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Biodegradation of dicyclopentadiene in the field

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Abstract

Dicyclopentadiene (DCPD) is formed during the pyrolysis of alkanes to produce olefins suitable for manufacturing synthetic polymers. DCPD has an irritating odor with a 5 ppb detection level that provides the impetus for remediation efforts. One method of destroying odors is to alter the structure of the chemical. This can be accomplished by biological oxidation using microorganisms. Field studies at two sites, where DCPD was a soil contaminant, indicated that biodegradation contributed significantly to DCPD removal. DCPD degradation was stimulated by decreasing bulk soil density and adding nitrogen and phosphorous nutrients. The presence of other easier degradable aromatic hydrocarbons may also be beneficial, suggesting that the process is cometabolic.

Introduction

The production of ethylene from ethane by pyrolysis yields other hydrocarbons as byproducts, which can be fractionated by distillation. One of these fractions is called "py gas" or C5+. The C5+ fraction typically consists of benzene (45% w/w), dicyclopentadiene (13% w/w), cyclopentadiene (7.0% w/w), toluene (6.0% w/w), styrene (3.0% w/w), pent-1-ene (2.5% w/w), cyclopentene (1.7% w/w), isoprene (1.5% w/w), naphthalene (1.3% w/w), 1,3-butadiene (1.1% w/w), indene (1.0% w/w), and others in minor quantities.

Purified DCPD is used for the manufacture of unsaturated polyester resins for marine and electrical applications (Stahl 1995). Production of DCPD in the United States was estimated to be in excess of 70×10^6 kg for 1994 and exports to the United States from Canada were in excess of 1.6×10^6 kg indicating significant transport of DCPD in North America (Stahl 1995). The risk of spilling any commodity increases with increased production and use, which has been increasing continually for DCPD. For this reason, it is important to understand the fate of this chemical in the environment.

The public perception of the severity of DCPD-containing spills is caused by its pungent smell and low odor threshold for DCPD of 5.7 ppb (Amoore & Hautala 1983) or 100 pg (Ventura et al. 1997). Although DCPD has only moderate oral toxicity (Verschueren 1983) its low odor threshold makes water unacceptable for drinking at concentrations in excess of 25 to 50 ppt (Ventura et al. 1997). Fortunately, DCPD derivatives (e.g., 8-dihydro DCPD and tetrahydro DCPD) are practically odorless at 1000-fold larger concentrations. From work at the Rocky Mountain Arsenal in Colorado Spanggord et al. (1979) concluded that biotransformation of DCPD in soil is slow with 50% conversion to carbon dioxide in four to seven years at 25 °C. However, oxygenated derivatives of DCPD were detected more readily. *In vitro* studies of DCPD biodegradation have shown that environmental microbial consortia can slowly degrade DCPD to oxygenated derivatives and to CO_2 (Stehmeier et al. 1996). *Pseudomonas* Q5, which was isolated from C5+ contaminated soil and can grow on naphthalene, has been shown to be able to form oxygenated DCDP derivatives *in vitro* (Shen et al. 1998).

In this study, we examine the fate of DCPD released into glacial till as part of a C5+ spill. Our results

show that DCPD is bioremediated, possibly through co-metabolic degradation that is stimulated by nutrient addition.

Materials and methods

Field sites

Two field studies were performed to determine the loss of DCPD at sites A and B within a petrochemical facility located in Alberta, Canada. At site A 149 m^3 of excavated, C5+ contaminated soil was placed in a lined pit to form a rectangular pile approximately 1 m in depth. The site was divided into three blocks (Figure 1A). Nutrients (14 kg of 12-3-5 fertilizer, Canadian Industries Ltd.; 4 kg of ammonium nitrate, So Green Corp.; 500 g of NaH_2PO_4 and K_2HPO_4, Fisher Scientific) were spread evenly over the surface of blocks 1 and 2 with a lawn spreader and were then mixed into the soil with a tractor-mounted backhoe. Block 1 was also amended with organic bulking agents (three 60 L bags of Oclansorb, a heat-treated peat product, Hi Point Industries, and four bales of straw). Block 3 did not receive bulking agents or nutrients. Soil pile construction and amendment was completed within a 10 hour period on September 3, 1992. Two volatile-collection boxes (50 cm × 50 cm; 17 L) were placed in each block (Figure 1). Six pairs of capped pipes (15 cm diameter) were placed along the north and south sides of the soil pile to monitor qualitatively, whether carbon dioxide was generated in the soil. The open end of each pair of pipes was set at a soil depth of 30 cm and 60 cm allowing diffusion of soil gas into the pipe.

Site B was on soil contaminated when C5+ spilled from an open valve. The objective at this site was to achieve degradation of DCPD without soil removal by enhancing air access. The contaminated soil was therefore extensively augered from July 20 to 24, 1995 resulting in an approximately 8 × 10 m area of well-loosened soil (Figure 1B). The augered holes were 30 cm in diameter and 1 m in depth. Before augering, nitrogen and phosphorus nutrients were spread over the entire surface (30 kg of ammonium nitrate and 4 kg of monoammonium phosphate; Alberta Wheat Pool bulk fertilizer), such that higher levels were present where contamination was greatest, based on previous analysis of the site. The site was divided into blocks 4, 5, 6 and 7 for analysis purposes based on hydrocarbon concentration. Five volatile-collection boxes were placed on the surface of the site as indicated in Figure 1B.

Analytical methods

Soil temperature was measured at 30 and 60 cm depth with Reo-Temp Model J dial thermometers (All Temp Sensors, Edmonton, AB) placed at various points over the sites. These probes remained in the soil during the analysis period.

Soil samples of 250 ml were taken with a split spoon soil auger (Hoskin Scientific Ltd., Vancouver B.C.) at evenly spaced intervals and at depths of 30 and 60 cm at site A and of 30, 60 and 90 cm at site B. The sampling points are indicated by dots in Figures 1A and 1B. For hydrocarbon measurement five grams of soil were weighed into a 40 ml crimp top vial and deionized water was added to a 10 ml volume (EPA 1986a). After incubation of the crimped vial at 90 °C for 30 min, 1 ml of the headspace was injected into a gas chromatograph coupled to a mass spectrometer (EPA 1986b). Hydrocarbon concentrations (mg/kg of soil) were calculated from these data for DCPD (C_{DCPD}) and other hydrocarbons (C_{other}). The latter could be split into components as in Figure 2.

Volatile organic carbon (VOC) was removed from the collection boxes (Figure 1) by passage through a charcoal tube (SKC Inc., Eighty Four, PA, Part #226-09) using a Gilian personal air sampler (Leavit-Safety, Calgary, AB) at an average flow rate F = 75 ml/min for an average time T = 2 h. Adsorbed hydrocarbons were extracted from the charcoal and analyzed (NIOSH 1972) to yield the amount of total VOC (w_{VOC}) and volatilized DCPD (w_{DCPD}). Concentrations of volatiles in the boxes ($c_{V,DCPD}$ or $c_{V,other}$, in mg/L for DCPD and other hydrocarbons) were calculated with Equations (1) and (2):

$$c_{V,DCPD} = w_{DCPD}/(F^*T), \tag{1}$$

$$c_{V,other} = (w_{voc} - w_{DCPD})/(F^*T). \tag{2}$$

Hydrocarbon degrading microorganisms were enumerated using a most probable number technique (Rodiha 1973), as described previously (Stehmeier et al. 1996) with 25 μl C5+ per tube. As an additional indicator of biological activity, carbon dioxide concentrations were determined at site A in the capped 15 cm diameter pipes, which were open ended at 30 and 60 cm depth (Figure 1A). Soil gas samples were collected in Tedlar bags (SKC Inc., Eighty Four, PA)

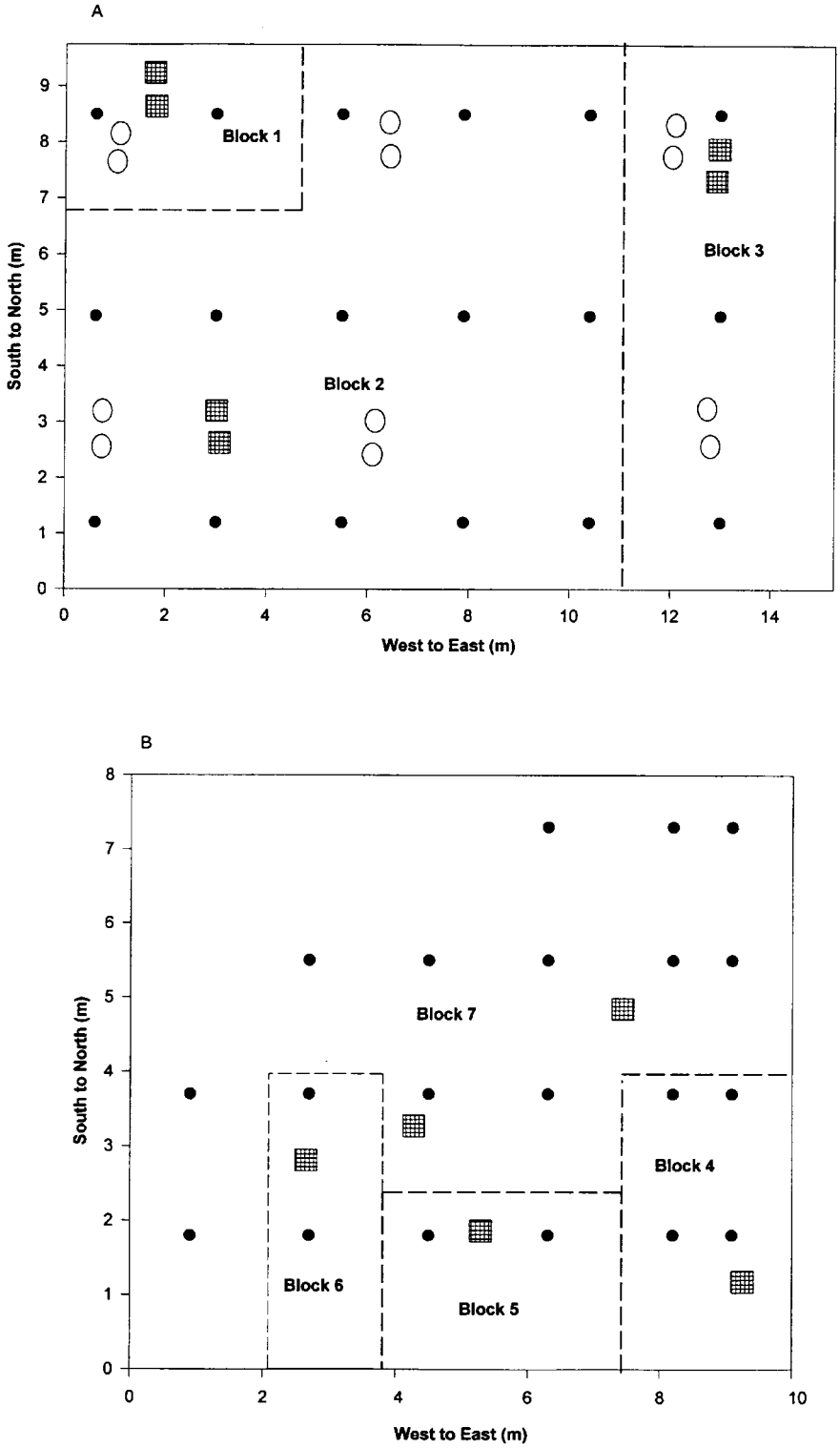

Figure 1. Survey of sites A and B. Block 1 was amended with organic bulking agents (straw and peat) and inorganic nutrients, block 2 with inorganic nutrients only, and block 3 was unamended. At site B blocks 4 through 7 were chosen on the basis of hydrocarbon concentration. Striped squares represent boxes for collecting volatile hydrocarbons. The open circles represent pipes used to collect and trap soil gas. The solid circles represent sampling points.

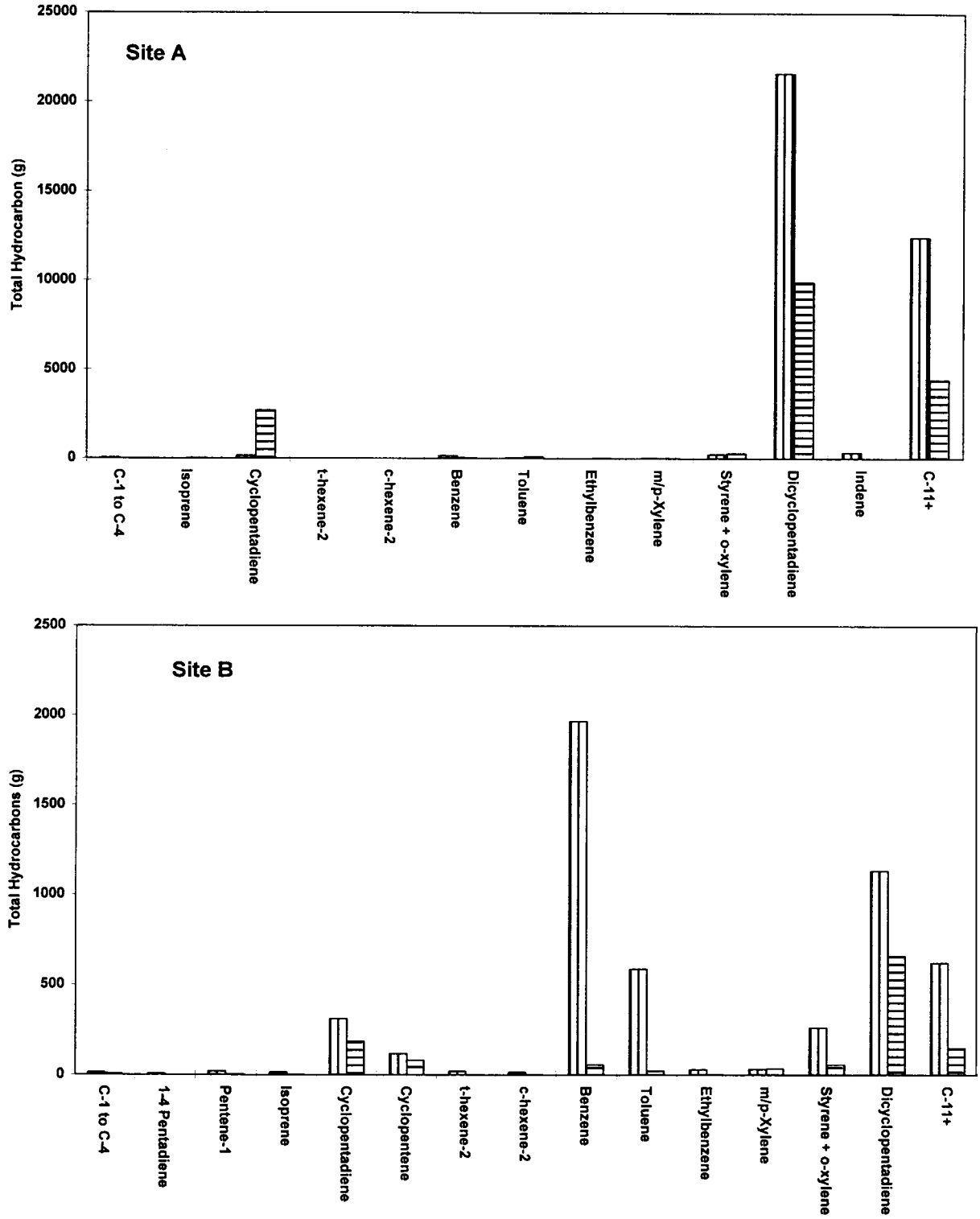

Figure 2. Hydrocarbon profiles for sites A and B. Bar values represent the estimated total weight for that particular component at site A on November 5 of 1992 (vertically striped bars) and after 266 days on July 28 of 1993 (horizontally striped bars) and at site B on July 24 of 1995 (vertically striped bars) and after 116 days on November 16 of 1995 (horizontally striped bars).

by first creating a vacuum in the bag with a Gilian personal air sampler and then opening the bag to soil gas. Carbon dioxide concentrations were measured using a Fisher Gas Partitioner Model 1200 (Fisher Scientific Ltd.)

Fluorescein diacetate (FDA) hydrolysis (Song 1988) was used as an indicator of biological activity at site B. Soil (1 g) was added to 25 ml of phosphate buffer (60 mM, pH 7.6) and 0.5 ml of FDA (2 mg/ml in acetone). After shaking at room temperature for 1 h, the reaction was stopped by addition of 25 ml acetone. The absorbance of the filtered supernatant was recorded at 490 nm in a Turner Model 330 spectrophotometer and related to a standard curve of absorbance versus thermally hydrolyzed FDA. Toxicity of the soil at site B was determined by MICROTOX^{TM} analysis (Environment Canada 1992) using reagents and lyophilized *P. phosphoreum* indicator bacteria from MICROTOX^{TM}.

For detection of oxygenated derivatives of DCPD, soil or groundwater from site B were extracted with ethyl acetate. The concentrated extract was used for GC-MS analysis as described elsewhere (Stehmeier et al. 1996; Shen et al. 1998).

Calculations

The average initial and residual concentrations of hydrocarbon (C_i and C_r) were analyzed statistically to determine if C_i and C_r differed significantly. The one-tailed Student's t test for two samples assuming unequal variances was chosen because this test corrects for small sample size by associating the distribution to the number of analyses (Shefler 1979).

$$t = [(C_i - C_r) - 0]/S_{C_i - C_r}. \tag{3}$$

Where, t = Student's t distribution, $C_i - C_r$ = sample statistic or difference between the means, 0 = mean of the sampling distribution, null hypothesis is initial population mean and residual population mean are equal, $S_{C_i - C_r}$ is the standard error of the statistic.

$$S_{C_i - C_r} = (S^2_{c_i}/n_{C_i} + S^2_{C_r}/n_{C_r})^{1/2}. \tag{4}$$

The sample variance is calculated as $S^2_{c_i} = ((n\Sigma x^2 - (\Sigma x)^2)/n^2$, where x is a headspace analysis and n is the number of analyses.

Initial and residual amounts of hydrocarbon (H_i and H_r) were determined for a 266 day period at site A and a 116 day period at site B by averaging the measured concentrations (Table 1: C_i and C_r) for the sampling points at each depth (Site A: 30 and 60 cm; site B: 30, 60, 90 cm) and multiplying this average value by the corresponding mass of soil (M) within each block. It was assumed in these calculations that the concentrations measured at 30, 60 or 90 cm were uniform for 0 to 0.333 m, 0.333 to 0.666 m, or 0.666 to 0.999 m depth, respectively. A value of 1700 kg/m^3 was used for the soil density.

The amount of hydrocarbon volatilized in these same periods was estimated as follows. First, the average concentrations of DCPD and other hydrocarbons ($C_{V,\mathrm{DCPD}}$ and $C_{V,\mathrm{other}}$ in mg/L), present in the boxes at a given time, were calculated for each block. These average concentrations are plotted in Figures 3 and 4. A time-average volatilized concentration ($\langle C_{V,\mathrm{DCPD}}\rangle$ and $\langle C_{V,\mathrm{other}}\rangle$) for each block for the entire 266 or 116 day period was then estimated from the relevant surface area of the curves in Figures 3 and 4. Little hydrocarbon volatilization was detected when a box was installed and hydrocarbon was collected immediately for 2 h. The lower limit of volatilized hydrocarbon was therefore near zero. The upper limit was calculated under the assumption that all hydrocarbon in the box emerged entirely during the 2 h collection period. The total amount of DCPD volatilized (V_{DCPD} in g) during Δt days was estimated as the median of these upper and lower limits as:

$$V_{\mathrm{DCPD}} = 0.5^*\langle C_{V,\mathrm{DCPD}}\rangle^*(15)^*(24^*\,\Delta t/2)^*(A/0.25)^*(1/1000), \tag{5}$$

where $15L$ is the total volume of the box, 2 h the collection period, 24 the number of hours in a day, A the surface area of a block and (1/1000) to convert mg to g.

The difference in total amount of hydrocarbon between two sampling dates ($H_i - H_r$) minus the estimated loss due to volatilization (V) in the same period was assigned as loss due to biodegradation (B).

$$B = (H_i - H_r) - V. \tag{6}$$

Biodegradation rates R_B (μg per kg of soil per day) were calculated as:

$$R_B = 10^6 B/(\Delta t M), \tag{7}$$

where Δt was either 266 or 116 days and M was the mass of soil in a block.

Table 1. Statistical analysis of hydrocarbon concentrations at site A (blocks 1 to 3) and B (blocks 4 to 7)[a]

	DCPD contamination									Other hydrocarbon contamination								
Block	C_i[a]	n[b]	$S^2_{C_i}$[c]	C_r[d]	n	$S^2_{C_r}$	$t-$[e]	t[f]	Δ[g]	C_i[a]	n[b]	$S^2_{C_i}$[c]	C_r[d]	n	$S^2_{C_r}$	$t-$[e]	t[f]	Δ[g]
1	74	2	399	24	2	27	3.08	3.40	Yes	96	2	773	22	2	97	3.08	3.52	Yes
2	85	10	2,715	47	10	354	1.36	2.15	Yes	67	10	1,535	43	10	563	1.34	1.66	Yes
3	50	4	1,809	28	4	621	1.48	0.91	No	63	4	948	20	4	237	1.53	2.47	Yes
Site A	72	16	2,321	39	16	448	1.32	2.45	Yes	69	16	1,272	35	16	508	1.32	3.29	Yes
4	35	12	1,261	13	12	29	1.37	2.08	Yes	140	12	8,885	8	12	10	1.36	4.86	Yes
5	8	6	118	2	3	2	1.48	1.35	No	30	6	123	3	3	8	1.44	5.59	Yes
6	3	6	28	1	6	1	1.48	0.71	No	16	6	165	1	6	0.3	1.48	2.86	Yes
7	3	36	2	5	15	49	1.33	−0.86	No	7	36	11	3	15	22	1.30	2.06	Yes
Site B	10	60	423	7	36	53	1.29	1.07	No	37	60	4,474	4	36	19	1.30	3.74	Yes

[a] Mean initial concentrations of DCPD or other hydrocarbons in a block or site (mg kg^{-1}) determined from headspace measurements.
[b] Number of analyses for determination of the mean.
[c] Variance of the sample determined as described in the text.
[d] Mean residual concentrations of DCPD or other hydrocarbons in a block or site (mg kg^{-1}) determined from headspace measurements.
[e] Critical t value for one tailed t-test with 90% confidence.
[f] Student's t-test value comparing C_i and C_r. Null hypothesis, $C_i = C_r$, $t \leq t$-crit. Alternative hypothesis, $C_i > C_r$, $t > t$-crit.
[g] C_i is significantly greater than C_r at 0.10 level of confidence.

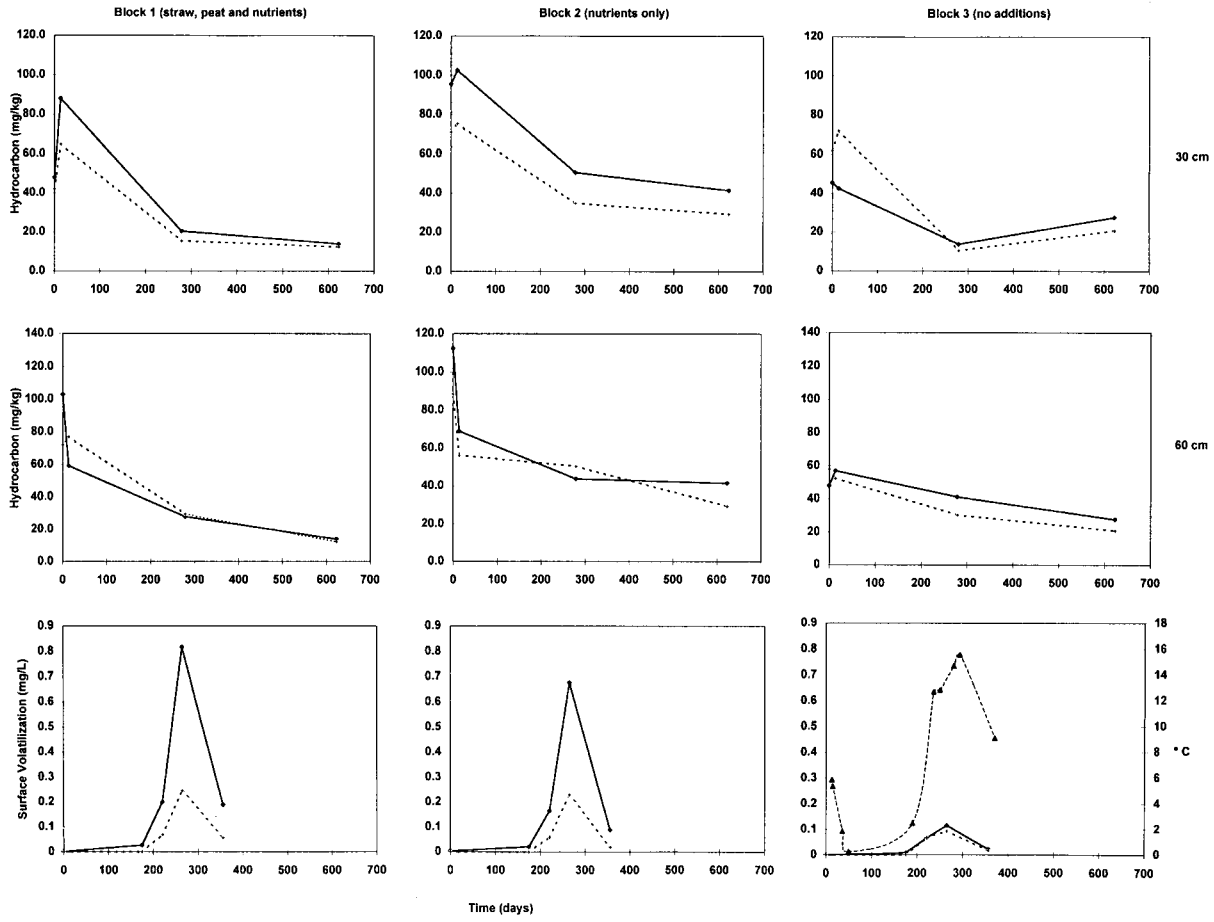

Figure 3. Fate of DCPD (solid line) and other hydrocarbons (dotted line) at site A. The calculated average concentrations (mg/kg) at 30 and 60 cm depth are plotted versus time. Average concentrations of volatilized hydrocarbons (mg/L) are also plotted versus time. The soil temperature at 60 cm depth is indicated for block 3 (▲).

Results

Ex situ remediation at site A

Site A was constructed on September 3, 1992 from previously excavated, contaminated soil piles. Mixing of these piles during construction of site A explains the relatively uniform contaminant concentrations in blocks 1, 2 and 3 (Table 1) when the first measurements were taken on October 28 and November 5, 1992. At that time the composition of the contaminating hydrocarbon had already changed significantly from that of the C5+ mixture spilled initially into the soil. DCPD was the main component and remained so during the course of the remediation process. Smaller amounts of cyclopentadiene and higher molecular weight hydrocarbons (C11+) were also present (Figure 2). The average concentrations of DCPD and other hydrocarbons in the soil are plotted as a function of time in Figure 3 for a 616 day period. DCPD and other hydrocarbons disappeared from all three blocks both at 30 and at 60 cm depth during this period. The concentrations measured at the start of the experiment (October 28 and November 5, 1992) were on average two- to five-fold higher than those found at the end (July 6, 1994). The rates of concentration decrease appeared higher in the first year than in the second year.

Hydrocarbon volatilization at site A was highly seasonal. Maximal volatilized concentrations for both DCPD and other hydrocarbons were sampled on July 28, 1993 in all three blocks, when the soil temperature at 60 cm reached 13 °C (Figure 3: block 3). Lower values were observed both earlier and later in the season. The volatilized concentrations in boxes installed

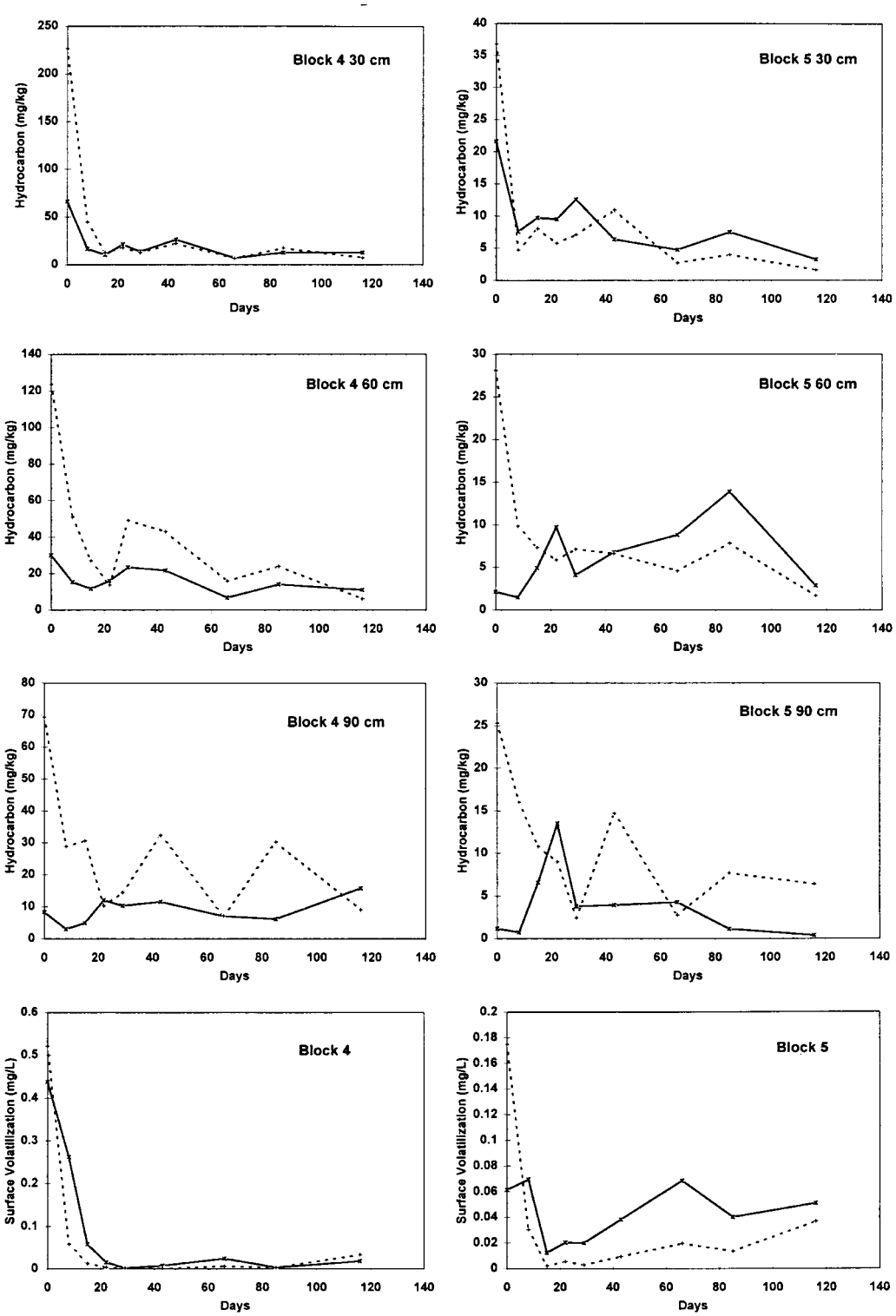

Figure 4. Fate of DCPD (solid line) and other hydrocarbons (dotted line) at site B. The average hydrocarbon concentrations (mg/kg) at 30, 60 and 90 cm depth are plotted versus time for blocks 4 and 5. Average concentrations of volatilized hydrocarbons (mg/L) for each block are also plotted versus time.

in block 3 were six to seven fold lower than those found for blocks 2 and 1, possibly because the addition of nutrients (block 2) and nutrients and bulking agents (block 1) resulted in increased porosity.

In situ remediation at site B

Site B represented a spill of C5+ that had entered undisturbed clay till at the south end of blocks 4 and 5 (Figure 1B). The initial hydrocarbon profile for site B showed benzene as the main component (Figure 2), while significant amounts of toluene and styrene were also present. This profile was similar to that of the originally spilled C5+ fraction. The difference in hydrocarbon contamination levels between blocks was high in site B (Table 1). These concentrations decreased in the order block 4, 5, 7 and 6 (Table 1) in proportion to the distance from the source of the spill (Figure 5). Because studies at site A indicated a beneficial effect of addition of nutrients on the DCPD bioremediation rate (see statistical analysis below) nutrients were added in proportion to the contamination level. A control block to which no nutrients were added was not included. Remediation at site B was started by nutrient addition and augering from July 20 to 24, 1995. Subsequent measurements were done on seven dates, ending on November 16, 1995. The composition of the hydrocarbon contamination at site B changed drastically during this period (Figure 2). After 116 days, DCPD was the primary residual contaminant with significant amounts of C11+ also present (Figure 2). The profile at site B following 116 days of treatment strongly resembled that found at site A throughout the remediation process (Figure 2).

The concentrations of DCPD and other hydrocarbons were monitored at 30, 60 and 90 cm depth and are plotted for blocks 4 and 5 as a function of time in Figure 4. In block 4, which had the highest hydrocarbon contamination levels of the site, a rapid decrease in the concentration of other hydrocarbons was seen at all three depths in a three week period (Figure 4, block 4: 30, 60 and 90 cm). This was due primarily to the removal of easily degradable C5+ components (benzene, toluene and styrene). DCPD concentrations decreased at 30 and 60 cm, but not at 90 cm. Similar data were obtained for block 5 (Figure 4). Blocks 6 and 7, which had very low hydrocarbon concentrations, did not show a statistically significant change in DCPD concentration (not shown). The concentration of other hydrocarbons did decrease in these blocks.

Boxes for measuring volatiles were installed on July 24, immediately following completion of augering. In block 4, the volatilized concentration of other hydrocarbons was highest on day 1 and dropped off sharply afterwards (Figure 4). A similar effect was seen in block 5 (Figure 4), but not in blocks 6 and 7, where the volatilized concentrations were much lower (not shown).

Because of the variability in hydrocarbon concentration at site B the presentation of data in terms of blocks with a constant concentration throughout is not accurate. Isopleth diagrams were, therefore, constructed with SigmaPlot® to better illustrate the distribution of DCPD and other hydrocarbons as a function of time using the values for C_i and C_r on day 1 and day 116, respectively, at 30 and 90 cm depths. Isopleths, constructed by interpolating three times between the data points, are shown in Figure 5A and B. Interestingly, the zero time isopleths for DCPD and other hydrocarbons at 30 cm (Figure 5A) are very similar, but those at 90 cm (Figure 5B) are not. This suggests that DCPD was retained more strongly by the soil when the C5+ spill moved downwards from the surface. After 116 days C_r for other hydrocarbons was smaller than C_i both at 30 and at 90 cm. C_r for DCPD was smaller than C_i at 30 cm, but not at 90 cm (Figure 5A and B). This could be due in part to material transport from the surface, because the data suggest the existence of a significant vertical DCPD concentration gradient at zero time.

Evidence for biological degradation of DCPD

C5+ hydrocarbon degrading bacteria were present at both sites throughout the testing period at 10^5 to 10^8 cells/g of soil. No correlation was found between cell numbers and rate of DCPD removal, block location or treatment.

At 60 cm depth in block 1, which contained organic bulking agents and nutrients, more CO_2 was produced per g of DCPD lost than in block 2, which only received nutrients, and in block 3, the unamended control (0.8, 0.2 and 0.1 g of CO_2 per g of DCPD lost, respectively). The additional CO_2 per g of DCPD formed in block 1, as compared to blocks 2 and 3, likely originated from breakdown of organic bulking agents. This increased value does not reflect a more complete conversion of DCPD to CO_2 in block 1.

The presence of oxygenated derivatives of DCPD could be shown by extraction of soil and groundwater from site B with ethyl acetate. GC-MS analysis

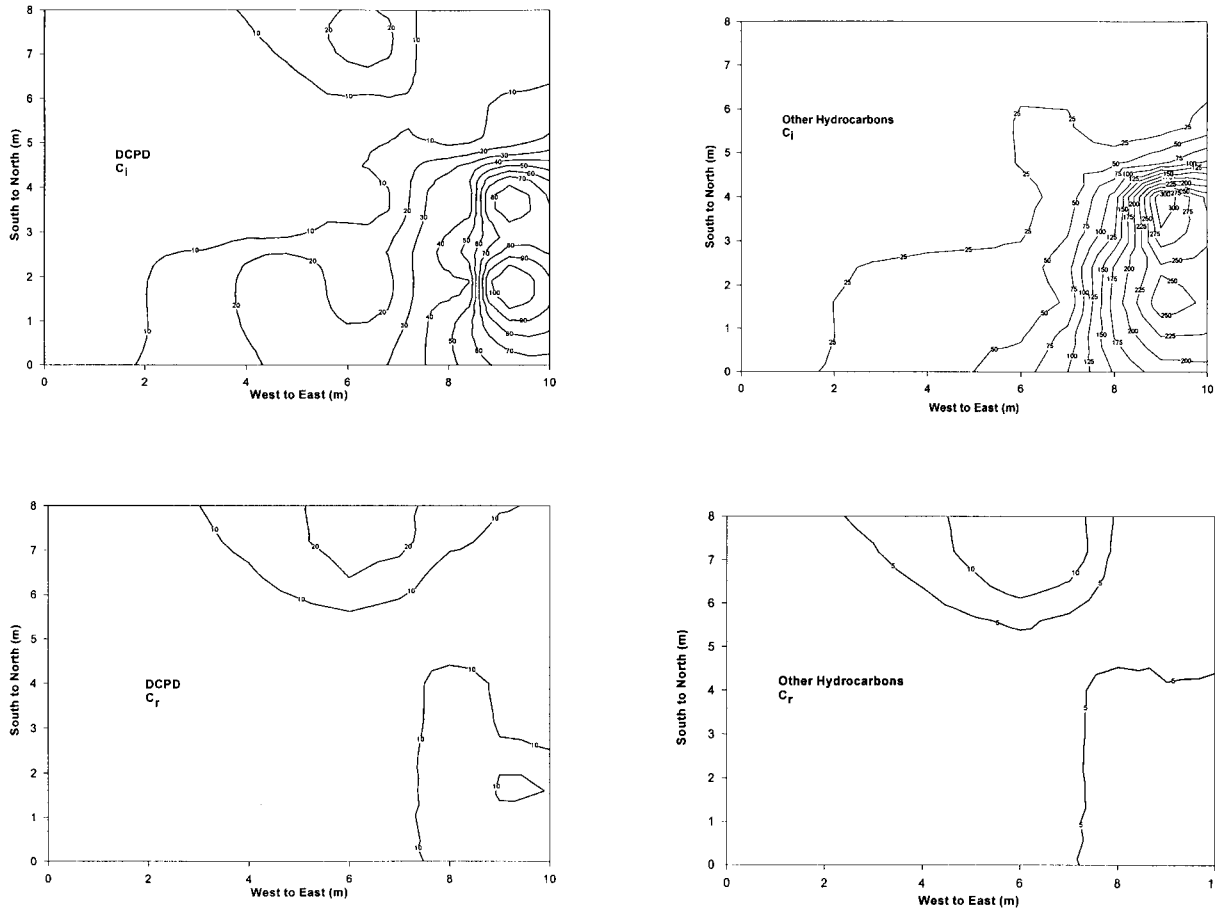

Figure 5a. Isopleth diagrams for the initial (C_i) and residual (C_r, after 116 days) concentrations of DCPD and other hydrocarbons at site B at (A) 30 cm and (B) 90 cm depth. The numbers are the concentrations in mg/kg soil.

of the concentrated extract indicated the presence of *endo*-tricyclo[5.2.1.$0^{2,6}$]deca-8-en-3-ol and *endo*-tricyclo[5.2.1.$0^{2,6}$]deca-4,8-diene-3-one. These compounds have been shown to form by microbial action (Stehmeier et al. 1996; Shen et al. 1998). Their detection thus provides strong evidence for microbially catalyzed oxidation of DCPD.

Biological activity at site B also was monitored by FDA hydrolysis. At 116 days FDA hydrolysis had increased over 600% (block 4), over 200% (blocks 5 and 6) and over 400% (block 7). The increased FDA hydrolysis for site B suggested the presence of an active microbial population at 116 days, although DCPD had become the predominant substrate.

Toxicity testing of soil taken at 30 cm depth from block 4, site B, generally indicated a 5- to 6-fold decrease in toxicity (defined as the amount of aqueous extract needed to reduce the luminescence of *P. phosphoreum* by 50%, as compared to a control) between start and end points. This may reflect removal of the toxic, low molecular weight hydrocarbons benzene and toluene, rather than the removal of DCPD.

Discussion

Statistical analysis of C_i and C_r

Although Figures 3 and 4 provide evidence for the disappearance of DCPD and other hydrocarbons with time from both sites A and B, it is important to analyze whether the suggested decreases are statistically significant. The average initial and residual concentrations of hydrocarbon (C_i and C_r) were calculated for a 266 day interval at site A (see Figure 3) and for a 116 day interval at site B (Figure 4: the entire interval for which data were collected) by averaging the headspace analyses taken at each depth within each block. C_i,

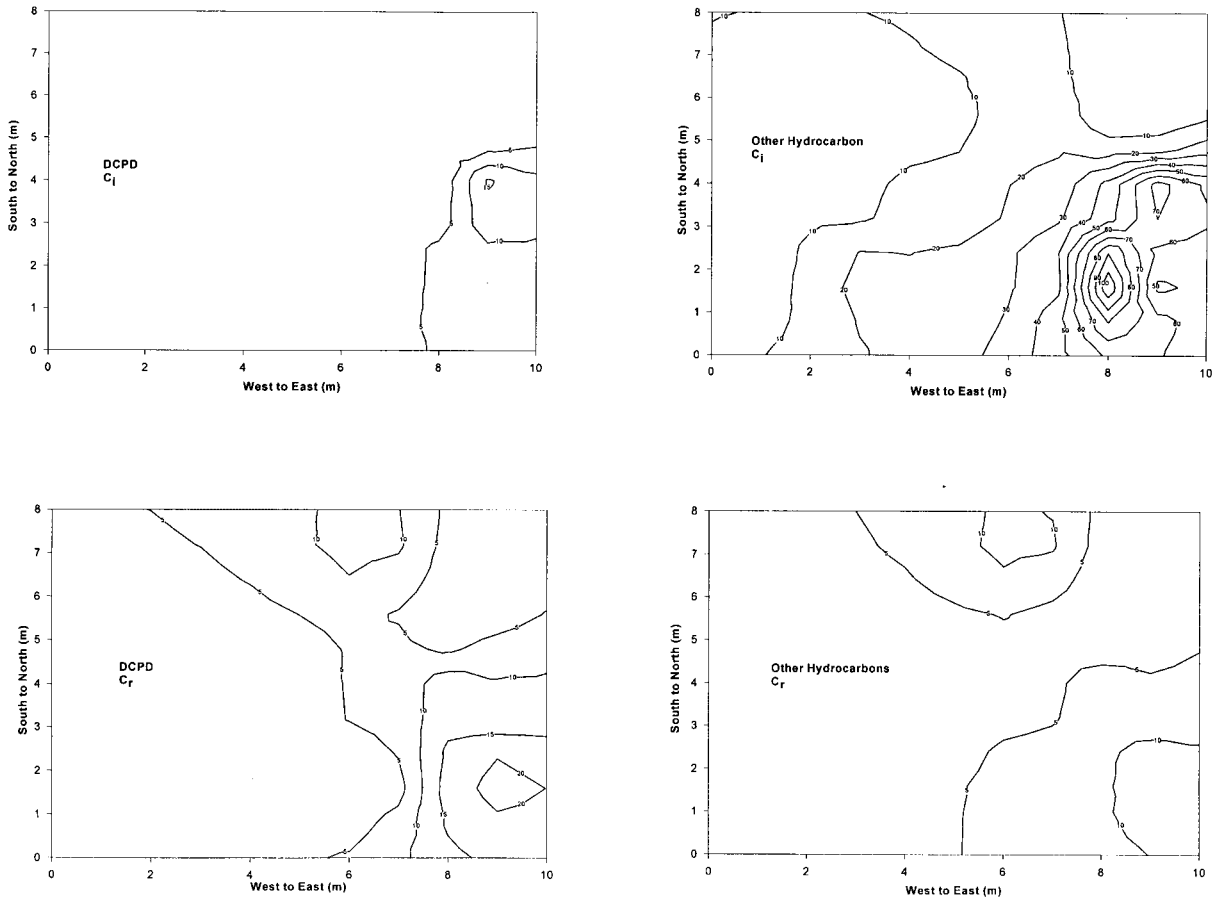

Figure 5b.

C_r and the number of analyses, n, are presented in Table 1.

At site A, the average concentrations of DCPD decreased 70%, 50% and 50% for blocks 1, 2 and 3, respectively. The Student's t test indicated that these decreases were significant at a confidence level of 90%, 95% and 79%. The lower confidence level for block 3 may indicate that there is no statistical difference between C_i and C_r. The probabilities that DCPD and other hydrocarbons were lost from site A in its entirety were, respectively, greater than 98% and 99% of being significant. Block 1 was treated with bulking agents and nutrients, block 2 with nutrients only and block 3 was the unamended control. It is probable that the loss of DCPD was larger in blocks 1 and 2 than in block 3, suggesting that nutrient addition is beneficial for DCPD removal.

Loss of other hydrocarbons at site B was highly probable with t values in excess of 2 for all four blocks. The probability that C_i for other hydrocarbons was greater than C_r at site B as a whole was 99.95%. However, for DCPD this probability was only 85%. The low t values for blocks 5, 6 and 7 for DCPD signify that the sample means for C_i and $C_r$did not differ significantly. The increase in DCPD concentration for block 7 had a negative t value and is therefore not statistically significant.

Calculation of biodegradation rates

The average concentrations, C_i and C_r, were used to calculate the total amounts (g) of initial (H_i) and residual (H_r) DCPD or other hydrocarbons (Table 2). The average concentrations of volatile DCPD and other hydrocarbons, for the 266 or 116 day periods, calculated from Figures 3 and 4, were used to estimate V, the total amount of volatilized material, with Equation (5). These values must be considered upper limits. Even so, V was not a large fraction of the total loss, e.g.

Table 2. Fate of hydrocarbons at sites A and B

			Dicyclopentadiene						
Block[1]	Area[2]	Mass[3]	H_i[4]	H_r[5]	V[6]	B[7]	f_r[8]	f_v[9]	f_b[10]
1	15	25,500	1,887	612	208	1,067	32	11	57
2	92	156,400	13,294	7,351	1,057	4,886	55	8	37
3	41	69,700	3,485	1,952	96	1,437	56	3	41
Site A	148	251,600	18,618	9,915	1,357	7,346	53	7	39
4	11.7	19,890	696	259	24	413	37	3	59
5	15.6	26,520	212	53	31	128	25	15	60
6	7.8	13,260	40	13	31	−4	NA	NA	NA
7	42.2	71,740	215	359	60	−204	NA	NA	NA
Site B	77.3	131,410	1,163	684	147	332	59	13	29
			Other hydrocarbons						
Block[1]	Area[2]	Mass[3]	H_i[4]	H_r[5]	V[6]	B[7]	f_r[8]	f_v[9]	f_b[10]
1	15	25,500	2,448	561	66	1,821	23	13	74
2	92	156,400	10,479	6,725	374	3,380	64	4	32
3	41	69,700	4,391	1,394	78	2,919	32	2	66
Site A	148	251,600	17,318	8,680	518	8,120	50	13	47
4	11.7	19,890	2,785	159	16	2,610	5	1	94
5	15.6	26,520	796	80	14	702	10	2	88
6	7.8	13,260	212	13	12	187	6	12	88
7	42.2	71,740	502	215	32	255	43	6	51
Site B	77.3	131,410	4,295	467	73	3,755	11	2	87

[1] The time interval (Δt) for initial and residual contamination is 266 days for site A and 116 days for site B. Blocks are indicated in Figure 1.
[2] Area of block or combined area of site (m^2).
[3] Total soil mass in block or site (kg) for 1 m depth and soil density 1700 kg m^{-3}.
[4] Total hydrocarbon (g) at the start of the experiment.
[5] Total hydrocarbon (g) at the conclusion of the experiment.
[6] Total volatilized hydrocarbon (g), determined with Equation (5).
[7] Total biodegraded hydrocarbon (g), determined with Equation (6|).
[8] Percentage of total hydrocarbon remaining, $f_r = (H_r/H_i) \times 100$.
[9] Percentage of total hydrocarbon volatilized, $f_v = (V/H_i) \times 100$.
[10] Percentage of total hydrocarbon biodegraded, $f_b = (B/H_i) \times 100$.

Table 3. Rates of loss for DCPD and other hydrocarbons (μg contaminant kg $soil^{-1}$ day^{-1}) at sites A and B for the duration of the field studies

	Block 1	Block 2	Block 3	Site A	Block 4	Block 5	Block 6	Block 7	Site B
$R_{T,\mathrm{DCPD}}$[1]	189	143	83	130	189	52	18	0*	31
$R_{T,\mathrm{other}}$[1]	278	90	162	129	1138	233	129	34	251
$R_{B,\mathrm{DCPD}}$[2]	157	117	78	110	179	42	0*	0*	22
$R_{B,\mathrm{other}}$[2]	268	81	157	121	1131	228	122	31	246

*No statistically significant loss.
[1]Total loss rate in μg per kg soil per day: $R_T = 10^6(H_i - H_r)/(\Delta t M)$.
[2]Biodegradation loss rate (Equation (7)).

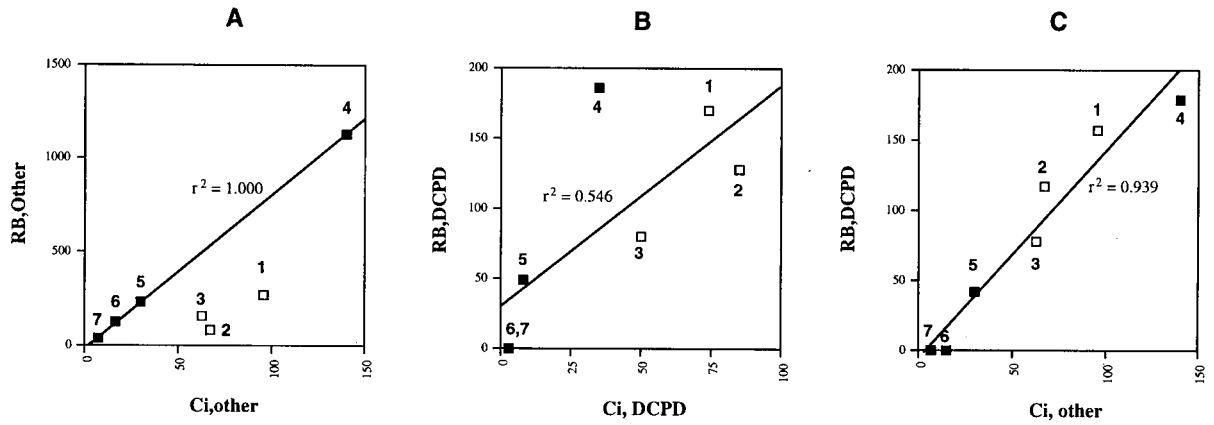

Figure 6. Relationships between initial concentrations of DCPD and other hydrocarbons ($C_{i,\mathrm{DCPD}}$, $C_{i,\mathrm{other}}$) and biodegradation rates ($R_{B,\mathrm{DCPD}}$, $R_{B,\mathrm{other}}$). (A) $R_{B,\mathrm{other}}$ versus $C_{i,\mathrm{other}}$. The solid line is for site B (blocks 4, 5, 6 and 7) only. (B) $R_{B,\mathrm{DCPD}}$ versus $C_{i,\mathrm{DCPD}}$. The solid line is for all data points. (C) $R_{B,\mathrm{DCPD}}$ versus $C_{i,\mathrm{other}}$. The solid line is for all data points.

the fractions volatilized (f_V) for DCPD at site A were 11, 8 and 3% for blocks 1, 2 and 3, respectively. The amounts of DCPD and other hydrocarbons removed through biodegradation were substantial (Table 2). For DCPD the biodegraded fraction (f_B) varied from 37 to 57% at site A and from 59 to 60% at site B. For other hydrocarbons f_B varied from 32 to 74% at site A and from 51 to 94% at site B. A higher proportion of other hydrocarbons was degraded at site B primarily because this fraction contained considerable amounts of easily degradable components (Figure 2).

The presence of a microbial community that actively degrades C5+ components other than DCPD at site B is suggested by the strong correlation between biodegradation rate and initial concentration of other hydrocarbons (Figure 6A: r^2 = 1.000). The data for site A (Figure 6A: 1, 2 and 3) fall below this line, reflecting a slower biodegradation rate due to a different composition of the other hydrocarbon component (Figure 2). The loss of single ring aromatics by biodegradation has of course been well documented by others (Barbaro et al. 1992; Barker et al. 1987; Dupont et al. 1994; Thierrin et al. 1993). In contrast, the biodegradation rate for DCPD does not correlate with initial DCPD concentration (Figure 6B: r^2 = 0.546). Interestingly, its correlation with the initial concentration of other hydrocarbons is much better (Figure 6C: r^2 = 0.939). This correlation could be fortuitous, because the biodegradation rates for DCPD in different blocks are not strictly comparable due to different treatments. If it is significant, it could indicate that DCPD degradation is cometabolic, requiring the presence of bacteria actively metabolizing other C5+ components. This suggestion is in agreement with the fact that pure or enrichment cultures able to use DCPD as sole carbon and energy source have sofar not been isolated. *Pseudomonas* Q5, isolated from C5+ contaminated soil and capable of growth on naphthalene, was found to be able to convert DCPD into oxidized derivatives (Shen et al. 1998), similar to those found in this study and to those described by others (Stehmeier et al. 1996; Van Breemen et al. 1987, 1993). Development of efficient methods for removal of DCPD odors is important in restoring water quality in sites contaminated with this compound (Ventura et al. 1997). Our field studies confirm that DCPD is not rapidly biodegraded compared to other hydrocarbons of the C5+ mixture (Figure 6A, B). Conversion of DCPD into odorless, oxidized derivatives by naphthalene- or BTEX-utilizing bacteria is the most likely mechanism for its removal from the field and the most promising avenue of future research.

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References

Amoore JE & Hautala E (1983) Odor as an aid to chemical safety: Odor thresholds compared with threshold limit values and volatilities for 214 industrial chemicals in air and water dilution. J. Appl. Toxicol. 3: 272–290

Barbaro JR, Barker JF, Lemon LA & Mayfield CI (1992) Biotransformation of BTEX under anaerobic denitrifying conditions: Field and laboratory observations. J Contam Hydrol 11: 245–272

Barker JF, Patrick GC & Major D (1987) Natural attenuation of aromatic hydrocarbons in a shallow sand aquifer. Water Monitor Rev, Winter Issue: 64–71

Dupont RR, Sorensen DL & Kemblowski M (1994) Evaluation of intrinsic bioremediation at an underground storage tank site in northern Utah. In: Proceedings of the EPA Symposium on Intrinsic Bioremediation of Ground Water, pp. 176–177. U. S. Environmental Protection Agency, Washington, DC

Environment Canada (1992) Biological Test Method: Toxicity Test Using Luminescent Bacteria (*Vibrio fischeri*). EPS 1/RM/24. Section 7, Specific Procedures for Testing Samples of Effluent, Leachate, and Elutriate. Ottawa, Ontario

EPA (1986a) Method 3810: Headspace. In: Test Methods for Evaluating Solid Waste. Volume 1B: Laboratory Manual Physical/Chemical Methods, Third Edition. United States Environmental Protection Agency, Office of Solid Waste and Emergency Response, SW 846, Washington, DC, Revised September, 1986, pp. 3810-1–3810-5

EPA (1986b) Method 8260: Volatile organic compounds by gas chromatography/mass spectrometry (GC/MS): Capillary column technique. In Test Methods for Evaluating Solid Waste. Volume 1B: Laboratory Manual Physical/Chemical Methods, 3rd Edn. United States Environmental Protection Agency, Office of Solid Waste and Emergency Response, SW 846, Washington, DC, Revised November, 1990, pp. 8260-1–8260-51

NIOSH. 1972. National Institute of Occupational Safety and Health, Physical and Chemical Analysis Branch Analytical Method 127, Organic Solvents in Air, Revised 1977

Rodina AG (1972) Methods in Aquatic Microbiology. University Park Press, Baltimore, MD, p. 179

Schefler WC (1979) Statistics for the Biological Sciences, 2nd Edn. Addison-Wesley Publishing Company, Don Mills, Ontario, p. 227

Shen Y, Stehmeier LG & Voordouw G (1998) Identification of hydrocarbon-degrading bacteria in soil by reverse sample genome probing. Appl. Environ. Microbiol. 64: 637–645

Song HG (1988) Petroleum hydrocarbons in soil: biodegradation and effects on the microbial community. PhD thesis, Rutgers, The State University of New Jersey, New Brunswick

Spanggord RJ, Chou TW & Mabey WR (1979) Studies of Environmental fates of DIMP and DCPD. Supported by the US Army Medical Research and Development Command, Fort Detrick, Frederick, MD. 21710. Contract No. DAMD 17-78-C-8053. SRI Project LSU-7551. SRI International Report AD-AO78 236/7. SRI International, Menlo Park, CA 94025

Stahl WF (1995) Cyclopentadiene/Dicyclopentadiene-United States. Chemical Economics Handbook-SRI International. 640.5000 A

Stehmeier LG, Jack TR & Voordouw G (1996) *In vitro* biodegradation of dicyclopentadiene by microbial consortia isolated from hydrocarbon contaminated soil. Can. J. Microbiol. 42: 1051–1060

Thierrin J, Davis GB, Barber C, Patterson BM, Pribac F, Power TR & Lambert M (1993) Natural degradation rates of BTEX compounds and naphthalene in a sulfate reducing groundwater environment. Hydrol. Sci. J. 38(4): 309–322

Van Breemen RB, Fenselau CC, Cotter RJ, Curtis AJ & Connolly G (1987) Derivatives of dicyclopentadiene in ground water. Biomed. Environ. Mass Spectrom. 14: 97–102

Van Breemen RB, Tsou Y & Connolly G (1993) Oxidation of dicyclopentadiene in surface water. Biol. Mass Spectrom. 22: 579–584

Ventura F, Romero J & Pares J (1997) Determination of dicyclopentadiene and its derivatives as components causing odors in groundwater supplies. Environ. Sci. Technol. 31: 2368–2374

Verschueren K (1983) Handbook of environmental data on organic chemicals. 2nd ed. Van Nostrand Reinhold Co., New York