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Project Summary

Isolation, Characterization, and Identification of Microorganisms from Laboratory and Full-Scale Landfills

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Studies were conducted to determine whether solid wastes in landfills contain human pathogens and whether these organisms survive landfill conditions and drain out with the leachate. Pilot-scale and commercial-sized landfill operations were evaluated, along with laboratory-sized lysimeters containing municipal solid waste, hospital waste, and sewage sludge.

Total and fecal coliforms in initial leachate tended to decrease rapidly with time, whereas the numbers of fecal streptococci decreased at a slower rate. These indicator organisms were generally below detectable limits in leachates after a year, but specific microorganisms (including pathogens) could be isolated from these leachates and from the solid wastes.

This Project Summary was developed by EPA's Municipal Environmental Research Laboratory, Cincinnati, OH, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

One of the concerns of landfill researchers is whether or not pathogenic microorganisms present in solid wastes are able to survive landfill conditions and drain with the leachate through the underlying soil to groundwater or to surface waters. To demonstrate how such migration could occur, it is first necessary to determine that municipal solid wastes contain infectious microorganisms, then to evaluate the evidence for their survival both in newly deposited landfills and those several years old, and finally to show that microbes could pass to groundwater below. If these microorganisms could survive for long periods of time in the landfill, they might be resistant enough to survive in leachate leaving the landfill and would be able to pollute the surrounding environment. Consequently, the working hypothesis for this study was that the pathogenic microorganisms present in solid waste could survive landfill conditions and can drain out with the leachate and contaminate the surrounding waters.

Experimental Approach

To carry out these objectives, the following experimental approach was taken: Methods were developed (Phase I), simulated landfills were studied (Phase II), and field studies were conducted on full-scale landfills (Phase III). Phase I examined problems such as the freezing of leachate samples, leachate toxicity, most probable number (MPN) test, nonspecificity, and bacterial stress resulting from leachate toxicity. Methods development involved (1) improving a *Salmonella* enrichment medium, (2) concentrating leachate for the enumeration of *Mycobacterium*, (3) a comparison of eight streptococcal enumeration procedures, (4) adapting clostridial enumeration procedures, and (5) developing methane-utilizing and methane-producing bacterial procedures. In Phase

II, six lysimeters were constructed and placed at the University of Cincinnati to test the survival of pathogens in various combination of hospital, sewage sludge, and municipal solid wastes. After 2 to 3 years, these six lysimeters were opened and the solid wastes within were examined for surviving fecal indicator bacteria and pathogens. In addition, leachates were collected and examined at experimental lysimeters at Boone County, Kentucky, and at the EPA Center Hill Facility in Cincinnati, Ohio. In Phase III, the leachate from a commercial landfill was used to isolate streptococci, salmonellae, mycobacteria, and clostridia. Also, closure studies at the Boone County landfill yielded an opportunity to compare similar lysimeter studies made previously. Finally, assays for methane-utilizing and methane-producing bacteria were made on leachate and on solid waste from this same landfill. Tables 1 and 2 describe the landfill sites and lysimeters.

Table 2. Description and Purpose of Laboratory Lysimeters

Number	Contents*+	Purpose
A	Sewage sludge	To provide a microbial control for the sewage sludge used in Lysimeter B.
B	Municipal solid waste plus sewage sludge	To determine the impact of municipal sewage sludge additions on the rates of decomposition and formation of gas leachate. Also, to elucidate microbial changes in the leachate.
C	Municipal solid waste	To provide a control for microbial numbers and species
D	Municipal solid waste	Same as Lysimeter C.
E	Municipal solid waste plus hospital waste	To determine the impact of pathogens on municipal solid waste and the ability of pathogens to survive landfill conditions
F	Hospital waste	To provide microbial control for the hospital waste used in Lysimeter E.

*208.2 liters in each 55 gal drum.
+Lysimeters maintained at 20°C in a constant-temperature room.

Methods

Methods selected for this work were initially derived from those in current use. However, these methods did not always work efficiently and were occasionally nonspecific and imprecise. Some of the problems encountered included the following:

1. Growth of fewer microbial colony types from leachate following laboratory freezing of leachate samples,
2. Problems with pure culture isolation,
3. Lack of a stock culture storage system,
4. No growth in higher concentrated leachate (some growth did appear in more dilute portions),
5. Nonspecific tests,
6. No isolation of pathogens from samples containing sewage sludge and other wastes,
7. Variations in streptococci concentrations detected by two or more methods,
8. Lack of comparability of MPN tests with plate counts, and
9. Bacterial stress.

Short-term studies were carried out to analyze the methods and to improve their performance. Though not all of the problems were solved, the analyses strongly suggest that further improvements would greatly increase the pathogen and fecal indicator identifications

Results and Discussion

Boone County and Center Hill Simulated Landfills

Leachate samples were examined periodically from the Boone County and Center Hill simulated municipal solid waste landfills to determine the fecal indicator levels, plate counts, and the identification of pathogenic microorganisms. The fecal indicator and plate count assays are presented in Table 3. The results show that the total and fecal coliforms had died out, but the streptococci remained viable. The eosin methy-

Table 1. Landfill Sampling Sites

Landfill	Leachate or Solid Waste Abbreviation	Dimensions and Placement	Solid Waste Added: Type	Solid Waste Added: Amount	Date Prepared	Date of Last Waste Deposit
Commercial, Full-Scale Operational	CL	600 Acres in Soil	Municipal, hospital commercial	Millions of Tons	1954	Daily (active)
Boone County, Kentucky	BC 1	30' x 149' x 8.5' in soil	Municipal	435 Tons	1971	June 1971 (inactive)
Boone County, Kentucky	BC 2 through 5	12' x 6' Steel Cylinders in Soil	Municipal	2 Tons	1972	August 1972 (inactive)
Center Hill, Cincinnati, Ohio	CH 1 through 19	12' x 6' Steel Cylinders	Municipal, sewage sludge, industrial	2 Tons	1974, 1975	Nov. 1974 & April, 1975 (inactive)
University of Cincinnati, Cincinnati, Ohio	A through F	2' x 2' in Steel 55-gal Drums	Municipal, sewage sludge, hospital	150 lbs	1978	August 1978 (inactive)

Table 3. *Microbial Counts* From Landfill Leachate*

Quantitative Tests	Landfills (Lysimeters)				
	Boone County		Center Hill	Commercial Landfills	
	BC-1 (6 years)δ	BC-2 (5 years)δ	CH-19 (2 years)δ	From Pipes+ (10 years)δ	From Landfill Surface‡ (Weeks ?)δ
MPN/100 ml:					
Total coliforms	< 30	< 30	< 30	< 30	1.8×10^4
Fecal coliforms	< 30	< 30	< 30	< 30	23
Fecal streptococci	1×10^2	< 20	80	50	3.5×10^4
Agar plate counts, CFU/100 ml:					
Blood-aerobic	7.3×10^5	5.0×10^4	1.7×10^5	5.0×10^5	6.2×10^6
Blood-anaerobic	8.0×10^5	$< 10^3$	1.8×10^4	1.9×10^5	1.3×10^5
Brain heart	4.0×10^5	1.0×10^4	6.0×10^5	1.8×10^4	2.7×10^6
Eosin methylene blue	3.0×10^5	1.0×10^4	9.0×10^3	6.0×10^3	5.0×10^6
Inhibitory mold**	1.7×10^4	7.0×10^3	ND++	$< 10^3$	$< 10^3$
KF streptococcal	$< 10^3$	$< 10^3$	7.0×10^2	5.0×10^2	2.5×10^3
Mycosel**	2.0×10^3	10^3	ND++	$< 10^3$	$< 10^3$
Sabouraud**	4.0×10^5	2.0×10^6	2.8×10^4	3.0×10^5	$< 10^3$
Tellurite	5.0×10^4	6.0×10^3	7.0×10^3	5.0×10^2	$< 10^3$

* = *Averages of two or more leachate samples.*
\+ = *The leachate came from pipes deep within the landfill ("old" leachate).*
‡ = *The leachate emerged from the soil at many sites ("fresh" leachate).*
δ = *Age of waste in landfills and lysimeters.*
** = *Incubated at ambient temperature.*
++ = *Not determined.*
? = *Exact time unknown.*

lene blue plate counts included not only the Gram-negative rods but also a number of Gram-positive rods. In addition the KF agar plate counts of 10^2 to 10^3 CFU/100 ml supported the MPN levels of fecal streptococci, which ranged from 10^1 to 10^4 MPN/100 ml. Finally, the fungal levels were among the highest counts found, indicating, perhaps, aerobic conditions or contamination with aerobic materials.

To give some measure of the pathogens that did survive, the microoganisms from the Boone County and Center Hill lysimeters were identified. The leachate bacteria isolated are presented in Table 4. The results demonstrated that most of these isolates were saprophytes such as *Penicillium*, but a few opportunistic pathogens were found. The majority of the streptococcal isolates identified were fecal streptococci, indicating the presence of fecal contamination.

Table 4. *Microorganisms Isolated From Boone County and Center Hill Leachate*

Site	Leachate Source	Microbial Isolates Identification	Number of Strains
Boone County	*TC2 (A-D)*	*Allescheria boydii*	
		Bacillus sp.	
		CDC Ve-1	
		Cephalosporium sp.	
		Corynebacterium sp.	
		Listeria monocytogenes	
		Micrococcus sp.	
		Moraxella sp.	
		Streptococcus durans	2
		Streptococcus, Type Q	8
		Streptococcus faecalis	12
Center Hill	*CH-9*	*Bacillus* sp.	
		Corynebacterium sp.	
		Listeria monocytogenes	
		Sepedonium sp.	
		Streptococcus sp.	
		Yeast	
	CH-19	*Acinetobacter* sp.	
		Alcaligenes sp.	
		Bacillus sp.	
		Clostridium sp.	
		Enterobacter cloacae	
		Fusarium sp.	
		Penicillium sp.	
		Pseudomonas-like	
		Staphylococcus sp.	
		Streptococcus sp.	
		Yeasts	
	CH-14	*Enteroccous*, Type D	8
		Lactic *Streptococcus*	2

University of Cincinnati Lysimeters

Six lysimeters were built at the University of Cincinnati to determine the survival of pathogenic microorganisms in landfills containing mixtures of sewage sludge, municipal solid waste, and hospital waste (see Table 2). These laboratory lysimeters were constructed on August 16, 1978. All lysimeters were incubated at 20°C. Tests to determine the survival of fecal indicators and pathogenic

microorganisms in the leachate were carried out at periodic intervals during a 2- to 3-year period. At the end of the test period, the lysimeters were opened and the contents were examined for microbes.

Microbial analyses of the three types of solid wastes used in the six lysimeters appear in Table 5. All three wastes had high levels of total coliforms, fecal coliforms, and fecal streptococci. The sewage sludge generally showed lower fungal levels than the others. This result would be expected because the microbial population was restricted to those coming from the digestive tract. The municipal solid waste, on the other hand, contained high fungal counts, probably because it contained wastes from more sources (soil, trash, and sweepings, for example).

The ratio of fecal coliforms to fecal streptococci (FC/FS) was used to deter- the origin of the wastes (animal or human). Environmental samples from human fecal material yield FC/FS ratios of >4 whereas animal ratios are <0.7. Sewage sludge resulted in a ratio of 7.27, indicating a human origin. The ratio in municipal solid waste was 0.19, which indicates nonhuman sources, including animals and vegetation. Hospital wastes had a ratio of 1.05, an intermediate value.

The microorganisms isolated and identified from each type of waste are presented in Table 6. Six of the 15 identified genera are listed as pathogens. Most of these bacteria are usually found in feces. Of the six pathogen types listed, three were found in sewage sludge, five in municipal waste, and four in hospital waste. Of the total number of pathogens isolated from each waste, 6 were from sewage sludge, 10 were from municipal waste, and 8 were from hospital waste.

Table 6. *Gram-Negative Bacteria Isolated From Solid Wastes Used in the Construction of the University of Cincinnati Lysimeters*

Bacterial Genera and Species	*Bacterial Numbers Isolated from*		
	Sewage Sludge	*Municipal Waste*	*Hospital Waste*
Oxidase Negative Lactose Fermenters:	*6*	*10*	*7*
Escherichia coli	*8*	*3*	*2*
Enterobacter *sp.*	*7*	*3*	*6*
Klebsiella* *sp.*	*3*	*3*	*5*
Citrobacter *sp.*	*4*	*10*	*11*
Subtotal	*22*	*19*	*24*
Non-lactose Fermenters			
Serratia *sp.*	*4*	+	*4*
Proteus *sp.*	*3*	*10*	*13*
Providencia *sp.*	+	1	1
Salmonella*	+	*3*	*1*
Subtotal	*7*	*14*	*19*
Oxidase Positive Nonfermenters:			
Aeromonas‡ *sp.*	+	*2*	*3*
Flavobacterium *sp*	*1*	+	+
Herellea* *sp*	+	*2*	*2*
Mima* *sp.*	*2*	*1*	+
Moraxella* *sp.*	+	*1*	*1*
Pasteurella hemolytica*	*1*	+	+
Pseudomonas *sp.*	*1*	*2*	*17*
Subtotal	*5*	*8*	*23*

+ *None detected.*
* *These bacteria appear on the Communicable Disease Center (1974) list (i.e., Classification of Etiologic Agents on the Basis of Hazard).*
‡ *A fermenter.*

The fecal indicator tests of the leachates taken over the 2-year span, presented in Table 7, show that most total coliform levels and fecal coliform MPN levels had decreased to <20 MPN/100 ml at the end of the 13th week, and the fecal streptococci had disappeared after 104 weeks.

Microorganisms found in the lysimeter leachate after 2 years are listed in Table 8. These microorganisms should be compared with those in Table 6. The only surviving pathogenic Gram-negative bacillus was *Acinetobacter* sp. Several fungi were found (*Phialophora* sp., *Monosporium* sp., *Aspergillus niger*), but they were not on the CDC list of hazardous agents. These fungi can be found in pathogenic lesions, however.

Final assays were made on the contents of the six lysimeters to determine the fecal indicator levels, plate counts, and the pathogen levels (e.g., *Clostridium*, *Salmonella*, and *Mycobacterium*). Two lysimeters were examined after 2 years, and four were examined after 3 years. An examination of the surface of the waste in lysimeters UC-B through F indicated that the bentonite packed around the edge of the waste and the sides of the lysimeter were intact. As expected, it kept the water away from the sides of the lysimeter and allowed only the center of the deposited solid waste to be moistened by the weekly water additions. Lysimeter UC-A, the sewage sludge lysimeter, had no bentonite to direct the water flow. There the sludge had separated from the sides of the lysimeter by at least 1 cm, and it was obvious that the water had run down into a pocket made between the sludge and the lysimeter wall. The marks made on the wall of the lysimeter indicated that the

Table 5. *Microbial Densities in Three Types of Solid Waste Added to Lysimeters*

Type of Test and Microbe	*Sewage Sludge*	*Hospital Waste*	*Municipal Waste*
Fecal indicator test (MPN/100g)			
Total coliforms	2.8×10^{11}	9.0×10^{10}	7.7×10^{10}
Fecal coliforms	2.4×10^{10}	9.0×10^{10}	4.7×10^{10}
Fecal streptococci	3.3×10^{9}	8.6×10^{10}	2.5×10^{11}
Agar plate counts (CFU/100g)			
Standard methods	1.7×10^{10}	3.8×10^{10}	4.3×10^{11}
Blood - aerobic	4.1×10^{10}	3.9×10^{10}	3.6×10^{11}
Blood - anaerobic	2.9×10^{10}	2.2×10^{10}	3.5×10^{11}
Eosin methylene blue	1.5×10^{10}	3.1×10^{10}	3.4×10^{10}
Inhibitory mold agar	1.0×10^{7}	3.8×10^{8}	6.9×10^{9}
KF streptococcal	3.6×10^{7}	3.0×10^{9}	4.2×10^{10}
Mycosel	3.6×10^{7}	7.5×10^{9}	1.6×10^{9}
Sabouraud	1.4×10^{10}	3.4×10^{10}	2.5×10^{11}
Tellurite	4.3×10^{7}	2.6×10^{8}	6.6×10^{9}

Table 7. *Microbial Concentrations in Leachates Obtained From the UC Laboratory Lysimeters 13 and 104 Weeks After Construction*

Quantitative Tests	UC Laboratory Lysimeters					
	A, sewage sludge	B, MSW plus sewage sludge	C MSW	D MSW	E, MSW plus hospital waste	F, hospital waste
MPN/100 ml:						
Total coliforms:						
13 weeks	20	86	40	<20	<20	<20
104 weeks	< 2	< 2	< 2	< 2	< 2	< 2
Fecal coliforms:						
13 weeks	<20	<20	<20	<20	<20	<20
104 weeks	< 2	< 2	< 2	< 2	< 2	< 2
Fecal streptococci:						
13 weeks	4.9×10^2	1.6×10^4	3.5×10^2	2.3×10^2	2.9×10^4	7.9×10^2
104 weeks	< 2	< 2	< 2	< 2	< 2	< 2
Agar plates, CFU/100 ml:						
Blood-aerobic:						
13 weeks	1.9×10^7	1.2×10^8	6.8×10^7	2.9×10^8	7.6×10^6	1.2×10^7
104 weeks	1.2×10^8	1.3×10^6	1.6×10^6	1.4×10^7	9.5×10^5	3.3×10^5
Blood-anaerobic:						
13 weeks	6.3×10^5	1.1×10^7	8.0×10^7	5.6×10^7	3.1×10^7	2.1×10^8
104 weeks	1.2×10^8	3.3×10^6	3.2×10^6	4.0×10^7	1.7×10^6	2.7×10^6
Standard methods:						
13 weeks	1.4×10^8	5.4×10^7	7.9×10^7	3.1×10^8	1.5×10^7	6.3×10^8
104 weeks	1.0×10^8	1.4×10^6	1.2×10^8	2.2×10^7	2.8×10^7	3.1×10^4
Sabouraud:						
13 weeks	2.2×10^7	4.9×10^7	2.2×10^7	6.6×10^7	4.6×10^7	5.2×10^6
104 weeks	3.1×10^6	4.2×10^5	4.2×10^5	1.7×10^6	3.5×10^5	1.9×10^4
Tellurite:						
13 weeks	1.1×10^6	1.6×10^6	7.8×10^6	4.5×10^7	1.7×10^6	5.9×10^6
104 weeks	4.0×10^6	2.9×10^4	4.0×10^3	1.6×10^4	6.4×10^4	$< 10^3$

Table 8. *The Identification of Leachate Microorganisms Taken From the Lysimeters During the 100th Week of Operation*

Lysimeter Designation	Microbial Group	Identification Name	Number of Cultures
UC-A	*Bacteria*	*Acinetobacter* sp.	2
		Alcaligenes faecalis	2
		Corynebacterium acquaticum	1
		Corynebacterium sp.	1
		Pseudomonas sp.	7
	Fungi	*Monosporium* sp	1
		Phialophora sp.	1
UC-B	*Bacteria*	*Bacillus* sp.	2
		Corynebacterium sp.	4
	Fungi	*Aspergillus niger*	1
		Monosporium sp.	1
		Yeast	1
UC-C	*Fungi*	Yeast	1
UC-D	*Fungi*	*Penicillium* sp.	1
UC-E	*Fungi*	Yeast	1
UC-F	*None*	*None*	0

sludge had settled about 3 cm during the 3 years.

The compactness of density of the waste was also examined. This parameter varied with the type of waste. The sewage sludge lysimeter waste appeared to have the consistency of pudding. The waste was easily scooped out during the sampling process. The other waste lysimeters were compacted, and all required considerable digging to remove the samples. For the most part, the waste did not appear to have decomposed or changed to any great degree. However, the compacted waste tended to become soil-like 30 cm from the surface of the waste—that is, it was gritty and had a darker hue compared with the waste on the surface.

The results of the assays on the solid waste samples contained within the UC-F (hospital waste) and UC-D (MSW) lysimeters, which were opened after 2 years, are listed in Tables 9 and 10. Levels of indicator organisms were higher in the upper solid waste layers than in samples taken at lower depths. An association appears to exist between microbial numbers and pH, especially in the MSW lysimeter. The bacterial concentration generally decreased with the decrease in pH levels. These results demonstrated that the coliforms had not died as was indicated by the low fecal indicator levels in the leachate; rather, they had survived for more than 2 years. The lower pH in the lower sections of the solid waste deposited within the lysimeters may have resulted from the carbon dioxide given off during the microbial fermentation of the waste materials. Pathogens isolated directly from the wastes were *Clostridium botulinum, Clostridium tetani, Klebsiella* sp., *Acinetobacter* sp., and *Monosporium apiospermum.*

Commercial and Full-Scale Lysimeters

Leachate samples were examined for fecal indicator levels and plate counts at an active commercial landfill and an inactive full-scale landfill at Boone County, Kentucky (see Table 1). The results are included in Table 3. The fecal indicator levels, (i.e., the total coliforms, fecal coliforms, and the fecal streptococci) from the Boone County leachate were

Table 9. *Microbial Levels in Solid Waste From a 2-Year-Old Lysimeter Containing Hospital Waste*

Quantitative Tests	Lysimeter Contents at Descending Levels		
	10 cm	20 cm	30 cm
MPN/100 g (100 ml):			
Total coliforms	2.2×10^4	9.2×10^3	1.6×10^2
Fecal coliforms	20	< 20	< 20
Fecal streptococci	3.4×10^2	1.1×10^3	70
Agar plate counts CFU/100 g (100 ml):			
Standard Methods	6.0×10^7	6.0×10^5	4.3×10^4
Chocolate (aerobic)	4.1×10^7	4.8×10^5	8.2×10^4
Chocolate (anaerobic)	1×10^7	7.1×10^7	8.0×10^6
PEA* (aerobic)	2.1×10^5	2.9×10^5	3.1×10^3
PEA (anaerobic)	5.4×10^6	5.8×10^6	1.6×10^5
Blood (aerobic)	1.7×10^6	1.1×10^6	2.7×10^4
MacConkey (aerobic)	9.3×10^6	4.2×10^4	6.0×10^3
MacConkey (anaerobic)	1.2×10^5	$<1 \times 10^3$	$<1 \times 10^3$
IMA	4.2×10^5	5×10^3	1.6×10^4
M-enterococcus	1.2×10^4	$< 1 \times 10^3$	$< 1 \times 10^3$
Sabouraud	3.9×10^7	1.7×10^5	1.2×10^5
Tellurite	2.0×10^4	$<1 \times 10^3$	$<1 \times 10^3$
Chemical Tests			
pH	5.15	5.2	4.95
Conductivity, μmho/cm	1519	874	826
Moisture, %	61.5	58.0	61.9

* Phenylethyl alcohol agar without blood.

Table 10. *Microbial Levels of Solid Waste From a 2-Year-Old Lysimeter Containing Municipal Solid Waste*

Quantitative Tests	Lysimeter Contents at Descending Levels		
	10 cm	20 cm	30 cm
MPN/100 g (100 ml):			
Total Coliforms	1.3×10^3	2.2×10^3	20
Fecal Coliforms	90	< 20	< 20
Fecal Streptococci	7.1×10^4	2.4×10^3	20
Agar plate counts, CFU/100 g (100 ml):			
Standard Methods	1.0×10^9	2.6×10^8	2.3×10^7
Blood Agar (aerobic)	2.5×10^9	2.3×10^8	4.7×10^7
Blood Agar (anaerobic)	spreader	spreader	spreader
PEA* (aerobic)	2.7×10^8	2.2×10^8	4.1×10^7
PEA (anaerobic)	3.5×10^7	1.5×10^7	4.9×10^6
MacConkey	$<1.0 \times 10^3$	1.0×10^3	$<1 \times 10^3$
IMA	4.0×10^3	2.0×10^3	$<1 \times 10^3$
Sabouraud	$<1.0 \times 10^3$	$<1 \times 10^3$	$<1 \times 10^2$
Tellurite	3.5×10^7	6.1×10^6	1.7×10^7
Chemical Tests:			
pH	7.1	6.2	5.15
Conductivity, μmho/cm	1780	1940	2230
Moisture, %	69.65	65.34	64.09

* Phenylethyl alcohol agar without blood.

usually 10^2 MPN/100 ml or less. The coliforms had apparently died out, but the streptococci remained present on an inconsistent basis. The Boone County landfill was 6 years old when these determinations were made.

On the other hand, the commercial landfill had fecal indicator levels ranging from 10^2 to 10^4 MPN/100 ml of leachate. Possibly these levels were higher because this was an active landfill and received waste daily. Later it was found that the CL-4 leachate levels decreased steadily whereas those from the three leachate springs (sites CL-1, CL-2, and CL-3) had higher levels of total coliforms, fecal coliforms, and fecal streptococci. The main difference was apparently that the CL-4 leachate came from a 10-year-old waste site by means of a pipe placed deep within that waste. No new waste had been placed on top of it since it was deposited. On the other hand, CL-1, CL-2, and CL-3 leachate samples apparently came from the new waste deposited daily on top of the landfill. This leachate drained downward and emerged from shallow surface leachate springs.

The plate counts are also listed in Table 3. One series of counts is from the Boone County BC-1 leachate measurements, and two are from the commercial landfill (i.e., from the older waste and from the newer waste described above). The Boone County leachate microorganisms had higher fungal and Gram-positive bacillus counts and thus were similar to the older commercial landfill leachate levels. The distinguishing marks of the newer leachate (commercial landfill) were the low levels of Gram-positive bacilli and fungi along with higher streptococcal levels as determined by the KF stretococcal plates. Finally, the aerobic blood agar plates had higher counts than the anaerobic plates, indicating that few anaerobes were present.

The identified microorganisms from the commercial landfill leachates include the following:

*Acinetobacter** sp.
Aspergillus niger
Cephalosporium sp.
Clostridium perfringens
Enterobactor agglomerans
Enterobacter cloacae
Enterobacter sp.
Fusarium sp.
*Mycobacterium** sp.
Neurospora sp.
Pencillium sp.
Proteus sp.
Providencia alcalifaciens
Providencia sp.
Pseudomonas sp.
Pseudomonas fluorescens
*Salmonella** sp.
Streptococcus faecalis

The bacteria significant to public health are marked with an asterisk. They are agents of ordinary potential hazard, such as staphylococci, that can cause disease when the agent penetrates the skin.

Solid Waste and Leachate From a Landfill at Closure

A 9-year-old landfill site was examined before closure in August 1980. The examination at various depths of the landfill solid wastes and the leachate yielded unexpectedly high populations of indicator organisms as shown in Table 11. The small numbers of streptococci detected previously in the leachate became significant when the solid wastes from the landfill were examined after excavation. These results show that there were high levels of fecal indicator

Table 11. Fecal Indicator Bacteria Levels in Solid Waste and Leachate From a 9-Year-Old Landfill at Boone County, Test Cell 1

Type of Sample	Samples Collected at These Descending Levels		Fecal Indicator Bacteria MPN(CFU)/100 g or 100 ml		
	Ft	(m)	Total Coliforms	Fecal Coliforms	Total Streptococci
Top soil	1.5	(0.46)	2.4×10^5	3.5×10^3	9.8×10^5
MSW	5.0	(1.52)	1.1×10^2	$<2.0 \times 10^1$	2.0×10^4
MSW	7.0	(2.13)	2.4×10^3	2.0×10^1	6.0×10^4
MSW	8.5	(2.60)	1.6×10^5	2.3×10^2	3.3×10^5
MSW	9.0	(2.74)	5.4×10^4	3.3×10^2	ND
Leachate	10.0	(3.01)	9.2×10^3	3.5×10^2	1.1×10^2
MSW	10.5	(3.20)	3.5×10^4	4.9×10^2	3.5×10^4
Clay liner	11.0	(3.35)	5.0×10^1	$<2.0 \times 10^1$	2.0×10^4
Soil beneath	13.0	(3.96)	5.0×10^1	$<2.0 \times 10^1$	$<2.0 \times 10^4$

organisms in the top 1.5-ft (0.046 m) soil sample. Below this, lower levels of fecal indicators occurred at the 5-ft (1.52 m) sample, and higher levels occurred for the rest of the wastes extending to the bottom of the landfill.

The levels of total coliforms, fecal coliforms, and fecal streptococci in all waste materials were relatively high. Results from the solid waste were not consistent with those from leachate samples over the years, which showed essentially no indicator organisms present. The higher levels in the top soil may have resulted from animals. Fecal indicator bacteria survived within the landfill, which suggests the possibility that leachate may transport these bacteria to the groundwater if the liner materials were penetrated. The clay liner and the soil beneath the plastic liner, however, showed that few bacterial indicators were present. These last assays demonstrated that the microbe-containing leachate was not able to penetrate the clay liner and soil below to contaminate the groundwater.

Tests for specific microorganisms in the solid waste included *Acinetobacter* sp., *Moraxella* sp., *Salmonella* sp., and *Klebsiella pneumoniae*— all pathogens.

Summary and Conclusions

Methods were modified by applying the bacterial stress procedures to bring about the improved enumeration and isolation of coliforms, streptococci, *Mycobacterium, Salmonella,* and *Clostridium* species.

Newly constructed experimental lysimeters containing MSW, hospital waste, and sewage sludge were studied for 3 years. Indicator organism densities and specific bacteria found were similar for all three types of solid waste added to the lysimeters. Total and fecal coliforms in the leachate decreased to <20 MPN/100 ml by the 13th week, the fecal streptococci disappeared after 2 years. However, several bacteria and fungi were isolated from the leachate after 2 years.

Leachate samples were collected from both active and inactive areas of a large commercial landfill. Total coliforms and fecal streptococci in the active seepage was on the order of 10^4 per 100 ml, with fewer fecal coliforms, whereas leachate from the inactive portion generally was free of indicator organisms. Total plate counts were generally one or two orders of magnitude higher for the active area compared with the inactive area. Numerous bacteria and fungi were isolated and identified from the commercial landfill leachate, including a number of pathogens.

Relatively low levels of indicator organisms were found in experimental lysimeter leachates that had been studied for a number of years. One 9-year-old site was examined closely at closure. Positive values were found at various depths for total coliforms, fecal coliforms, and fecal streptococci. This result shows that low levels of microbes in leachates do not always mean that these organisms are absent within the solid waste.

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The complete report, entitled "Isolation, Characterization, and Identification of Microorganisms from Laboratory and Full-Scale Landfills," (Order No. PB 84-212 737; Cost: $35.50, subject to change) will be available only from:
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