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Bacterial Abundance and Activity in Hazardous Waste-Contaminated Soil

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The Office of Technology Assessment has estimated that there may be as many as 10,000 abandoned hazardous waste disposal sites across the United States, many of which have the potential to adversely impact drinking water supplies (OTA, 1985). Of the many options available for the remediation of these sites, the use of microorganisms to degrade organic chemicals offers many advantages in terms of speed, efficiency and cost (Lee and Ward, 1985). Bioremediation may involve either the augmentation of the ability of the *in situ* microorganisms to utilize the waste or the application of microorganisms acclimated to degrade particular wastes or combinations of wastes.

Bioremediation has been used with success to treat soils contaminated by kerosene and other hydrocarbon products (Dibble and Bartha, 1979; Bossert et al., 1984). In these studies, the indigenous soil populations of the contaminated soils were effective in removing the spilled hydrocarbons and returning the soil to productivity. The effectiveness of bioremediation for nonhydrocarbon compounds or the complex mixtures of chemicals likely to occur at abandoned hazardous waste sites has received less attention. The present study was designed to determine the activity of indigenous microbial populations at a hazardous waste site, their degree of adaptation to toxic chemicals present in the waste mixture, and their ability to degrade toxic organic compounds, using phenol as a model substrate.

MATERIALS AND METHODS

Samples of soil cores for microbiological analysis were obtained from a hazardous waste site in Northern Indiana. The site is located in an area of low ground surrounded by wetlands. The surface deposits consist of sand intermixed with waste materials. The water table is high, usually within 3-5 feet of the surface. Wastes from used construction debris such as timbers, concrete blocks and bricks were deposited in low areas on the site with chemical wastes such as used oil, acids and paint solvents and sludges being poured around the debris. Tires and car batteries have also been found on the site. Foundry sand was deposited over the site as a cover material. Samples were collected under sterile conditions and maintained under refrigeration prior to analysis. Five test cores were selected for study.

Results of chemical analysis (*James Hill, personal communication*) of the well water are presented in Table 1. Depths of the water samples were 29, 33, 25, 28 and 25 feet for wells A, B, C, D and E respectively.

Table 1. Summary of chemical analysis of the monitoring wells

Well	Location	Aromatic Hydrocarbons	Chlorinated Solvents	Phenol
A	Offsite	Absent	Absent	Absent
B	On site	Toluene, 80,000 ppm	Methylene chloride, 21,000 ppm	630 ppm[1]
C	On site	Absent	Absent	Absent
D	On site	Toluene detected[2]	Methylene chloride, 14,000 ppm	Absent
E	On site	Toluene 1,100 ppm	Methylene chloride detected[2]	1500 ppm

[1] chlorinated and/or methylphenols also present
[2] could not be quantified

Soil slurries were prepared from the core samples by weighing approximately 25 g of soil (wet weight) and suspending in 250 mL of sterile 0.1% sodium pyrophosphate. Viable heterotrophic bacterial counts were performed by the spread plate technique using three media: half strength nutrient agar (Difco), peptone-yeast extract-tryptone-glucose agar at 1/20 strength (Ghiorse and Balkwill, 1983) and mineral salts medium unsupplemented with a carbon source. The composition of the mineral salts medium was a follows, expressed on a per liter basis: KH_2PO_4, 0.11 g; K_2HPO_4, 0.65 g; NH_4NO_3, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $CaCl_2 \cdot 2H_2O$, 0.025 g; $FeCl_3 \cdot 6H_2O$, 2.5 mg; $MnCl_2 \cdot 4H_2O$, 1 mg; $ZnSO_4 \cdot 7H_2O$, 3.5 mg. Total bacterial counts were performed using acridine orange epifluorescence microscopy according to the method of Scheraga et al. (1979). Heterotrophic activity was determined using a radiolabeled amino acid mixture (ICN, specific activity 50 mCi/m-mole) by the one-concentration method of Gocke (1977). Dry weights were determined on the soil core samples by drying overnight at 102 C. All results have been expressed on a dry weight basis.

Phenol degrading ability was assessed by placing 10 mL of the soil suspension into a serum vial, and adding 50 μL of a solution containing 0.0225 μCi uniformly labeled phenol (ICN, specific activity 43 mCi/mmole) and unlabeled phenol to achieve final concentrations of 0.01, 0.10, 0.25, 1.0, 10, 100, and 250 μg/mL. The serum vials were capped with a rubber stopper into which a collection well had been inserted. The well contained 0.2 mL of 1N KOH absorbed onto a filter paper strip. After 24 hours incubation, the reaction was halted by the addition of 2 mL of 2N H_2SO_4. After overnight incubation, the papers were removed and placed in 15 mL of liquid scintillation cocktail (BudgetSolve, Research Products International Corp.) and counted on a Beckman Model 7000 liquid scintillation counter. Killed controls were run simultaneously with experimental vials and were prepared identically except that the sulfuric acid was added at the beginning of the incubation.

The number of phenol degraders in the soil suspension was estimated by the most probable number (MPN) method of Lehmicke et al. (1979). Dilutions of the soil suspension were placed in five replicate tubes, a phenol stock containing approximately 2000 dpm of radiolabeled phenol was added to each tube (total volume 5 mL), and the tubes were capped by rubber stoppers into which a plastic well had been inserted. After 6 weeks, the reaction was stopped by the addition of 1 mL of 2N H_2SO_4, and 0.2 ml of 1N KOH was added to the filter paper in the center well. After overnight incubation, the paper strips were removed and counted as described above. Tubes were scored positive if the number of counts in the experimental tubes was more than twice the number of counts in a killed suspension tube prepared simultaneously with the experimental tubes but to which the sulfuric acid was added at the beginning of the incubation. Number of phenol degraders was determined according to the tables of Rodina (1972).

RESULTS AND DISCUSSION

Bacterial numbers as determined by viable plate counts ranged from 3×10^5 colony forming units (CFU)/g to 1.7×10^8 CFU/g (Table 2). Although there is a tendency for higher counts to be observed in the upper soil layers, this trend is by no means universal in this set of cores. These differences in CFU may reflect local differences in substrate availability and physical and chemical environment. It is noteworthy that bacteria were more abundant in the cores taken on site when compared to the off-site core. While bacteria in on-site samples would be expected to have been exposed to a mixture of potentially toxic chemicals, this exposure has apparently not had a deleterious effect on bacterial abundance; rather, there seems to have been a stimulation.

Table 2. Bacterial abundance and activity in soil cores

Well and Depth (ft)	Viable Bacterial Numbers (x 10^5 per g)			Total Bacterial Numbers (x 10^5/g)	Heterotrophic Activity (hours)
	Nutrient Agar	PYTG Agar	Mineral Salts Agar		
A-5	33.7	30.1	154	330	4.63
-10	24.2	19.1	12.0	825	33.34
-15	70.7	71.9	32.6	1,190	33.34
-20	8.3	8.1	13.8	213	35.27
B-10	408	590	471	1,130	4.37
-15	206	164	167	5,000	48.75
-20	86	63.8	138	464	25.31
C-5	327	313	877	12,500	6.23
-10	82.1	31.4	95.0	5,540	133.4
-15	472	199	133	7,470	28.21
-20	4.8	5.6	3.1	2,330	1181
D-10	592	370	425	8,670	11.12
-15	529	550	541	9,580	10.91
-20	196	157	159	3,740	17.74
-25	1190	768	1024	9,610	5.51
E-3	1710	696	735	21,600	14.88
-8	230	224	266	8,530	9.60
-13	585	805	524	7,320	25.68

Of the three media used for plate counts, half-strength nutrient agar yielded higher counts than the other two media, although PYTG agar usually gave comparable although generally lower counts. Both of these media represent dilutions of nutrient rich media. Mineral salts medium, which can be characterized as a nutrient poor medium because of its lack of carbon sources other than impurities in the agar, yielded inconsistent counts in comparison to the other two media, sometimes yielding higher counts, sometimes lower. It is probable that these media, and especially mineral salts agar, may be counting different subsets of the bacterial population, and these subsets may make varying contributions to the total community, depending on local conditions.

Total bacterial numbers were higher than CFU by factors which ranged from 2.4 to 583, the exact factor varying with soil core and depth. Such differences between total bacterial numbers and CFU have been observed in other subsurface samples (Ghiorse and Balkwill, 1983) and may reflect differing nutritional requirements of bacterial communities at different locations within the site.

Heterotrophic activity demonstrated a similar degree of heterogeneity with respect to location and depth as did the measures of bacterial abundance. Although there was a tendency for those sites yielding the lowest turnover times to exhibit higher bacterial numbers, this was by no means universal, and may reflect local differences in nutrient supply and growth conditions.

Phenol biodegradation, expressed as the fraction of added phenol utilized in 24 hours (Table 3), is observed to decrease with increasing phenol concentration, indicating inhibition of biodegradation by phenol itself. Thus, both the concentration at which phenol degradation is inhibited and the fraction of added phenol utilized are important parameters to consider in evaluating adaptation of the population to biodegrade phenol. A third parameter to be considered is the number of phenol degraders present in the bacterial community. In respect to these three parameters, well D and the lowest depth of well A demonstrated the highest phenol biodegradation capacity, yet these wells had no detectable levels of phenol. Well E, which had the highest level of phenol, had moderate phenol degrading ability and low numbers of phenol degraders. Well B, which had an intermediate level of phenol present, had no significant phenol biodegradation but moderate numbers of phenol degraders. Wells A and C demonstrated phenol biodegradation capabilities in the uppermost layers, but low numbers of phenol degraders. Thus, no clear relationship exists between the number of degraders present, the extent of phenol biodegradation and the presence of phenol in the well water samples.

It is likely that phenol itself may have reached inhibitory levels at wells B and E and therefore is limiting its own biodegradation. In contrast, phenol concentrations at well D may never have achieved inhibitory concentrations, and so an active population of phenol degraders developed at this site. In addition to phenol, the presence of other organic compounds at wells B and E may be exerting an additional inhibitory effect on phenol biodegradation.

In summary, hazardous waste contaminated soils possess active populations of soil microorganisms, which in this particular case are more abundant and

Table 3. Phenol degrading activity in soil cores

Well and Depth (ft)	Phenol biodegradation: fraction of added phenol utilized at indicated concentrations (ppm)							Number of phenol utilizers x 10^3
	0.01	0.10	0.30	1.0	10	100	250	
A -4.5	0.226	0.271	0.255	0.2390	0	0	0	3.0
-9.5	0.010	0	0	0	0	0	0	6.0
-14.5	0.187	0	0	0	0	0	0	0.9
-19.5	0.128	0.201	0.161	0.056	0.014	0	0	120.0
B -10	0	0	0	0	0	0	0	85.0
-15	0.019	0	0	0	0	0	0	25.0
-20	0.068	0.064	0	0	0	0	0	8.0
C -5	0.100	0.198	0.185	0.118	0.124	0	0	3.0
-10	0	0	0	0	0	0	0	4.5
-15	0	0	0	0	0	0	0	5.0
-20	0	0	0	0	0	0	0	25.0
D -10	0	0	0	0	0	0	0	140.0
-15	0.260	0.285	0.245	0.231	0.112	0.18	0.022	110.0
-20	0.171	0.267	0.254	0.268	0.144	0.008	0	150.0
-25	0.141	0.213	0.204	0.092	0	0	0	50.0
E -3	0.014	0.007	0	0	0	0	0	3.0
-8	0.171	0.170	0.230	0.130	0.198	0	0	14.0
-13	0.147	0.298	0.193	0.179	0.200	0	0	12.0

more active than adjacent, noncontaminated soil. Abundance and activity of bacterial populations varied considerably from site to site and may be related to differences in nutrient status or the presence of inhibitory chemicals. Phenol degrading capacity of the soils varied from location to location and could not be directly correlated with either total numbers of bacteria present, numbers of phenol degrading bacteria present, or phenol concentration at the site. It is proposed that phenol itself as well as other contaminants at the site may be exerting inhibitory effects on bacteria with phenol degrading capabilities. The observed heterogeneity will have to be taken into consideration in implementing bioremediation of contaminated soils.

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