, CA.
- Wan J., Wilson J.L., and Kieft T.L., 1994.** Influence of the gas-water interface on transport of microorganisms through unsaturated porous media. *Applied and Environmental Microbiology*, 60, 509-516.
- Yates M.V. and Ouyang Y., 1992.** VIRTUS, a model of virus transport in unsaturated soils. *Appl. Environ. Microb.*, 58, 1609-1616.