APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1982, p. 981–986
0099-2240/82/050981-06$02.00/0
Vol. 43, No. 5

Survival of *Salmonella adelaide* and Fecal Coliforms in Coarse Sands of the Swan Coastal Plain, Western Australia

WILLIAM F. PARKER* AND BRIAN J. MEE

Department of Microbiology, University of Western Australia, Queen Elizabeth II Medical Centre, Nedlands Western Australia, 6009, Australia

Received 12 November 1981/Accepted 11 January 1982

The survival of *Salmonella adelaide* and fecal coliforms in two coarse sands influenced by two sources of septic tank effluent was studied. The experiments were conducted in conditions that reflected the soil environment beneath functioning septic tank systems. Significant differences in survival were found with different effluent sources. In one experiment the survival of *S. adelaide* was similar to that of fecal coliforms; in the other it was not. The nonuniform, multiphasic nature of survival curves and variability observed in these experiments suggests that the application of such survival data for establishing management criteria for septic tank systems—by, for example, the use of soil moisture characteristic curves to give estimates of movement in the soil—is inappropriate.

Not all soils are ideally suited for septic tank operation, and this is related to soil texture and topography. Soils of low permeability are unsuitable because of problems with premature hydraulic failure and surface ponding of effluent. The management of septic tanks in coarse soils, from an engineering viewpoint, is less troublesome because of adequate infiltration rates. However, if a septic system is sited without an adequate depth of unsaturated soil, then a public health hazard might arise. These two situations of considerable public health importance have been reviewed by Bouma (2) and Wall and Webber (18). Predicting a health hazard in such situations is a difficult task, for field studies with tracers are often impossible, especially in suburban environments. Instead, some assessment of the potential health hazard associated with septic tank operation can be made by laboratory studies.

It was suggested by Bouma et al. (3) that survival data for fecal organisms could be compared with soil-moisture characteristic curves, and hence the distance of soil filtration necessary for removal could be defined as a function of moisture content. There are a number of possible sources of variability that may limit the usefulness of the concept, such as the culture conditions of the strain(s) studied, the physiological differences between genera, the recovery methods, the moisture content, the soil type, the indigenous microbial flora, and the effluent source itself.

The primary purpose of this study was to assess the survival of *Salmonella adelaide* and fecal coliforms (FC) under conditions as close as possible to those found soon after a septic system is installed or where high water tables prevail. The soil tested were from the Perth, Australia, metropolitan area, where significant numbers of septic tanks are in use. These septic tanks are frequently located in areas with high water tables.

MATERIALS AND METHODS

Bacterial strains. The *S. adelaide* strain used was isolated from an ocean sewage outfall and subsequently made resistant to 50 μg of nalidixic acid per ml by ethyl methane sulfonate (Sigma) mutagenesis by the procedure described by Miller (10). FC were the natural effluent populations.

Effluent sources. Septic tank effluents were obtained from the effluent infiltration systems (soak wells or leach drains) of two families of five persons (two adults, three children). No specific tests were conducted to determine chemical or physical differences between the effluents, but it was known that one of the families (effluent B) had a predominantly vegetarian diet.

Soils. Both soils are coarse sands (8), aeolian in origin, and form extensive dune systems. Particle size analyses have been performed by Whelan and Barrow (19). Bassendean sand (96.9% coarse sand, 2.7% fine sand, 1.2% silt, 0% clay) is a weakly acidic, siliceous podzol with some iron-humus banding. Spearwood sand (76.9% coarse sand, 18.4% fine sand, 2.5% silt, 2.8% clay) is a weakly acidic, siliceous yellow sand, with some iron oxide and is less heavily leached. These two dune systems form the major part of the Swan Coastal Plain of Western Australia.

Approximately 5 kg of each soil was removed from a depth of 2 m, which is approximately equivalent to the point at which septic tank effluent enters the soil. After collection, a sub-sample was air dried for 60 min at 37°C, and moisture contents were determined on both fresh and air-dried portions by oven drying at 105°C for 24 h. The bulk soils to be used in survival studies

were not sterilized. After storage overnight at 15°C, the soils were air dried and, just before setting up, passed through a 1.68-mm sieve to remove debris.

Preparation of soil tests. FC were concentrated 40-fold by centrifugation and resuspended in the same effluent to give a concentration of approximately 10^5 cells per ml. *S. adelaide* cells were grown to stationary phase in Vogel and Bonner (17) minimal salts medium with 0.2% glucose and added to effluent suspensions to give a concentration of approximately 4×10^6 cells per ml. It has been shown (6) that differing bacterial concentrations have no effect on survival. Concentrated effluents (1.2 ml) with and without *S. adelaide* were added to 20-g samples of air-dry soils to restore the soils to 5% moisture. One-half of the samples were brought to saturation (23% moisture) by adding coliform-free nonsterile groundwater. All soil samples were set up in airtight (wax-sealed) plastic pots and stored at 15°C in the dark. Each soil type was run in duplicate at each moisture content, and the entire procedure was repeated with another effluent source. Soil pH determination, by placing 5 g of soil sample in 45 ml of distilled water, was carried out at the start of the experiments and again 32 days later.

Enumeration of surviving bacteria. Samples were taken at 0, 1, 2, 4, 8, 16, 32, and 64 days, and FC and *S. adelaide* organisms were enumerated. Soil samples were shaken in dilute buffer (17), omitting glucose, and the supernatant was assayed in triplicate both for FC, by membrane filtration, and for *S. adelaide*, by spread plate. FC were grown on mFC medium (Difco) for 22 h at 44.5°C, and *S. adelaide* was grown on XLD medium (BBL) supplemented with 50 μg of nalidixic acid per ml for 22 h at 37°C.

Statistical analyses. Multiway factorial analyses were performed with a computer program for analysis of variance (Genstat V Mark 4.01, Lawes Agricultural Trust, Rothamsted Experimental Station, England, 1977).

RESULTS

Survival curves for *S. adelaide* and FC are shown in Fig. 1. The curves demonstrate that the organisms survived for extended periods in moist or saturated sands. In the majority of cases in this study, the die-off was minimal (<99.9%). The greatest die-offs occurred in three situations (FC, effluent B, Spearwood sand, saturated and unsaturated; *S. adelaide* effluent A, Spearwood sand, saturated).

The phenomenon of apparent "regrowth" was frequently observed during the first 16 days, giving rise to oscillations in the curves. Because of this, no attempt was made to fit equations. A further consequence of this variability was that membranes or plates were on some occasions uncountable. In these cases, the data have been represented by open symbols in Fig. 1 and omitted from statistical analysis.

Estimates of 90% die-off (T_{90}) values obtained graphically are shown in Table 1. These values were taken from rates calculated over the first 32 days and ignore oscillations in the curves. The T_{90} values for FC were generally higher than those for *S. adelaide*. There appeared to be no differences due to effluent source, but both types of organisms survived better in Bassendean sand and survived less in saturated conditions.

The soil pH data were similar to those expected in the soil profiles beneath septic systems for these soils. The initial pH values were neutral (range, 6.4 to 7.1), and after 32 days they became more acidic (range, 4.9 to 5.9). In saturated samples the pH range was narrower and less acidic (5.7 to 5.9).

Analyses of variance. For analyses of variance, interaction terms were chosen according to expected effects and analyses were performed at each time point. The effects were considered real if a trend appeared in the variance ratios overall rather than if they appeared as isolated but significant values.

Table 2 shows the variance ratios obtained when the data from effluents A and B were combined. The bacteria behaved differently in the different effluents, although the variation due to effluent source diminished by day 16. The variance due to soil type was consistent throughout the experiment, but the variance due to moisture content was not. The interactions of effluent with bacteria and effluent with moisture were significant sources of variation compared with soil-moisture, soil-bacteria and soil-effluent interactions. Further separate analyses were performed on the data from each effluent. The pattern established from the combined analysis was confirmed. There was a significant difference between survival of bacteria in effluent A and that in effluent B (Table 3). Although moisture content was a significant source of variation in the experiment with effluent A, no effect was seen with effluent B. Neither interaction between soil type and bacteria nor that between soil type and moisture was consistently significant.

To obtain a more controlled measure of the environmental influences on enteric bacterial survival, a further analysis was done by omitting data for FC, thus eliminating differences in response due to indigenous population. Table 4 shows the variance ratios obtained for *S. adelaide* where the effects of effluent, soil type, moisture content, and the interaction of effluent with moisture were tested. There was a consistently significant effect of effluent source on survival of *S. adelaide* throughout the experiment. Neither moisture content nor the interaction of effluent with moisture had any significant effect on the survival of *S. adelaide*. Soil type appeared to have some effect, but this was not consistent. The effect of soil type appeared to be significant only for the combined analyses (Table 2). An identical analysis, not included here,

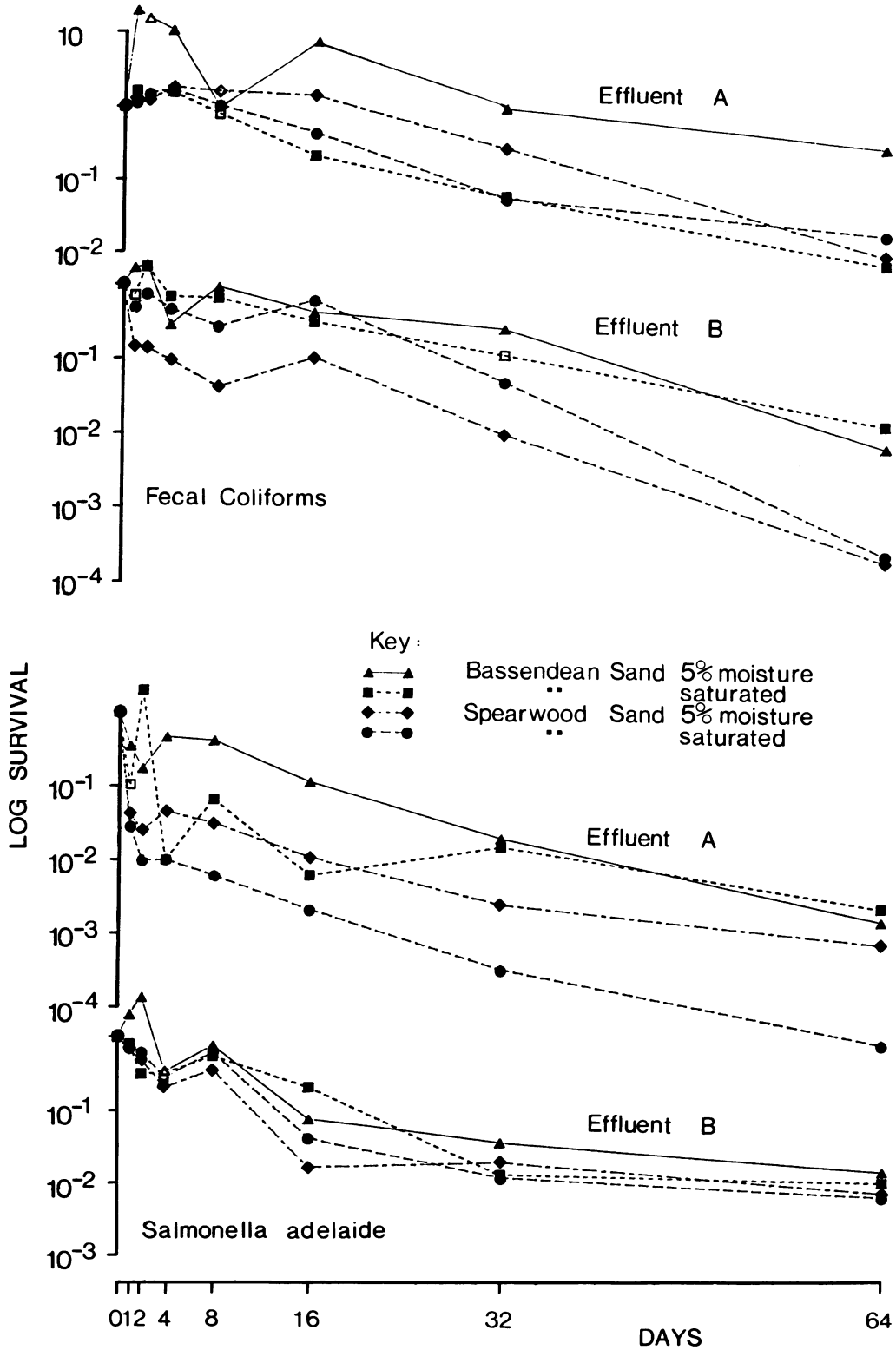

FIG. 1. Survival of *S. adelaide* and FC in two coarse sands at two moisture levels influenced by two sources of septic tank effluent. (Open symbols indicate values omitted from statistical analyses; see text for explanation.)

TABLE 1. T_{90} values for *S. adelaide* and FC in Spearwood and Bassendean sands

Soil	Effluent	T_{90}[a]	
		S. adelaide	FC
Unsaturated			
Bassendean	A	46.5	>64
	B	31.5	51.5
Spearwood	A	12.5	59
	B	17	16
Saturated			
Bassendean	A	18.5[b]	26
	B	16.5	34.5
Spearwood	A	7	26
	B	16.5	27.5

[a] Calculated from the overall die-off rate between days 0 and 32.
[b] Calculated over 64 days.

was performed with FC data only, and the results were not significant for any of the treatment terms.

DISCUSSION

In this study we have shown that significant differences in survival patterns of both indigenous FC populations and laboratory-grown *S. adelaide* were due to the effects of two sources of household septic tank effluent. The fact that *S. adelaide* had a survival pattern similar to that of FC for one effluent, but not the other, suggests the need to evaluate a wider range of organisms, as well as effluent sources, before general conclusions about the survival of enteric bacteria in this particular soil environment can be made.

We chose in this study to compare fecally excreted "wild" populations and organisms grown under laboratory conditions. In the latter case, the use of minimal media was intentional to ensure that the organisms had little metabolic reserve. It has been shown, for example, that enteric bacteria grown on nutrient-rich media survive better in aqueous suspensions than those grown on minimal media (13). Chandler and Craven (4) obtained equivalent T_{90} values for *Escherichia coli* cells whether they were directly inoculated into soil from rich nutrient media (T_{90} = 15 days) or after having been stored at 4°C for 7 days in 0.1% peptone (T_{90} = 14 days). *Salmonella typhimurium*, however, exhibited different behavior, with T_{90} values of 14 and 10 days, respectively. Van Donsel et al. (16) observed that minimal media do not necessarily yield organisms in the same physiological state as fecally excreted wild strains. The results reported here confirm that for *S. adelaide*, this observation is important.

The survival of bacteria in soils after discharge from septic tank systems is not likely to be limited by moisture in coarse sands under operational field conditions. It is only when appreciably lower moisture contents are encountered that survival is significantly reduced (5). At the levels of moisture in the experiments reported here, the differences were not critical for survival. There was some evidence that cells did not survive as well under saturated conditions if T_{90} values (Table 1) are considered. However, analyses of variance (Tables 2 through 4) showed that moisture content differences overall had no significant effect on survival.

The application of survival data involves the fitting of equations to curves and the derivation of expressions for die-off rates. In practice, simple equations may be impossible to fit. Survival data, for example, from field studies have frequently shown regrowth phenomena and curves are multiphasic (1, 4, 5, 7, 15, 16). We have observed a similar phenomenon in this study, where external influences, such as light and temperature, were carefully controlled.

TABLE 2. Variance ratios for effluents A and B combined[a]

Sampling day	Variance ratio of:									Residual degrees of freedom[b]
	B	E	S	M	E × B	E × M	S × M	S × B	S × E	
1	56.8[c]	12.1[d]	20.1[c]	11.9[d]	91.6[c]	10.4[d]	24.6[c]	3.9	0.2	6
2	44.0[c]	18.1[d]	22.7[c]	1.9	69.6[c]	7.3	6.4	0.1	0.2	4 (2)
4	37.6[c]	1.6	39.9[c]	0.2	124.4[c]	34.3[c]	4.9	7.1	0.2	3 (3)
8	5.9	92.5[c]	77.3[c]	7.1	—[f]	19.5[d]	5.3	10.6[d]	17.7[d]	3 (3)
16	130.4[c]	0.2	20.1[c]	5.9[d]	26.8[c]	33.5[c]	5.2	7.3[d]	0.6	6
32	19.8[c]	0.1	7.2[d]	6.6[d]	4.7	5.5	2.4	0.1	0.4	5 (1)
64	4.1	0	8.6[d]	2.2	25.6[c]	2.7	0.2	1.5	0.4	6

[a] Sources of variation were bacteria (B), effluent source (E), soil type (S), and moisture content (M).
[b] Number in parentheses indicates number of omitted values (see text).
[c] Significant at the 0.1% level.
[d] Significant at the 5% level.
[e] Significant at the 1% level.
[f] Data omitted from analyses (see text).

TABLE 3. Variance ratios for effluents A and B separated[a]

Effluent	Sampling day	Variance ratio of:					Residual degrees of freedom[b]
		B	S	M	S × B	S × M	
A	1	186.55[c]	15.17	28.32[d]	4.01	25.49[d]	2
	4	106.96[c]	16.47	14.02	3.05	2.33	2
	16	96.04[d]	9.62	23.50[d]	3.39	2.79	2
	32	432.09[d]	217.67[d]	215.89[d]	113.89	9.56	1 (1)
	64	15.28	1.61	2.96	0.02	0.37	2
B	1	1.81	7.33	0.02	0.91	5.66	2
	2	1.13	10.58	0.66	0.01	1.75	2
	8	3.28	11.63	1.68	5.91	5.63	2
	16	14.68	5.20	4.27	1.97	1.12	2
	32	5.00	4.19	0.05	1.97	1.85	2
	64	44.19[d]	61.09[d]	0.14	33.06[d]	0.09	2

[a] Sources of variation were bacteria (B), soil type (S), and moisture content (M). Data for effluent A on days 2 and 8 and effluent B on day 4 were omitted from the analyses (see text).
[b] Number in parentheses indicates number of omitted values (see text).
[c] Significant at the 1% level.
[d] Significant at the 5% level.

TABLE 4. Variance ratios for *S. adelaide*[a]

Sampling day	Variance ratio of:				Residual degrees of freedom[b]
	E	S	M	E × M	
1	19.82[c]	0.74	3.03	1.24	3
2	128.11[d]	29.08[c]	5.15	1.90	2 (1)
8	14.22[c]	4.69	1.69	2.17	3
16	52.29[d]	85.93[d]	4.29	49.39[d]	3
32	1.54	2.16	3.16	1.28	2 (1)
64	50.85[d]	5.49	7.92	5.05	3

[a] Sources of variation were effluent (E), soil type (S), and moisture content (M). Data from day 4 were omitted from the analyses (see text).
[b] Number in parentheses indicates number of omitted values (see text).
[c] Significant at the 5% level.
[d] Significant at the 1% level.

The outcome of survival experiments with enteric bacteria in natural, unsterilized soils is subject to a number of influences apart from soil texture and moisture, such as indigenous flora. For example, McCambridge and McMeekin (9) and Tate (14) have described the effects of protozoan predators. Before any application of data on the survival of enteric bacteria can be made, it will be necessary to ascertain the effects of other organisms. There still remains the need to understand more about the microbiology of soil disposal systems, largely because they are uncontrolled and their impact on the public health is difficult to assess epidemiologically. Data from field studies (3, 12) and tracer studies (11), though invaluable, may not offer enough information about bacterial survival and movement.

It is doubtful whether survival data can be directly applied, as suggested by Bouma and his colleagues (3). Apart from biological variability, such an application would be restricted by soil heterogeneity. It is unlikely that any useful relationship between survival and the soil moisture characteristic, even for coarse sands with no profile development, could be postulated.

ACKNOWLEDGMENTS

We thank I. James of the Department of Mathematics, University of Western Australia, for help with the manipulation of the Genstat V programs and advice on the interpretation of the results.

W.F.P. received a postgraduate scholarship from the Department of Conservation and Environment, Western Australia, which is gratefully acknowledged.

LITERATURE CITED

1. **Bergner-Rabinowitz, S.** 1956. The survival of coliforms, *Streptococcus faecalis* and *Salmonella tennessee* in the soil and climate of Israel. Appl. Microbiol. 4:101–106.
2. **Bouma, J.** 1975. Unsaturated flow during soil treatment of septic tank effluent. J. Environ. Eng. Div. Am. Soc. Civ. Eng. 101:967–983.
3. **Bouma, J., W. A. Ziebell, W. G. Walker, P. G. Olcott, E.**

McCoy, and F. D. Hole. 1972. Soil absorption of septic tank effluent. University of Wisconsin-Extension Geological and Natural History Survey, Information Circular no. 20.

4. **Chandler, D. S., and J. A. Craven.** 1980. Relationship of soil moisture to survival of *Escherichia coli* and *Salmonella typhimurium* in soils. Aust. J. Agric. Res. **31**:547–555.
5. **Kibbey, H. J., C. Hagedorn, and E. L. McCoy.** 1978. Use of fecal streptococci as indicators of pollution in soil. Appl. Environ. Microbiol. **35**:711–717.
6. **Klein, D. A., and L. E. Casida.** 1967. *Escherichia coli* die-out from normal soil as related to nutrient availability and the indigenous microflora. Can. J. Microbiol. **13**:1461–1470.
7. **Mallman, W. L., and W. Litsky.** 1951. Survival of selected enteric organisms in various types of soil. Am. J. Public Health **41**:38–44.
8. **McArthur, W. M., and E. Bettenay.** 1960. TITLE. Commonwealth Scientific and Industrial Research Organization Publication no. 16. The development and distribution of the soils of the Swan coastal plain, Western Australia. Melbourne, Australia.
9. **McCambridge, J., and T. A. McMeekin.** 1980. Relative effects of bacterial and protozoan predators on survival of *Escherichia coli* in estuarine water samples. Appl. Environ. Microbiol. **40**:907–911.
10. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 138. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
11. **Rahe, T. M., C. Hagedorn, E. L. McCoy, and G. F. Kling.** 1978. Transport of antibiotic-resistant *Escherichia coli* through Western Oregon hillslope soils under conditions of saturated flow. J. Environ. Qual. **7**:487–494.
12. **Reneau, R. B., and D. E. Pettry.** 1975. Movement of coliform bacteria from septic tank effluent through selected coastal plain soils of Virginia. J. Environ. Qual. **4**:41–44.
13. **Strange, R. E., F. A. Dark, and A. G. Ness.** 1961. The survival of stationary phase *Aerobacter aerogenes* stored in aqueous suspension. J. Gen. Microbiol. **25**:61–76.
14. **Tate, R. L., III.** 1978. Cultural and environmental factors affecting the longevity of *Escherichia coli* in histosols. Appl. Environ. Microbiol. **35**:925–929.
15. **Temple, K. L., A. K. Camper, and G. A. McFeters.** 1980. Survival of two enterobacteria in feces buried in soil under field conditions. Appl. Environ. Microbiol. **40**:794–797.
16. **Van Donsel, D. J., E. E. Geldreich, and N. A. Clarke.** 1967. Seasonal variations in survival of indicator bacteria in soil and their contribution to storm-water pollution. Appl. Microbiol. **15**:1362–1370.
17. **Vogel, J. H., and D. M. Bonner.** 1956. Acetyl ornithase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97–106.
18. **Wall, G. J., and L. R. Webber.** 1970. Soil characteristics and subsurface sewage disposal. Can. J. Public Health **61**:47–54.
19. **Whelan, B. R., and N. J. Barrow.** 1980. A study of a method for displacing soil solutions by centrifuging with an immiscible liquid. J. Environ. Qual. **9**:315–319.