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Associations of free-living bacteria and dissolved organic compounds in a plume of contaminated groundwater

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ABSTRACT

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Associations of free-living bacteria (FLB) and dissolved organic contaminants in a 4-km-long plume of sewage-contaminated groundwater were investigated. Abundance of FLB in the core of the plume (as delineated by maximum specific conductance) steadily decreased in the direction of flow from a point *0.25* km downgradient from the source to the toe of the plume. At *0.25* km downgradient, FLB comprised up to *31 % of* the total bacteria] population, but constituted < *7%* of the population at *2* km downgradient. Abundance of FLB correlated strongly *(r= 0.80, n = 23)* with total dissolved organic carbon (DOC) in contaminated groundwater between 0.64 and *2.1* km downgradient, although distributions of individual contaminants such as di-, tri- and tetrachloroethene were highly variable, and their association with FLB less clear. Numbers of FLB in the downgradient portion of the plume which is contaminated with branched-chain alkylbenzenesulfonate (ABS) surfactants were low ($< 5 \bullet 10^8$/L) in spite of relatively high levels of DOC (up to 4 mg/L). However, abundance of FLB correlated strongly with non-surfactant DOC along vertical transects through the plume. The ratio of FLB to DOC and the ratio of FLB to attached bacteria generally decreased in the direction of flow and, consequently, with the age of the organic contaminants.

INTRODUCTION

Increasing pollution of drinking-water aquifers by organic contaminants has led to considerable interest in the ability of groundwater bacteria (both surface-attached and unattached) to degrade and mineralize allochthonous organic compounds. It is often assumed that the smaller, unattached segment of the bacterial population in an aquifer contributes little to biorestoration, since a majority of the bacteria in contaminated aquifer sediments have been reported to be attached to solid surfaces (Harvey et al., 1984; Godsy et al.,

1992). However, it has been shown that potential subsurface biodegradation rates do not necessarily correlate with microbial density (Hickman and Novak, 1989) and in samples of at least one hydrocarbon-contaminated aquifer, most of the degradation potential appeared to be associated with the FLB and not the adherent population (Aamand et al., 1989). Despite their potential importance, the role of FLB in the breakdown of organic groundwater contaminants has largely been ignored in models which describe the fate of organic compounds in groundwater.

Degradation of highly mobile and persistent organic groundwater contaminants may be enhanced by co-transport of waste-adapted, FLB. It has been demonstrated that the on-soil application of unattached, pentachlorophenol-degrading bacteria *(Arthrobacter* sp.) resulted in a reduction in the half-life of this pesticide from 2 weeks to < 1 day (Edgehill and Finn, 1983). However, there have been few studies which have addressed the potential for interactions between FLB and organic contaminants in aquifer sediments. Laboratory microcosm studies by themselves may not be sufficient to assess this potential, because of the difficulty involved in recreating in situ conditions in the laboratory or in conducting experiments over the long time frame required for mineralization of some refractory contaminants. The objective of our investigation was to evaluate associations of FLB and dissolved organic contaminants in the field. This included studies of bacterial partitioning between groundwater and particulate surfaces and correlations between FLB, specific conductance (used as an indicator of contamination), dissolved organic carbon (DOC), and selected dissolved constituents within a 4-km-long, well-studied plume of contaminated groundwater on Cape Cod, Massachusetts, U.S.A.

STUDY AREA

The study area involves an unconfined aquifer near Falmouth, Massachusetts. The plume of organically-contaminated groundwater (-4 km in length, up to 1 km in width and 30 m in thickness) was formed by the on-land disposal of secondary-sewage effluent from Otis Air Force Base (Fig. 1). The contaminant plume is characterized by elevated levels of specific conductance (up to 400 μS), DOC (up to 4 mg/L) and temperature (up to 18'C) relative to adjacent uncontaminated groundwater (< 80 μS, < 1 mg/L and I0 ºC, respectively). The Pleistocene sediments through which the plume travels were deposited in layers as glacial outwash and are composed largely of quartz and feldspar. There is little clay. Mean grain size, average porosity, and hydraulic conductance are ~ 0. 5 mm, 0.38 and ~ 0. 1 cm/s, respectively (LeBlanc, 1984).

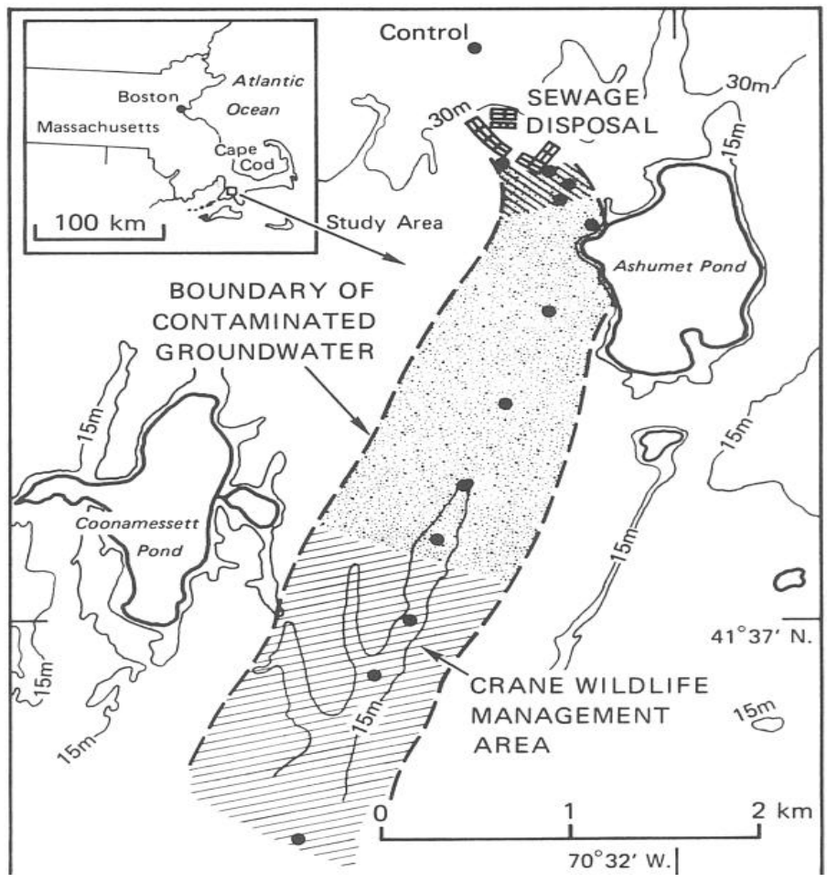

Fig. 1. Groundwater study area in Cape Cod, Massachusetts. The *(lashed lines* indicate the approximate boundaries of the contaminant plume as deter-mined by specific conductance > 100 μS/cm. *Dots* represent sampled well clusters. Different *shading* indicates approximate boundaries of the three major zones of the plume with respect to distribution of free-living bacteria (FLB) and their correlation to other dissolved plume constituents. The three zones (listed in order of proximity to the sewage disposal area) are characterized by: increases in abundance of FLB with distance downgradient (Zone 1, *stippled/hatched);* linear correlations between FLB, specific conductance and DOC (Zone 2, *stippled area);* and linear correlations between FLB and non-surfactant DOC (Zone 3, *hatched).*

EXPERIMENTAL

Groundwater samples were obtained during the summer of 1985 from a network of well clusters (polyvinylchloride [PVC], 250-tim slot width, 5.1 and 3.2 cm in diameter) that were located along the path of the plume at 0.08, 0.25, 0.38, 0.45, 0.64, 1.0, 1.6, 2.1, 2.4, 3.0 and 4.4 km downgradient from the disposal beds. Each well in a cluster was screened at a different depth allowing

sampling along a vertical profile from just below the water table into the anoxic to near-anoxic core of the plume. The 5.1-cm-diameter wells were sampled using a stainless-steel submersible pump (Keck geophysical Instruments, Inc., Okemos, Michigan, U.S.A.) attached to Teflon"' tubing. The 3.2-cm-diameter wells were evacuated with a gasoline-driven pump and subsequently sampled using a peristaltic pump, Samples for bacteria, DOC, volatile organic compounds (VOC) and surfactants were taken after 3-5 well volumes had been pumped and specific conductance and pH had stabilized, and stored at 0-4 ºC. Samples used for total bacterial counts were collected in sterile glass or plastic bottles and fixed immediately with formaldehyde (final concentration, 2% w/v). Samples for DOC and surfactants were filtered (0.45-μm pore size, silver) and samples for VOC were collected in gas-tight bottles stored without headspace. Since groundwater close to the infiltration beds can be subject to changes in chemical conditions in response to fluctuations in loading, the well cluster at 0.25 km downgradient was also sampled in June and November, 1984. This allowed assessment of temporal variability in FLB within the core of the plume.

Specific conductance, dissolved oxygen (DO), temperature and pH were measured in the field by standard methods (Greenberg et al., 1985) with probes fitted into tightly sealed in-stream containers. Bacterial abundances were determined by the acridine-orange direct-counting (AODC) procedure (Hobbie et al., 1977), using a Leitz Dialux 20 microscope modified for epifluorescence as described by Harvey (1987). DOC was measured by persulfate oxidation followed by infrared detection (Wershaw et al., 1983; Greenberg et al., 1985). Total anionic surfactants and their breakdown products (Field and Leenheer, 1988) were measured as methylene blue active substances (MBAS) according to standard procedures (Wershaw et al., 1983; Greenberg et al., 1985). The MBAS method operationally determines hydrophobic organic compounds that contain a strong-acid functional group (ionized at pH 2); organic or inorganic interferences from non-surfactant compounds appeared to be minimal. Volatile and semivolatile organic compounds were analyzed using purge and trap followed by gas chromatography-mass spectrometry (GC-MS) (Longbottom and Lichtenberg, 1982; Wershaw et al., 1983) or closed-loop stripping followed by GC-MS (Grob and Zurcher, 1976; Barber et al., 1984).

Samples of contaminated aquifer sediments for determination of adherent bacteria were taken adjacent to well clusters located 0.08, 0.25, 0.38, 0.45 and 2.15 kin downgradient from the disposal beds. Cores of aquifer sediments were taken at depths corresponding to existing well screens with a piston-type coring device (Zapico et al., 1987) that was used in conjunction with a hollow-stem auger drill. Core material was processed using an aseptic, wet-sieving technique to separate out the larger (> 100 μm) grains, which harbor

TABLE I

Specific conductance (Spec. cond.), dissolved oxygen (D.O.), dissolved organic carbon (DOC), methylene-blue-active substances (MBAS), dichloroethylene (DCE), trichloroethylene (TCE), tetrachlorocthylene (PCE) and bacteria in outfall effluent and groundwater collected along the core of the contaminant plume

Distance from source (km)	Depth (m)	Spec. Cond. (μS)	D.O. (mg/L)	DOC (mg/L)	MBAS (mg/L)	Chlorinated hydrocarbons (μg/L)			Bacterial abundance*[1] ($\times 10^9$/L)
						DCE	TCE	PCE	
0.00	0.0	n.a.	n.a.	6.4	0.24	0.7	0.2	0.8	n.a.
0.08	22.9	280	0.5	2.6*[2]	n.a.	n.a.	n.a.	n.a.	2.37 $\pm$ 0.39
0.25	20.1	345	0.2	1.1*[2]	n.a.	n.a.	n.a.	n.a.	4.50 $\pm$ 0.57
0.38	18.6	305	$\leqslant$0.1	1.0*[2]	n.a.	n.a.	n.a.	n.a.	3.05 $\pm$ 0.17
0.45	14.0	334	$\leqslant$0.1	2.2*[2]	n.a.	n.a.	n.a.	n.a.	1.58 $\pm$ 0.35
0.64	15.2	360	$\leqslant$0.1	2.6	0.45	0.0	0.1	1.0	1.37 $\pm$ 0.19
1.0	22.0	255	$\leqslant$0.1	1.1	0.07	278	2.6	6.8	1.08 $\pm$ 0.13
1.6	19.8	240	$\leqslant$0.1	1.0	0.25	18.0	21.1	160	0.69 $\pm$ 0.16
2.1	21.0	245	$\leqslant$0.1	1.1	0.49	3.5	1.0	1.6	0.39 $\pm$ 0.04
3.0	25.6	215	0.3	1.3	2.38	0.5	3.7	1.9	0.22 $\pm$ 0.04
4.4	21.6	140	1.3	0.2	0.02	0.0	0.0	0.0	0.07 $\pm$ 0.03

n.a. = not analyzed.
*[1] Free-living (unattached) bacteria (FLB). Numbers of FLB in uncontaminated groundwater were $< 5 \cdot 10^7$/L.

few bacteria and make direct counting procedures problematic (Harvey et al., 1984). Adherent bacteria in a suspension of the fine (< 100-μm diameter) sediment fraction were then enumerated using AODC. Partitioning of the bacterial populations between aquifer sediment surfaces and pore water was determined by comparing abundances of unattached bacteria in pore (well) water with total bacterial populations present in adjacent sediments (cores).

RESULTS AND DISCUSSION

Distributions

Concentrations of FLB, DOC, MBAS, the major halogenated hydrocarbons, specific conductance and DO in groundwater collected along a longitudinal transect through the core of the contaminant plume are listed in Table 1. Values reported represent groundwater sampled from the well in each cluster having the highest specific conductance. With the exception of the well farthest downgradient (4.4 km) from the disposal beds, specific conductances

TEMPORAL VARIABILITY IN BACTERIAL ABUNDANCE
(Contaminated Groundwater at SDW316, Cape Cod, Massachusetts)

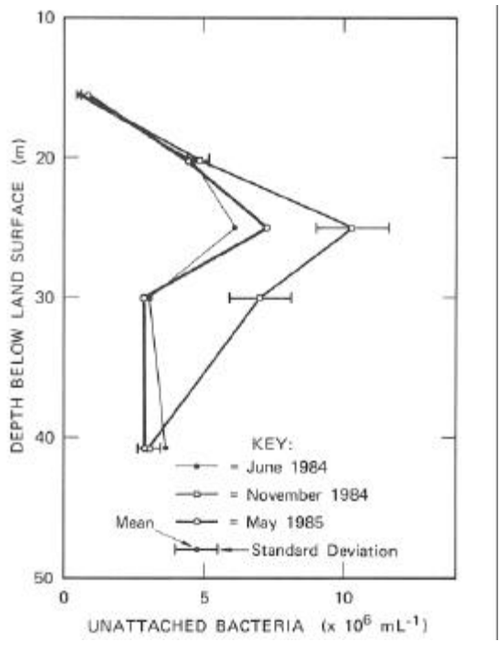

Fig. 2. Temporal variability in abundance of free-living bacteria along a vertical transect through the core of the contaminant plume at 0.25 km downgradient from the infiltration beds. *Error bars* reflect counting precision for single samples.

were > 200 ,uS, which is typical for the oxygen-depleted core of the contaminant plume.

Since the contaminant plume may not be steady state, temporal variations in abundances of FLB were investigated in order to better interpret spatial trends. Fig. 2 depicts changes observed over the course of a year in numbers of FLB along a vertical profile through the plume, 0.25 km downgradient from the infiltration beds. Although little variation was observed for the shallower, more dilute portion of the plume (15- and 20-m depths), numbers of FLB in the anoxic core of the plume (30- and 40-m depths) varied about two-fold between June and November of 1984. However, abundance of FLB in the core of the plume at ~ 3 km downgradient did not vary significantly between the summers of 1983 (Harvey et al., 1984) and 1985 (Table 1). In general, temporal variations in FLB within the core of plume appear to be small relative to spatial variations in the direction of flow.

Numbers of FLB increased slightly from 0.08 to 0.25 km downgradient from the infiltration beds, then decreased steadily in the direction of flow to

the toe of the plume (-4 km downgradient). The decline can be reasonably fit ($r=0.98$, $P<0.001$) using an exponential decay function, i.e. [FLB] = $3.82 - 10^6$ exp [-0.91 x distance (km)]. Although other functions may also be invoked, exponential decay is consistent with mathematical descriptions of bacterial immobilization during their downgradient transport through sandy aquifer sediments (Harvey and Garabedian, 199 1) and descriptions of bacterial dieoff in the groundwater environment (Bitton et al., 1983). The decrease in FLB with increasing distance downgradient from the source of contamination was observed in other studies at the Cape Cod site (Harvey et al., 1984; Harvey and George, 1987), and in an investigation involving a contaminated, sandy aquifers in Fulda, Germany (Marxsen, 198 1). Decreases in abundance of FLB with increasing distance downgradient from a point source of groundwater contamination seems reasonable, since the more-readily degraded compounds in complex contaminant mixtures appear to be preferentially degraded during transport through aquifer sediments (Godsy et al., 1992).

The pattern of spatial variation in DOC along the same transect appeared to be more complex (Table 1). In another study, DOC maxima of ~4 mg/L were observed both near the disposal beds and at ~ 3 km downgradient (Thurman et al., 1986). The variability in DOC along longitudinal transects through the plume partly reflects the uneven distribution of refractory compounds. The organic matrix that comprises the DOC is a very complex mixture that includes nonionic and anionic surfactants and their metabolites (see Field et al., 1992 in this issue) and a wide variety of volatile and semivolatile compounds, including alkyl and chlorinated aliphatic and aromatic hydrocarbons, alkylphenols, aldehydes, and phthalate esters (Barber et al., 1988; Barber, 1992).

Changes in the ratio of unattached to attached bacteria along the longitudinal transect through the plume are evident from Table 2. The FLB comprise > 30% of the population in at least one part of the contaminant plume near the loading beds, but very little of the population in the more distal portion. The partitioning of the bacterial population between pore water and particle surfaces appears to be highly variable within the plume. However, a general increase in partitioning of the bacterial population to the aqueous phase with decreasing distance to the source of contamination is evident, apparently unrelated to the silt content of the sediments (Table 2). In a study involving anaerobic degradation of creosote contaminants in a sandy, unconfined aquifer in Pensacola, Florida, U.S.A., it was found that up to 49 % of the methanogens, as determined by MPN, in a portion of the contaminated sediments were free-living (Godsy et al., 1992). However, almost all of the bacteria in uncontaminated aquifer sediments were attached to particle surfaces, similar to what was observed earlier for the Cape Cod aquifer (Harvey et al., 1984). Although a number of factors can affect attachment and

Partitioning of the bacterial population between sediment surfaces and pore water along a longitudinal 3-km transect through the plume of contaminated groundwater at Cape Cod

Distance (km)	Depth (m)	Silt (%)	Number of cores	Partitioning (% free-living)	
				avg.	range
0.08	11–30	0.6–1.8	4	13.9	9.1–21.6
0.25	16–31	0.3–5.3	4	30.8	9.6–64.1
0.38	12–31	0.3–1.7	4	22.1	1.2–36.8
0.45	6–11	0.4–2.9	9	6.6	0.9–14.7
2.1	21	0.8	1	6.3	–
2.9*	32		1	0.3	–

– = no data.
*Data for 2.9 km downgradient are from Harvey et al. (1984).

detachment of bacteria from solid surfaces, it is clear that the presence of organic contaminants in aquifer sediments may lead to a redistribution of the population between the solid and aqueous phase.

Correlations

Correlations involving DOC and FLB were investigated for specific sections of the plume, since distribution patterns of DOC and FLB differ over the length of the 4.4-km transect. Three major regions of the plume were identified on the basis of DOC and FLB distributions and characteristics (Fig. 1), i.e. the first 200 in downgradient from the outfall, which is characterized by a relatively sharp decline in DOC with distance (>2 mg/L per 100 m), moderately labile compounds (including degradation products of alkylphenol polyethoxylate nonionic surfactants, carboxylated LAS degradation products and phthalate esters; Barber, 1992), and high potential bacterial growth rates (p,≥0.02; Harvey and George, 1987); the region between 0.2 and 2 km downgradient, characterized by older, more recalcitrant DOC (such as chlorinated aliphatic and aromatic hydrocarbons; Barber, 1992) and steady declines in FLB along the direction of flow; and the most distal portion of the plume (2-3 km downgradient), characterized by low abundances (<0.4-10^9/L) of FLB and DOC which is largely composed of ABS surfactants and breakdown products (Thurman et al., 1986).

Correlations between FLB and specific conductance along vertical transects through the plume at two locations were reported in a previous investigation (Harvey et al., 1984). In the present study, it was found that there was a significant linear correlation between FLB and specific conduc-

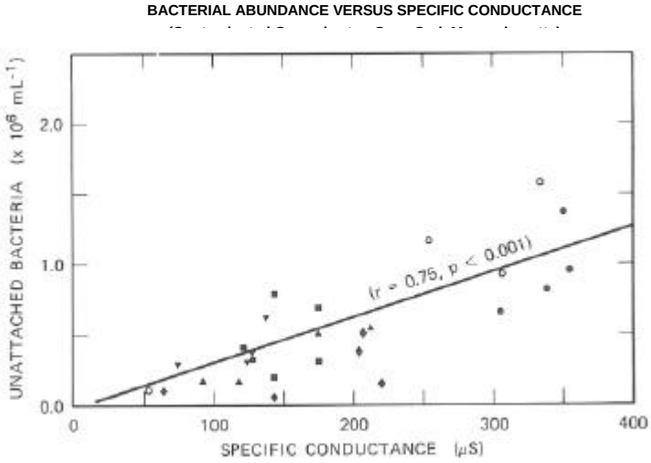

Fig. 3. Linear correlation between numbers of free-living bacteria vs. specific conductance for groundwater collected at various depths within the plume between 0.45 and 2.4 km downgradient from the disposal beds (○ = samples taken at 0.45 km, ● = at 0.64 km, ▲ = at 1.0 km, ■ = at 1.6km, ◆ = at 2.1 km and ▼ = at 2.4 km downgradient).

tance for the entire sample population *(n* = 29) collected from the central section of the plume (delineated by the stippled region in Fig. 1). The correlation was most significant between 0.45 and 2.44 km (r = 0.75, p < 0.001; Fig. 3). This correlation may result from the fact that specific conductance is a good indicator for the degree of dilution of the sewage effluent by native groundwater and, consequently, dilution of the organic contaminants that affect bacterial growth (Harvey et al., 1984). The linear correlation between DOC and specific conductance for the central region of the plume is depicted in Fig. 4. Although the DOC that is being transported downgradient within the plume is subject to sorption and biodegradation, its distribution nevertheless correlated strongly (r=0.89, p<0.001) with specific conductance. However, a slope for the regression less than unity (i.e. 0.7) suggests sorptive and/or degradative loss of DOC. For the sample population represented in Fig. 4, a strong (r=0.80, p<0.001) linear relationship was also observed between numbers of **FLB and DOC, i.e. [FLB] = (0. 386[DOC]** +0.11)-106 . A coefficient of determination (r 2) of 0.64 for the above regression suggests that about two-thirds of the spatial variability in numbers of unattached bacteria may be explained simply by spatial distribution of DOC.

By comparison, the regression between FLB and DOC in the ABS-contaminated section (2-3 km downgradient) of the plume was weak (r = 0. 63 at p < 0. 1, *n* = *9*). Since ABS is highly recalcitrant, as evidenced by its presence after 30-50 yr travel time in the aquifer, and even inhibitory to bacterial

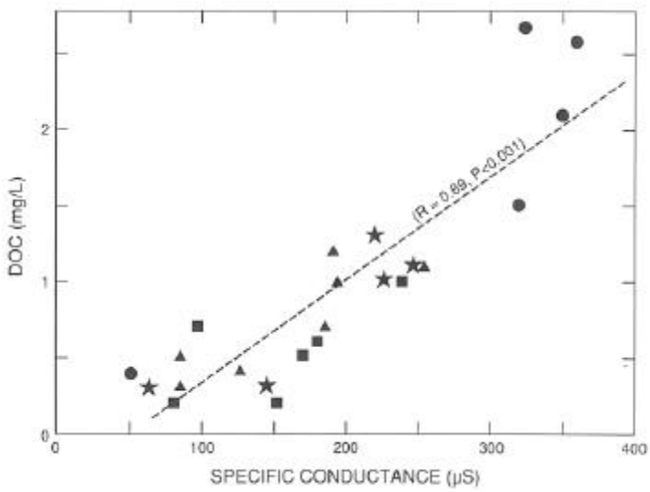

Fig. 4. Linear correlation (1985) between dissolved organic carbon and specific conductance for groundwater collected at various depths within the plume between 0.64 and 2.1 km downgradient from the disposal beds (● = samples taken at 0.64 km, ▲ = at 1.0 km, ■ = at 1.6 km and ★ = at 2.1 km downgradient).

growth (Urano and Salto, 1985), DOC may not be a good aggregate measure of biologically available organic carbon in ABS-contaminated groundwater. However, by subtracting the ABS carbon (assumed to be ~ 0. 5 [M BAS]) from measured values of DOC, a better estimate of potentially bioavailable organic carbon may be obtained. Fig. 5 depicts the regression between FLB and surfactant-corrected DOC concentrations along vertical transects taken at 4 locations within the distal portion of the plume, 2-4 km downgradient from the infiltration beds. In general, the correlations between FLB and dissolved organic contaminants were stronger when surfactant DOC was subtracted from total DOC. The coefficient of determination for the regression between FLB and (DOC-0.5 [MBAS]) along a vertical transect through the plume at 2.1 km downgradient was 0.93 at $p \leq 0.01$, but was only 0.76 at $p \leq 0.1$ for the regression between FLB and total DOC.

Although no correlation was found between FLB and total DOC for the entire longitudinal transect, the ratio of FLB to DOC generally decreased in the direction of flow; in the distal, surfactant-contaminated portion of the plume, ratios of FLB/DOC were on the order of 10^8 cells/mg, whereas between 10^9 and 10^{10} cells/mg were generally observed for groundwater near the infiltration beds. It is possible that the older, more-refractory DOC in the distal portion of the plume may not be capable of supporting as much biomass as the more recent, more-labile DOC near the outfall. However, the ability of FLB to degrade the various contaminants under in situ conditions has not yet

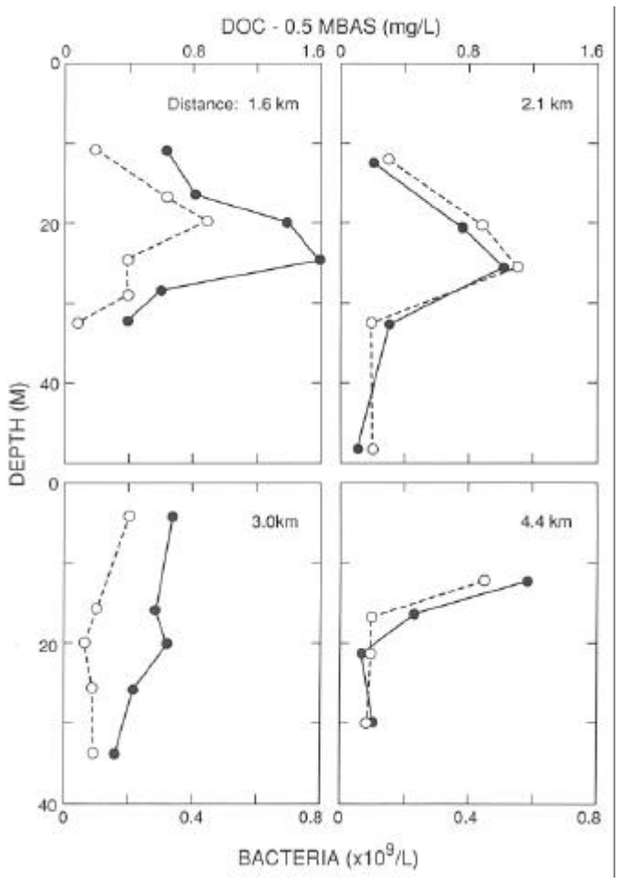

Fig. 5. Vertical profiles of abundance of free-living bacteria and DOC concentrations (corrected for highly-refractory surfactants), in the contaminant plume from 1.6 to 4.4. km downgradient from the disposal beds. Surfactant-corrected DOC depicted by *open circles*, bacterial abundance by *shaded circles.*

been studied. In general, mineralization of DOC by FLB in samples of low-nutrient groundwater appears to be slow, even for simple substrates that have short half-lives in surface-water environments. Ventullo and Larson (1985) report turnover times for glucose (1.3 g/L, 10'C) in groundwater collected from Ohio, U.S.A., and Ontario, Canada, that range from 4 to 52 days. Rates of glucose assimilation by FLB in samples of the Cape Cod plume are also quite low (36 and I I nmol L-'day-'at 0.64 and 1.80 km downgradient, respectively; Harvey et al., 1984). It is likely that both in situ and laboratory investigations will be needed to delineate the potential for interaction and

co-transport between FLB and the complex mixture of highly mobile and persistent organic contaminants within the plume.

In summary, although free-living bacteria appear to constitute an insignificant fraction of the total bacterial population in uncontaminated aquifer sediments, relatively large numbers of FLB may be found organicallycontaminated groundwater. At the Cape Cod site, FLB correlated strongly with DOC in the central portion (0.6-2 km downgradient) of the plume and with the non-surfactant DOC in the ABS-contaminated zone further downgradient (2-3 km), suggesting at least some potential for interaction. Potential contributions of the FLB in biotransformation and mineralization of the contaminant DOC may be greatest within several hundred meters of the outfall, where FLB are in highest abundance and constitute a significant (up to 31 %) fraction of the total bacterial population. It is clear that more research is needed to more clearly delineate the role of FLB in the fate of organic pollutants in contaminated aquifers. However, based upon the data presented and in the absence of further more-detailed study, it may be premature to neglect the contributions of non-adherent bacteria.

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