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High Levels of Microbial Contamination of Vegetables Irrigated with Wastewater by the Drip Method

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The public health aspects of the use of wastewater in agriculture and the effects of the drip irrigation method on the contamination of vegetables were studied. The method used was to simulate enteric microorganisms' dissemination by contaminated irrigation water in the field. The vegetables were irrigated with an effluent inoculated with a high titer of traceable microorganisms: poliovirus vaccine and a drug-resistant *Escherichia coli*. The dissemination of the marker organisms in the field was followed, and the effects of certain manipulations of the drip irrigation method on the contamination of the crops by the effluent were examined. It was shown that drip irrigation under plastic sheet cover with the drip lines placed either on the soil surface or buried at a depth of 10 cm significantly reduced crop contamination from inoculated irrigation water even when massive doses of bacteria and viruses were used. The microbial contamination was found to persist in the irrigation pipes and in the soil for at least 8 and 18 days, respectively. The data indicate that the recovery of the marker organisms was affected by soil texture and environmental conditions.

The continuous interest in the problem of wastewater irrigation arises from the limited water resources in many parts of the world. This water constraint restricts agricultural production, which is under pressure to expand in the face of the world's growing population. In many countries, wastewater use is under the regulation of public health authorities (12). However, the formulation of microbiological criteria for the evaluation of the potential use of such water is sometimes difficult due to incomplete data on the dissemination and survival of pathogenic microorganisms from contaminated irrigation water.

The method of irrigation may be of primary importance in the contamination of crops and environment. The effects of spray irrigation have been characterized by several workers (5, 8, 11, 14), but information on other methods of irrigation is scarce. In a recent report by Sadovski et al. (9), it was shown that drip irrigation (3, 4) is an advantageous method for wastewater use. The results of that study indicated that drip irrigation with sewage of effluent under a plastic sheet soil cover, below the soil surface, or during a limited growth period of the crops (up to the flowering stage) significantly reduced crop contamination. Complete assessment of the public health significance of these observations is difficult due to incomplete recovery of pathogenic microorganisms from contaminated vegetables and variable amounts of enteroviruses in sewage effluent.

To overcome these problems, a more challenging approach was developed. The plan was to simulate the conditions which occur upon use of highly contaminated irrigation water. For this purpose, vegetables were grown by wastewater irrigation, the effluent used was inoculated with high titers of traceable microorganisms (drug-resistant *Escherichia coli* and poliovirus vaccine), and the dissemination of the marker organisms in the field was followed.

The results of this study are reported herein.

MATERIALS AND METHODS

Agricultural methods. Field experiments were conducted in two different locations in Israel: at Kibbutz Eilot (a communal agricultural settlement), situated in the Arava Desert near the Red Sea port of Elath (in an area of approximately 500 m^2); and at the Kfar Hayarok (agricultural school), situated in the Mediterranean coastal plain (in an area of approximately 1,400 m^2).

The experimental plots were planted with cucumbers (*Cucumis sativum*) according to farming procedures common to the region. The plots were irrigated by a drip system, using three variations: (i) the drip

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lines were exposed on the soil surface in the conventional manner; (ii) the soil and drip lines were covered with transparent or dark polyethylene sheets 0.03 mm thick and 1.1 m wide; (iii) the drip lines were buried at a depth of 10 cm in the soil, covered with polyethylene sheets. (The latter manipulation was only used in the field experiments at the Kfar Hayarok.) The soil cover was installed before planting, which was subsequently carried out via small openings in the centers of the polyethylene sheets. Strict measures were taken to minimize cross-contamination between the different irrigation treatment plots. They included a proper layout to reduce wind effects as well as fencing and regulation of the agricultural activities in the fields.

Irrigation water. The sewage effluent used for irrigation originated from local (city of Elath or Kfar Hayarok) sewage treatment plants, each of which contains a settling pond and two consecutive oxidation ponds of 3 days' detention time each. The effluent was pumped to the agricultural experimental plots and passed through gravel and a screen filter (3). The effluent volume in each irrigation was 8 to 10 m^3, applied over 2 to 3 h. In some of the irrigations, the effluent was inoculated with marker bacterium and poliovirus via a 50-liter fertilizer tank connected to the irrigation lines. Such containers are designed to allow a continuous and even addition of fertilizers to the water throughout the irrigation period.

Two types of simulation experiments were performed. In one trial, at Kibbutz Eilot, two parallel replicate field studies were conducted successively. In this set of trials, the contamination process was the result of a single irrigation with inoculated effluent. In the second stimulation, at Kfar Hayarok, three successive inoculation irrigations were performed.

Collection and handling of samples. Effluent samples were collected from the drip lines during irrigation. Soil (from surface layers) and vegetables were collected after termination of irrigation on the days of inoculation or before irrigation on the days when inoculation was not performed. Sampling was random; samples were taken at several locations of each treatment area. All samples were transported to the laboratory in ice chests and treated on the same day.

Microbial methods. (i) Bacteria. An *E. coli* (Nal^r) mutant resistant to nalidixic acid was isolated from mixed fecal coliform cultures grown in EC medium (Difco) at 44.5°C and treated with *N*-methyl-*N'*-nitrosoguanidine according to the method of Adelberg et al. (1). The mutants were tested for their stable growth characteristics after multiple transfers on violet red bile agar (VRBA, Difco) containing 200 μg of nalidixic acid (Calbiochem) per ml; for their indole, methyl red, Voges-Proskauer, and citrate reactions; and for their survival rates in both saline and effluent suspensions exposed to direct sunlight. The growth appearance of the selected mutant was stable, and its response was similar to that of *E. coli* isolated from sewage. In addition, resistance transfer to other bacteria in sewage was tested and found to be of low probability.

The mutant was propagated by incubation at 37°C in shake cultures of Trypticase soy broth (TSB, Baltimore Biological Laboratory) containing 200 μg of nalidixic acid per ml. It was concentrated by centrifugation followed by suspension in 2% peptone–water containing 0.5% $CaCO_3$ and refrigerated at 5°C until use.

(ii) Viruses. Poliovirus vaccine types 1, 2, and 3 were propagated in BGM cell cultures (2) and incubated at 33°C for 24 to 48 h. The viruses were harvested and frozen at −80°C until use.

Enumeration of microorganisms. Microbial contamination on vegetable surfaces (in 800- to 1,400-g samples) was eluted by two consecutive elutions, each of 1,000 ml of 0.85% NaCl in 0.02 M phosphate buffer, pH 8.0, according to a previously described procedure (9). Soil samples were suspended in the buffer solution (20 or 50 g in 80 or 450 ml, respectively), and the heavy particles were allowed to settle.

The numbers of resistant mutants, measured as colony-forming units (CFU) per 100 ml of sewage effluent or CFU per gram of soil or vegetables in their respective eluents, were determined on VRBA containing 200 μg of nalidixic acid per ml.

For enumeration of viruses, the vegetable eluents (ca. 2 liters) were treated with antibiotics in the following manner: 1 ml of antibiotic solution containing 200 mg of streptomycin, 2×10^5 U of penicillin, 4 mg of neomycin, and 5 mg of kanamycin was added to each 9 ml of eluent. The treated sample was directly inoculated and assayed for plaque-forming units (PFU). In the single-inoculation experiments, the remaining volume of eluent was concentrated by the polyelectrolyte (PE 60) method (13). In the multiple-inoculation experiments, the remaining eluents were concentrated in two stages: first, the viruses were absorbed onto 0.45-nm Cox filters and eluted by washing the filter three times with 30 ml of 3% beef extract, pH 9.0; then, the three washes were pooled and reconcentrated by the organic flocculation method (7).

The enumeration of viruses in soil from the single-inoculation experiments was performed as follows. A 25-g soil sample was transferred into a centrifuge tube, and 10 ml of 0.02 M glycine buffer, pH 11.5, was added. The tube was shaken for 2 to 3 min and centrifuged at $12,000 \times g$ for 2 min. The pH of the supernatant was adjusted to 7.2. This procedure was repeated twice, and the supernatants were pooled. Antibiotics were added as above, and the sample was assayed for PFU. In the multiple-inoculation experiments, a 25-g soil sample was transferred to a centrifuge tube, and 150 ml of 3% beef extract, pH 9.0, was added. The contents of the tube were mixed with the aid of a magnetic stirrer for 15 min and centrifuged ($8,000 \times g$, 20 min). The slightly turbid supernatant was transferred to a second centrifuge tube and centrifuged ($12,000 \times g$, 10 min) to obtain a clear solution. The supernatant was then concentrated by the organic flocculation method (7).

The enumeration of viruses in the sewage effluent was performed as follows: 1 ml of antibiotic solution was added to each 9-ml sample of sewage effluent as described previously, and the treated sample was directly inoculated and assayed for PFU.

The numbers of viruses present in all samples were determined by seeding on BGM cells and assaying as described by Shuval et al. (10).

Environmental conditions. The soil of the Arava Desert near the Red Sea is an alluvial, desert type;

that of the coastal plain is a brown-red, sandy type. Water infiltration in these two soils is different, and it is rather restricted in the former.

In accordance with the harsh climatic conditions in the Arava Desert, the main agricultural seasons in that area are spring and fall. Our experiments in that region were performed in November (fall); those in the coastal plain were performed in July (summer).

Some data on the climatic conditions in these areas during the experimental periods are shown in Table 1. In general, the conditions prevailing during the coastal plain experiments were less favorable to enteric microorganism survival. The duration of sunlight and the global radiation in that area were higher than those in the Arava Desert and reached 124 and 210% of the latter values, respectively.

RESULTS

Figures 1 through 4 illustrate the course of microbial dissemination in the field (at the Arava Desert experimental site) as a result of a single irrigation with inoculated effluent. Two variations of drip irrigation were used: irrigation on the exposed soil surface and irrigation below transparent polyethylene sheets which covered the soil and the irrigation system.

The marker bacteria (Nal^r) could not be detected in uninoculated effluent, and the mean enteroviral count in this effluent was 800 PFU/100 ml. The microbial counts in the effluent emerging from the systems of the different irrigation treatments (Fig. 1) and in covered and uncovered soil (Fig. 2) were similar. Therefore, the data from the different plots were averaged and presented as mean values. The densities of microorganisms in the inoculated effluent entering the irrigation system were 1.0×10^7 CFU of marker bacteria and 9.0×10^6 PFU of enteroviruses per 100 ml (Fig. 1).

The maximal values of microbial density in effluent emerging from the irrigation system (9.1×10^6 CFU and 6.4×10^6 PFU/100 ml) were reached after 100 min of irrigation. Sixteen hours later, during a follow-up irrigation with uninoculated effluent, the microbial density in the water which emerged from the irrigation system was 1000-fold lower than the initial level. During the observation period of 8 days, we were able to detect low levels of marker organisms in the effluent which emerged from the irrigation lines. The small peaks in bacterial colony counts, on days 2 and 8, or in viral counts, on day 8, indicated that the elution of microorganisms from the irrigation system was affected by two possible factors: first, propagation of bacteria; second, adsorption of viruses and bacteria to the organic matter which lined the inside walls of the irrigation system. The repeated irrigations

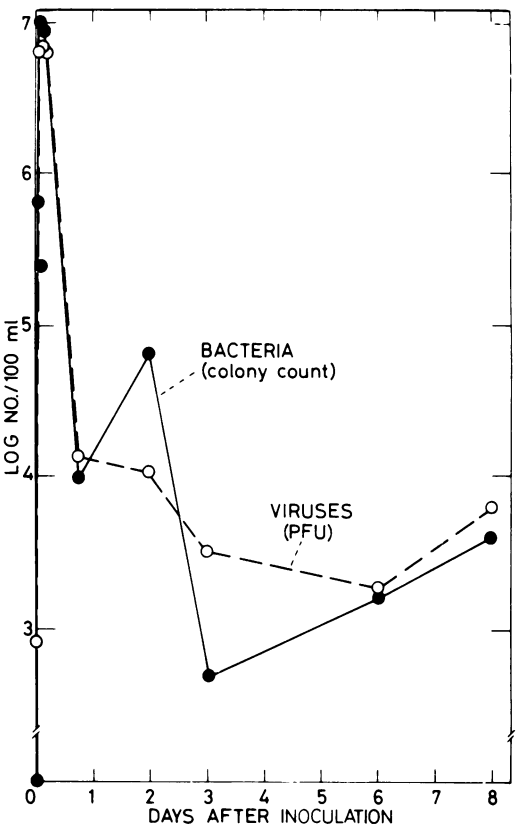

FIG. 1. *Marker bacterium and virus contamination in effluent from drip irrigation lines. Inoculation of the effluent was performed in a single irrigation. The data shown are log mean values of two experiments.*

TABLE 1. *Climatic conditions during the experimental periods*[a]

Location	Period	Temp (°C)				Sunlight duration (mean h/day)	Global radiation (mean daily kJ/cm² per h)	Relative humidity (%) at:		
		Soil (noon)		Air				8:00 a.m.	2:00 p.m.	8:00 a.m.
		Mean	Range	Maximal	Minimal					
Kibbutz Eilot	Nov. 1974	26	22–30	30	13	9.5	1.31	55	27	41
Kfar Hayarok	July 1976	42	40–43	28	23	11.8	2.76	68	62	70

[a] Data were provided by the Israel Meteorological Service, Bet Dagan.

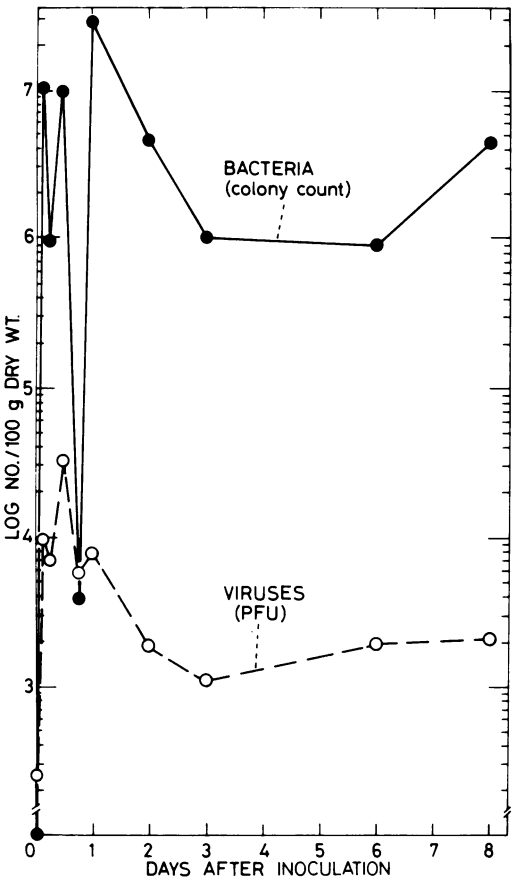

FIG. 2. *Marker bacterium and virus contamination in effluent-irrigated soil. Experimental details are given in the legend to Fig. 1.*

may have resulted in the release of the organisms as shown.

The soil contamination (Fig. 2) immediately after irrigation with inoculated effluent was 1.0×10^7 CFU of marker bacteria and 1.0×10^4 PFU of enteroviruses per 100 g of soil (dry weight). The contamination levels on the following days were only slightly lower and persisted for the entire observation period. The variations in microbial counts in the soil samples (such as the drop in bacterial count in samples collected 16 h after inoculation) may be attributed to the lack in homogeneity of microbial dispersion and elution from the soil.

Figures 3 and 4 illustrate the course of vegetable contamination from the inoculated irrigation water. The data indicate that the plastic sheet cover over the soil and drip lines was an effective barrier against contamination of the vegetables. Cucumbers grown in soil which was not covered were positive for bacterial and viral contamination throughout the observation period. The maximal contamination densities were recorded on the day the inoculated irrigation was carried out: 1.0×10^4 CFU of marker bacteria and 2.2×10^3 PFU of enterovirus per 100 g of vegetables. This was followed by a gradual decrease to 65 CFU and 30 PFU per 100 g of vegetables on day 8.

The cucumbers from soil which was covered

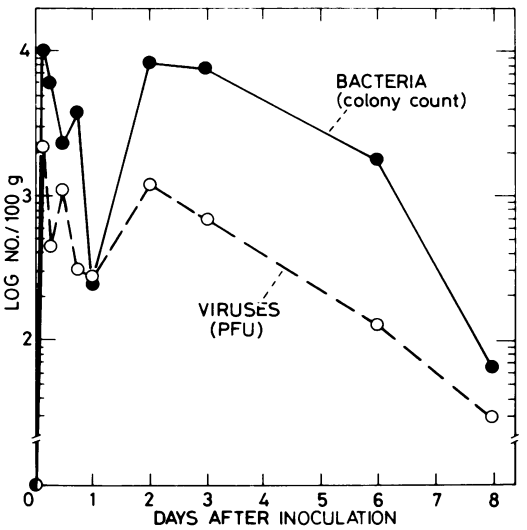

FIG. 3. *Marker bacterium and virus contamination in effluent-irrigated cucumbers grown in exposed soil. Experimental details are given in the legend to Fig. 1.*

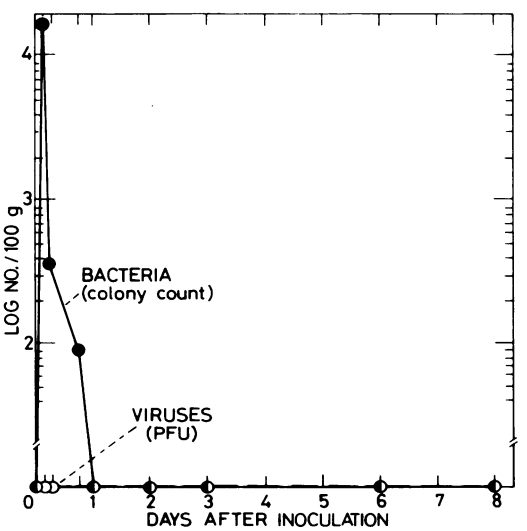

FIG. 4. *Marker bacterium and virus contamination in effluent-irrigated cucumbers grown in soil covered with polyethylene sheets. Experimental details are given in legend to Fig. 1.*

with plastic sheets were positive for bacterial contamination immediately after irrigation with inoculated effluent in only one of the two replicated experiments, but all samples on the following days or throughout the other experiment were negative.

Viral contamination of vegetables from the covered plot was generally undetectable, and the few positive samples found were collected shortly after the irrigation with inoculated effluent (with only a few PFU per 100 g of vegetables).

In an epidemic situation, the effluent which might be used for irrigation would be contaminated with excessive titers of enteric microorganisms for several days. In such an instance, the dissemination of these organisms in the field would be continuous, and contamination of the vegetables would result from much more massive doses.

This situation was simulated (at the coastal plain experimental site) by three irrigations with inoculated effluent on 3 successive days with 1-day breaks among them. Variations in drip irrigation included surface irrigation and irrigation below dark polyethylene covers, the drip lines either placed on the soil surface or buried at a depth of 10 cm.

Compared with the previous experiments, the inoculation levels of the effluent entering the irrigiation system were higher. The average counts per 100 ml were 4.4×10^{11} CFU of marker bacteria and 2.2×10^{11} PFU of enteroviruses. The effluent emerging from the different irrigation treatment systems was similar. In the first, second, and third inoculations, respectively, the systems contained 6.6×10^7, 2.5×10^8, and 2.6×10^8 CFU of marker bacteria and 2.7×10^6, 7.2×10^6, and 3.0×10^6 PFU of enteroviruses per 100 ml (Fig. 5 and 6).

Recoveries of the inoculated microorganisms from the soil or the vegetables were generally small. After the first irrigation with inoculated effluent, the concentration of marker bacteria in the soil was 5.7×10^4 CFU/100 g (dry weight). It increased to 2.1×10^6 and 1.3×10^6 CFU/100 g after the second and third irrigations, respectively. After the last irrigation with inoculated effluent, a continuous decrease in counts was observed, reaching 1.3×10^2 CFU/100 g of soil after 14 days. This level of contamination was maintained for at least 11 additional days. The contamination of enterovirus in soil after the first irrigation was 1.2×10^3 PFU per 100 g of soil (dry/weight), increasing to 1.2×10^4 and 9.1×10^3 PFU/100 g of soil after the second and third inoculations, respectively. The enterovirus concentration decreased to 47 PFU/100 g of soil 10 days after the last inoculation; no virus could be detected after 5 additional days.

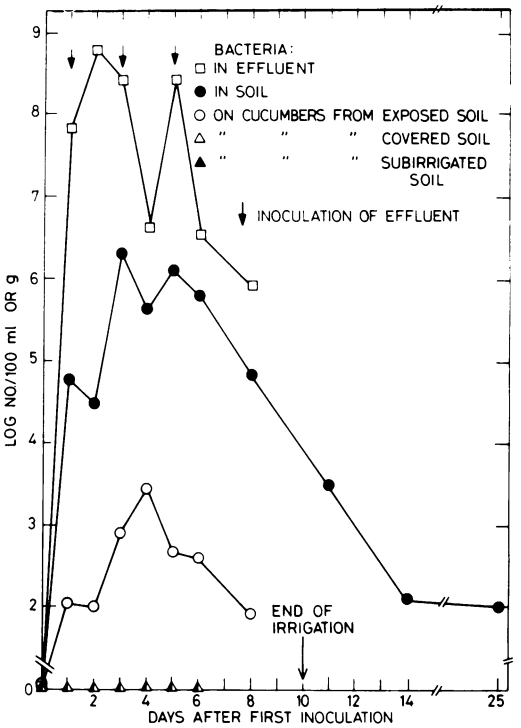

FIG. 5. *Marker bacterium contamination in effluent and soil and cucumbers irrigated with effluent by the drip method. Contamination occurred from three irrigations with inoculated effluent. The results are mean values of samples from three experimental plots.*

It is noteworthy that irrigation was ceased 5 days after the last inoculation. This resulted in a drop in soil moisture content from an average of 15.0% during the irrigation period to an average of 3% in samples collected after 16 days.

The contamination densities on cucumbers grown in exposed soil reached maximal values (1.7×10^3 CFU of marker bacteria and 0.13 PFU of enteroviruses per 100 g of vegetables) after the second inoculation. The contamination decreased immediately after the last inoculation.

The microbial contamination on cucumbers grown in covered soil was undetectable. This was in accord with the observations from previous experiments. The soil cover alone was sufficient to prevent the contamination of the vegetables. Burying the drip lines in the soil beneath the polyethylene cover had no noticeable effect on contamination.

DISCUSSION

The results of this investigation support the previous observation (9) that certain manipulations of drip irrigation, such as irrigation under

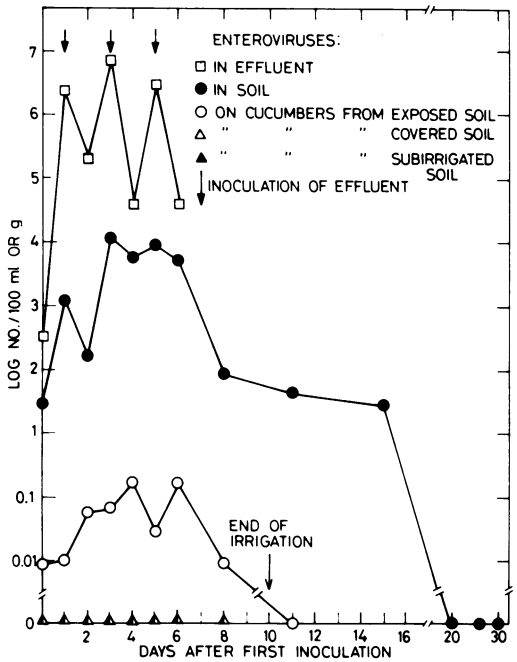

FIG. 6. *Virus contamination in effluent and soil and cucumbers irrigated with effluent by the drip method. Experimental details are given in legend to Fig. 5.*

plastic sheet cover, significantly reduce crop contamination from contaminated irrigation water even under conditions of massive doses of bacteria and viruses which may exist in an epidemic situation. The control of contamination is attributed to the special characteristics of the drip irrigation method (4, 9) which minimize the spread of pathogens through surface flow and aerosolization (14). The physical presence of the plastic sheet soil cover also controls the contamination. All agrotechnical manipulations which have been tried, especially covering the soil with plastic sheets, are quite common and acceptable farming procedures. Thus, the practical advantages of this method for wastewater irrigations are self-evident.

The results of our studies on the simulation of microbial contamination of vegetables through irrigation water draw attention to several problems. The first is the persistence of the contamination in the irrigation pipes and in the soil. As shown in Fig. 1, the effluent emerging from the irrigation system during irrigation for at least 8 days after contaminated wastewater had been used contained a high titer of both bacteria and enteroviruses. This sustained the infection in the field. The persistence of pathogenic bacteria and viruses in the soil is an important cause for concern, since contaminated soil may serve as a reservoir for numerous cross-contaminations of crops and agricultural machinery. The second problem is the effect of environmental conditions and soil composition on the recovery of pathogenic microorganisms from soil surface layers. The Arava Desert experiments were conducted under moderate climatic conditions in alluvial-type soil, which restricts water infiltration (Table 1). Both factors encouraged the viability of pathogenic microorganisms in the upper soil layers. Their recovery for at least 8 days after contaminated wastewater had been used for irrigation was only slightly lower than that obtained immediately after irrigation was performed (i.e., 10^7 CFU of marker bacteria and 10^4 PFU of enteroviruses per 100 g [Fig. 2]). In the coastal plain experiments, a far more massive inoculation was used: 4.4×10^{11} CFU of marker bacteria and 2.2×10^{11} PFU of enteroviruses per 100 ml in three successive irrigations. However, microbial recoveries from the soil and vegetables were low and declined rapidly after the inoculation (Fig. 5 and 6). These observations may be related to the ease of water infiltration in the light-textured soil of the coastal plain, to the restricting climatic conditions, and especially to the higher global radiation values which prevailed during the experimental period. Nevertheless, the *E. coli* mutant could be recovered from the soil 20 days after the last inoculation and 15 days after irrigation was completely terminated and the soil moisture had declined to 3%.

Thus, climatic conditions and soil type should be considered as important factors affecting the survival of microbial contaminations in the field. But even under extreme conditions, pathogenic microorganisms may persist. Although infiltration of water into the soil may help reduce vegetable contamination, the increased danger of contamination of the underground water should not be overlooked.

Our results indicate that mulching of the soil with plastic sheets did not affect the survival of contaminating enteric microorganisms. There were similar concentrations of these organisms in the soil covered with plastic sheets (both dark and transparent) and in uncovered soil.

The differences in the contaminations of the vegetables from these two soil treatments should be attributed to the mechanical barrier effect of the plastic sheet soil cover. In a recent report by Katan et al. (6), it was shown that mulching of the soil with transparent polyethylene before planting resulted in the control of dissemination of soil-borne plant pathogens via biological and thermal processes. In our experiments, the soil surface (both covered and uncovered) was protected from direct sunlight by the foliage and

differences in the soil temperatures were insignificant. However, the disinfection effect of the soil cover recorded by Katan et al. (6) should be considered for controlling enteric contaminants in soil.

The reduction of crop contamination by burying the drip lines in the soil without mulching was demonstrated previously (9). In this work, burying of the drip lines was combined with plastic sheet mulching. Since mulching by itself reduced crop contamination almost entirely, the effect of the subsurface irrigation could not be evaluated.

In conclusion, we would like to point out the advantage of simulation experiments for studying public health aspects of the use of contaminated wastewater in agriculture. This approach enables the most sensitive testing of the effects of different irrigation methods under extreme and highly challenging conditions.

The safe reuse of wastewater for agriculture is a desired goal in many arid-zone countries. We have shown that the drip irrigation method, particularly with plastic soil covering, may promote this cause.

We make no recommendation for using highly contaminated water for practical agricultural production, even with the irrigation methods used in this work. It must be stressed that agricultural manipulation of the above-described irrigation method can be used as an auxiliary means of public health protection. This method is not meant to replace proper microbiological standards.

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LITERATURE CITED

1. **Adelberg, E. A., M. Mandel, and G. Chein-Ching-Chen.** 1965. Optimal conditions for mutagenesis by N-methyl-N'-nitro-N-nitroso-guanidine in *Escherichia coli* K^{12}. Biochem. Biophys. Res. Commun. **18**:788–795.
2. **Barron, A. L., C. Olshevsky, and M. M. Cohen.** 1970. Characteristics of the BGM line of cells from African green monkey kidney. Arch. Gesamte Virusforsch. **32**: 389–392.
3. **Goldberg, D., B. Gornat, and D. Rimon.** 1976. Drip irrigation. Scientific Publications, Kfar Shmaryhahu, Israel.
4. **Goldberg, D., and M. Shmueli.** 1970. Drip irrigation—a method used under arid and desert conditions of high water and soil salinity. Trans. ASAE **13**:38–41.
5. **Hickey, J. L. S., and P. C. Reist.** 1975. Health significance of airborne microorganisms from wastewater treatment process. Part II. Health significance and alternative for action. J. Water Pollut. Control Fed. **47**: 2758–2773.
6. **Katan, J., A. Greenberger, H. Alon, and A. Grinstein.** 1976. Solar heating by polyethylene mulching for the control of diseases caused by soil-borne pathogens. Phytopathology **66**:638–688.
7. **Katzenelson, E., B. Fattal, and T. Hostovesky.** 1976. Organic flocculation: an efficient second-step concentration method for the detection of viruses in tap water. Appl. Environ. Microbiol. **32**:638–639.
8. **Katzenelson, E., and B. Teltch.** 1976. Dispersion of enteric bacteria by spray irrigation. J. Water Pollut. Control Fed. **48**:710–716.
9. **Sadovski, A. Y., B. Fattal, and D. Goldberg.** 1978. Microbial contamination of vegetables irrigated with sewage effluent by the drip method. J. Food Protect. **41**:336–340.
10. **Shuval, H. I., A. Thompson, B. Fattal, S. Cymbalista, and Y. Wiener.** 1971. Natural virus inactivation processes in seawater. J. Sanit. Eng. Div. Am. Soc. Civ. Eng. **97**:587–600.
11. **Sorber, C. A., S. A. Schaub, and H. T. Bausum.** 1974. An assessment of a potential virus hazard associated with spray irrigation of domestic wastewaters, p. 241–252. *In* J. S. Malina, Jr., and B. P. Sagik (ed.), Virus survival in water and wastewater systems. University of Texas Press, Austin.
12. **Sullivan, R. H., H. M. Cohn, and S. S. Baxter.** 1973. Survey of facilities using land application of wastewater. American Public Works Association report to the Environmental Protection Agency. EPA 430/9-73-006, Washington, D.C.
13. **Wallis, G., and J. L. Melnick.** 1970. Detection of viruses in large volumes of natural waters by concentration on insoluble polyelectrolytes. Water Res. **4**:787–796.
14. **Teltsch, B., and E. Katzenelson.** 1978. Airborne enteric bacteria and viruses from spray irrigation with wastewater. Appl. Environ. Microbiol. **35**:290–296.