APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1987, p. 633–638
0099-2240/87/040633-06$02.00/0

Vol. 53, No. 4

Survival of Pathogenic Bacteria in Various Freshwater Sediments

G. ALLEN BURTON, JR.,[1]† DOUGLAS GUNNISON,[2] AND GUY R. LANZA[1]*

Graduate Program in Environmental Sciences, University of Texas at Dallas, Richardson, Texas 75083,[1] and Waterways Experiment Station, U.S. Army Corps of Engineers, Vicksburg, Mississippi 39180[2]

Received 24 March 1986/Accepted 26 December 1986

Four human-associated bacteria, *Pseudomonas aeruginosa*, *Salmonella newport*, *Escherichia coli*, and *Klebsiella pneumoniae*, were tested for survival in five freshwater sediments. Bacterial survival in continuous-flow chambers was monitored over 14-day periods on sediments ranging from organically rich high-clay fractions to organically poor sandy fractions. Bacterial die-off ranged from 1 to 5 orders of magnitude in sediments. *E. coli* survived as long as or longer than *S. newport*. *P. aeruginosa* and *K. pneumoniae* tended to survive longer than *E. coli*. Survival of *E. coli* and *S. newport* was greater in sediments containing at least 25% clay. Good reproducibility allowed the development of linear models to describe die-off rates.

Studies of the survival of fecal coliforms (FC) are numerous (7, 9, 16, 24, 32). Most investigations have involved either soil or marine environments and have concentrated only on reduction in bacterial numbers over time. Studies in recent years have frequently revealed much higher numbers of indicator and pathogenic bacteria in sediments than in overlying waters. Apparently, higher concentrations of indicator and pathogenic bacteria in the sediments are due to a combination of sedimentation, sorption (which provides protection from bacteriophage and microbial toxicants [30, 31, 38]), and the phenomenon of extended survival in sediments (13, 15, 35).

Several studies of enteric survival have been done with sediment systems (5, 13, 15, 21, 25, 31, 35). It is difficult to translate information on the survival of indicator bacteria in sediments from studies done with soils or water. This problem is a result of the many conflicting conclusions, varied methodologies, and considerable differences between ecosystems. In addition, sediment survival studies have involved the use of static systems, inhibitory recovery media, protective chambers, dialysis bags, sterile sediments, and shaking incubation systems. These experimental design factors reduce the significance of relationships of data to in situ phenomena. The first sediment study by Van Donsel and Geldreich (35) reported a 90% die-off of both FC and *Salmonella* spp. in 7 days in various sediments. This rate is much lower than die-off in water, which often occurs within 3 days (32). Also, no studies have been done to compare the survival of FC or pathogens in different types of lake or stream sediments, and few have been done to compare water types (37). Therefore, effects of environmental parameters are poorly defined. The role of heterotroph starvation in survival in sewage, lake water, and marine environments has been studied (28, 33).

In most survival studies, *Escherichia coli* was the only organism tested. The use of *E. coli* as an indicator of fecal pollution for all ecosystems has been questioned. Some studies have indicated that *E. coli* dies off faster than *Salmonella* spp. (10, 19, 34, 36) and therefore is not a suitable indicator for the presence of this pathogen.

The water quality testing criteria in use at present do not take into account sediments as a potential reservoir of pathogens. The higher numbers of pathogens occurring in sediments, along with increasing usage of recreational waters, creates a potential health hazard from resuspension and subsequent ingestion (13, 17, 18, 26). Thus, there is a need to obtain additional information on the survival of indicator and pathogenic bacteria in sediments and the factors which contribute to their survival.

The present study was undertaken to investigate the survival of several resistance-labeled bacterial pathogens in various types of sediment in continuous-flow laboratory microcosms. The suitability of *E. coli* as an indicator species was assessed by comparing its survival rate with those of *Salmonella newport*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

Survival chambers. Survival studies were conducted in continuous-flow Plexiglass chambers (30 by 30 by 60 cm; 52 liters). Fresh soil or sediment was collected, stored under sample site water at 4°C, and tested within 2 weeks. Sediment was placed into each of four chambers to a depth of 8 cm and flooded 1 week prior to an experiment. Water was pumped into the chamber through Tygon tubing (Norton) into an opening 20 cm above the sediment surface. The outflow was located at the water surface, 55 cm above the chamber bottom. Reverse osmosis-purified water was used as overlying water, with salts added to equal the concentrations of the major cations and anions occurring in the natural waters from which the sediments were taken (Table 1). Cation and anion concentrations were based on water quality data previously collected by the Army Corps of Engineers, the U.S. Environmental Protection Agency, or the Texas Department of Water Resources. Logistical problems associated with shipment and storage of large quantities of site water necessitated the use of reconstituted water. Salts were added to a polyethylene reservoir which was continuously aerated. Water was aged for at least 3 days, and the pH was adjusted to in situ levels. Flow rates of 4 ml/min were used, resulting in approximately a 7.3-day retention time for the water column in each chamber. Dissolved oxygen (meter model 54; Yellow Springs Instrument Co., Yellow Springs, Ohio), temperature (YSI meter model 54), pH (meter model 125; Corning Glass Works, Corning, N.Y.), and conductivity (YSI meter model 33) were monitored throughout the experiment (Table 2) under conditions similar to those in the aquatic systems from which the sediments were removed.

* Corresponding author.
† Present address: Biological Sciences Department, Wright State University, Dayton, OH 45435.

TABLE 1. Reconstituted water chemistry

Test system[a]	Concn (mg/liter) of:						
	CO_3^{2-}	SO_4^{2-}	Cl^-	Na^+	K^+	Ca^{2+}	Mg^{2+}
WES[b]/Eagle Lake	70.0	41.0	1.8	17.7	5.3	34.4	7.3
Lake DeGray	30.0	4.0	4.0	1.9	4.0	13.0	4.0
Lake Lavon	140.0	41.0	10.8	11.0	31.8	69.0	14.6
Red River	0.1	229.4	346.5	222.9	8.4	252.8	32.1

[a] Major ion concentrations approximate those occurring in the respective natural waters.
[b] WES, Waterways Experiment Station. Used water quality of the Mississippi River at Vicksburg, Miss., as model.

Test sediments. Sediments and soil were collected from Lake DeGray, Arkadelphia, Ark.; Lake Lavon, Dallas, Tex.; Eagle Lake, Vicksburg, Miss.; Red River, Dennison, Tex.; and Waterways Experiment Station, Vicksburg, Miss. (soil). Sediment texture was determined by the hydrometer method of Day (8). Approximate particle sizes were as follows: sand, >62 μm; silt, 4 to 62 μm; and clay, <4 μm. Organic matter in the sediment was estimated from the weight loss from dried sediment upon overnight combustion in a muffle furnace at 550°C. Total Kjeldahl nitrogen was determined following acid digestion as described by Bremner (4). Total phosphorus was determined after sediments were digested with red fuming nitric acid for 4 h. All analyses were performed, at a minimum, in triplicate with Technicon Auto-analyzers (Technicon Instruments Corp., Tarrytown, N.Y.). Total viable bacteria were estimated by spread plating sediment dilutions on standard methods agar (SMA [Plate Count Agar]; Difco Laboratories, Detroit, Mich.).

Test bacteria. *E. coli* SR3078, *S. newport* SRM, *K. pneumoniae* PC278, and *P. aeruginosa* ATCC 27853, all resistant to 740 μg of streptomycin per ml, were tested in each sediment. Streptomycin-resistant strains were obtained by the method of Danso et al. (6). Cultures were grown overnight at 35°C in standard methods broth (Plate Count Agar) (Difco) containing streptomycin (Sigma Chemical Co., St. Louis, Mo.) on a rotary shaker at 120 rpm. Actively growing cultures were centrifuged (4,229 × *g* for 15 min at 4°C) and washed three times with sterile phosphate-buffered saline (0.067 M, pH 7.4) before use as inocula.

Chamber inoculation. Immediately before inoculation of the test bacteria, all but 2 cm of the overlying water was withdrawn from each test chamber. The washed suspension of bacteria was then added to the chamber at an approximate concentration of 10^7 to 10^9 CFU/ml (data presented as CFU per milliliter of sediment) and gently mixed for 10 min to ensure even distribution of the bacteria across the sediment surface. During mixing, approximately 1 to 3 mm of sediment was resuspended in the overlying 2 cm of water. Replicate cores initially taken showed a relatively homogeneous distribution of inoculum across the sediment surface (coefficient of variation, <25%). At approximately 18 h after the resuspended sediment had settled, the chambers were refilled with water to a depth of 47 cm in a manner minimizing the disturbance of the sediment surface. No bacterial inoculum was added to the water column. A continuous flow of water was initiated, and monitoring of pH, temperature, conductivity, and dissolved oxygen were maintained until the experiment was terminated. At no time during the experiments was it necessary to adjust the pH, temperature, conductivity, or dissolved oxygen concentration, since relatively stable conditions persisted (Table 2).

TABLE 2. Chamber monitoring data[a]

Sediment test	Dissolved oxygen (mg/liter)	pH	Conductivity (μmho)	Temp (°C)
WES[b]				
1	7.5–8.9	7.1–7.8	189–264	18.4–20.8
2	6.6–8.4	7.4–7.6	226–290	18.0–19.7
3	5.5–8.9	7.2–7.7	235–300	19.3–20.4
4	6.4–7.6	7.5–7.7	280–312	19.5–21.2
Lake DeGray				
1	5.8–8.0	6.8–7.2	100–160	16.0–21.0
2	5.7–8.5	7.0–7.8	120–190	17.0–19.0
3	5.4–8.3	7.1–7.8	100–132	18.0–19.2
Lake Lavon				
1	6.8–8.9	7.4–8.4	210–300	18.2–19.8
2	6.4–8.0	7.6–8.2	263–292	18.8–19.7
3	7.2–8.3	7.8–8.7	250–280	17.0–20.0
Eagle Lake	7.0–8.7	7.2–8.1	190–640	17.8–19.8
Red River				
1	5.8–9.2	7.3–7.8	1,100–2,100	19.0–20.1
2	6.4–8.8	6.9–7.6	880–990	18.5–19.8
3	6.6–8.8	6.9–7.9	910–1,120	18.0–20.0

[a] Range over a 14-day test period.
[b] WES, Waterways Experiment Station.

Sampling procedure. During each sampling period, triplicate sediment cores (depth, 1.7 cm) were removed with a sterile 9-mm diameter glass tube and combined, resulting in a total of 3 ml of wet sediment. Pooled sediment samples were vortexed and serially diluted with phosphate-buffered saline. Sampling of overlying water was minimized to avoid enumeration of test pathogens in the water. Owing to the significant dilution of pathogen numbers in the overlying 42.3 liters and flowthrough conditions, the contamination of sediment counts by overlying-water pathogens was minimal. Initial samples for test bacteria in the water column were not collected until the chambers had filled, at approximately 18 h, to negate any dilution which occurred. Appropriate dilutions were placed on spread plates in duplicate on SMA containing 740 μg of streptomycin per ml and incubated at 35°C. Tracing the survival of test bacteria in the overlying water was of secondary importance, although it was of interest for comparing die-away events between the water and sediments; however, numbers of total viable bacteria and viable test bacteria in the overlying water were enumerated by plate counts during each sample period. For each experiment, there was a control chamber in which the sediment was examined at each sample period for background levels of naturally occurring streptomycin-resistant

TABLE 3. Sediment characteristics

Sediment	Organic matter (%)	TKN[a] (ppm)	TP[b] (ppm)	Clay/silt/sand ratio	TPC[c] (CFU/ml)
Lake Lavon	14.8	3.18	9.98	75:25:0	10^6
Lake DeGray	6.2	16.32	6.90	28:55:18	10^6
WES[d] soil	9.0	24.10	7.70	12:76:11	10^7
Eagle Lake	5.2	13.97	14.30	25:51:24	
Red River	0.7			2:0:98	10^6

[a] Total Kjeldahl nitrogen, 3 to 11 replicates.
[b] Total phosphorus, three to nine replicates.
[c] Total plate count of bacteria on SMA.
[d] WES, Waterways Experiment Station.

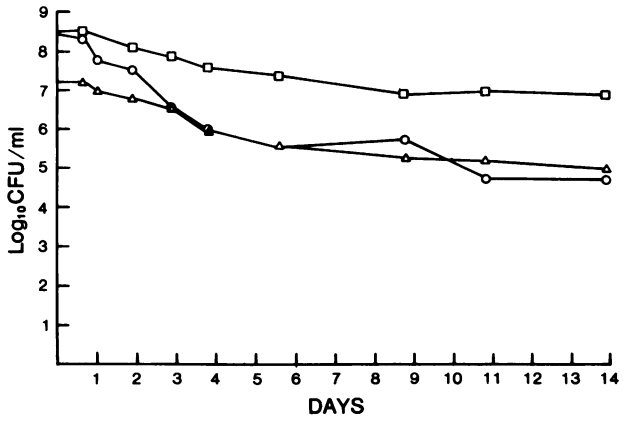

FIG. 1. Survival of *P. aeruginosa* (□), *S. newport* (○), and *K. pneumoniae* (△) in Lake Lavon sediment.

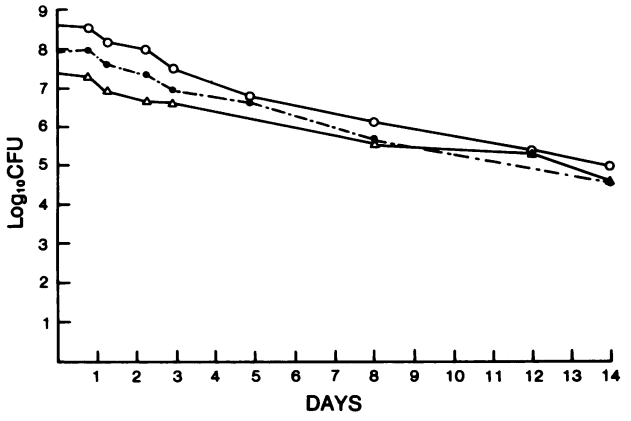

FIG. 3. Survival of *S. newport* (○), *E. coli* (●), and *K. pneumoniae* (△) in Lake DeGray sediment.

bacteria. Streptomycin-resistant test bacteria were checked periodically to ensure the stability of the streptomycin resistance. Viable counts for total bacteria were obtained by plating on SMA and incubation at 25°C for 10 days.

Statistical analysis. Plates containing less than 20 CFU/ml or more than 400 CFU/ml were discarded. Variances between replicates were checked to ensure acceptable levels (coefficient of variation, ≤25%). To better compare the biological relationships under study, the bivariate relationship of bacterial counts with time was fitted to a least-squares regression by using 36 combinations of logarithmic and square-root transformations. Statistical comparisons were made by using the paired Student *t* test or the Spearman rank correlation (r_s) at the 95% level ($P \leq 0.05$). Statistical tests on survival models were done with the Statistical Analysis System (SAS, Raleigh, N.C.). Linearized survival slopes were compared by using the analysis of covariance procedure (39).

RESULTS

The four test bacteria had extended survival in sediments in the laboratory microcosms compared with that in overlying waters. Survival rates varied among test bacteria and among test sediments; however, reproducibility between replicate tests was good. Physical and chemical characteristics of the reconstituted waters and sediments are given in Table 1 to 3. Sediments varied from the high organic matter and high clay content of Lake Lavon to the low (0.7%) organic matter and 98% sand of the Red River (Table 3). The only sediment characteristic for which there was an apparent relationship with bacterial survival was particle size. *E. coli* and *S. newport* survived longer (r_s = 0.80) in sediments containing at least 25% clay (Lavon, DeGray, and Eagle Lakes; Fig. 1 to 4). *E. coli*, *S. newport*, and *K. pneumoniae* exhibited greatest die-off in the sandy Red River test systems (Table 4; Fig. 5).

Preliminary survival tests with the test bacteria were conducted with sediments which were pretested by autoclaving, dried at 180°C, or sterilized with ethylene oxide. In all tests, we noted initial increases in test bacterium numbers followed by erratic and extended survival, compared with untreated sediment microcosms (data not shown).

Monitoring of the sediment microcosms indicated stable conditions throughout the 2-week test period. Dissolved oxygen, temperature, pH, conductivity, flow rates, and water chemistry were kept at near-constant levels (Table 2). Benthic macroinvertebrate activity (from tubifex worms) was observed throughout the tests with DeGray, Eagle, and Lavon Lake sediments. Heterotrophic bacterium levels also remained within an order of magnitude of initial levels in overlying water (data not shown). No background strepto-

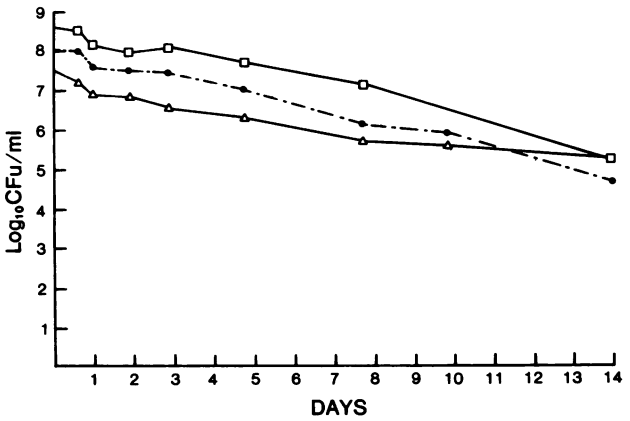

FIG. 2. Survival of *P. aeruginosa* (□), *E. coli* (●), and *K. pneumoniae* (△) in Lake DeGray sediment.

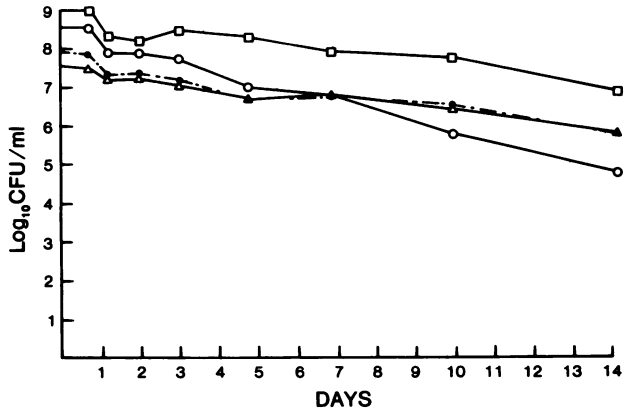

FIG. 4. Survival of *P. aeruginosa* (□), *E. coli* (●), *K. pneumoniae* (△), and *S. newport* (○) in Eagle Lake sediment.

TABLE 4. Survival model statistics for sediments

Bacterium and sediment	r^2	Intercept	Slope	Rate[a]
E. coli				
WES	0.95 (0.01)[b]	19.1 (0.1)	−4.2 (0.2	0.678
Lake DeGray	0.91 (0.04)	19.5 (0.2)	−2.8 (0.2)	0.764
Eagle Lake[c]	0.93	19.0	−1.9	0.833
Red River	0.90 (0.07)[d]	18.4 (0.6)	−4.0 (0.4)	0.681
Lake Lavon	0.82 (0.20)[d]	18.6 (0.6)	−1.4 (0.9)	0.874
S. newport				
WES	0.96 (0.01)	21.4 (0.1)	−4.5 (0.2)	0.649
Lake DeGray	0.94 (0.01)[d]	20.6 (0.6)	−3.2 (0.1)	0.736
Eagle Lake[c]	0.89	21.0	−3.1	0.743
Red River	0.90 (0.05)	20.3 (0.5)	−3.9 (0.6)	0.688
Lake Lavon	0.87 (0.07)	20.0 (0.4)	−2.6 (1.1)	0.779
K. pneumoniae				
WES	0.83 (0.06)	19.0 (0.4)	−1.9 (0.6)	0.833
Lake DeGray	0.96 (0.04)[d]	17.6 (0.1)	−2.2 (0.4)	0.810
Eagle Lake[c]	0.90	17.9	−1.3	0.883
Red River	0.92 (0.04)	17.2 (1.0)	−3.3 (0.7)	0.729
Lake Lavon	0.92 (0.04)[d]	17.8 (0.6)	−2.2 (0.1)	0.810
P. aeruginosa				
WES	0.97 (0.01)	19.0 (0.1)	−1.4 (0.1)	0.874
Lake DeGray	0.92 (0.08)[d]	20.1 (0.3)	−1.8 (0.1)	0.841
Eagle Lake[c]	0.86	21.4	−1.6	0.858
Red River	0.85 (0.08)[d]	16.4 (0.1)	−1.0 (0.5)	0.909
Lake Lavon	0.91 (0.06)	19.8 (0.4)	−1.5 (0.4)	0.866

[a] Survival rate per hour.
[b] Standard deviation (n = 3).
[c] n = 1.
[d] n = 2.

mycin-resistant organisms were recovered from the microcosms during the tests. Streptomycin resistance was maintained by the test bacteria. Recovery of test bacteria on SMA without streptomycin was not significantly different ($P < 0.05$) from that on media with streptomycin, indicating adequate recovery of any stressed test bacteria. Monitoring of test bacteria in the overlying water column showed decreasing numbers with time, which were directly related to dilution rates (data not shown).

Figures 1 to 6 give representative survival data in sediments. Other replicate tests produced similar die-off rates which were statistically significant ($P < 0.05$) (Table 4).

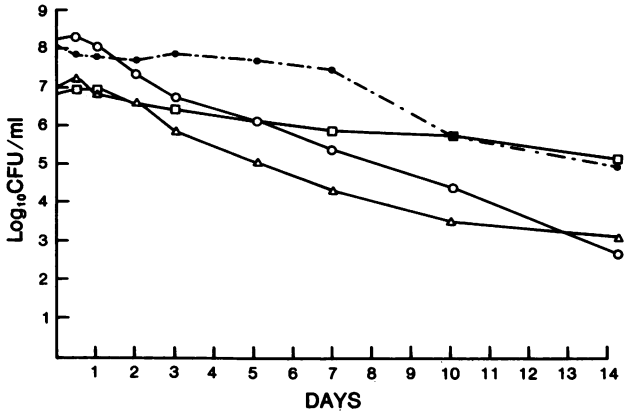

FIG. 5. Survival of *P. aeruginosa* (□), *E. coli* (●), *S. newport* (○), and *K. pneumoniae* (△) in Red River sediment.

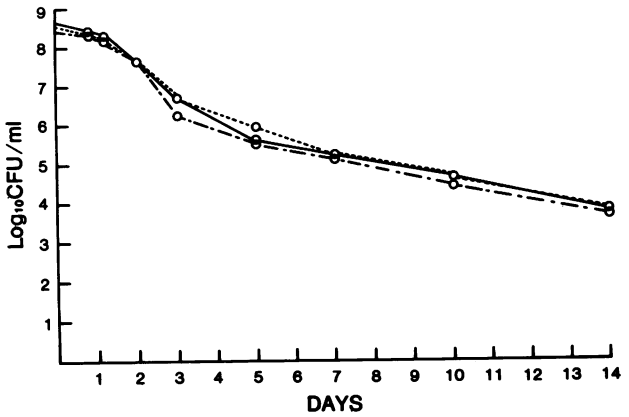

FIG. 6. Survival of *S. newport* in flooded Waterways Experiment Station soil. Each line represents replicate determinations.

Variance between replicate microcosms and replicate trials were small. Four replicate microcosms of *S. newport* produced near identical die-off patterns (Fig. 6). Replicate trials also produced little survival model variation (Table 4). Trend differences in sediment survival were observed between test bacteria. *P. aeruginosa* tended to survive best in each of the sediment types examined, followed by *K. pneumoniae*. *P. aeruginosa* survival varied little between sediments (1 to 2.3 orders of magnitude die-off over 14 days). *S. newport* die-off tended to be faster than that of the other test bacteria, ranging from 2.8 to 5.5 orders of magnitude during the tests. *E. coli* survived as well as or better than *S. newport* in sediments in all tests.

To better compare and predict survival rates, the bivariate relationship of the bacterial count (as a dependent variable) with time (as an independent variable) was fitted to a least squares by using 36 combinations of transformations. The general model $y = Ae^{-\beta \ln x}$ was developed, from which the following equation was derived: $\ln C = \beta_0 + \beta_1[\ln (T + 1)] + \epsilon$, where $\ln C$ is the natural logarithm of the initial bacterial density, β_0 is the intercept, β_1 is the die-off slope, T is time, and ϵ is residual error.

The model described survival data of the 45 tests very well, with a coefficient of determination greater than 0.82 (Table 4). Combination of survival data from all sediment tests for each test bacterium produced lower coefficients of determination: *E. coli*, $r^2 = 0.58$; *K. pneumoniae*, $r^2 = 0.66$; *P. aeruginosa*, $r^2 = 0.74$; and *S. newport*, $r^2 = 0.48$. Survival slopes were compared by analysis of covariance (39). This method, which tends to be robust, did not show statistically significant differences among any of the 45 slopes. Each of the analyzed slopes comprised 9 to 10 datum points.

DISCUSSION

This study showed extended survival of enteric bacteria in freshwater sediments. These results support the findings of other studies indicating levels of FC and pathogens manyfold higher in the sediments than in overlying water (14, 17, 18, 20, 24, 26, 29, 35; S. A. Winslow, M. S. thesis, University of Arizona, Tucson, 1976). Van Donsel and Geldreich (35) sampled various streams and lakes and found 100- to 1,000-fold more FC in the sediments than in overlying waters. Similar ratios of FC in sediment and water were found in the

Mississippi River (18). The present study shows that these reported higher densities are due, in part, to greater survival in sediments than in overlying waters and supports the work of others (5, 21, 25, 35). At initial concentrations of 10^8 viable cells per ml, such as are found in feces, these pathogens could survive in sediments for months, which is in contrast to a faster die-off in waters. A *Salmonella* die-off of 99% between 6 h and 3 days in water has been reported (32).

The use of resistance-labeled bacteria for ecosystem analysis is well documented (6, 27, 33, 34). This method allows direct contact and interaction of the test organism with its natural environment with subsequent easy recovery. Other approaches, such as the use of dialysis sacs and membrane chambers, impede transport of organic and inorganic complexes and protect the test organisms from factors such as protozoan predation and benthic grazing. The bacteria used in these studies exhibited no detectable spontaneous reversion to nonresistant forms. Recovery of test bacteria on SMA with and without streptomycin showed no difference in numbers over time. This is noteworthy in light of the numerous studies that have revealed the large percentage of bacteria which become physiologically injured in aquatic systems and are not recoverable on selective media. Bissonnette et al. (3) found that a significant proportion of *E. coli* cells lose their ability to produce colonies on selective media, yet are recoverable on nutritionally rich nonselective medium. The recovery procedure used in this microcosm study apparently was sufficient for recovery of stressed test bacteria while inhibiting the growth of indigenous microflora.

Although testing in situ usually yields more environmentally relevant results than typical laboratory studies, this flowthrough microcosm system yielded reproducible results, which eliminates design weaknesses of earlier survival studies. This was in part evidenced by stable heterotrophic plate count numbers throughout the tests and by similarity between replicate survival tests. The initial inoculum density (approximately 10^7 to 10^9 CFU/ml) is higher than that found in natural waters; however, similar levels of *E. coli* and *K. pneumoniae* organisms may occur per gram of human feces (11). Studies of *Vibrio cholerae* showed no difference between survival rates at high and low inoculum densities (21). Thus, the test system mimicked a heavily contaminated freshwater body with a turnover rate of 7.3 days.

Few studies of bacterial survival in aquatic ecosystems have correlated survival rates with environmental parameters (9, 24). Survival of bacteria in water is affected by numerous interacting factors including protozoa, antibiosis, organic matter, algal toxins, dissolved nutrients, heavy metals, temperature, and the physicochemical nature of the aquatic environment (9). In a marine study, LaBelle et al. (24) measured 12 environmental variables, none of which could be correlated to numbers of indicator bacteria numbers in the sediments. Gerba and McLeod (13) attributed the longer survival of *E. coli* in estuarine sediments to the greater content of organic matter present in the sediment than seawater. Grimes (18) suggested that higher FC numbers occur in silty clay sediments than sandy sediments as a result of surface area or particle charge differences; however, results failed to show particle size effects. Chan et al. (5) found extended *Enterobacter aerogenes* survival in nutrient-rich, fine-grained sediments. Our studies showed greater survival of *E. coli* and *S. newport* in sediments of higher clay content. This may be due to higher concentrations of organic matter and nutrients; however, survival in the Waterways Experiment Station system (a flooded soil), which contained high organic matter and nutrients, was similar to that in the sandy Red River system, which was low in organic matter. Inability to correlate survival with the total organic matter measure is not surprising, considering the varying nature of the organic matter and the multitude of environmental factors which affect survival.

This study has shown *E. coli* to be an adequate indicator of *S. newport* in various freshwater sediments and supports many investigations on *Salmonella* spp. and *E. coli* survival in water and soil ecosystems (1, 2, 12, 22, 32). Although rates vary greatly among soil, water, and sediment media, *E. coli* has usually been observed to survive as long as or longer than *Salmonella* spp., thus fulfilling an essential requirement for an indicator of pathogenic bacteria. However, some studies, particularly those conducted with soils, have shown *Salmonella* spp. to survive longer than *E. coli* (19, 34). *Salmonella* spp. have also been shown to persist longer than *E. coli* in water (10, 19, 36). The inconsistency of these findings with those of the majority of studies, which showed greater persistence of the indicator, may be attributed to varied strain characteristics, different methodologies, and unknown environmental variables. Although our study of freshwater sediments is unique, the results suggest that in various sediment types, survival of enteric bacterial species does not show the great differences observed in soil tests by other investigators. The greatest difference, in our tests, occurred with the consistently longer survival of *P. aeruginosa* and *K. pneumoniae* than of *E. coli* and *S. newport*. Extended survival of pseudomonads in water (23) and sewage has been observed (33). The longer survival of different species suggests underlying physiological characteristics, as reported in other studies (23, 28, 33). In water, enteric bacteria appear to be unable to adequately compete with natural microflora for low concentrations of nutrients (33). This inability to compete, plus antagonistic factors, result in a faster die-off than for indigenous strains and is probably a factor in sediments.

The lack of variation in survival between replicate tests permits the estimation of die-off rates for test bacteria. Although statistically significant differences did not exist among survival slopes in most cases, constant trends were observed, i.e., *P. aeruginosa* and *K. pneumoniae* survived longer than *E. coli* and *S. newport*. The inability to detect significant differences is most probably due to the robust nature of the analysis of covariance and an inadequate number of datum points. Theoretical calculations of bacterial densities that involve the use of the survival models show that significant differences will exist between the test bacteria with increasing time.

Adsorption, sedimentation, and extended survival can contribute to increased levels of enteric bacteria in sediments, creating a potential health hazard. Indicator and pathogenic bacteria and viruses occur at the highest concentrations in the upper layers of sediment, which may be resuspended by turbulence (17, 18, 26; W. F. Horak, M. S. thesis, University of Arizona, Tucson, 1974). This study suggests that the sediment reservoir allows enteric and pathogenic bacteria to survive, possibly for several months; thus, resuspension and human ingestion in primary-contact waters is a real possibility. Resuspension of bacteria may account, in part, for the erratic FC levels often encountered in water monitoring programs, since grab samples of water would give only an immediate picture of bacterial levels. State bacteriological standards and monitoring procedures currently fail to address these problems. A more meaningful and accurate indication of water-quality conditions would be

obtained by also monitoring indicator bacteria and virus levels in surface sediments.

ACKNOWLEDGMENTS

This work was supported primarily by the U.S. Army Corps of Engineers Environmental and Water Quality Operational Studies Program. Partial funding was provided by the University of Texas at Dallas.

We thank John Seamans and Bob Meglin for assistance in model development.

LITERATURE CITED

1. **Andre, P. A., H. H. Weiser, and G. W. Malaney.** 1967. Survival of bacterial enteric pathogens in farm pond water. J. Am. Water Works Assoc. **59**:503–508.
2. **Beard, P. J.** 1940. Longevity of *Eberthella typhosus* in various soils. Am. J. Public Health **30**:1077–1082.
3. **Bissonnette, G. K., J. J. Jezeski, G. A. McFeters, and D. G. Stuart.** 1975. Influence of environmental stress on enumeration of indicator bacteria from natural waters. Appl. Microbiol. **29**:186–194.
4. **Bremner, M. J.** 1965. Total nitrogen, p. 1324–1345. *In* C. A. Black (ed.), Methods of soil analysis, part 2. American Society of Agronomy, Madison, Wis.
5. **Chan, K.-Y., S. H. Wong, and C. Y. Mak.** 1979. Effects of bottom sediments on the survival of *Enterobacter aerogenes* in seawater. Mar. Pollut. Bull. **10**:205–210.
6. **Danso, S. K. A., M. Habte, and M. Alexander.** 1973. Estimating the density of individual bacterial populations introduced into natural ecosystems. Can. J. Microbiol. **19**:1450–1451.
7. **Dawe, L. L., and W. R. Penrose.** 1978. "Bactericidal" property of seawater: death or debilitation? Appl. Environ. Microbiol. **35**:829–833.
8. **Day, P. R.** 1956. Report of the committee on physical analyses, 1954–1955. Soil Sci. Soc. Am. Proc. **20**:167–169.
9. **Faust, M. A., A. E. Aotaky, and M. T. Hargadon.** 1975. Effect of physical parameters on the in situ survival of *Escherichia coli* MC-6 in an estuarine environment. Appl. Microbiol. **30**: 800–806.
10. **Gallagher, T. P., and D. F. Spino.** 1968. The significance of coliform bacteria as an indicator of enteric pathogens. Water Res. **2**:169–175.
11. **Geldreich, E. E., J. M. Allen, and R. H. Taylor.** 1978. Interferences to coliform detection in potable water supplies, p. 13–20. *In* C. W. Hendricks (ed.), Evaluation of the microbiology standards for drinking water. EPA-570/9-78-OOC. U.S. Environmental Protection Agency, Washington, D.C.
12. **Geldreich, E. E., L. C. Best, B. A. Kenner, and D. J. Van Donsel.** 1968. The bacteriological aspects of stormwater pollution. J. Water Pollut. Control Fed. **40**:1861–1872.
13. **Gerba, C. P., and J. S. McLeod.** 1976. Effect of sediments on the survival of *Escherichia coli* in marine waters. Appl. Environ. Microbiol. **32**:114–120.
14. **Gerba, C. P., E. M. Smith, G. E. Schaiberger, and T. D. Edmond.** 1979. Field evaluation of methods for the detection of enteric viruses in marine sediments, p. 64–75. *In* C. D. Litchfield and P. L. Seyfried (ed.), Methodology for biomass determinations and microbial activities in sediment. American Society for Testing Materials, Philadelphia.
15. **Goyal, S. M., and W. N. Adams.** 1984. Drug-resistant bacteria in continental shelf sediments. Appl. Environ. Microbiol. **48**:861–862.
16. **Greenberg, A. E.** 1956. Survival of enteric organisms in sea water. Public Health Rep. **71**:77–86.
17. **Grimes, D. J.** 1975. Release of sediment-bound fecal coliforms by dredging. Appl. Microbiol. **29**:109–111.
18. **Grimes, D. J.** 1980. Bacteriological water quality effects of hydraulically dredging contaminated upper Mississippi River bottom sediment. Appl. Environ. Microbiol. **39**:782–789.
19. **Gudding, R., and O. Krogstad.** 1975. The persistance of *Escherichia coli* and *Salmonella typhimurium* in fine-grained soil. Acta Agric. Scand. **25**:285–288.
20. **Hendricks, C. W.** 1971. Increased recovery rates of salmonellae from stream bottom sediments versus surface waters. Appl. Microbiol. **21**:379–380.
21. **Hood, M. A., and G. E. Ness.** 1982. Survival of *Vibrio cholerae* and *Escherichia coli* in estuarine waters and sediments. Appl. Environ. Microbiol. **43**:578–584.
22. **Hood, M. A., G. E. Ness, and N. J. Blake.** 1983. Relationship among fecal coliforms, *Escherichia coli*, and *Salmonella* spp. in shellfish. Appl. Environ. Microbiol. **45**:122–126.
23. **Kurath, G., and R. Y. Morita.** 1983. Starvation-survival physiological studies of a marine *Pseudomonas* sp. Appl. Environ. Microbiol. **45**:1206–1211.
24. **LaBelle, R. L., C. P. Gerba, S. M. Goyal, J. L. Melnick, I. Cech, and G. F. Bogdan.** 1980. Relationships between environmental factors, bacterial indicators, and the occurrence of enteric viruses in estuarine sediments. Appl. Environ. Microbiol. **39**:588–596.
25. **LaLiberte, P., and D. J. Grimes.** 1982. Survival of *Escherichia coli* in lake bottom sediment. Appl. Environ. Microbiol. **43**:623–628.
26. **Matson, E. A., S. G. Hornor, and J. D. Buck.** 1978. Pollution indicators and other microorganisms in river sediment. J. Water Pollut. Control Fed. **50**:13–19.
27. **McCoy, E. L., and C. Hagedorn.** 1979. Quantitatively tracing bacterial transport in saturated soil systems. Water Air Soil Pollut. **11**:467–469.
28. **Morita, R. Y.** 1982. Starvation-survival of heterotrophs in the marine environment. Adv. Microb. Ecol. **6**:171–198.
29. **Pellet, S., D. V. Bigley, and D. J. Grimes.** 1983. Distribution of *Pseudomonas aeruginosa* in a riverine ecosystem. Appl. Environ. Microbiol. **45**:328–332.
30. **Roper, M. M., and K. C. Marshall.** 1974. Modification of the interaction between **Escherichia coli** and bacteriophage in saline sediment. Microb. Ecol. **1**:1–13.
31. **Roper, M. M., and K. C. Marshall.** 1978. Effects of salinity on sedimentation and of particulates on survival on bacteria in estuarine habitats. Geomicrobiol J. **1**:103–116.
32. **Rudolfs, W., L. L. Falk, and R. A. Ragotzkie.** 1950. Literature review on the occurrence and survival of enteric pathogenic, and relative organisms in soil, water, sewage, sludges, and on vegetation. Sewage Ind. Wastes **22**:1261–1281.
33. **Sinclair, J. L., and M. Alexander.** 1984. Role of resistance to starvation in bacterial survival in sewage and lake water. Appl. Environ. Microbiol. **48**:410–415.
34. **Temple, K. L., A. K. Camper, and G. S. McFeters.** 1980. Survival of two enterobacteria in feces buried in soil under field conditions. Appl. Environ. Microbiol. **40**:794–797.
35. **Van Donsel, D. J., and E. E. Geldreich.** 1971. Relationships of salmonellae to fecal coliforms in bottom sediments. Water Res. **5**:1079–1087.
36. **Vasconcelos, G. J., and R. G. Swartz.** 1976. Survival of bacteria in seawater using a diffusion chamber apparatus in situ. Appl. Environ. Microbiol. **31**:913–920.
37. **Verstraete, W., and J. P. Voets.** 1976. Comparative study of *Escherichia coli* survival in two aquatic ecosystems. Water Res. **10**:129–136.
38. **Weiss, C. M.** 1951. Adsorption of *Escherichia coli* on river and estuarine silts. Sewage Ind. Wastes **23**:227–237.
39. **Zar, J. H.** 1974. Biostatistical analysis. Prentice-Hall, Inc., Englewood Cliffs, N.J.