APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 1976, p. 537-546
Vol. 32, No. 4

Survival of Coliform Bacteria in Sewage Sludge Applied to a Forest Clearcut and Potential Movement into Groundwater

ROBERT L. EDMONDS
College of Forest Resources, University of Washington, Seattle, Washington 98195

Received for publication 17 February 1976

Anaerobically digested dewatered sludge (10 to 15 cm thick) was applied to a forest clearcut as a fertilizer source in northwest Washington on gravelly glacial outwash soil. This sludge is not microbiologically sterile and may contain pathogenic organisms. Fecal coliform bacterial counts in sludge applied in summer (July) fell from 1.08×10^5 to 358/g in 204 days and to 0/g in 267 days. Dieoff appeared more rapid in winter (January)-applied sludge, when counts fell from 1.2×10^5 to 20/g in 162 days. Initial death rates were related to sludge temperature, moisture, pH, physical composition, and microbial competition. Aftergrowth of fecal coliforms occurred in warm summer and fall months, but counts were of similar magnitude to background levels in forest soils, where a maximum count of 54/g was recorded. Total coliform counts in fresh sludge ranged from 1.4×10^6 to 1.9×10^6/g. Numbers stabilized at 10^3 to 10^4/g in spring, fall, and summer, with lower numbers in winter. Both total and fecal bacteria moved from the sludge to the soil beneath, but few penetrated past the first 5 cm. The soil acts as an effective biological filter. Few fecal coliform bacteria were recorded in the groundwater, generally being less than 5/100 ml and mostly 0/100 ml. A maximum count of 52/100 ml was recorded. Groundwater contamination from vertical movement of potential pathogens appears unlikely, but hazards from surface runoff and direct handling in the first year may arise.

Disposal of sewage sludge into water systems is generally thought to be environmentally unsound, and for some years alternative disposal methods have been tried (9). Such alternatives include composting, land filling, and agricultural fertilization. Use of sludge as a fertilizer has been uncommon in forest ecosystems. Many areas of forest land, however, produce poor-quality forests, particularly those situated on the glacial outwash soils in the Puget Sound region in Washington. Sewage sludge is a potential source of plant nutrients, and thus it is reasonable to believe that forest productivity could be improved by sludge applications.

Anaerobically digested sludge, however, is not microbiologically sterile and may be a potential health hazard. Fecal coliform bacteria counts are high (10^5/g of dry sludge), and the spread of pathogens from the sludge into the soil and groundwater systems is of concern since it is important to maintain the high quality of the spring and river waters in the vicinity of forests.

Numerous field and laboratory experiments indicate that most bacteria are removed after passage through less than a meter of soil and many do not pass the first few centimeters (5, 8). Removal is accomplished by mechanical straining and sedimentation on grain surfaces (5). Most studies of bacterial removal have been conducted in sand, loam, and clay soils. No studies have been conducted on the gravelly outwash soils of the Pacific Northwest, which should provide the least resistance to movement of pathogens. Groundwater systems are usually close to the surface of these soils because of underlying hardpans, and they are generally located in areas of moderate to high rainfall.

Another concern is the length of survival of fecal coliform bacteria in the sludge and the soil beneath, since direct human contact with sludge is possible. There is also a likelihood of pathogen movement through storm runoff. Mallmann and Litsky (6) studied the survival of selected enteric organisms in various soil types. They found that coliform bacteria survived longer in loam and muck soils than in sandy soil. Van Donsel et al. (11) also found that survival of fecal coliform bacteria (*Escherichia coli*) in soil plots varied seasonally.

This study was initiated with the following objectives: (i) to determine the environmental soundness of utilizing dewatered sludge on clearcut areas in forests on gravelly glacial outwash soils; (ii) to determine the length of survival of total and fecal coliform bacteria in the sludge and soil systems in relation to environmental conditions and time of year of application; and (iii) to determine whether fecal coliform bacteria in the sludge are capable of moving into the soil and groundwater systems and eventually into streams.

MATERIALS AND METHODS

Study area. The study area was located at the College of Forest Resources, University of Washington's Pack Forest. Pack Forest is located approximately 110 km south of Seattle near Eatonville, Wash. The specific study plots were located in a 60-ha clearcut area and in an adjacent 45-year-old Douglas fir (*Pseudotsuga menziesii*) forest. The plots were on poor-quality gravelly glacial outwash soil of the Everett series. This coarse soil has a thin A-horizon and a B-horizon containing large rocks up to 25 cm in diameter. The C-horizon also contains large rocks, and an impermeable hardpan occurs at depths varying between 3 and 10 m. The 0- to 15-cm depth of the horizon consists of 82.5% gravel (>2 mm), 10.8% sand, 5.5% silt, and 1.2% clay. The gravel content increases with increasing depth, and organic matter is low. All groundwater reaching the hardpan flows laterally into the Mashel or Nisqually rivers, which meet near the study area, through a series of springs. The springs are located approximately 0.5 km from the edge of the clearcut. Anaerobically digested dewatered sludge (20 to 40% solids) from the city of Seattle's METRO West Point Treatment Plant was transported to the clearcut in tanker trucks. A small pilot plot was set up in November 1972, and large-scale delivery commenced in December 1973. A total of 55,000 m^3 of sludge was applied to 22 ha of the clearcut up to August 1975.

Four plots were set up in the clearcut and one in the forest area. In plot 1 (clearcut), 10 cm of sludge was applied in November 1972; in plot 2 (clearcut), 10 cm of sludge was applied in January 1974; in plot 3 (clearcut), 15 cm of sludge was applied in July 1974; plot 4 (clearcut) was a control (no sludge); and plot 5 (forest) contained no sludge. Plots 2 and 3 were raked and spread by bulldozer in September 1974 in order to enhance drying and decomposition, and tree seedlings were plant in winter 1975. Plot 1 was planted in 1973 and received a herbicide (Atrazine) treatment in summer 1974.

Sampling of sludge, soil, and water for coliform bacteria. Sewage sludge, the soil beneath the sludge, and control soils in the clearcut and forest were sampled at approximately 2-month intervals for a 12-month period commencing 29 July 1974. Some earlier samples were taken in plots 1 and 2. A final sample in all plots was taken in November 1975. In each plot, a hole was dug with a shovel at three locations (replications) exposing both the sludge and the soil beneath it. Five samples were taken around the sides of each hole 5 cm below the sludge surface with an alcohol-sterilized and flamed trowel. These five samples were pooled in a gas-sterilized Whirlpak plastic bag. The soil beneath the sludge was sampled in a similar way 5 cm below the soil/sludge interface. The soil was sampled first in order to avoid contamination from above. Soil in the control plots was sampled 5 cm below the litter/soil interface.

Groundwater was sampled in a well drilled before sludge application beneath the sludge area at a depth of 8 m, in a spring draining into the Mashel River from beneath the sludge area (spring 1) 90 m from the sludge boundary and in a spring 30 m from the clearcut boundary draining into the Nisqually River that did not drain beneath the sludge (control spring 2). Water samples were collected monthly starting in January 1974. In addition, soil solution samples were taken intermittently, when flows allowed, from 15-cm-diameter tension lysimeter plates (0.1 atm of suction) developed by Cole and Gessel (2) at a depth of 180 cm. Sludge, soil, and water samples were transferred as soon as possible to the laboratory, and total and fecal coliform counts were determined by using serial dilutions and the most-probable-number technique (1). Sludge and soil moisture contents were determined gravimetrically on a wet-weight basis, using the following expression:

$$\text{percent moisture content} = \frac{\text{g of wet} - \text{g of dry}}{\text{g of wet}} \times 100$$

Bacterial counts were expressed on a dry-weight basis. Bacterial counts in water samples were expressed per 100 ml.

Determination of rainfall, soil, and sludge temperatures and pH. Rainfall was determined with a raingage set in the approximate center of the clearcut. Soil temperatures in the clearcut without sludge were measured continuously with a thermograph (The Instrument Co., Baltimore, Md., no. 5-1100). The thermograph probe was set 8 cm beneath the soil surface. Spot checks of soil and sludge temperatures were carried out with a soil thermometer (Weston, model 4303). In the period 17 July 1975 to 1 October 1975, sludge temperatures in plot 3 were monitored in a similar manner to the clearcut soil with a thermograph (Kahlsico, model 38AM580, two probe; Kahl Instrument Co., El Cajon, Calif.). Temperatures were monitored in an adjacent 15-cm-deep fresh sludge pile with the same thermograph using the second probe. Soil and sludge pH were determined with a Fisher Accumet pH meter in a 2:1 distilled water-soil or sludge slurry.

RESULTS

Survival of bacteria. Initial total coliform counts in the sludge were approximately 10^6/g (Table 1). The quality of the sludges dumped varied throughout the study period, however, with the November 1972 and January 1974 sludges containing more sand than the sludge dumped in July 1974.

Total coliform counts in the sludge applied in summer (July) were reduced from 1.96×10^6 to 7.20×10^4/g after 204 days (Table 1). Counts then rose in the spring and fell again in summer, finally reaching a level of 2.04×10^3/g after 469 days (approximately 15.5 months). Fecal coliform counts fell more rapidly, and after 204 days counts had fallen from 1.08×10^5/g to 358/g. Counts then went to zero in the winter months and increased in the spring, summer, and fall, finally reaching a count of 147/g on 10 November 1975 (469 days). Survival of coliform bacteria that had moved from the sludge to the soil beneath showed a similar seasonal trend, but numbers were generally lower (Table 1). Table 1 also indicates that sampling variability was high because the standard errors of the means were high.

In the sludge applied in winter (January), total and fecal coliform counts dropped to 709 and 20/g, respectively, after 162 days (Table 2). An increase in total coliform counts then occurred in late summer and fall, followed by a winter decrease, spring increase, summer decrease, and fall increase again. Fecal counts remained at zero except for the spring increase to 153/g. Table 3 shows percent survival of fecal coliforms as a function of time in sludge applied in both winter and summer.

Total coliform counts in the soil beneath the sludge were lowest in the spring, summer, and early fall and highest in the late fall and winter, when a maximum count of 1.05×10^5/g was recorded. Fecal coliform counts in the soil were essentially zero throughout the study period (Table 2).

To assess long-term survival and seasonal trends, the sludge and the soil beneath the sludge applied in November 1972 were sampled. Total coliform counts were still high in the sludge, with numbers as high as 5.42×10^4/g (Table 4). Counts were low in the winter months, but as spring approached they increased. A slight decrease occurred in late sum-

TABLE 1. *Fecal and total coliform bacteria (mean ± standard error) in sludge and soil beneath sludge applied in July 1974*

Sampling date	Days	Sludge		Soil	
		TC[a]	FC[b]	TC	FC
1974					
29 July	0	$(1.96 \pm 1.47) \times 10^6$	$(1.08 \pm 0.99) \times 10^5$	20.0	0.3
30 Sept.	64	$(1.68 \pm 1.56) \times 10^5$	$(2.21 \pm 2.13) \times 10^4$	$(9.66 \pm 9.55) \times 10^5$	981 ± 947
9 Dec.	134	$(4.71 \pm 2.52) \times 10^5$	$(1.09 \pm 0.51) \times 10^4$	$(9.94 \pm 9.86) \times 10^4$	45 ± 38
1975					
18 Feb.	204	$(7.20 \pm 5.92) \times 10^4$	358.0 ± 352.0	$(2.56 \pm 1.55) \times 10^3$	0.3 ± 0.3
22 Apr.	267	$(3.44 \pm 3.23) \times 10^5$	0.0	$(1.35 \pm 0.95) \times 10^3$	0.0
30 June	336	$(1.58 \pm 1.58) \times 10^4$	28.0 ± 28.0	$(1.08 \pm 0.83) \times 10^4$	3.5
30 July	366	$(2.35 \pm 2.23) \times 10^4$	6.4 ± 6.0		
10 Nov.	469	$(2.04 \pm 1.68) \times 10^3$	147.0 ± 147.0	62.0 ± 41.0	0.2 ± 0.1

[a] TC, Total coliforms/gram of dry sludge or soil.
[b] FC, Fecal coliforms/gram of dry sludge or soil.

TABLE 2. *Fecal and total coliform bacteria (mean ± standard error) in sludge and soil beneath sludge applied in January 1974*

Sampling date	Days	Sludge		Soil	
		TC[a]	FC[b]	TC	FC
1974					
1 Jan.	0	$(1.40 \pm 1.32) \times 10^6$	$(1.20 \pm 1.10) \times 10^5$		
11 June	162	709.0 ± 600.0	20.0 ± 10.0		
29 July	210	$(4.16 \pm 3.33) \times 10^4$	43.0 ± 43.0	232.0	0.0
30 Sept.	273	$(6.38 \pm 6.36) \times 10^3$	195.0 ± 195.0	176.0 ± 97.0	0.2 ± 0.2
1975					
9 Dec.	343	$(5.42 \pm 4.40) \times 10^4$	0.0	$(1.05 \pm 0.80) \times 10^5$	0.0
18 Feb.	414	23.0 ± 13.0	0.0	$(1.98 \pm 0.82) \times 10^4$	0.0
22 Apr.	477	$(1.40 \pm 0.55) \times 10^3$	153.0 ± 72.0	137.0 ± 98.0	0.0
30 June	546	6.0 ± 3.0	0.0	11.0 ± 8.0	0.0
10 Nov.	648	$(1.60 \pm 1.27) \times 10^3$	0.0	258.0 ± 205.0	0.0

[a, b] See Table 1.

mer, followed by a fall increase and a winter decrease. This pattern was similar to that observed for the January 1974 treatment. Total coliform counts in the soil beneath the sludge remained high in 1974, but did not reach high levels in the 1975 samples. Herbicide application in 1975 may have affected numbers. Trends shown in the soil beneath the sludge applied in January 1974, when winter counts were high, were not repeated.

Fecal coliform counts, once they approached zero in the sludge, apparently do not increase, and the highest count recorded in the sludge applied in November 1972 was 25/g (Table 4). Fecal coliform counts in the soil beneath the sludge also remained low.

Table 5 shows the levels of total and fecal coliform bacteria in the forest and clearcut soils without sludge. Numbers were considerably lower than those associated with the sludge, with total coliforms ranging from 2 to 1,886/g in the forest and 0.4 to 310/g in the clearcut. A definite seasonal pattern was observed, with lowest numbers in summer and winter and highest numbers in spring and fall. Fecal coliform counts were generally less than 1/g, with one exception of 54/g in the forest in September 1974.

TABLE 3. *Percent survival of fecal coliform bacteria as a function of time for sludge applied in winter (1 January) and summer (29 July) 1974*

Winter application		Summer application	
Days	Survival (%)	Days	Survival (%)
0	100.0	0	100.0
162	0.02	064	20.46
210	0.04	134	10.09
273	0.16	204	0.33
343	0.0	267	0.0
414	0.0	336	0.03
477	0.13	366	0.01
546	0.0	469	0.14
648	0.0		

The survival of coliform bacteria in a soil or sludge system is related to pH, temperature, and moisture conditions. Table 6 shows the pH of the sludges and soils as a function of time of year. Fresh sludge has a high pH, with the sludge applied in July 1974 having an initial pH of 7.8. However, this high pH rapidly dropped, and after approximately 4 months the pH was 5.6. The pH then rose to 6.1 in February and fell to 5.1 by September. Generally the sludge and the soil beneath the sludge are more acid than either the untreated clearcut and forest soils. The soils beneath the sludge are apparently acidified as a result of the decomposition of the sludge.

The sludge temperature is also important to the survival of coliform bacteria. Spot checks in the late morning and early afternoon, when temperatures were expected to approach their maxima, revealed that the temperatures in the sludge throughout the year were generally higher than the temperatures in the untreated forest and clearcut soils (Table 7). A temperature of 22°C was recorded in the sludge dumped in January 1974, compared with 19°C in the clearcut without sludge. Mean daily temperatures averaged weekly in the soil in the clearcut ranged from 25°C in August 1974 to 4°C in February 1975 (Fig. 1). Although some frosts did occur, the soil did not freeze at the 8-cm depth. The minimum daily temperature was 3°C. Temperatures in the sludge applied in July 1974 and fresh sludge applied in July 1975 were continuously monitored from mid-July to the beginning of October 1975 to determine whether the temperature relationships indi-

TABLE 4. *Fecal and total coliform bacteria (mean ± standard error) in sludge and soil beneath sludge applied in November 1972*

Sampling date	Sludge		Soil	
	TC[a]	FC[b]	TC	FC
1974				
31 May	$(2.60 \pm 2.10) \times 10^3$	0.0	4.10×10^4	0.0
29 July	$(1.85 \pm 1.71) \times 10^4$	0.6 ± 0.6	4.75×10^4	0.0
30 Sept.	$(1.67 \pm 0.81) \times 10^3$	0.0	$(2.54 \pm 1.84) \times 10^3$	0.0
9 Dec.	$(5.42 \pm 4.40) \times 10^4$	25.0 ± 22.0	$(3.86 \pm 3.64) \times 10^3$	9.5 ± 9.2
1975				
18 Feb.	179.0 ± 78.0	4.1 ± 1.4	606.0 ± 428.0	1.4 ± 1.2
22 Apr.	$(1.88 \pm 1.64) \times 10^4$		248.0 ± 4.0	0.0
30 June	2.43 ± 2.08	0.0	22.0 ± 19.0	0.0
24 Nov.	624.0	0.0	307.0	2.8

[a], [b] See Table 1.

TABLE 5. *Fecal and total coliform bacteria (mean ± standard error) in forest and clearcut soils from July 1974 to November 1975*

Sampling date	Forest		Clearcut	
	TC[a]	FC[b]	TC	FC
1974				
29 July	2.0	0.0		0.0
30 Sept.	1886.0 ± 1712.0	54.0 ± 32.0	8.0 ± 7.0	0.0
9 Dec.	118.0 ± 87.0	0.17 ± 0.16	13.0 ± 10.6	0.0
1975				
18 Feb.	39.0 ± 24.0	0.1 ± 0.1	0.4 ± .065	0.0
22 Apr.	5.10 ± 37	0.25 ± 0.25	37.0 ± 16.0	0.25 ± 0.25
30 June	7.0 ± 5.0	0.3 ± 0.15	0.17 ± 0.17	0.0
24 Nov.	43.0 ± 22.0	0.12 ± 0.12	310.0 ± 160.0	0.0

[a, b] See Table 1.

TABLE 6. *pH of sludge and soil at Pack Forest*

Sample	Sampling date							
	7/31/74	12/9/74	2/20/75	4/24/75	6/27/75	8/5/75	8/28/75	9/25/75
Sludge								
Nov. 1972	5.8	6.2	5.7	6.0	6.0	5.5	5.1	5.1
Jan. 1974	6.3	6.0	6.1	6.5	5.6	6.6	6.6	6.5
July 1974	7.8	5.6	6.1	6.1	5.7	5.4	5.4	5.1
Soil								
Beneath Nov. 1972 sludge		6.0	6.0	5.7	5.2			5.0
Beneath Jan. 1974 sludge		5.2	5.8	5.9	5.1			5.5
Beneath July 1974 sludge		6.1	5.5	5.2	5.2	5.3	5.3	4.6
Clearcut		6.2	6.3	6.4	6.2			6.6
Forest		6.0	6.1	6.4	6.2	5.6		6.1

TABLE 7. *Temperature (C) in sludge and soil at Pack Forest*

Sample	Sampling date							
	12/6/74 (10:51, 11:06)[a]	2/13/75 (12:00, 3:10)	4/22/75 (11:35, 13:05)	6/27/75 (1:13, 2:33)	7/17/75 (10:24, 1:56)	7/3/75 (10:02, 11:40)	8/28/75 (11:02, 1:24)	9/25/75 (10:16, 1:30)
Sludge								
Nov. 1972	8	6	10	16	16[b]	16	19	17
Jan. 1974	7	6	11	16	22	16	19	17
July 1974	7	6	8	15	17[b]	16	19	17
Clearcut	7	5	6	14	19	13	19	16
Forest	7	4	5	13	17	12	15	14

[a] Time of sampling.
[b] Temperatures monitored early in the day resulting in lower temperatures than clearcut and January 1974 sludge, which were monitored in the afternoon.

cated by the spot checks were the same over daily periods in the summer. Temperatures in the July 1974 sludge were slightly higher than soil temperatures (Fig. 1), and fresh-sludge temperatures were even higher, reaching a weekly maximum of 26°C. This supported the spot-check temperature data. A maximum daily temperature of 40°C was recorded in the fresh sludge as opposed to a maximum of 32°C in the July 1974 sludge and a similar maximum of 32°C in the soil.

Moisture levels in the sludge also influenced survival. Percent moisture in the sludge applied in July 1974 dropped from its initial monitored value of 54% (wet-weight basis) as drying proceeded in the summer (Fig. 2). However, the moisture content rapidly increased with the onset of the fall rains. The sludge

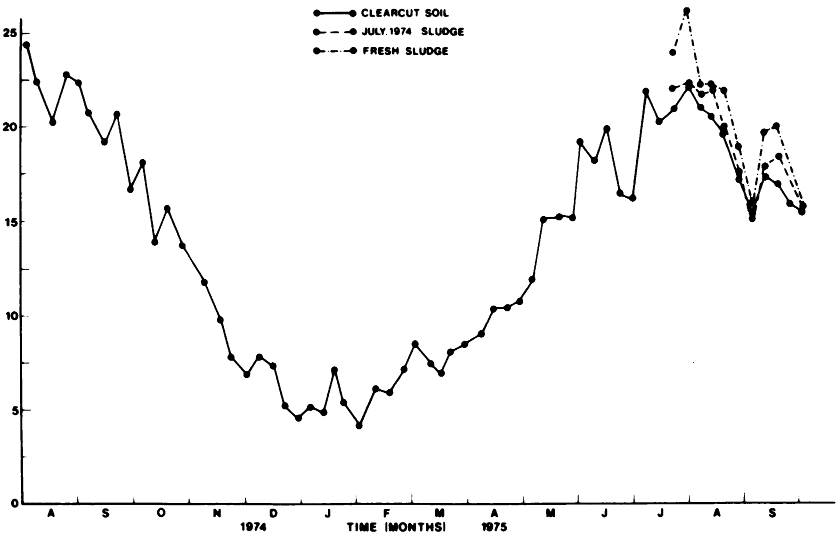

FIG. 1. *Average weekly soil and sludge temperatures in the clearcut at Pack Forest.*

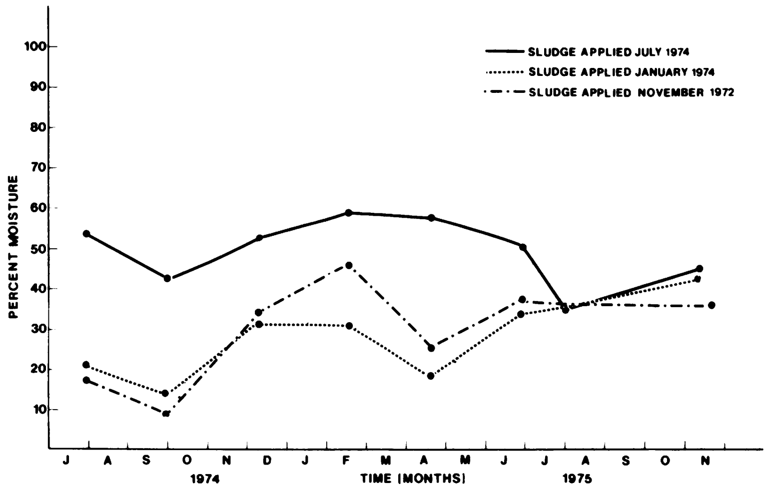

FIG. 2. *Percent moisture content of sludge applied in November 1972, January 1974, and July 1974.*

began to dry again in the spring and summer of 1975 and reached a low of 34% before the moisture content once again increased. The moisture contents of the other sludge treatments showed similar trends, but peaks were lower, perhaps due to the fact that there was more sand in these sludges and decomposition had proceeded further. The moisture contents of these sludges, however, remained higher than the moisture contents of the clearcut soil without sludge (Fig. 3). Figure 3 also shows that the soils beneath the sludges dumped in January and July 1974 were wetter in the summer months and drier in the winter months than the clearcut soil, indicating the hydrophobic nature of the sludge. The moisture content of the forest soil was generally higher than that of all the other soils. Figure 4 shows seasonal rainfall patterns.

Movement of coliform bacteria into soil and groundwater systems. Both fecal and total coliform bacteria moved from the sludge into the soil beneath. Total coliform bacteria increased from 20/g to 9.66×10^5/g of soil in the period from July 29 to 30 September 1975 (Table 1). Fecal coliforms also increased from 0.3 to 981/g of soil in this same period. This was two orders of magnitude less than the numbers in the sludge. Numbers of fecal coliforms were markedly reduced in the December sample to 45/g and went to zero in February.

Data from the tension lysimeter plates indicate that some total and fecal coliform bacteria had penetrated to 180 cm under the sludge applied in July 1974, with 370/100 ml (total) and 160/100 ml (fecal) being detected in January 1975. By 3 February, however, no coliform bacteria were detected. In later samples throughout the year, no fecal coliforms were detected in any lysimeter sample, although total coliforms ranging up to 470/100 ml were detected.

Few coliform bacteria move from the soil into the groundwater system (Table 8). Total coliform bacteria in the well beneath the sludge area reached a maximum of 955/100 ml in October 1975, with numbers being near zero in the summer, spring, and winter months and highest in the period after the fall rains began. Similar trends are apparent in the data for the control spring and the spring draining beneath the sludge area. The control spring had higher counts than the treated spring (maximum of 3,460/100 ml compared with 115/100 ml on 30 September 1974), perhaps due to the fact that the spring water flowed over land and plant surfaces for a short distance before it was sampled.

Fecal coliform counts were generally near zero with the exception of one high count of 52/100 ml in the well (Table 8), which may have been an actual count or contamination.

DISCUSSION

Fecal coliform bacteria present in the sludge at the time of application apparently remain viable for many months, although populations decrease over time in the sludge applied in both summer and winter, with survival being longer in the summer-applied sludge. Standard errors given with the sample means indicate that the variability between replicate samples was large in both the sludge and soil. Emphasis has been placed on trends in the data rather than absolute numbers. Total coliform and fecal coliform colonies were not verified to ensure that the differential counts were correct. Thus, bacterial data concerning groundwater in particular are of a presumptive nature.

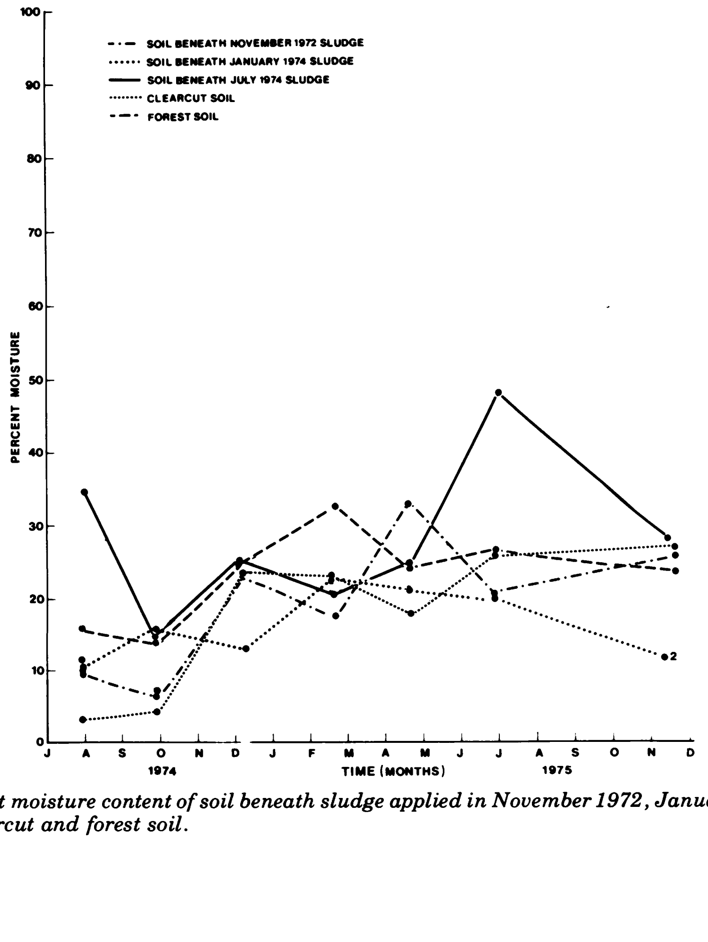

FIG. 3. *Percent moisture content of soil beneath sludge applied in November 1972, January 1974, and July 1974 and of clearcut and forest soil.*

The fecal coliform populations in the July 1974 sludge decreased slowly in the summer and fall so that after 134 days the fecal count was still 1.09×10^4 (Table 1) or 10% of the original (Table 3). However, at the onset of winter the fecal coliform population declined rapidly until after 204 days survival was 0.33% (Table 3), and after 267 days none were detected (Table 1). Fecal coliform survival is influenced by sunlight, temperature, moisture, organic matter, and the presence of competitive organisms (7). Warm summer sludge temperatures (Fig. 1), high moisture levels (Fig. 2), and relatively high pH (>5.6) (Table 6) during the summer and early fall period enabled the fecal coliforms to survive. Cuthbert et al. (3) indicated that fecal coliforms could survive for 110 days at pH 5.5 to 6.3 in soil incubated at 18°C, but death was rapid in very acid peat soil (pH 2.9 to 3.7) in the dark.

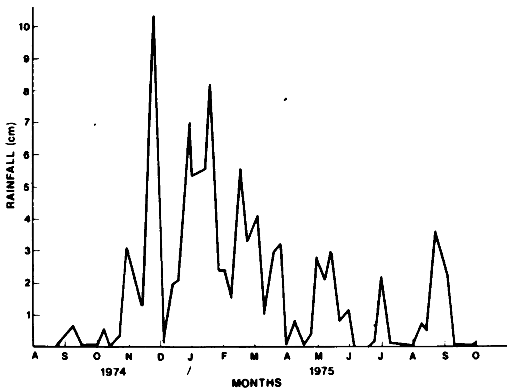

FIG. 4. *Weekly rainfall in the clearcut at Pack Forest.*

Soil temperatures in the sludge were generally higher than those in the clearcut soil (Table 7). Sludge temperatures greater than 25°C in the fresh sludge in the summer (Fig. 1) would enhance fecal coliform survival. Average weekly soil temperatures did not fall below 4°C, and the minimum daily temperature recorded was 3°C. Occasional frosts, however, did occur, and the top layer of sludge was observed to freeze. These cold temperatures no doubt reduced the number of fecal coliforms in winter, but perhaps not as rapidly as would have oc-

TABLE 8. *Total and fecal coliform bacteria counts from spring 1 (draining beneath the sludge), spring 2 (control), and a well beneath the sludge*[a]

Sampling date	Location	Coliform counts/100 ml Total	Fecal	Sampling date	Location	Coliform counts/100 ml Total	Fecal
7 Jan. 1974	Spring 1	0	0	3 Mar. 1975	Spring 1	0	1
13 Feb. 1974	Spring 1	50			Spring 2	52	0
16 Apr. 1974	Well	0	0		Well	3	0
14 May 1974	Spring 1	18	0	2 Apr. 1975	Spring 1	0	0
	Spring 2	18	0		Spring 2	24	0
11 June 1974	Spring 1	18	0		Well	60	0
	Well	18	0	22 Apr. 1975	Spring 1	0	0
9 July 1974	Well	11	0		Spring 2	0	0
23 July 1974	Spring 1	7	0		Well	0	0
	Well	280	52	2 June 1975	Spring 1	0	0
20 Aug. 1974	Spring 1	12	0		Spring 2	52	0
	Well	12	1		Well	0	0
9 Sept. 1974	Spring 1	40	5	30 June 1975	Spring 1	1	0
	Spring 2	165	0		Spring 2	4	0
	Well	210	0		Well	0	0
30 Sept. 1974	Spring 1	115	0	29 July 1975	Spring 1	0	0
	Spring 2	3460	0		Spring 2	170	0
	Well	225	0	3 Sept. 1975	Spring 1	14	0
3 Dec. 1974	Spring 1	49	0		Spring 2	70	0
	Spring 2	640	0	6 Oct. 1975	Spring 1	124	1
	Well	0	0		Spring 2	500	0
6 Jan. 1974	Spring 1	130	0		Well	955	0
	Spring 2	685	0	3 Nov. 1975	Spring 1	27	0
	Well	0	0		Spring 2	330	0
3 Feb. 1975	Spring 1	5	0		Well	0	0
	Spring 2	60	0	1 Dec. 1975	Spring 2	360	0
	Well	10	0		Well	0	0

[a] Sludge dumping in the area began in December 1973.

curred had the soils frozen. Weiser and Osterud (12) indicated that freezing temperatures cause mortality of *E. coli* in culture and repeated freezing and thawing increases mortality. There appeared to be some aftergrowth in the warm spring, summer, and fall in 1975 (Tables 1 and 3), indicating that some fecal coliforms survived the first winter. Increased acidification of the sludge down to pH 5.1 (Table 6), however, would not enhance bacterial survival, perhaps due to microbial competition.

Survival of fecal coliforms in the sludge applied in January 1974 was 0.02% after 162 days (Table 3). Initial survival in the sludge applied in the winter was less than that in summer, probably due to the unfavorable cold temperatures as discussed above. This sludge also contained a small amount of sand from the storage beach at the treatment plant, and this may have also aided in decreasing survival. Mallmann and Litsky (6) noted poorer coliform survival in sandy soils than loamy soils. However, this may be offset by the pH, which remained higher than that of the July 1974 sludge (Table 6).

There did, however, appear to be some aftergrowth of the fecal coliforms in the following warm summer and fall, when counts reached 195/g (Table 2). A winter decline to zero was noted with an increase in spring. Counts returned to zero in the summer and fall of 1975. It would appear that some fecal coliforms were capable of surviving for at least 477 days (Table 3). Some fecal coliforms could still be detected in the November 1972 sludge in the summer, fall, and winter of 1975 (Table 4), with a maximum of 25/g on 9 December 1974.

These numbers, however, approached background numbers of fecal coliforms detected in the clearcut and forest soils uncontaminated with sludge. A maximum count of 54/g was recorded in the forest soil on 30 September 1975 (Table 5). These fecal coliforms are no doubt from a variety of natural sources, including plants and animals. Geldreich et al. (4), for example, found fecal coliforms on a variety of different vegetation types.

Fecal coliforms in the soil beneath the July 1974 sludge were reduced to background levels after 204 days (Table 1), but they could still be detected in the soil beneath the sludge after several years (Table 4). Numbers were approximately the same or slightly higher than background levels (Table 5). Moisture levels in the soil beneath the sludge were slightly higher than levels in the clearcut soil (Fig. 3), and this may enhance survival and reproduction of fecal coliforms.

There was considerably greater reduction in numbers of fecal coliforms than total coliforms moving from the sludge to the soil (Table 1), and this may indicate that the fecal coliforms are poorer competitors than total coliforms in the soil environment.

Van Donsel et al. (11) carried out a series of experiments examining seasonal survival of *E. coli* in exposed and shaded soil plots. They found only 0.001% survival after 65 days. Results from the current study indicate that fecal coliforms in the sludge can survive for longer periods and that the season during which sludge is applied affects the initial survival rates. Van Donsel et al. also found that survival varies with season, although their results differed from the results obtained in this study. They noted that survival in exposed plots was greatest in winter and lowest in summer, during hot, dry conditions. In shaded plots, survival was greater in autumn than winter. However, conditions were still too hot and dry in the summer to allow for good survival.

Within at least 12 months, the fecal coliform bacteria in the sludge and soil beneath the sludge declined to densities approaching levels in the clearcut-area soil. To achieve an initial 99% kill of fecal coliforms required approximately 130 days during the winter and approximately 230 days during the summer (Table 3).

Total coliform bacteria in the sludge showed an initial reduction and then stabilized at 10^3 to 10^4/g in spring, summer, and fall, with lower values in winter (Tables 1, 2, and 4). The numbers in the sludge and soil beneath were several orders of magnitude more than those recorded in the untreated forest and clearcut soils (Table 5). Numbers in the clearcut were generally lower than those found in the forest.

Although fecal coliform bacteria can apparently survive many months in the sludge, very few viable ones appear to move from the sludge to the soil. Of an initial count of 1.08×10^5/g in the July 1974 sludge, only 981/g could be detected on 30 September 1974 (Table 1) after the onset of the rain (Fig. 4). Only 45/g were detected on 9 December, after considerable rainfall in November.

Fecal coliforms were detected in the lysimeter leachates at 180 cm, with 160/100 ml being detected in January 1975. Only on a few occasions were fecal coliforms detected in the well and spring waters draining beneath the sludge (Table 8). A maximum of 52/100 ml was recorded, but other counts were less than 5/100 ml.

These results indicate that few viable fecal coliforms penetrate to soil depths greater than 5 cm beneath the sludge and virtually none reach the groundwater system. These results agree with those of Krone et al. (5) and Pound and Crites (8), which indicated that few bacteria pass the first few centimeters of soil.

Although the movement of viable fecal coliforms from the sludge to the soil was minimal, large numbers of total coliforms (9.66×10^5/g) moved to the 5-cm depth (Table 1). As previously discussed, the low numbers of fecal coliforms may be a result not only of filtration and sedimentation but also of poor competitive ability in the soil.

Few of the total coliforms penetrate to greater depths, with a maximum of 370/100 ml being detected in the lysimeter leachate at 180 cm. Counts of similar magnitude were recorded in the well waters beneath the sludge (0 to 955/100 ml) (Table 8) and spring 1 (0 to 130/100 ml). Interestingly, higher counts for total coliforms were observed from the control spring 2 (0 to 3,460/100 ml). Spring 2 runs over vegetation and soil, and this no doubt accounts for the higher numbers in the control spring. In Montana, Stuart et al. (10) found that the total and fecal coliform counts were higher in a mountain stream from a watershed closed to public use than in a stream from a similar watershed that was open to public use. Animal contamination was assumed to account for the higher total and fecal coliform counts.

The seasonality in the total coliform counts in the groundwater, with high numbers in the fall and low numbers in the winter (Table 8), corresponds to the activity in the soil and sludge above. Most of the movement of water through the soil profile occurs in the winter months, when the rainfall is high (Fig. 4) and bacterial activity is low.

Thus it appears that although fecal and total coliforms can survive in the sludge for long periods of time, there is very little movement of either into the groundwater systems through the gravelly outwash soil. This soil is effective in acting as a biological filter for potentially hazardous pathogens. Very little danger exists for contamination of groundwater and streams due to vertical movement of bacteria. However, because fecal coliforms can remain viable in the surface layers, stormwater runoff and direct contact with contaminated soil could still pose a health problem.

ACKNOWLEDGMENTS

Sincere thanks go to the Municipality of Metropolitan Seattle (METRO) for funds to conduct this study, to Ray Dalseg of the METRO Water Quality Laboratory for all coliform analysis, and to Hans Riekerk, College of Forest Resources, University of Washington, for collecting spring, well water, and lysimeter leachates involved in this investigation.

LITERATURE CITED

1. **American Public Health Association.** 1965. Standard methods for examination of water and wastewater, 12th ed. American Public Health Association Inc., New York.
2. **Cole, D. W., and S. P. Gessel.** 1968. Cedar River research – a program for studying pathways, rates, and processes of elemental cycling in a forest ecosystem. Forest Resources monogr. no. 4. University of Washington, Seattle.
3. **Cuthbert, W. A., J. J. Panes, and E. C. Hill.** 1955. Survival of *Bacterium coli* Type I and *Streptococcus faecalis* in various soils. J. Appl. Bacteriol. 18:408–414.
4. **Geldreich, E. E., B. A. Kenner, and P. W. Kabler.** 1964. Occurrence of coliforms, fecal coliforms, and streptococci on vegetation and insects. Appl. Microbiol. 12:63–69.
5. **Krone, R. B., G. T. Orlob, and C. Hodgkinson.** 1958. Movement of coliform bacteria through porous media. Sewage Ind. Wastes 30:1–13.
6. **Mallmann, W. L., and W. Litsky.** 1951. Survival of selected enteric organisms in various types of soil. Am. J. Public Health 41:38–44.
7. **Miller, R. H.** 1973. The soil as a biological filter, p. 71–94. *In* W. E. Sopper and L. T. Kardos (ed.), Recycling treated municipal wastewater and sludge through forest and cropland. Pennsylvania State University Press, University Park.
8. **Pound, C. E., and R. W. Crites (ed.).** 1973. Wastewater treatment and reuse by land application, vol 11. Environmental Protection Technical Ser., EPA-660/2-73-0066. EPA Office of Research and Development, Washington, D.C.
9. **Sopper, M. E., and L. T. Kardos (ed.).** 1973. Recycling treated municipal wastewater and sludge through forest and cropland. Pennsylvania State University Press, University Park.
10. **Stuart, D. G., G. K. Bissonnette, T. D. Goodrich, and W. G. Walter.** 1971. Effects of multiple use on water quality of high-mountain watersheds: bacteriological investigations on mountain streams. Appl. Microbiol. 22:1048–1054.
11. **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.
12. **Weiser, R. S., and C. M. Osterud.** 1945. Studies on the death of bacteria at low temperature. I. The influence of the freezing temperature, repeated fluctuations of temperature, and the exposure to freezing temperatures in the mortality of *Escherichia coli*. J. Bacteriol. 50:413–439.