The Survival of Coliforms, *Streptococcus faecalis* and *Salmonella tennessee* in the Soil and Climate of Israel

S. Bergner-Rabinowitz

Sewage Research Laboratory, Central Laboratories, Ministry of Health, Jerusalem, Israel

Received for publication November 17, 1955

The utilization of sewage in agriculture has lately been receiving increasing attention in many countries. The question is especially important in a country with limited water resources like Israel where insufficient rainfall must be supplemented by irrigation. In order to evaluate the public health hazard involved, it is necessary, among other things, to obtain information on the survival of enteric organisms in the soil. This subject has been reviewed extensively and summarized by Rudolfs *et al.* (1950).

The purpose of the present investigation, which was started early in 1951, is to study the survival of enteric bacteria in the soil under the field conditions obtained in Israel. The climate is characterized by long, hot, and dry summers and resembles that of the San Joachim Valley in California.

Materials and Methods

Coliforms, *Streptococcus faecalis* and *Salmonella tennessee* were chosen for study. The streptococcus was of particular interest in the light of Mallmann and Litsky's (1951) suggestion that enterococci might be good indicators of fecal pollution. The survival of these species was studied at the soil surface and at a depth, in summer and winter, in cultivated and uncultivated plots. The survival of *S. tennessee* was also studied in open storage tanks of the type commonly used in irrigation practice in Israel.

The types of sewage used were either raw domestic sewage, trickling filter effluent (Rigbi *et al. In Press*) or raw domestic sewage mixed with a suspension of *S. tennessee*.

The experiments were carried out in plots situated in a valley near Jerusalem, the soil consisting of *terra-rossa* (Mediterranean red limestone soils poor in organic matter). The plots were prepared for irrigation experiments under the supervision of the Water Department of the Ministry of Agriculture in 1951. The agricultural part of the work was carried out by the Soils Department of the Agricultural Research Station of Rehovot. The experiments involving the survival of coliforms and *S. faecalis* in soil irrigated with raw sewage and trickling filter effluent were carried out in the summer of 1951 and repeated in 1953. All of the other experiments were carried out in 1952.

Jerusalem sewage is strong. The B.O.D. usually ranges between 800 to 1200 ppm; and the most probable number (MPN) of coliforms is generally of the order of 10^6 to 10^7 per ml. Pathogenic bacteria can occasionally be isolated. Thus, out of 18 samples of sewage examined, *Salmonella* sp. (Type Meleagris) according to Bergey's Manual was found in two samples, *Salmonella typhimurium* in one, and *Shigella dysenteriae* in another. Intestinal parasites are frequent, and include eggs of *Ascaris, Trichuris, Trichostrongylus, Taenia, Hymenolepis*, rhabditoidic larvae and cysts of *Giardia lamblia* and *Endamoeba*. The trickling filter effluent has a B.O.D. lying generally between 150 and 250 ppm. The MPN of coliforms is usually of the order of 10^5 or 10^6. Parasites are found less frequently and in smaller numbers in the trickling filter effluent than in raw sewage.

The procedure generally adopted was to run the sewage in the furrows of the experimental plots. Samples were subsequently taken from various depths of soil over a given period, and the bacterial numbers determined. When *S. tennessee* was studied, cultures of this organism were added to sewage to give a count roughly equal to the coliform MPN.

Soil samples were taken from the surface of the soil and at depths down to 8 in. In the latter case the overlying and surrounding soil was first carefully removed with a scoop to prevent particles from falling in.

Each sample examined was composited from three samples taken at random from the plot studied. Twenty grams of each composite sample were placed in sterile Erlenmeyer flasks, 180 ml of sterile saline were added, and the resulting mixture was then shaken for two minutes. After settling for 30 seconds, a series of dilutions was prepared from the suspension. Three such composite samples were taken for each plot and the results averaged.

The MPN of coliforms was determined by inoculating the dilution into lactose fermentation tubes. *Salmonella* was enumerated by the method of Heukelekian and Shulhoff (1935): One ml of dilution was poured and spread on *Salmonella-Shigella* plates (Difco SS). The plates were dried in an incubator, then covered and placed upside down. After 24 hours the colonies could be counted with ease. Counting was still easier after 48

TABLE 1. *MPN of coliform and Streptococcus faecalis per gram of soil irrigated with raw sewage and trickling-filter effluent*

Days after Irrigation	Plot Irrigated with			
	Raw sewage		Trickling-filter effluent	
	Coliforms	*Streptococcus faecalis*	Coliforms	*Streptococcus faecalis*
1	6,200,000	360,000	1,100,000	70,000
4	700,000	24,000	240,000	7,000
6	240,000	24,000	70,000	2,900
8	24,000	7,000	95,000	3,300
10	50,000	2,400	29,000	2,400

hours, by which time the center of the colonies had usually darkened somewhat, owing to the formation of sulfides. Individual colonies were identified by agglutination tests with Kauffmann sera (1950). The MPN of *S. faecalis* was determined by using Rothe's azide dextrose broth (Rothe, 1931) as recommended by Mallmann and Seligman (1950). Positive findings were verified microscopically, and specific tests were occasionally carried out to confirm that the organism was indeed *S. faecalis*. These tests comprised the ability (1) to grow in tryptose broth containing 6.5 per cent sodium chloride; (2) to survive at a temperature of 60 C for 30 minutes; and (3) to decolorize and clot litmus milk.

Preliminary experiments were made in order to determine whether the method employed gave low counts because of the adsorption of bacteria by the soil. It was shown that on shaking *Salmonella* suspensions with soil and allowing to settle, the count of the suspension remained essentially unchanged.

RESULTS

A comparison was made of the survival in summer of coliforms and *S. faecalis* in soil irrigated with raw sewage and trickling-filter effluent, respectively. Raw sewage and trickling-filter effluent were run in the furrows of two uncultivated plots. The numbers of coliforms and streptococci found in the soil at a depth down to 4 in on successive days are given in table 1.

It may be seen that the number of coliforms and streptococci dropped rapidly for both plots. After a few days, differences between the two plots ceased to be very marked. The rate of disappearance is roughly similar for raw sewage and trickling-filter effluent. This suggests that from the point of view of bacterial survival no advantage is gained by irrigation with trickling-filter effluent.

A study was made of the survival of *S. tennessee* and coliforms in uncultivated soil in winter. A calculated amount of *S. tennessee* culture was added to raw sewage. The mixture so obtained was found to contain 2×10^6 *Salmonella* organisms and 2.4×10^6 coliforms per ml. The mixture was applied to a plot 160 sq ft in area which had been irrigated with raw sewage during the previous summer. The coliform and *Salmonella* populations in subsequent days are represented graphically in

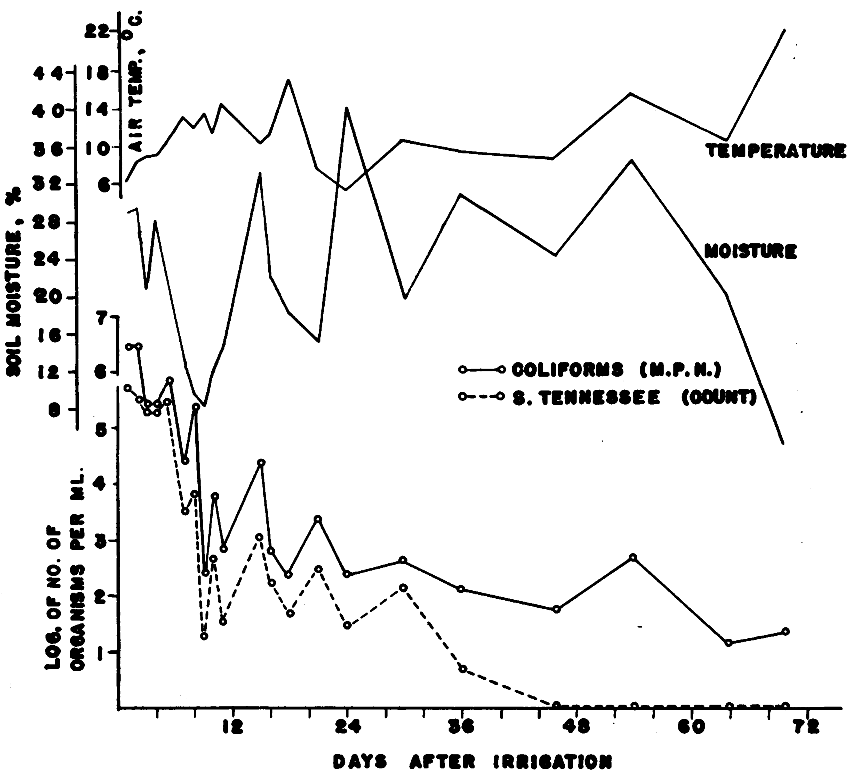

FIG. 1. Numbers of *Salmonella* and coliforms at surface of plot irrigated in winter with raw sewage containing *Salmonella tennessee* suspension.

figures 1 and 2. The figures also contain graphs of weather readings and soil moisture, taken concurrently with the determination of bacterial numbers.

Rain fell frequently during the experiment. Air temperatures at that period ranged from 2 to 21 C, the daily mean temperature varying from 3.5 to 17 C, with a median value of 10.5 C. Soil moisture at the surface ranged between 8 to 39 per cent, with a median value

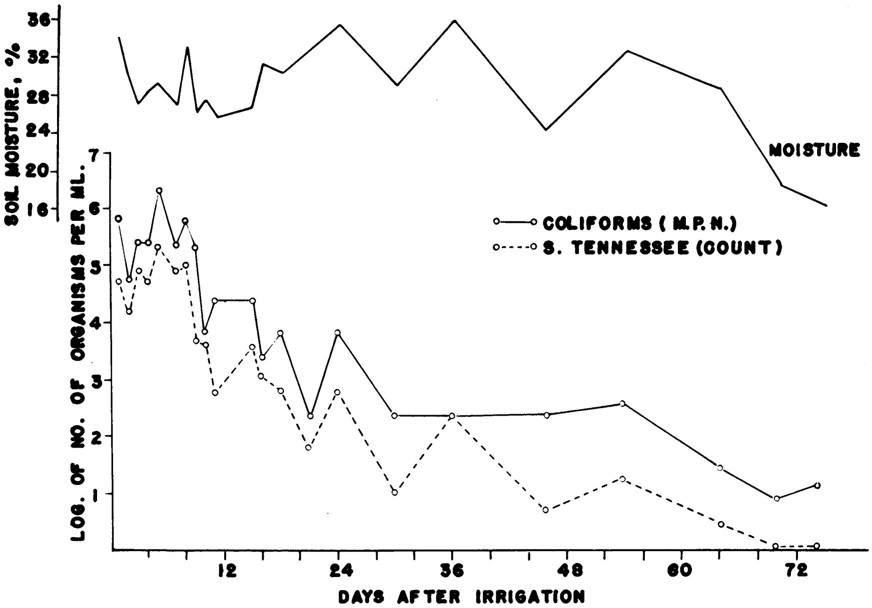

FIG. 2. Numbers of *Salmonella* and the coliforms at depth of 4 inches in plot irrigated in winter with raw sewage mixed with *Salmonella tennessee* suspension.

TABLE 2. *Salmonella tennessee, coliforms, and Streptococcus faecalis per gram of soil. Plot irrigated in summer with raw sewage mixed with Salmonella tennessee suspension*

Days after Irrigation	Soil Moisture Per cent (Dry Basis)		*Salmonella tennessee* (Count)		Coliforms (MPN)		*Streptococcus faecalis* (MPN)	
	Surface	4 to 8 in depth	Surface	4 to 8 in depth	Surface	4 to 8 in depth	Surface	4 to 8 in depth
1	23	29	725,000	30,000	700,000	70,000	10,000	4,000
3	14	27	70,000	1,300	70,000	24,000	11,000	1,200
5	9	25	550	500	12,000	10,000	1,300	425
8	7	21	45	3	1,300	4,500	240	450
11	6	17	2	0	600	3,700	400	450
15	4	15	0	0	240	4,700	450	110
17	3.8	15.5	0	0	500	3,600	500	230
19	3.2	15.1	0	0	700	1,200	500	220
22	3.3	14.3	0	0	130	370	240	240
38	3.1	12.5	0	0	80	425	13	110

of 22.4 per cent. At the two depths, soil moisture never fell below 19 per cent, nor did it rise above 30 per cent.

As may be seen from figures 1 and 2, there was a big initial reduction in numbers of both *S. tennessee* and coliforms. Thus, at the surface, the numbers of both genera dropped from an initial number of millions and several hundred thousand organisms per gram of soil, to about 100, in 9 days. At depths of 4 in and 8 in this drop was somewhat smaller: The *Salmonella* population dropped from a few hundreds of thousands to several hundreds, in 16 to 18 days, while for coliforms, a similar drop occurred in 16 to 21 days. The number of *Salmonella* organisms continued to decrease as long as the experiment lasted; for coliforms, the graph flattened out at a figure of thousands or hundreds at the surface (after 9 days), and ten thousands or thousands at a

TABLE 3. *Numbers of Salmonella tennessee in open storage tank and in the soil*

	Days after Irrigation									
	0	1	3	5	8	11	15	17	19	22
Storage tanks: count per ml of sewage	10,000,000		100,000	1,300	450	300	100	30	1	0
Soil: count per gram										
Surface	—	725,000	70,000	550	15	2	0	0	0	0
4 to 8 in depth	—	30,000	1,300	500	3	0	0	0	0	0

depth (after 16 days). Once these figures were reached, the population appeared to become relatively stable, decreasing only slowly with time. The experiment lasted for 74 days.

Salmonella disappeared by the 46th day at the surface, and the 70th day at a depth. Coliforms persisted throughout the experiment, though by the 64th day they numbered no more than a few tens per gram of soil, out of an original number of millions or hundreds of thousands. For both genera the numbers decreased more rapidly at the surface, and differences between the two depths do not appear to be significant.

A study of the survival of *S. tennessee*, coliforms and *S. faecalis* was carried out in uncultivated soil in summer. Sewage was allowed to stand for a few days in 500-gallon storage tanks made of galvanized iron sheet. On the day of the experiment, a suspension of *S. tennessee* was added. After mixing, the numbers were as follows: *Salmonella*, 10^7; coliforms, 1.76×10^5; streptococci, 1.6×10^4. The uncultivated plots were then irrigated with the sewage mixture, samples being taken at various intervals from the surface and from a depth of 4 to 8 in. The bacterial numbers obtained are given in table 2.

TABLE 4. *The survival of Salmonella tennessee in cultivated plot (sunflowers) irrigated in summer once with sewage mixed with Salmonella suspension and subsequently with raw (uninoculated) sewage*

Sewage Used	Day of Irrigation (Number of Days Since the Begining of the Experiment)	Day of Soil Sampling (Number of Days Since the Beginning of the Experiment)	Soil Moisture Per Cent (Dry Basis)		*Salmonella tennessee*, Count per Gram of Soil	
			Surface	4 to 8 in depth	Surface	4 to 8 in depth
Sewage mixed with Salmonella suspension	0	0	29	26.5	1,500,000	40,000
		1	24	25	1,900,000	4,500
		3	14	24	10,000	750
		5	9	22	80	14
		7	7	20	5	5
Raw uninoculated sewage	7	7	31	28	3	6
		9	16	29	120	80
		12	9.5	23.5	35	350
		14	8	20.5	5	105
	14	15	23	25	0	13
		21	6.5	19	2	31
		23	4.0	17.5	0	20
	24	24	32	28	0	10
		26	14	25	0	15
		28	10.5	19	0	5
		30	8.5	19	0	5
	31	31	33	30	0	3
		37	12	21	0	0
	38	38	32	30	0	0
		44	13.5	22	0	0

Table 2 shows that *Salmonella* organisms died rapidly. In spite of the huge initial count at the surface, no organisms were found there after 15 days. At a depth of 4 to 8 in, and out of an initial 30,000 organisms per gram of soil, none remained after 11 days. Coliforms and streptococci persisted throughout the experiment, which lasted 38 days.

In summer as in winter, after the number of coliforms had fallen to a figure of hundreds at the surface and thousands at a depth, the population of this organism became relatively stable, decreasing but little with time. The decrease was similar for streptococci, the corresponding figure for a stable population being hundreds, both for the surface and for a depth.

Judging by the rate of disappearance of both genera from the soil, streptococci do not appear to be preferable to coliforms as indicators of fecal pollution.

In the course of the above experiment, the storage tanks containing the sewage mixed with the *Salmonella* suspension remained exposed in the field. In order to compare the survival of *Salmonella* in the open storage-tanks and in the soil, samples of sewage were taken from the tanks at various intervals, and the numbers of *Salmonella* determined. The results are given in table 3, together with the concurrent figures for *Salmonella* in the soil, reproduced from table 2.

It may be seen that *S. tennessee* survived in the storage tanks longer than in the soil, disappearing by the 22nd day in the former and the 11th to the 15th day in the latter.

Figures 1 and 2 and tables 2 and 3 relate to the survival of *Salmonella* in the soil after a single application of sewage mixed with *Salmonella* suspension. In actual practice, however, it is common to irrigate every 6 to 8 days. It was therefore decided to determine the survival time of *Salmonella* in the soil on repeated irrigation as follows:

Sewage mixed with *Salmonella* suspension was applied in furrows to a sunflower plot. Following this, the soil was irrigated every 6 to 8 days with raw sewage to which *Salmonella* had not been added. The *Salmonella* count of the soil was determined at regular intervals. The results of this experiment, carried out between June and August 1952, are given in table 4. As may be noted from table 4, *Salmonella* disappeared by the 23rd day at the surface and the 37th day at a depth of 4 to 8 in. The figures during the first half of the experiment were obtained when the crop was still low and the soil well exposed to the sun. It appears that the survival time of *Salmonella* in a cultivated plot in the summer, lies between the survival times for summer and winter in uncultivated plots.

DISCUSSION

The results reported above are in general agreement with the viability of *Salmonella typhosa* as observed by other authors. In general *S. typhosa* has been found to persist in soil less than 100 days. Thus Melick (1917) showed that *S. typhosa* survived from 29 to 58 days, depending on the soil and strain. Sedgwick and Winslow (1902) found that survival was longer in moist soil than in dry soil. Kligler (1921) found that in moist soil *Salmonella* survived up to 80 days, but that in dry soil it survived for only 20 days.

The prolonged survival of the *Coli-Aerogenes* group in soils is well established (Young and Greenfield, 1923; Skinner and Murray, 1926; Mallmann and Litsky, 1951) and is also brought out in the results presented here.

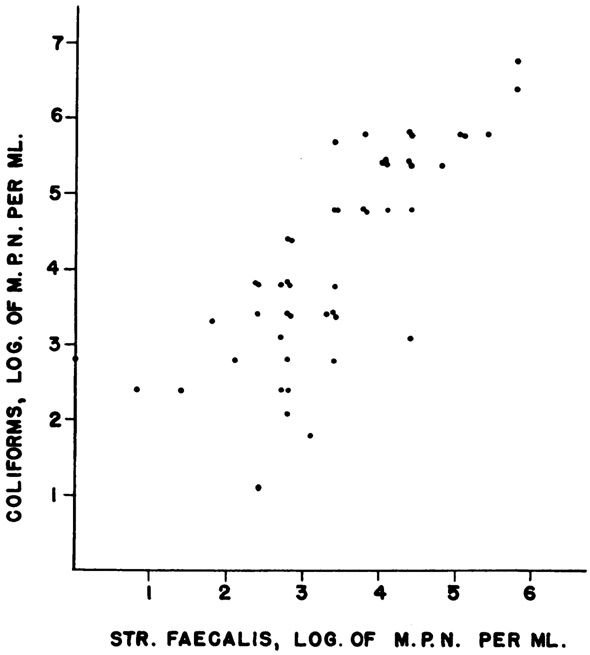

FIG. 3. Relationship between *Streptococcus faecalis* and coliforms surviving in the soil following sewage irrigation.

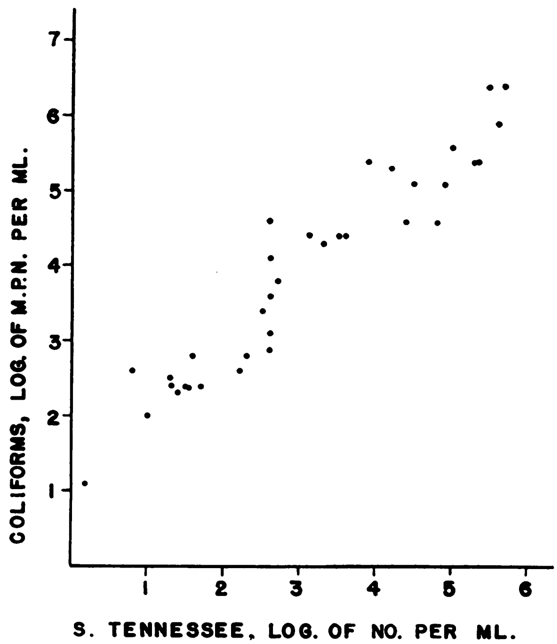

FIG. 4. Relationship between *Salmonella tennessee* and coliforms surviving in the soil following sewage irrigation.

Figure 3 shows the relationship between the MPN of streptococci and coliforms surviving in soil, and figure 4 shows a corresponding relationship between *Salmonella* and coliforms. The relationship is linear in both cases. The coefficients of correlation have been calculated to be positive. For streptococci and coliforms the coefficient was found to be $r = +0.76$ with a standard error of 0.15. The corresponding values for *Salmonella* and coliforms were found to be 0.95 and 0.23. This shows that in soil following irrigation a decrease of one genus is closely related with a decrease of the other two, and, as the coefficient is more than 4 to 5 times the standard error in each case, the coefficient may certainly be accepted as significant.

ACKNOWLEDGMENT

The author wishes to express her gratitude to Mr. A. Amrami, Director of the Sanitation Division, Ministry of Health, who initiated, planned and guided the sewage research project; to Dr. W. Silberstein, Director, the Central Laboratories, Jerusalem, for his guidance; to Prof. H. Heukelekian for valuable advice and criticism.

The work was begun under the part-sponsorship of the Research Council of Israel and carried out in the Sewage Research Laboratory donated by Mr. Sol Pincus and his family of New York.

Mrs. B. Orenstein assisted in the technical part of the work.

SUMMARY

Uncultivated plots were irrigated in summer with raw sewage and trickling-filter effluent. The rate of disappearance of coliforms and streptococci from *terra rossa* soil irrigated with raw sewage or trickling-filter effluent was found to be the same. This suggests that from the point of view of bacterial survival no advantage is gained by irrigation with trickling-filter effluent instead of raw sewage.

A study was made of the survival of *Salmonella tennessee* and coliforms in soil, during the winter. In soil irrigated once with sewage to which a *Salmonella* suspension had been added, there was a big initial reduction in numbers of both genera. *Salmonella* organisms continued to decrease throughout the experiment, disappearing by the 46th day at the surface of the soil, and the 70th day at a depth. The coliform population, however, decreased until it became relatively stable, showing but little change with time. This condition was generally obtained at a figure of thousands or hundreds at the surface, and ten thousands or thousands at a depth.

A study similar to that described above, but including

Streptococcus faecalis was carried out also in summer. *Salmonella* organisms disappeared from the surface of the soil by the 15th day, and from a depth by the 11th day. Coliforms and streptococci persisted throughout the experiment which lasted 38 days. Judging by the rate of disappearance of both genera, it seems likely that streptococci are not to be preferred to coliforms as indicators of fecal pollution of the soil.

A suspension of *Salmonella tennessee* was added to raw sewage kept in open storage tanks in the field. A portion of the mixture was applied to uncultivated plots, the rest remaining in the tanks. *Salmonella tennessee* survived in the storage tanks longer than in the soil, disappearing by the 22nd day in the former and the 11th to the 15th day in the latter.

A study of the survival of *Salmonella tennessee* was carried out in summer in the soil of a growing sunflower crop under sewage irrigation. This pathogen disappeared by the 23rd day at the surface and the 37th day at a depth, that is, between the corresponding summer and winter survival times for uncultivated plots.

There is a straight-line relationship between the decrease in numbers of *Salmonella*, coliforms, and streptococci. The coefficients of correlation have been found to be positive and significant.

REFERENCES

HEUKELEKIAN, H., AND SHULHOFF, H. B. 1935 Studies on the survival of *B. typhosus* in surface waters and sewage. N. J. Agr. Expt. Sta. Bull. 589. New Brunswick, New Jersey.

KAUFMANN, F. 1950 *The Diagnosis of Salmonella Types*. Charles C Thomas, Springfield, Illinois.

KLIGLER, I. J. Oct. 10, 1921 Investigations of soil pollution and the relation of the various types of privies to the spread of intestinal infections. International Health Board, Monograph 15, p. 1. Rockefeller Institute of Med. Research.

MALLMANN, W. L., AND LITSKY, W. 1951 Survival of selected enteric organisms in various types of soil. Am. J. Public Health, **41**, 38–44.

MALLMANN, W. L., AND SELIGMANN, E. B. 1950 A comparative study of media for the detection of streptococci in water and sewage. Am. J. Public Health, **40**, 286–289.

MELICK, C. O. 1917 The possibility of typhoid infection through vegetables. J. Infectious Diseases, **21**, 28–38.

RIGBI, M., AMRAMI, A., AND SHUVAL, H. Efficiency study of a small high-rate trickling filter plant in Jerusalem, Israel. Sewage Ind. Wastes (*In Press*).

ROTHE, W. C. Personal communication to the Difco Laboratories. Quoted from Mallmann and Seligmann, 1950.

RUDOLFS, W., FALK, L. L., AND RAGOTSKIE, R. A. 1950 Literature review on the occurrence and survival of enteric, pathogenic and relative organisms in soil, water, sewage and sludges, and on vegetation. I. Bacterial and virus diseases. Sewage and Ind. Wastes, **22**, No. 10, 1261–1277.

SEDGWICK, W. T., AND WINSLOW, C. E. A. 1902 Mem. Am. Acad. Arts and Sci., **12**, 508.

SKINNER, C. E., AND MURRAY, T. J. 1956 The viability of *B. coli* and *B. aerogenes* in soil. J. Infectious Diseases, **38**, 37–41.

YOUNG, C. C., AND GREENFIELD, M. 1923 Observations on the viability of the *Bacterium coli* growing under natural and artificial conditions. Am. J. Public Health, **13**, 270.