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Importance of closely spaced vertical sampling in delineating chemical and microbiological gradients in groundwater studies

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ABSTRACT

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Vertical gradients of selected chemical constituents, bacterial populations, bacterial activity and electron acceptors were investigated for an unconfined aquifer contaminated with nitrate and organic compounds on Cape Cod, Massachusetts, U.S.A. Fifteen-port multilevel sampling devices (MLS's) were installed within the contaminant plume at the source of the contamination, and at 250 and 2100 m downgradient from the source. Depth profiles of specific conductance and dissolved oxygen at the downgradient sites exhibited vertical gradients that were both steep and inversely related. Narrow zones (2–4 m thick) of high N_2O and NH_4^+ concentrations were also detected within the contaminant plume. A 27-fold change in bacterial abundance; a 35-fold change in frequency of dividing cells (FDC), an indicator of bacterial growth; a 23-fold change in ^{3}H-glucose uptake, a measure of heterotrophic activity; and substantial changes in overall cell morphology were evident within a 9-m vertical interval at 250 m downgradient. The existence of these gradients argues for the need for closely spaced vertical sampling in groundwater studies because small differences in the vertical placement of a well screen can lead to incorrect conclusions about the chemical and microbiological processes within an aquifer.

INTRODUCTION

A large degree of homogeneity is often ascribed to subsurface environments. Consequently, groundwater monitoring wells are frequently spaced with little attention to potential small-scale variation. However, heterogeneity is a common phenomenon in hydrologic regimes and needs to be considered

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when designing sampling networks (Gillham et al., 1983). This is especially true along a vertical axis. It is becoming evident that vertical chemical gradients can exist in groundwater, especially in contaminated aquifers. Pickens et al. (1978) designed multilevel sampling devices with sampling points at ~ 0.5-m intervals and installed them in a groundwater contaminant plume originating from a sanitary landfill. They observed steep vertical concentration gradients within the contaminant plume and regions where the contaminant plume itself was quite narrow. Likewise, Ronen et al. (1987a, b) reported significant changes in the concentrations of nitrate and oxygen within vertical intervals only a few centimeters thick in an aquifer in Israel. Gradients of this type could easily affect the geochemical nature of an aquifer and the microbial processes that occur therein. Therefore, it is essential to determine whether sharp gradients are a common characteristic of contaminant plumes and what their effect is upon the microorganisms within the aquifer. Whenever gradients are present, their vertical location and their steepness would dictate the sampling intervals necessary for describing a plume at any given site.

In this study we used multilevel sampling devices (MLS's) to determine the nature and the extent of vertical gradients of chemical and microbiological parameters in a large-scale contaminant plume. The plume is located in a sand and gravel aquifer on Cape Cod, Massachusetts, U.S.A. Significant changes in the concentration of dissolved constituents occurred in vertical intervals of < 1 m at sampling sites situated along a transect extending from the source of contamination to > 2000 m downgradient. Large changes in the microbial populations were also evident within the same vertical intervals. The results of this study argue for the need to collect samples at closely spaced vertical intervals when delineating contaminant plumes of this type.

MATERIALS AND METHODS

Study site

The study was conducted in a contaminant plume in a sand and gravel glacial outwash aquifer located on Cape Cod near the town of Falmouth. The contaminant plume is the result of more than 50 years of disposal of secondarily-treated sewage onto rapid-infiltration sand beds. The treated sewage percolates to the water table, which is located 6 m beneath the surface of the sand beds, and is subsequently transported in a southern direction by regional groundwater flow. Currently, the contaminant plume is > 3500 m long, 100 m wide, and 23 m thick (Fig. 1). The nature of the contaminant plume and the hydrology of the aquifer have been previously described (LeBlanc, 1984; Thurman et al., 1986; Barber et al., 1988).

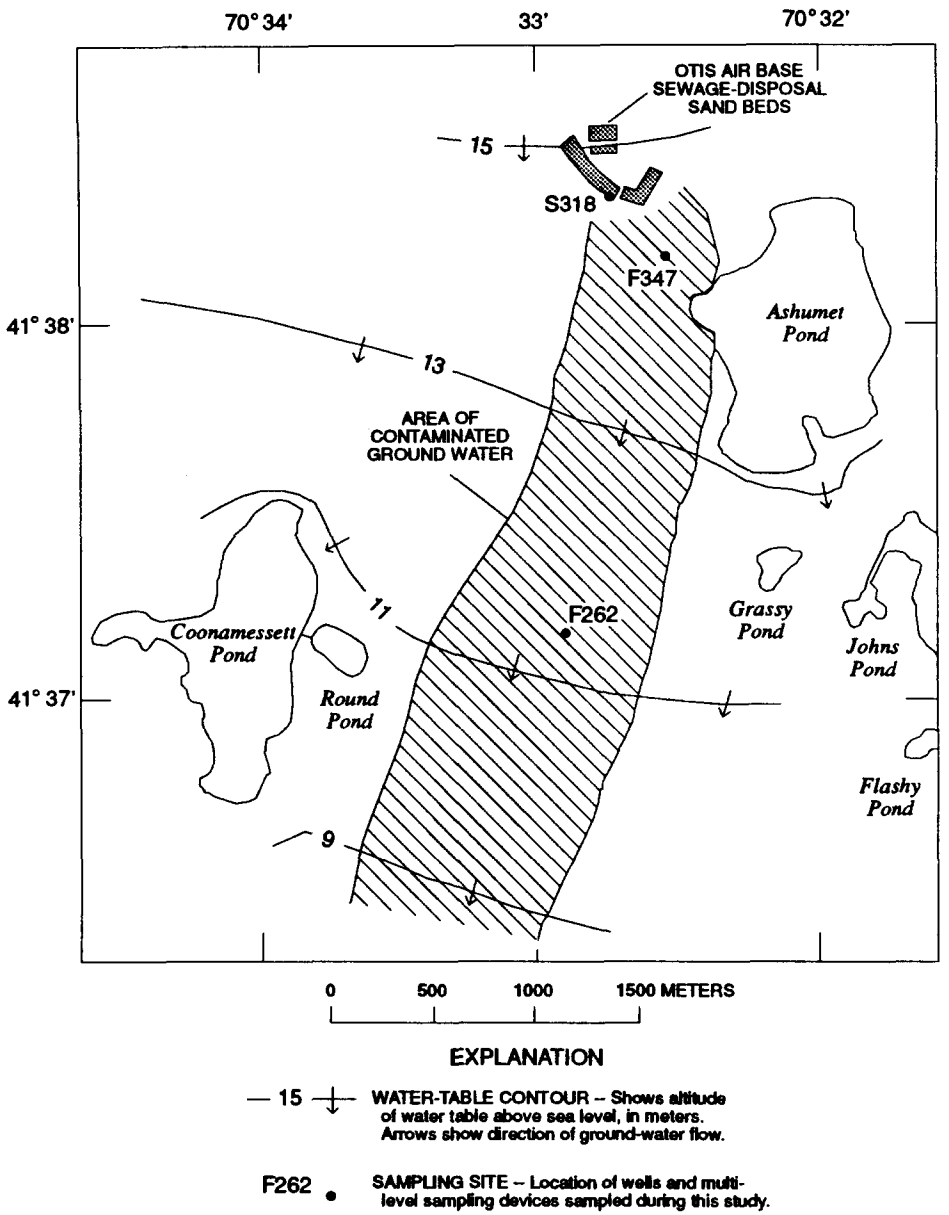

Fig. 1. Map showing the contaminant plume on Cape Cod, Massachusetts, and the locations of the wells and MLS's sampled during this study. The areal extent of the contaminant plume is based on specific conductance measure in 1985.

Sampling

Groundwater samples were obtained from three sites within the contaminant plume, S*318*, F*347* and F*262* (Fig. 1). Three to five 5-cm-diameter

observation wells, constructed with polyvinyl chloride (PVC) pipe and 0.6–0.9-m-long PVC slotted screens were installed at each site. The wells were installed by hollow-stem auger.

One or two MLS's were also located at each site. The MLS's consisted of 15 color-coded polyethylene tubes (6.5-mm diameter) encased in a PVC pipe (3.2-cm diameter). The lower ends of the tubes exit through holes in the PVC pipe at selected points spaced 0.6–1.2 m apart vertically and are capped with Nylon® screens. Each MLS was installed by hollow-stem auger drilling. The augers (8.3-cm inside diameter, 16.8-cm outside diameter) were drilled to the final depth, using only water as drilling fluid, and the MLS was set inside the augers. The augers were then pulled out and the sediments were allowed to collapse against the MLS. Because the sand and gravel at this site contains almost no silt and clay and is cohesionless, collapse was almost immediate. Seals were not placed between sampling points because there was no evidence that voids remained along the MLS's after installation (R. Morin, pers. commun., 1985) or that short-circuiting of flow occurred vertically along the MLS's during sampling (Garabedian et al., 1988).

Observation wells were sampled using a stainless-steel submersible pump (model SP*81*; Keck Geophysical Instruments, Inc., Okemos, Michigan, U.S.A.). Samples were taken after 3 to 4 well volumes were pumped and the specific conductance had stabilized. The MLS's were sampled with a peristaltic pump (Geotech Environmental Equipment, Inc., Denver, Colorado, U.S.A.). The tubing in the peristaltic pump head was connected directly to the tops of the MLS tubes at land surface. Direct suction of samples was possible because the water table at the three sites was only 3–6 m below land surface. At least 3 tube volumes were pumped before samples were collected.

For dissolved oxygen analysis, groundwater samples were collected in 300 mL BOD (biological oxygen demand) bottles positioned in 2-L glass jars. Each jar was connected in line between the MLS and the peristaltic pump. Inflowing sample entered the BOD bottle first, then overflowed into the larger vessel; 2–3 L were pumped through the BOD bottle, which was then stoppered while still submerged in the jar. Samples to be analyzed for dissolved ions were filtered through 0.45-μm pore size filters (Metricel®; Gelman Sciences, Inc., Ann Arbor, Michigan, U.S.A.); aliquots were frozen for nitrate determination or acidified with H_2SO_4 (pH 2) for ammonium determination. Water samples (16 mL) for N_2O determination were collected by syringe and injected into 20-mL stoppered serum bottles that had been sparged with Ar. For microscopic counts, samples were collected and preserved with formaldehyde as described by Harvey et al. (1984). For microbial activity measurements, 1-L glass bottles were over-filled with 3–5 L of water and capped to avoid an air headspace. The bottles were transported to a laboratory in an ice bath and incubations were initiated the following day.

Chemical analyses

Specific conductance was assayed by electrode (Hach Chemical Co., Loveland, Colorado, U.S.A.), oxygen by the iodometric titration method, using the azide modification (APHA–AWWA–WPCF, 1985). Nitrate was determined with an Autoanalyzer® (Technicon Instruments Corp., Tarrytown, New York, U.S.A.) using the cadmium reduction method (APHA–AWWA–WPCF, 1985) and ammonium by the salicylate hypochlorite method (Skougstad et al., 1979). Nitrous oxide was assayed by gas chromatography with an electron capture detector (Smith and Duff, 1988) and phosphate and chloride by ion chromatography (Dionex Corp. model *14* ion chromatograph; Dionex HPIC AS*3* column and ASC*2* guard column with 3 m*M* $NaHCO_3$–2.4 m*M* Na_2CO_3 eluent at 1.6 mL min^{-1} and 25°C).

Microscopy

Total numbers of bacteria in groundwater samples (unattached bacteria) were enumerated at 1000 × magnification in separate acridine-orange-stained preparations using black-stained polycarbonate filters (25-mm diameter, 0.2-μm pore size, Nuclepore Corp. No. *110656*) and a Dialux® *20* microscope (Leitz/Opto-Metric®, Division of E. Leitz, Inc.). The microscope was modified for epifluorescence as described by Harvey (1987). Frequency of dividing cells (FDC), indicative of bacterial growth, was calculated as the percentage of total unattached bacterial populations with clear invaginations in the cell wall between the dividing cells. Bacterial enumeration and determination of FDC for unattached groundwater bacteria are described in more detail by Harvey et al. (1984) and Harvey and George (1987).

Average cell length and total biomass for each unattached bacterial population were estimated from measurements made from scaled photomicrographs. Each bacterium in the photomicrographs was placed into 1 of 21 categories: coccoidal rods (0.2-, or 0.4-μm diameter), cocci (0.6-, 0.8-, or 1.0-μm diameter), rods (0.6-, 0.8-, 1.0-, 1.2-, 1.4-, 1.6-, 1.8-, 2.0-, 2.2-, 2.4-, 2.6-, 2.8-, or 3.0-μm length), or filaments (3-, 5-, or 10-μm length). Average cell length was determined from the size frequency distributions of the largest cell dimension. The equations of Palumbo et al. (1984) were used to calculate the width of rods. Individual cell volumes were then estimated from cell lengths and widths and converted to cell carbon using a conversion factor of $2.2 \cdot 10^{-13}$ g μm^{-3} C (Bratbak and Dundas, 1984). Total biomass (expressed as cell carbon) for the unattached bacterial population at each depth was estimated from the individual cell volumes and total bacterial abundance.

^{3}H-glucose uptake

Duplicate 60-mL syringes were filled with each groundwater sample; the sample bottles were flushed with O_2-free N_2 while the syringes were being filled. The syringes were sealed with rubber-stoppered injection hubs. After a 12-hr preincubation at 10°C, 0.5 mL of ^{3}H-*6*-glucose was added (specific activity adjusted to 30.3 Ci mol^{-1}, 3.6 μCi total, New England Nuclear Corp.) by syringe. The final glucose concentration was 2 μM. The syringes were shaken and incubated without headspaces at 10°C. A 20-mL subsample from each syringe was filtered through a 0.45-μm Gelman Metricel® filter after 2, 24 and 48 hr. The filters were washed two times with 5 mL of filtered groundwater (4°C), placed in capped scintillation vials, and digested in 1 mL 1 *N* HCl for 15 min at 100°C. Then, 1 mL of ethyl acetate was added to dissolve the filter, followed by 10 mL of scintillation cocktail (Beckman Ready Solve®, Beckman Instruments, Downers Grove, Illinois, U.S.A.). Radioactivity was determined with a Beckman® liquid scintillation counter (LS *7800*), and corrected for counting efficiency as determined with ^{3}H-toluene as an internal standard.

RESULTS

Chemical gradients

The wells used for this study are situated at locations that represent three different conditions within the contaminant plume (Fig. 1). Site S*318* is located within one of the infiltration sand beds, site F*347* is located 250 m downgradient (1.5–2-yr groundwater travel time), and site F*262* is much farther downgradient (2100 m, 12.5–17-yr groundwater travel time).

The specific conductance (SPC) of groundwater at site S*318* was relatively constant and $> 320\,\mu$S for the entire vertical interval sampled by the MLS (Fig. 2). In contrast, nitrate concentrations varied five-fold within the same interval (260–1300 μM) and dissolved oxygen (DO) decreased significantly (from 218 to 4 μM) within the upper 3 m of the interval. At site F*347*, SPC and DO had inversely-shaped profiles in the depth interval from 1–4 m below the water table (bwt) (Fig. 3a). SPC increased from 40 μS (indicative of uncontaminated groundwater) to 350 μS in the core of the contaminant plume. DO decreased throughout this same interval from 320 μM to the limit of detection ($\sim 4\,\mu M$). A similar relation between DO and SPC was evident at site F*262* (Fig. 4), though at a deeper vertical interval. The SPC gradient at site F*347* was the result of gradients of inorganic ionic species such as phosphate and chloride (Fig. 3b). However, the shape of the profiles of the

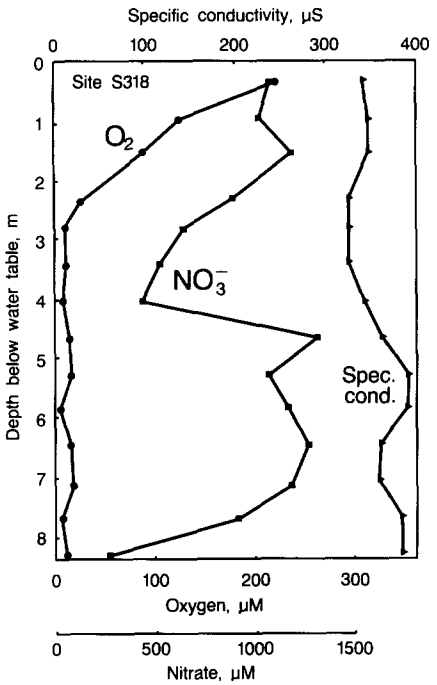

Fig. 2. Vertical profile of dissolved constituents from the MLS at site S*318* in 1986.

inorganic species was not necessarily the same as that of the SPC throughout the entire vertical interval. For example, there was a maximum in the phosphate concentrations at 3.5–4 m bwt (Fig. 3b). The close-interval sampling also detected narrow zones of relatively high concentrations of nitrous oxide at site F*347* (~2 m thick) and ammonium at site F*262* (~4.5 m thick) (Figs. 3a and 4). The entire nitrous oxide-containing zone and the phosphate maximum at site F*347* were not detected in the monitoring wells located at that site (Fig. 3a and b). Groundwater samples obtained from site F*347* as deep as 26 m bwt were contaminated (SPC = 260 µS).

Microbiological gradients

Samples were also collected from the MLS at site F*347* to determine if significant differences existed in the populations and activities of groundwater microorganisms over short vertical intervals. Bacterial abundance in groundwater samples increased over 27-fold in the depth interval from 1.2 to 6.4 m bwt, with the highest population density ($2.4 \cdot 10^6$ organisms mL^{-1}) located at 6.4 m bwt (Fig. 5). However, more pronounced was the change in community structure within this interval. With increasing depth, the general cell

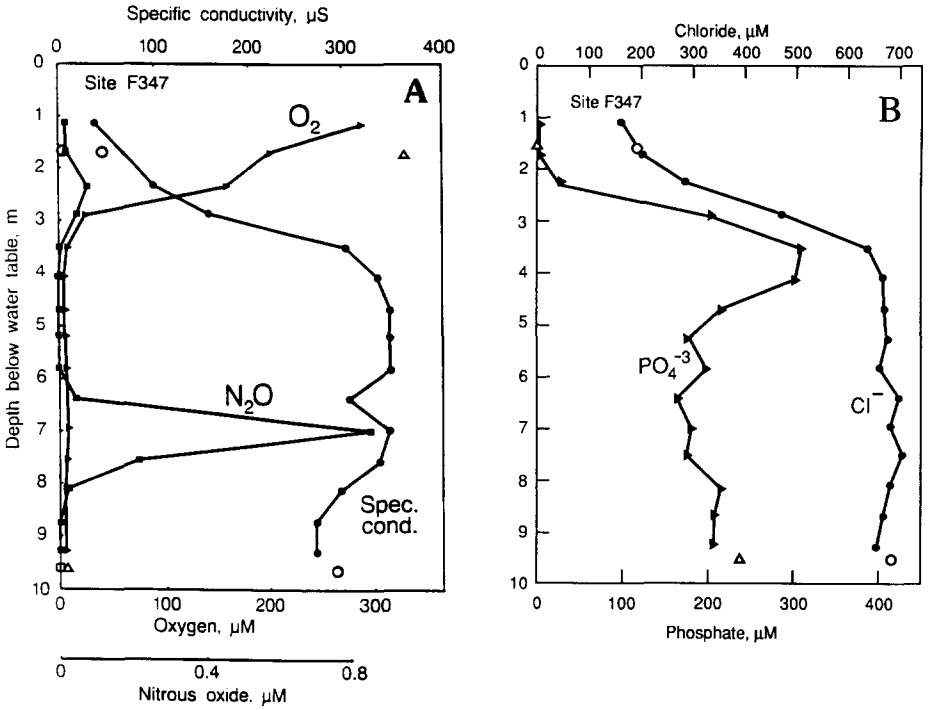

Fig. 3. Vertical profile of dissolved constituents from site F*347* in 1985/1986. *Closed symbols* are samples collected from an MLS; *open symbols* are samples collected from monitoring wells.

morphology of the bacterial population changed from small, relatively uniform cells to much larger, more morphologically diverse organisms (Fig. 6a–c). At 1.7 m bwt the average cell length was 0.3 μm and the estimated bacterial biomass (unattached) was 1.5 $\mu g\, L^{-1} C$. Both parameters increased with depth. Average cell length and biomass were 0.52 μm and 28 $\mu g\, L^{-1} C$, respectively, at 6.4 m bwt, as compared with 0.84 μm and 96 $\mu g\, L^{-1} C$, respectively, at 9.6 m bwt.

Fine-scale vertical variations were also evident in the frequency of dividing bacteria (Fig. 7) and uptake of ^{3}H-glucose (Fig. 8) in groundwater samples from site F*347*. Both parameters increased with depth, but not uniformly. A maximum of 7.1% of the unattached bacterial population was visibly in the process of cell division at 8.1 m bwt, as compared with only 0.2% of the population at 1.1 m bwt. This maximum was three times higher than the values for populations only 1 m above and below the maximum (Fig. 7). Glucose uptake by the microbial population generally increased about 3- to 4-fold with depth from 1.1 to 6.4 m bwt, but the rate of increase accelerated significantly below 7 m. When normalized for bacterial abundance, the

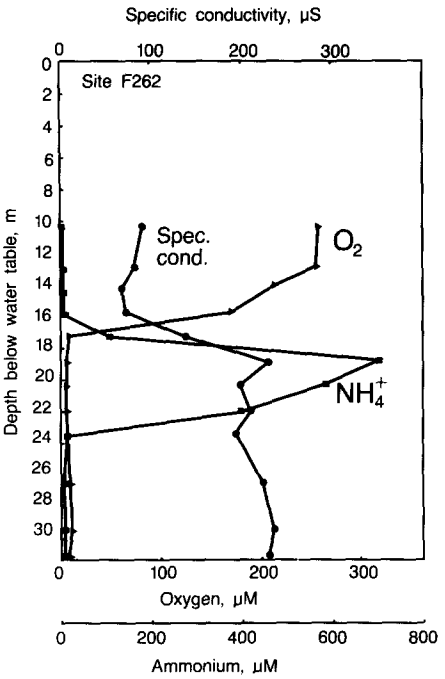

Fig. 4. Vertical profile of dissolved constituents from the MLS at site F*262* in 1987.

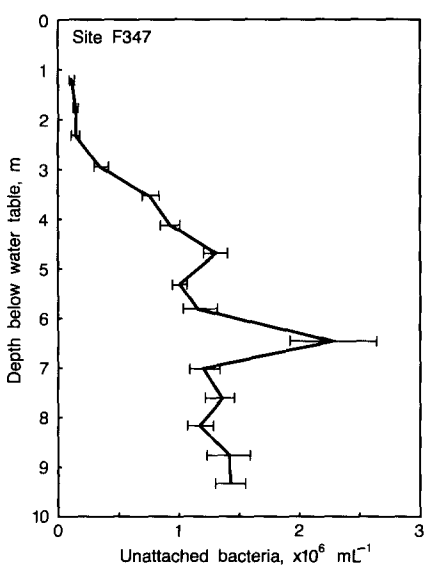

Fig. 5. Vertical profile of the number of free-living bacteria in groundwater collected from the MLS at site F*347* in 1985. *Error bars* represent ± one standard deviation.

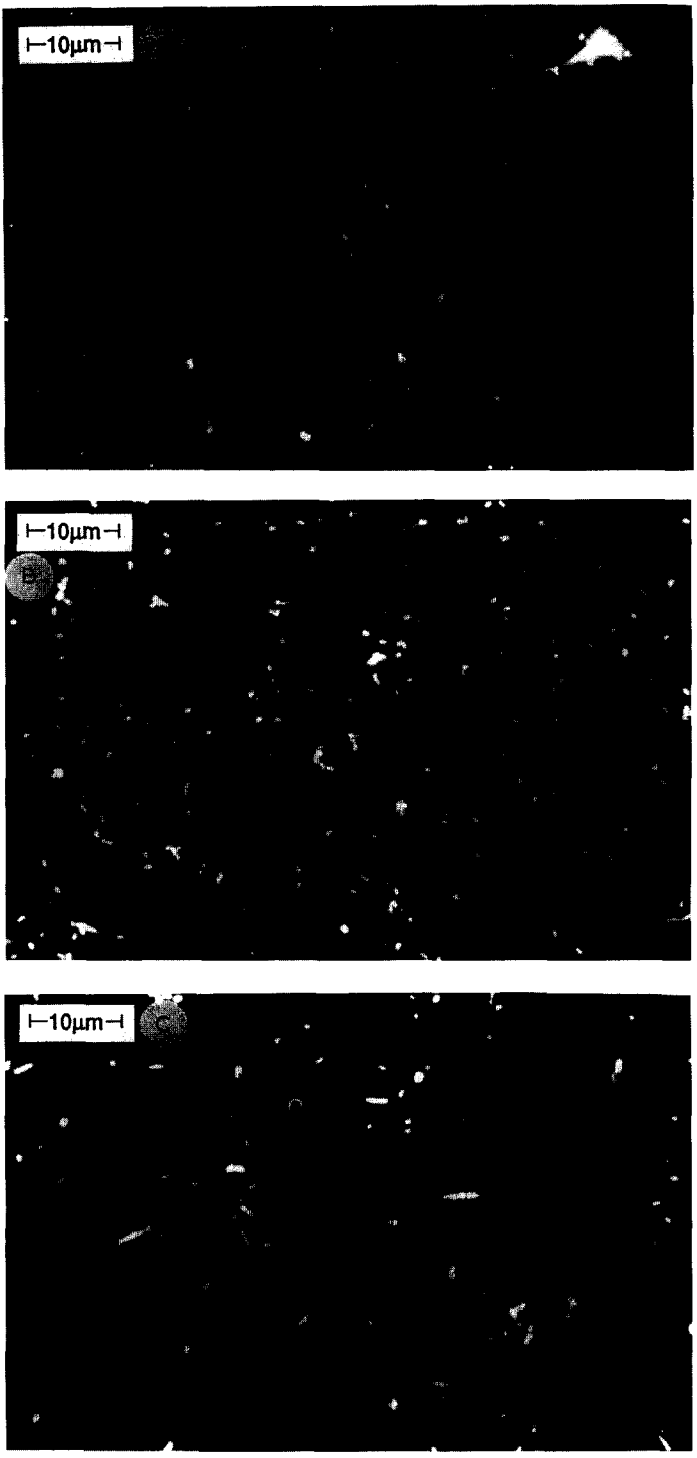

Fig. 6. Photomicrograph of acridine-orange-stained bacteria collected from site F*347* at: (A) 1.68 m; (B) 6.40 m; and (C) 9.60 m, below the water table in 1985. *Bars* are 10 μM.

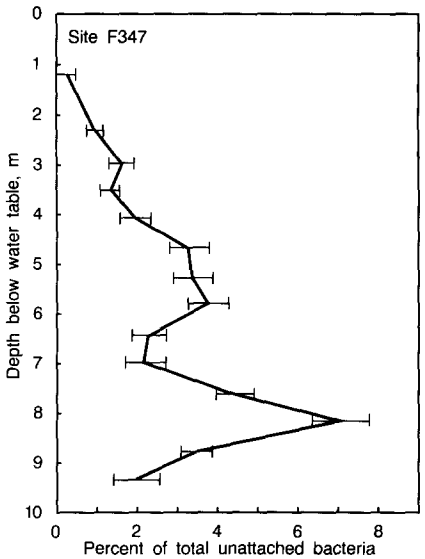

Fig. 7. Vertical profile of the frequency of dividing bacteria in groundwater collected from the MLS at site F*347* in 1985. *Error bars* represent ± one standard error.

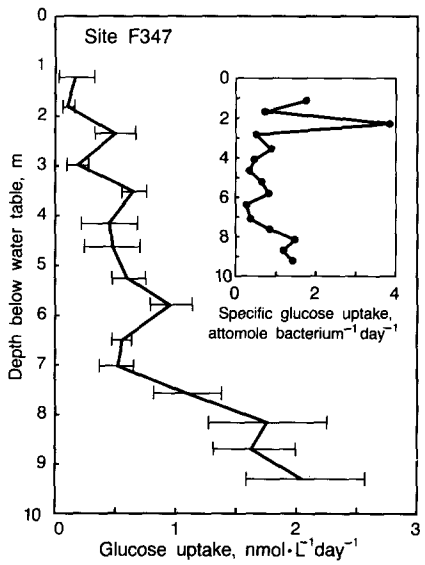

Fig. 8. Vertical profile of the rate of ^{3}H-glucose uptake by bacteria in groundwater collected from the MLS at site F*347* in 1986. Samples were incubated at 10°C with a glucose concentration of 2 μM. Each rate estimate was calculated from a best-fit linear regression of six time points (0–48 hr); *error bars* represent ± one standard deviation.

microbial population at 2.3 m bwt had a much higher rate of specific glucose uptake than did the populations at any other depth (Fig. 8, insert).

DISCUSSION

The three sampling sites were chosen for this study because the groundwater chemistry at these sites represents the general nature of this Cape Cod contaminant plume. As the contaminated groundwater is transported away from the infiltration beds by regional groundwater flow, it sinks and becomes overlain by uncontaminated water. The source of this uncontaminated groundwater is rainfall recharge (LeBlanc, 1984), and consequently the thickness of this uncontaminated layer increases with distance away from the infiltration beds (see Figs. 2, 3a and 4). The result is that throughout the entire length of the contaminant plume (except within the infiltration beds themselves) there is a chemical gradient established on a vertical axis between the uncontaminated groundwater and the underlying contaminated groundwater. This gradient is best delineated by specific conductance. Although the interface between the contaminated and uncontaminated groundwater has been subjected to several years of vertical dispersion, the chemical gradients remain relatively steep with depth at sampling sites located at considerable distances away from the contaminant source. For example, at site F*262*, which is up to 17 years of groundwater travel time away from the source, the zone containing the steep SPC gradient is only 3 m thick. This is also the case for wells located nearly 3000 m away (LeBlanc et al., 1988). The implication is that vertical dispersion is very limited within this aquifer. This conclusion is consistent with the results of a large-scale tracer experiment conducted at the Cape Cod site (Garabedian et al., 1987) in which the measured vertical dispersivity was only 0.15 cm, or > 600 times smaller than the longitudinal dispersivity. Furthermore, Molz and Widdowson (1988) used numerical models of chemical gradients similar to those observed at the Cape Cod site to demonstrate that the steep gradients can persist for long travel distances only if vertical mixing by dispersion is very limited.

The presence of these steep vertical gradients in groundwater points out the need for closely spaced sampling on a vertical axis to adequately delineate the gradients. This is especially important for key constituents such as DO. The O_2 gradients at the Cape Cod site are the inverse of the SPC gradients (except at the infiltration beds) and usually are steeper than the SPC gradients (Figs. 3 and 4). These O_2 gradients are the result of both limited vertical mixing and increased O_2 demand within the contaminant plume. The net result is that O_2 is not present at significant concentrations within the core of the contaminant plume for at least 3000 m from the infiltration beds.

However, the need for closely spaced vertical sampling is not restricted to

boundaries between contaminated and uncontaminated zones. The depth profiles of other dissolved constituents exemplify this point. There was a distinct peak of a dissolved gas, N_2O, within the contaminant plume and below the SPC gradient at site F*347* (Fig. 3a). There were also high concentrations of NO_3^- within this interval (Smith et al., 1987), so the N_2O was likely the result of denitrification (Smith et al., 1989). Likewise, a 6-m thick ammonium-containing zone was evident within the contaminant plume at site F*262* (Fig. 4). Ammonium is a common constituent of the contaminant plume (LeBlanc, 1984; Ceazan et al., 1989); however, because NH_4^+ occurs in thin zones south of site F*262*, MLS's are being used to determine the maximum extent of its movement. In contrast, a NO_3^- minimum was evident in the contaminant plume beneath the sewage infiltration beds (Fig. 2, 4 m bwt). In this case, nitrate concentrations of up to 1 m*M* were decreased in a zone that was only 2.5 m thick. This zone may be the result of either nitrate reduction, because it was located just below the oxygen gradient, or it may represent a spatially variable composite of several contaminant plumes from the eight sandbeds that were being loaded (sequentially on a 3-hr rotation) during this study. In any event, the data clearly demonstrate that both maxima and minima of various parameters can occur within narrow vertical intervals.

As might be expected in a system characterized by chemical gradients, populations of microorganisms change in response to those gradients. Large-scale changes were evident in the number and morphology of microorganisms present in groundwater at site F*347* within the depth interval sampled by the MLS. As already noted, this interval also contains gradients of oxygen, chloride, phosphate and inorganic nitrogen. Bacterial abundance generally increased with depth in this interval, but the shape of the profile did not reflect the profile of any single chemical constituent. There was also considerable fine-scale variation in the frequency of dividing cells (Fig. 7), which is an indication of growth, and in uptake of ^{3}H-glucose, which can be considered as a relative indicator of total heterotrophic activity (Fig. 8). It is interesting to note that the depths of the highest bacterial numbers, the fastest rate of growth, and the highest rate of ^{3}H-glucose uptake were not the same. Each parameter is measuring a different type of response by the free-living microbial populations to the changing geochemical regime within the sampling interval. It is evident that significant changes can occur in these parameters within vertical intervals in the aquifer as narrow as 1 m.

In surface water systems, geochemical gradients often coincide with changing redox boundaries. The same is true in groundwater, at least with respect to oxygen and nitrogen (see Figs. 2, 3a and 4, and Ronen et al., 1987a, b). Determining the location and steepness of redox gradients is critical when attempting to identify the geochemical processes that might be operating

within a particular region of an aquifer. It is also important when considering microbially-mediated processes because the predominant geochemical redox couple is usually the predominant terminal electron acceptor for the microbial community. The physiological capacity of the microbial population in situ is ultimately determined by the terminal electron acceptor. This is particularly relevant when attempting to predict whether contaminants will be subjected to biotransformation or biodegradation. For example, dichlorobenzene, which can be biologically degraded under aerobic conditions, is persistent within this Cape Cod contaminant plume because much of the plume is anoxic (Barber, 1988). As a second example, Kuhn et al. (1989) have demonstrated that the pathway of degradation for hydroxybenzoate isomers in aquifer sediments differs depending upon the electron acceptor involved. Thus, identifying the redox gradients within an aquifer, especially on a vertical axis, is an important aspect in predicting the fate and the speciation of organic or inorganic contaminants entering an aquifer.

The source of this contaminant plume on Cape Cod is a dilute sewage effluent ($\sim 400\,\mu S$) which recharges a glacial sand and gravel aquifer. Therefore, the contaminant plume is not an extreme example; rather, it is representative of the large number of contaminant plumes in the U.S.A. caused by disposal of human and animal wastes to shallow, permeable aquifers. It seems likely that the steep chemical gradients observed at this site are typical of contaminant plumes in similar hydrologic settings. For example, Pickens et al. (1978) used MLS's to delineate steep gradients of chloride in a sandy aquifer contaminated by landfill leachate, while Ronen et al. (1987a, b) reported concentration gradients of oxygen, nitrate, sulfate and chloride on a centimeter scale using dialysis samplers placed in a coastal plain aquifer under a fertilized field.

The particular MLS design used in this study was chosen because the sand and gravel collapsed immediately and completely, so no seals were needed between sampling points, and because the water table was shallow, so the small-diameter tubes could be pumped by suction from land surface. It is likely that steep concentration gradients occur in other geohydrologic settings. Various designs are available for MLS construction and installation (Gillham et al., 1983), and an appropriate design that is tailored to the site-specific conditions should be used.

This study has shown that vertical concentration gradients must be anticipated in shallow unconfined aquifers, even in contaminant plumes that are several kilometers long. Detection of these gradients requires closely spaced vertical sampling. Otherwise, interpretation of data from a few monitoring wells with screens set far apart in the vertical direction may lead to incorrect conclusions about the geochemical and microbiological processes in the aquifer.

CONCLUSIONS

This study has demonstrated that relatively large changes occur on a vertical axis in the concentrations of some of the constituents in a contaminant plume and in the characteristics of the unattached bacterial populations in a sand and gravel aquifer. These vertical gradients exist within the contaminant plume at least 3000 m downgradient from the source of contamination and demonstrate the need for closely spaced vertical sampling when characterizing a contaminant plume of this type. In many cases, sampling intervals of < 1 m may be necessary to adequately characterize the gradients.

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