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In cooperation with the
U.S. Army Garrison, Aberdeen Proving Ground
Environmental Conservation and Restoration Division
Aberdeen Proving Ground, Maryland

Preliminary Assessment of Microbial Communities and Biodegradation of Chlorinated Volatile Organic Compounds in Wetlands at Cluster 13, Lauderick Creek Area, Aberdeen Proving Ground, Maryland

Water-Resources Investigations Report 03-4119

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by Michelle M. Lorah, Mary A. Voytek, and Tracey A. Spencer

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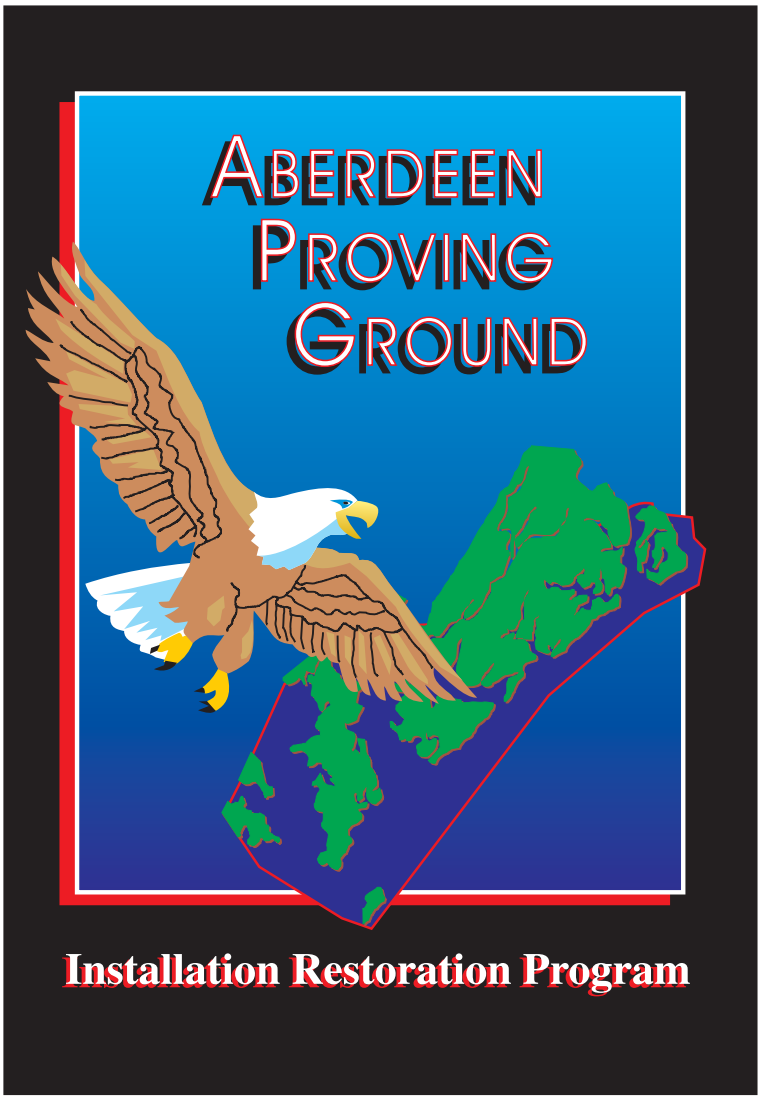

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Aberdeen Proving Ground, Edgewood Area

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Conversion Factors, Vertical Datum, and Abbreviations

Multiply	By	To obtain
	Length	
centimeter (cm)	0.3937	inch
millimeter (mm)	0.03937	inch
meter (m)	3.281	foot
meter (m)	1.094	yard
	Volume	
liter (L)	33.82	ounce, fluid
liter (L)	2.113	pint
liter (L)	1.057	quart
liter (L)	0.2642	gallon
liter (L)	61.02	cubic inch
	Flow rate	
meter per year (m/yr)	3.281	foot per year

Temperature in degrees Celsius ($^{\circ}$C) may be converted to degrees Fahrenheit ($^{\circ}$F) as follows:

$$^{\circ}F = (1.8 \times {}^{\circ}C) + 32$$

Concentrations of chemical constituents in water are given either in micrograms per liter (µg/L) or micromoles per liter (µmol/L).

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Abstract

A preliminary assessment of the microbial communities and biodegradation processes for chlorinated volatile organic compounds was conducted by the U.S. Geological Survey in wetlands at the Cluster 13, Lauderick Creek area at Aberdeen Proving Ground, Maryland. The U.S. Geological Survey collected wetland sediment samples from 11 sites in the Lauderick Creek area for microbial analyses, and used existing data to evaluate biodegradation processes and rates. The bacterial and methanogen communities in the Lauderick Creek wetland sediments were similar to those observed in a previous U.S. Geological Survey study at the West Branch Canal Creek wetland area, Aberdeen Proving Ground. Evaluation of the degradation rate of 1,1,2,2-tetrachloroethane and the daughter compounds produced also showed similar results for the two wetlands. However, a vertical profile of contaminant concentrations in the wetlands was available at only one site in the Lauderick Creek area, and flow velocities in the wetland sediment are unknown. To better evaluate natural attenuation processes and rates in the wetland sediments at Lauderick Creek, chemical and hydrologic measurements are needed along ground-water flowpaths in the wetland at additional sites and during different seasons. Natural attenuation in the wetlands, enhanced bioremediation, and constructed wetlands could be feasible remediation methods for the chlorinated volatile organic compounds discharging in the Lauderick Creek area. The similarities in the microbial communities and biodegradation processes at the Lauderick Creek and West Branch Canal Creek areas indicate that enhanced bioremediation techniques currently being developed for the West Branch Canal Creek wetland area would be transferable to this area.

Introduction

Cluster 13 of the Lauderick Creek area, Aberdeen Proving Ground (APG), Maryland was used for various chemical warfare-related training activities from 1920 until the 1950s (General Physics Corporation, July 1999) (fig. 1). Although historical records are incomplete, it is known that decontamination of chemical warfare material and training activities on clothing impregnation took place. One of the common military decontaminants and components of clothing-impregnating solutions was 1,1,2,2-tetrachloroethane (TeCA) (Nemeth, 1989; Lorah and others, 1997). The major volatile organic compounds (VOCs) detected in ground water in the Cluster 13, Lauderick Creek area are TeCA and trichloroethene (TCE), which are present at maximum concentrations of 86,000 and 6,000 µg/L (micrograms per liter), respectively (General Physics Corporation, July 1999) (fig. 2). Ground-water contamination extends to wetland and creek-bottom areas along Lauderick Creek, especially along the south tributary (figs. 2 and 3).

Since 1992, the U.S. Geological Survey (USGS) has studied natural attenuation of VOCs, primarily TeCA and TCE, in a freshwater tidal wetland along West Branch Canal Creek, which is southwest of Lauderick Creek in the Edgewood area of APG (Lorah and others, 1997; Lorah and Olsen 1999a, 1999b; Lorah and others, 2003) (fig. 1). Field and laboratory data showed that rapid anaerobic biodegradation is a major process in the wetland sediments at the West Branch Canal Creek wetland area and that monitored natural attenuation could be an effective ground-water remediation method at this site. In these wetland sediments, TeCA is biodegraded by simultaneous dichloroelimination and hydrogenolysis reactions, resulting in the production of 1,2-dichloroethene (both *cis* and *trans* isomers) (12DCE) and 1,1,2-trichloroethane (112TCA), respectively. These intermediate compounds biodegrade to vinyl chloride (VC) and 1,2-dichloroethane (12DCA), and ultimately to non-

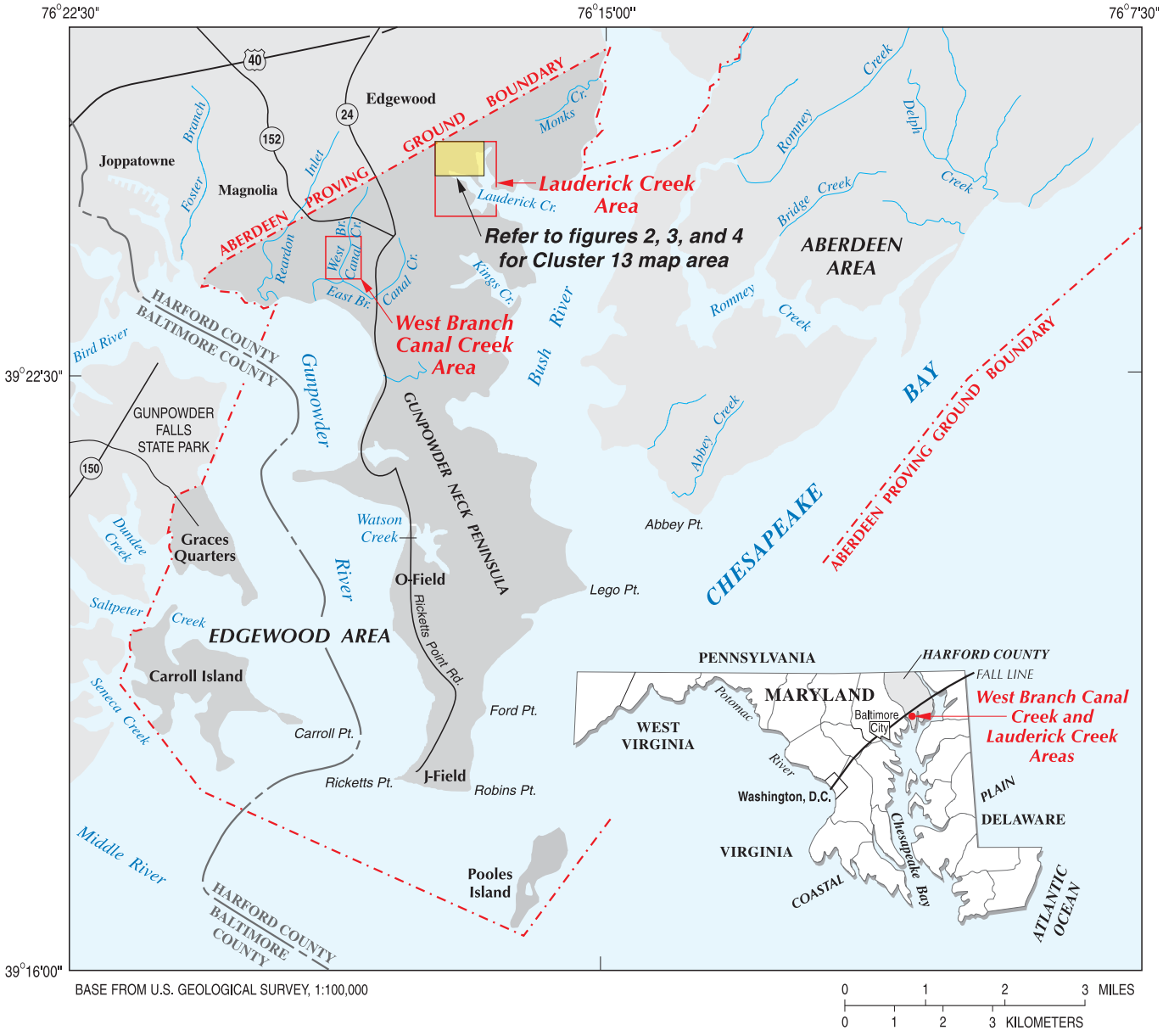

Figure 1. Location of the Cluster 13, Lauderick Creek, and West Branch Canal Creek areas, Aberdeen Proving Ground, Maryland (modified from Lorah and others, 1997, p. 5).

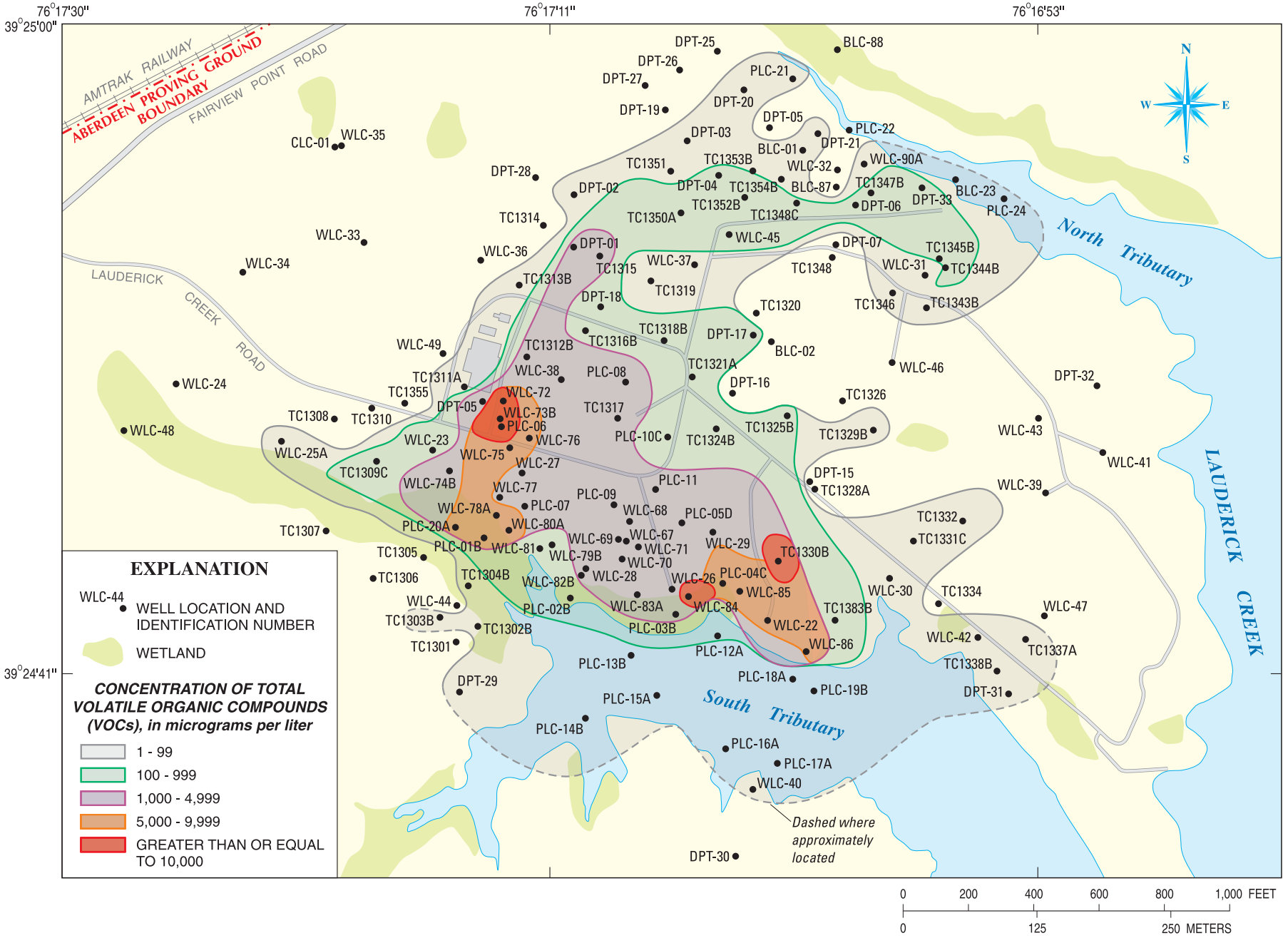

Figure 2. Concentration of total volatile organic compounds in ground water at Cluster 13, Lauderick Creek area (from General Physics Corporation, July 1999).

Figure 3. Well locations and wetland areas at Cluster 13, Lauderick Creek area (from General Physics Corporation, July 1999).

chlorinated, non-toxic VOCs (Lorah and others, 1997). Abiotic degradation of TeCA to TCE also occurs, but this reaction accounted for less than 10 percent of the TeCA removed in laboratory experiments (Lorah and others, 1997; Lorah and others, 2003). In 1999, the USGS began an intensive study of the microbial communities associated with biodegradation reactions in the wetland sediments at the West Branch Canal Creek study area (Lorah and others, 2003). A microbial consortium, rather than one individual microbial species or group, is involved in the degradation of TeCA and TCE in the wetland sediments at West Branch Canal Creek (Lorah and others, 2003). Critical microbial species that are involved in the degradation of TeCA and TCE have been tentatively identified by obtaining terminal-restriction fragment length polymorphism (TRFLP) profiles of the bacterial and methanogen communities during microcosm experiments with the wetland sediment (Lorah and others, 2003). This TRFLP technique is commonly referred to as DNA fingerprinting. Molecular probes also were used to determine the presence of the anaerobic dehalorespiring bacteria *Dehalococcoides ethenogenes* and *Desulfuromonas* species in the wetland sediment at some sites in the West Branch Canal Creek wetland area. These dehalorespiring bacteria can use chlorinated VOCs as terminal electron acceptors to derive energy and grow, allowing more rapid biodegradation of the VOCs than through cometabolic reduction reactions (Maymó-Gatell and others, 1997, 2001).

The microbial communities in the wetland sediments at the Cluster 13, Lauderick Creek area have not been characterized previously, although wetland porewater sampling has been conducted to determine the distribution of TeCA and TCE degradation products and to characterize the redox conditions (General Physics Corporation, July 1999). From October 2001 through September 2002, the USGS, in cooperation with the Environmental Conservation and Restoration Division at APG, conducted a preliminary investigation in the wetlands at Cluster 13, Lauderick Creek area. The objectives of this study were to characterize the microbial communities present in the surficial wetland sediments at the Cluster 13, Lauderick Creek area, and to conduct a preliminary evaluation of the technical feasibility of using natural attenuation or enhanced bioremediation as a remediation method for the ground-water plume. Microbial communities in the Cluster 13 wetland area were compared to available USGS data from the West Branch wetland study area to evaluate the presence or absence of critical microbial species involved in TeCA degradation. This evaluation was conducted to determine the validity of using available data on degradation pathways and rates from the West Branch wetland area (Lorah and others, 2003) to evaluate wetland remediation methods at Lauderick Creek. In addition to the use of monitored natural attenuation in the wetlands as a ground-water remediation method, ongoing studies at the West Branch wetland area are focused on developing methods to enhance *in situ* bioremediation of VOCs in "hotspots," including ground-water seeps. Possible enhancements include the addition of nutrients or electron donors, or the addition of critical microbial species (bioaugmentation) to the existing wetland sediments. The preliminary characterization of the microbial communities and biodegradation rates in the Lauderick Creek wetland area presented in this report was used to evaluate the transferability of the ongoing West Branch wetland remediation studies to Lauderick Creek.

Purpose and Scope

The purpose of this report is to (1) present the results of microbial community analyses of surficial wetland sediment samples from the Cluster 13, Lauderick Creek area, and (2) provide a preliminary assessment of the chlorinated VOC biodegradation processes and rates in the wetlands of the Lauderick Creek area using the collected microbial community data and existing ground-water data for this area. The microbial community and biodegradation in wetlands of the Lauderick Creek and West Branch Canal Creek areas are compared. Sediment samples for microbial community analyses were collected three times from the Lauderick Creek area (December 2001, April 2002, and June 2002). Microbial community analyses for the Lauderick Creek wetland sediments are compared to similar analyses conducted for the West Branch Canal Creek wetland area during 1999–2000 (Lorah and others, 2003). An initial evaluation of the biodegradation processes and potential for use of wetlands for ground-water remediation of VOCs in the Lauderick Creek area is given using available data for the Lauderick Creek area (General Physics Corporation, July 1999) and the West Branch Canal Creek area (Lorah and others, 1997; Lorah and others, 2003).

Description of Study Area

The Cluster 13, Lauderick Creek area is on a peninsula bordered by two tributaries (north and south tributaries) of Lauderick Creek, a tidal creek of the Bush River estuary (fig. 2). The area consists of approximately 67 percent secondary growth upland forest, 25 percent open grassy fields, 5 percent wetlands, and 3 percent developed areas (General Physics Corporation, July 1999). The wetlands primarily are along the northern sides of the north and south tributaries (fig. 3) and consist of freshwater (palustrine) forested wetlands, brackish (estuarine) intertidal emergent wetlands, and palustrine emergent wetlands (General Physics Corporation, July 1999). Wetland plants common to the palustrine emergent areas include phragmites (or common reed), cattails, and rushes, whereas the estuarine emergent species include cordgrass, three squares, and rushes (General Physics Corporation, November 1999).

A shallow surficial sand aquifer that is generally about 6 m (meters) thick is contaminated with chlorinated VOCs (fig. 2). The surficial aquifer consists of silty sands and well-sorted sands in the upper section, and gravelly sands and gravels in the lower section (General Physics Corporation, July 1999). A thick clay unit and an uncontaminated lower confined aquifer underlie the surficial aquifer (General Physics Corporation, July 1999). Three possible source areas in the surficial aquifer have total VOC concentrations

that are indicative of the presence of dense non-aqueous phase liquids (DNAPLs) (areas greater than 10,000 µg/L in fig. 2). All three areas are along the northern side of the south tributary of Lauderick Creek, and wetlands are located immediately downgradient from two of the three source areas (figs. 2 and 3). Chlorinated VOC contamination, including TeCA, TCE, and their anaerobic daughter compounds (primarily 12DCE and VC), also exists in the shallow ground water in the wetland and south tributary (General Physics Corporation, July 1999). One surface-water sample collected offshore in the south tributary had a TeCA concentration of 72 µg/L (total VOCs of 100 µg/L) (General Physics Corporation, November 1999), and one ground-water seep sample collected on the wetland surface adjacent to the south tributary had a TeCA concentration of 41 µg/L (total VOCs of 70 µg/L) (General Physics Corporation, July 1999).

Methods and Data Analysis

Microbial Community Analysis

Grab samples of the surficial wetland sediment were collected manually from 11 sites in the Cluster 13, Lauderick Creek area during three different seasons—winter (December 18, 2001), spring (April 9, 2002), and summer (June 18, 2002). Sediment sampling sites along the south tributary to Lauderick Creek were selected to cover a range of contaminant concentrations using available site data (General Physics Corporation, July 1999) (table 1; fig. 4). Only the south tributary wetlands were sampled because they are the most extensive and are in the most contaminated area in the Cluster 13, Lauderick Creek area. Sediment samples were placed in plastic bags and immediately frozen for later microbial analyses by TRFLP analysis on targeted gene products. Molecular analyses of the microbial communities were done in the USGS laboratory in Reston, Virginia.

Total genomic DNA was extracted from the sediment samples using the Bio101 FastDNA spin kit for soil according to manufacturer's instructions (Bio101 Inc., Vista, California). The bacterial and methanogen communities in the samples were characterized using a polymerase chain reaction (PCR) procedure to amplify specific targeted DNA and performing TRFLP analysis on the PCR products (Clement and others, 1998). In order to evaluate bacterial communities, 16S rDNA was targeted for analysis because it codes for a molecule present in all prokaryotes and can differentiate individual species and groups. PCR was performed to amplify a portion of the bacterial 16S rDNA using the primers 46forward with a fluorescent tag (FAM) and 519reverse (Brunk and others, 1996) at a concentration of 0.2 µmol/L (micromoles per liter). To characterize the methanogenic community, a key functional gene involved in methanogenesis, *mcr*A, was amplified and analyzed. *Mcr*A DNA amplifications were performed using the degenerate *mcr*A oligonucleotide primer pair at 0.2 µmol/L (Klein and others, 1988), except the forward primer was labeled with the fluorescent tag FAM. PCR conditions for bacterial and *mcr*A amplifications are given in Lorah and others (2003).

To obtain molecular fingerprints after amplification, bacterial 16S rDNA amplicons were digested overnight at 37 °C (degrees Celsius) with the restriction enzyme *Mnl*I, whereas *mcr*A amplicons were digested with *Rsa*I (New England Biolabs, Beverly, Massachusetts). Digests were precipitated in ethanol, centrifuged, dried, and resuspended in Tris-EDTA buffer. Samples were electrophoresed on an ABI310 sequencer (Applied Biosystems, Foster City, California), generating a plot of the base-pair size (using internal standards TAMRA 500) and the relative intensity of each fluorescent fragment. Undigested *mcr*A amplicons also were analyzed to determine the proportion of Methanosarcinaceae in the methanogen population on the basis of fragment length heterogeneity. The size of the PCR *mcr*A products differs between the Methanosarcinaceae (464 base pairs), the only group that contains acetotrophs, and the other main methanogen groups (481 base pairs)—Methanococcales, Methanobacteriales, and Methanomicrobiales. Because of this fragment length heterogeneity, the proportion of Methanosarcinaceae in the total methanogen community can be determined.

Table 1. *Location and background information on wetland sediment grab samples collected in the Cluster 13, Lauderick Creek area*

[VOCs, volatile organic compounds; total concentrations in micrograms per liter given for adjacent well sites as reported by General Physics Corporation (July 1999); if the well site included several screened intervals, the highest concentration of total VOCs is given here. Different well types reported by General Physics Corporation (July 1999) included: WLC, monitoring well; PW, U.S. Geological Survey porewater sampler; PLC, Waterloo Multilevel Samplers]

Sediment site number	Site number of adjacent well	Total VOCs in adjacent well
SS1	WLC-25	1.7
SS2	PW-12	2,212
SS3	PW-11; PLC-01	1,904; No data
SS4	PW-10	3,311
SS5	WLC-28; WLC-82	1,335; 15
SS6A	PLC-02	318
SS6B	PW-07	106
SS7	PW-08	636
SS8	PW-08	636
SS9	PW-06; PLC-03	No data; 1,130
SS10	PW-05; PLC-03	1,117; 1,130

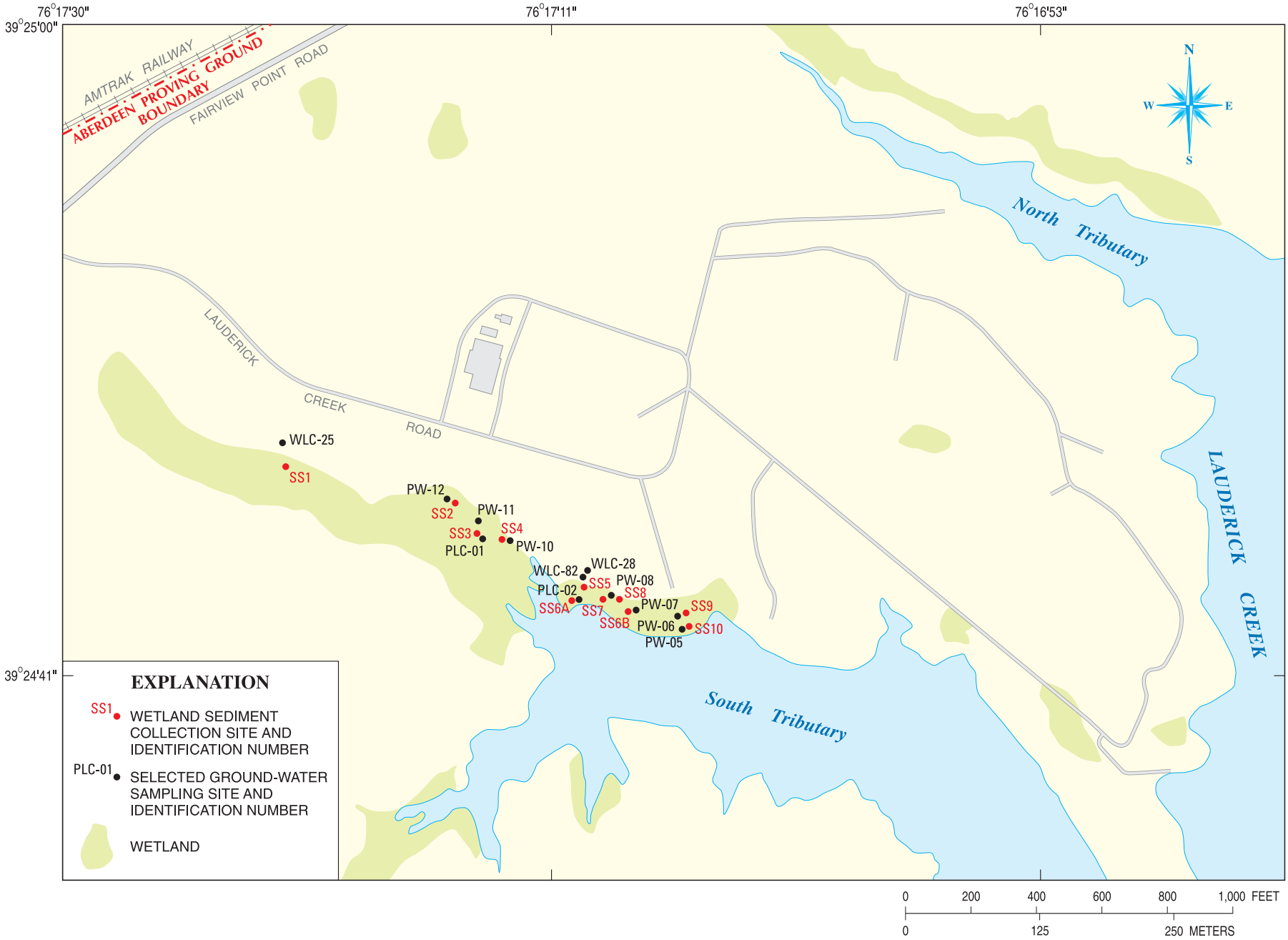

Figure 4. U.S. Geological Survey wetland sediment sampling locations in the Lauderick Creek area and selected previous ground-water sampling sites.

Each sediment sample also was amplified with primers (dhc 730f and dhc 1350r) specific for two groups of dehalorespiring bacteria—*Dehalococcoides* group (*Dehalococcoides ethenogenes* and *Dehalococcoides* sp. strain FL2) and *Desulfuromonas* group (*Desulfuromonas* sp. strain BB1 and *Desulfuromonas chloroethenica*). These primers were obtained from Löffler and represent an updated methodology from the nested primer set reported in Löffler and others (2000), which was determined to have a specificity problem. Positive controls analyzed for each group were 16S fragments that had been cloned into a plasmid by Löffler. DNA negative controls also were analyzed.

Biodegradation Data Analysis

Because ground-water flow directions in discharge wetlands are predominantly vertically upward, biodegradation processes and rates in the wetland sediments can be evaluated using flow velocities and concentrations of VOCs and redox constituents along a vertical flowpath. A review of available data for wetlands in the Cluster 13, Lauderick Creek area, however, showed that water-quality and hydrologic data generally were collected only at one depth in the wetland sediment at all piezometer or other porewater sampling sites located in the wetland (General Physics Corporation, July 1999). Only one site, PW-12, had screens placed at three depths in the wetland sediment (fig. 4). PW-12 consists of a bundle of three Teflon tubes (0.64-cm (centimeter) outside diameter), each with an approximately 2.5-cm-long screened interval vertical. VOC and chloride concentration data collected from site PW-12 in October 1998 were used to estimate field biodegradation rates of TeCA at Lauderick Creek, using the same method previously used at the West Branch Canal Creek area (Lorah and others, 2003). Measured TeCA concentrations were corrected for the effects of advection, dispersion, and dilution by normalization with the conservative tracer chloride, using the correction method outlined by Wiedemeier and others (1998). This tracer-corrected rate can be assumed to represent biodegradation rates if volatilization and sorption effects are assumed to be negligible. Because the shallowest screen at PW-12 is at a depth of 1 m, volatilization is likely to be minimal. Sorption also would have a negligible effect on the estimated field rates if the plume is at steady state (Lorah and others, 2003; Chapelle and Bradley, 1998). The first-order biodegradation rate was estimated by linear regression of the natural logarithm of the normalized TeCA concentration against traveltime along the flowpath. The traveltime was calculated using the linear ground-water flow velocity measured in the wetland sediments at West Branch Canal Creek (Lorah and others, 1997) because wetland flow velocities were not available for Lauderick Creek. Linear ground-water flow velocities from 0.6 to 0.9 m/yr (meters per year) were estimated using flow-net analyses, measured horizontal hydraulic conductivities, and estimated vertical hydraulic conductivities at West Branch Canal Creek (Lorah and others, 1997). The maximum of this estimated velocity range (0.9 m/yr), which was used to estimate field degradation rates for TeCA at West Branch Canal Creek (Lorah and others, 2003), was also used in this report to estimate a degradation rate at Lauderick Creek.

Assessment of Wetland Microbial Communities

The TRFLP analyses of the wetland sediments at Lauderick Creek showed that the bacterial and methanogen communities are similar to those observed at the West Branch Canal Creek wetland area. The TRFLP profiles for the bacterial communities on three sampling dates are shown in the Appendix. Each peak on the TRFLP profiles corresponds to a specific bacterial group, characterized by the fact that the restriction enzyme used in the DNA sample preparation produced the same DNA fragment length (measured by base-pair (bp) size on the *x* axis of the TRFLP profile). To compare the bacterial communities at the Lauderick Creek and West Branch Canal Creek wetland areas, the occurrence and peak area of three of the bacterial groups identified as important in the biodegradation of TeCA at West Branch Canal Creek are shown for wetland sediment samples collected from the two areas—bacterial groups that had a peak bp size from 89 to 90 (89/90), 172, or from 198 to 200 (198/200) (figs. 5a–b). The three bacterial groups were shown to be potentially important in TeCA degradation reactions by their increase in prominence during production of specific daughter compounds in wetland sediment microcosms amended with TeCA (Lorah and others, 2003). The 89/90 peak potentially is involved in the hydrogenolysis of TeCA to 112TCA; and the 172 and 198/200 peaks potentially are involved in the dichloroelimination reactions of TeCA to 12DCE and 112TCA to VC (Lorah and others, 2003). The three peaks generally were detected at all sites where wetland sediment samples were collected at West Branch (Lorah and others, 2003) and at Lauderick Creek. At three of the nine sites sampled at West Branch Canal Creek, all three peaks had approximately equal prominence in the bacterial community, with percent peak areas between 3 and 5 percent (fig. 5a). At four sites sampled at West Branch Canal Creek, the 198/200 peak was more prominent than the 89/90 and 172 peaks, with percent peak areas as high as about 14 percent (fig. 5a). The three bacterial peaks also were present in the Lauderick Creek wetland sediment, although their relative prominence in the bacterial community differs from that in the West Branch Canal Creek sediments. At Lauderick Creek, the 89/90 and 172 peaks had percent peak areas between 3 and 6 in several of the samples; this range is similar to the range observed at West Branch Canal Creek. However, the 172 peak had percent peak areas as high as 10 or 11 in several samples, whereas the 198/200 peak had percent peak areas less than 3 in all sediment samples. Although the 172 and 198/200 peaks are believed to be associated with the same dichloroelimination reactions (Lorah and others, 2003), the predominant bacterial group involved appears to differ between the West Branch Canal Creek and Lauderick Creek wetland areas.

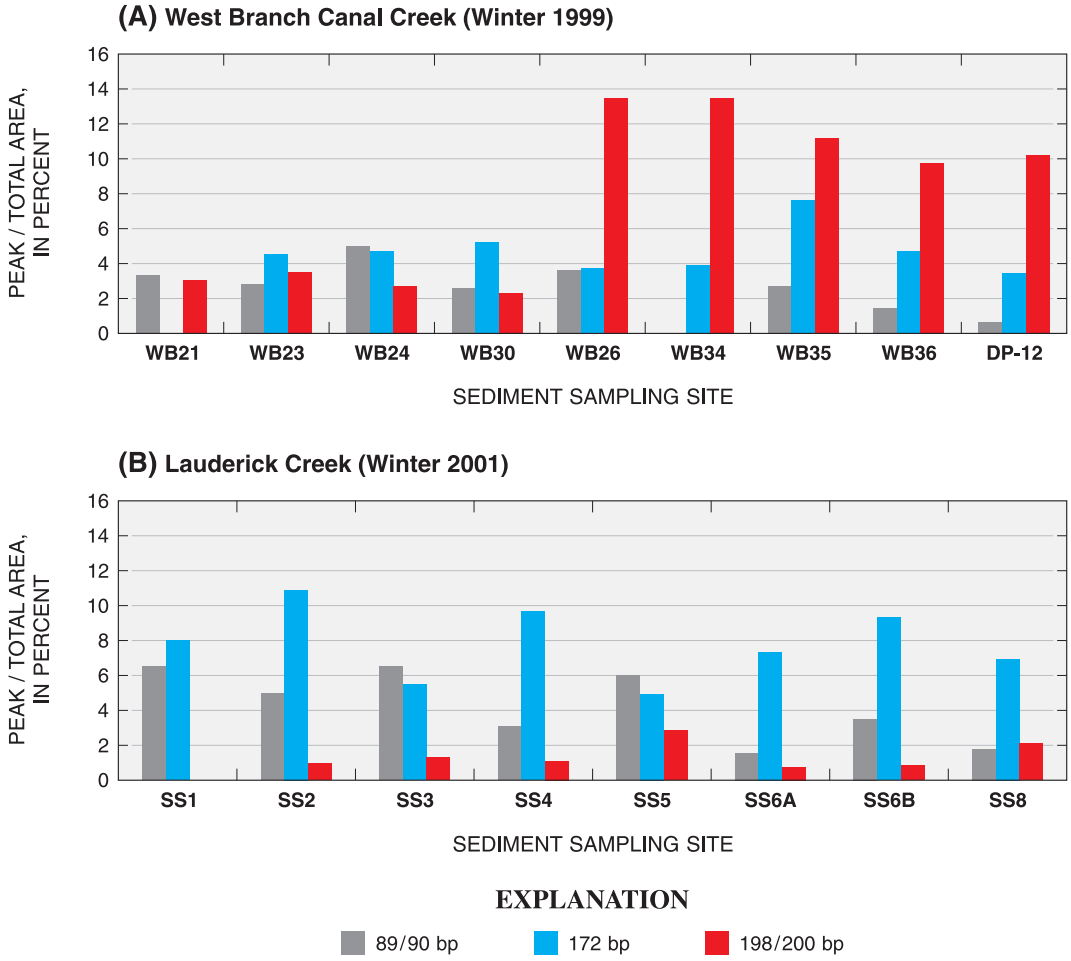

Figure 5. Percent peak areas [area of peak on terminal-restriction fragment length polymorphism (TRFLP) profile divided by the total area of all peaks on the profile] for three bacterial peaks [represented by their base pair (bp) size] that are believed to be associated with 1,1,2,2-tetrachloroethane (TeCA) biodegradation and were observed in wetland sediment samples collected at **(A)** West Branch Canal Creek, winter 1999 and **(B)** Lauderick Creek, winter 2001.

The bacterial communities at West Branch Canal Creek and Lauderick Creek also showed similar seasonal changes. In general, bacterial biomass was lower in the summer than in the winter at West Branch Canal Creek, even though microbial activity generally increases with warmer temperatures (Lorah and others, 2003). The same general biomass decrease was observed at Lauderick Creek, resulting in lower percent peak areas of important degradation-association bacteria in the summer than in the winter at many sampling locations (figs. 6a–b). For example, the bacterial peak at 89/90 bp had percent peak areas of less than 1 in four of the summer samples, but had percent peak areas as high as about 6 in samples collected from the same sites in the winter (fig. 6a). Although the reason for this decrease in importance of bacterial groups in the summer is unknown, it is possible that the growth of the wetland vegetation in the summer affects the microbial community (Lorah and others, 2003). In microcosms constructed with wetland sediment from West Branch Canal Creek and incubated at the same temperature, lower biodegradation rates of TeCA and its daughter products were observed when sediment was collected in the summer than in the winter/early spring (Lorah and others, 2003). The lower biodegradation rates measured in the summer microcosms support the observation that the TRFLP analyses indicate that important bacterial peaks are lower in the summer than in the winter.

The analyses using primers specific for the dehalorespiring bacteria in the *Dehalococcoides* (table 2) showed a similar seasonal trend to that seen for the general bacterial population observed from the TRFLP profiles (figs. 6a–b).

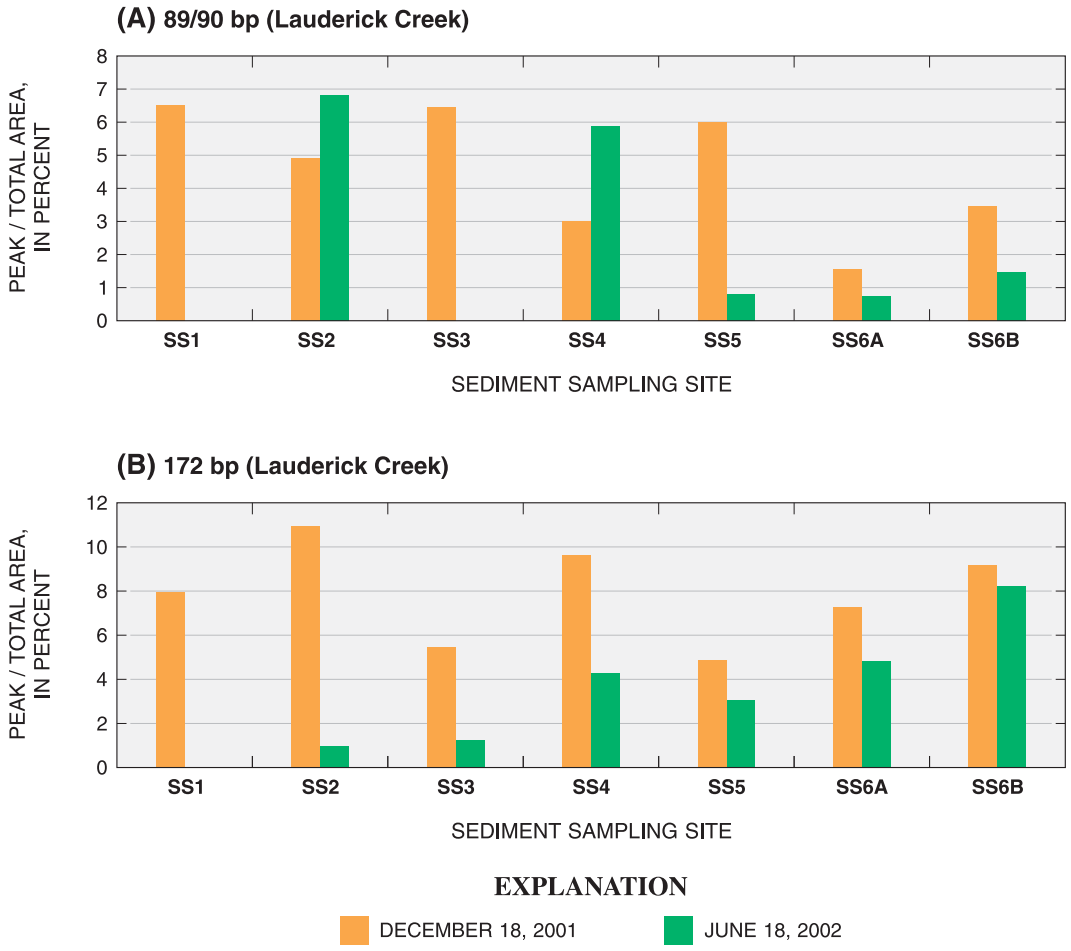

Figure 6. Percent peak areas [area of peak on terminal-restriction fragment length polymorphism (TRFLP) profile divided by the total area of all peaks on the profile] of bacterial peaks [represented by their base pair (bp) size] **(A)** 89/90 bp and **(B)** 172 bp for wetland sediment samples collected at Lauderick Creek in winter (December 2001) and summer (June 2002).

Dehalococcoides species were detected at two sites in the winter and spring but were not detected at any site in the summer sampling period (table 2). *Desulfuromonas*, however, was detected at the same four sites in the spring and the summer, and only two samples showed positive detections in the winter (table 2). The detection level of this qualitative procedure to detect these two groups of dehalorespiring bacteria is unknown; a more quantitative method could assist in discerning seasonal trends. These dehalorespiring bacteria also have been detected in the wetland sediments at West Branch Canal Creek (Lorah and others, 2003), although their seasonal distribution currently is unknown.

For the methanogen community, the relative proportion of Methanosarcinaceae, a family of methanogens that includes all those capable of utilizing acetate as a substrate, was evaluated (table 3). The Methanosarcinaceae are believed to be important in degradation of VC to non-chlorinated compounds in the wetland sediment at West Branch Canal Creek (Lorah and others, 2003). The percentage of Methanosarcinaceae in the total methanogen community ranged from 4.2 to 31 and averaged 14, 14, and 16, respectively, in winter, spring, and summer. The percentage of Methanosarcinaceae showed little seasonal change in these wetland sediments. The percentage of Methanosarcinaceae in the Lauderick Creek sediments was similar to that observed at the West Branch Canal Creek wetland area, which averaged 12 percent (table 4). Thus, both the bacteria and methanogen communities at the Lauderick Creek area generally are similar to those at the West Branch Canal Creek area.

Table 2. *Detections of dehalorespiring bacteria in the Dehalococcoides and Desulfuromonas groups in wetland sediment grab samples collected in the Cluster 13, Lauderick Creek area, 2001–02*

[Positive signs (+) indicate that the bacteria group was detected in the sediment sample, whereas negative signs (-) indicate that it was not detected.]

Sediment site number	*Dehalococcoides*			*Desulfuromonas*		
	12/18/2001	4/9/2002	6/18/2002	12/18/2001	4/9/2002	6/18/2002
SS1	-	-	-	-	-	-
SS2	-	-	-	-	-	-
SS3	-	-	-	-	+	+
SS4	-	-	-	-	-	-
SS5	+	+	-	-	+	+
SS6A	+	+	-	-	-	-
SS6B	-	-	-	-	-	-
SS7	-	-	-	+	+	+
SS8	-	-	-	+	+	+
SS9	-	-	-	-	-	-
SS10	-	-	-	-	-	-

Table 3. *Abundance of Methanosarcinaceae as a percentage of the total methanogen community in wetland sediment grab samples collected in the Cluster 13, Lauderick Creek area, 2001–02*

[Dashes indicate data were not available]

Sediment site number	Percentage of Methanosarcinaceae		
	12/18/2001	4/9/2002	6/18/2002
SS1	15	–	–
SS2	5.2	12	–
SS3	22	16	20
SS4	16	10	10
SS5	6.1	14	12
SS6A	14	7.8	11
SS6B	10	16	20
SS7	15	18	14
SS8	4.2	4.5	14
SS9	17	8.8	9.1
SS10	25	31	31

Table 4. *Abundance of Methanosarcinaceae as a percentage of the total methanogen community in wetland sediment grab samples collected in the West Branch Canal Creek area, March 1999*

[From Lorah and others, 2003]

Site number	Percentage of Methanosarcinaceae
WB19	14
WB23	11
WB24	12
WB30	15
WB35	13
WB36	6.1

Assessment of Biodegradation of Chlorinated Volatile Organic Compounds

The similar microbial communities in the wetland sediments at Lauderick Creek and West Branch Canal Creek indicate that similar biodegradation processes are occurring in both areas. The distribution of chlorinated VOCs with depth in the wetland porewater at site PW-12 shows that TeCA is degrading in the Lauderick Creek area and producing daughter compounds in proportions similar to those observed in the West Branch Canal Creek area (figs. 7a–b). TeCA concentrations decreased by a factor of 10 with decreasing depth along a 2-m vertical profile in the wetland sediments at site PW-12 (fig. 7a). The production of daughter compounds observed along this profile could account for the entire decrease in TeCA concentration. At both the Lauderick Creek and West Branch Canal Creek areas, 12DCE and VC are the predominant, persistent daughter compounds produced in the wetland porewater (figs. 7a–b), which is consistent with observations from microcosm experiments (Lorah and others, 1997; Lorah and Olsen, 1999a; Lorah and others, 2003). Relatively low concentrations of 112TCA also are observed at both sites. Microcosm experiments with West Branch Canal Creek sediments

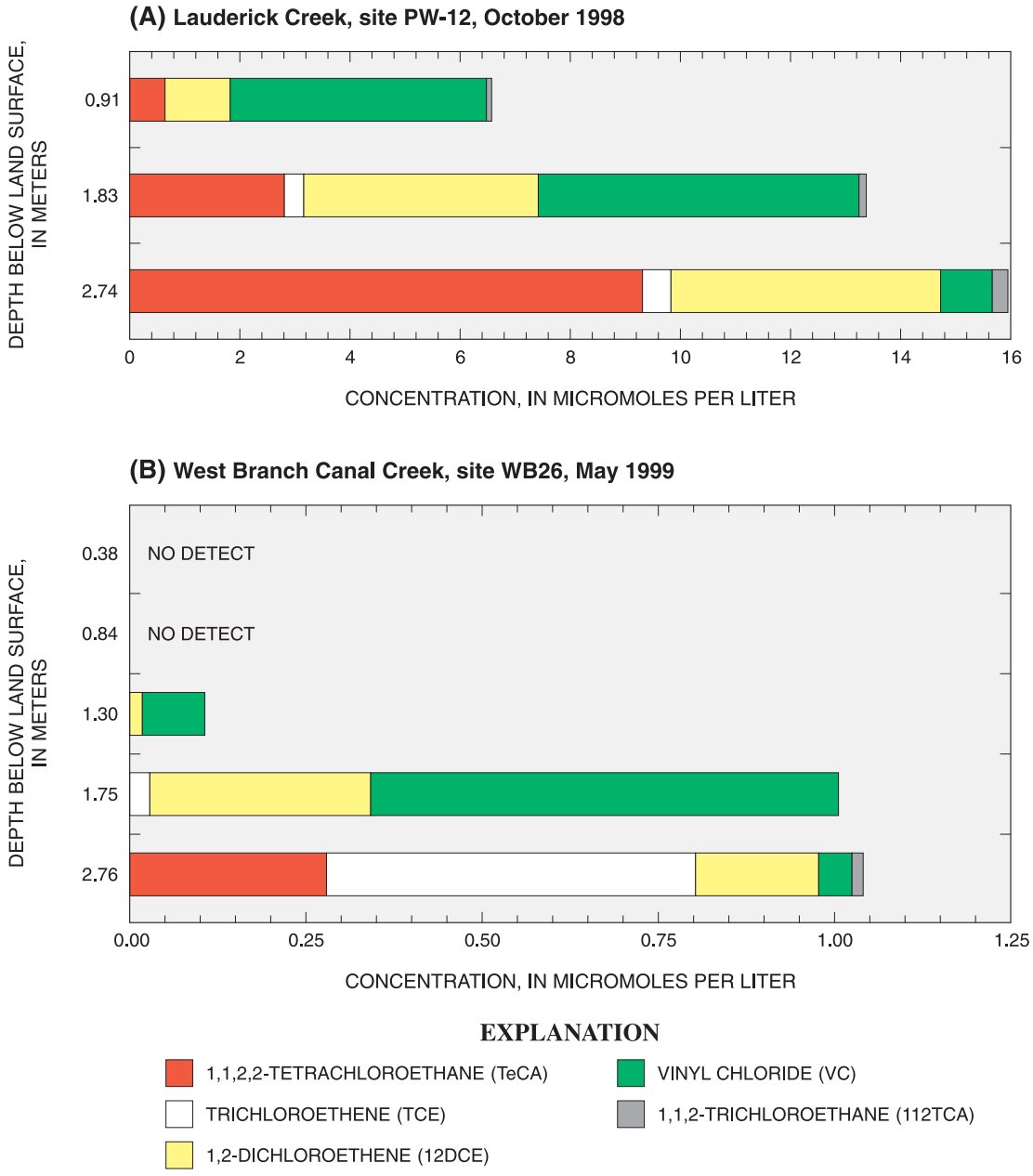

Figure 7. Distribution of 1,1,2,2-tetrachloroethane (TeCA) and its anaerobic daughter products in wetland porewater from **(A)** site PW-12, Lauderick Creek, October 1998, and **(B)** site WB26, West Branch Canal Creek, May 1999.

indicate that 112TCA is produced from TeCA degradation at the same or a higher molar concentration than 12DCE, but that the 112TCA then is degraded more rapidly than the 12DCE (Lorah and Olsen, 1999a; Lorah and others, 2003). Field and laboratory data from the West Branch Canal Creek area show that 112TCA is degraded to VC and 12DCA, but 12DCA analyses were not reported for the wetland porewater at Lauderick Creek (General Physics Corporation, July 1999). The relatively low proportion of TCE present in the wetland porewater at Lauderick Creek (figs. 7a–b) is consistent with results of microcosm experiments with the West Branch Canal Creek wetland sediments, which indicate that production of TCE accounts for less than 10 percent of the TeCA degraded (Lorah and others, 2003).

TeCA concentrations near the base of the wetland sediments at Lauderick Creek are about a factor of 10 higher than those generally observed in the West Branch Canal Creek sediments, and not all the TeCA has been degraded (or attenuated by other processes) by the time the shallowest sampling depth is reached (fig. 7a). However, the shallowest wetland porewater samples in the Lauderick Creek area were collected from a depth of about 1 m (General Physics Corporation, July 1999), whereas passive diffusion samplers, called peepers, were used at West Branch Canal Creek to obtain detailed vertical profiles of concentrations between 0 and 60 cm (Lorah and Olsen, 1999a, b). The peeper profiles at West Branch Canal Creek typically have shown that total VOC concentrations are near or below detection levels before land surface is reached (Lorah and others, 1997; Lorah and Olsen, 1999a, b). Because of the higher TeCA concentrations at Lauderick Creek, it is unclear whether similar complete attenuation would occur before land surface (or the surface of the creek bottom sediments) is reached.

Using the wetland porewater flow velocity of 0.9 m/yr that was calculated for the West Branch Canal Creek area, a TeCA biodegradation rate for the Lauderick Creek wetland sediment was calculated from the measured TeCA concentrations at site PW-12 (fig. 8). These calculations give a first-order degradation rate constant of 0.0034 per day, which corresponds to a half-life of about 200 days. For the West Branch Canal Creek area, field-derived TeCA degradation rates ranged from 0.0065 to 0.0085 per day at four sites, corresponding to a half-life of about 100 days (Lorah and others, 2003). The estimated degradation rates for both areas, therefore, are within the same order of magnitude. If flow velocity was determined for the Lauderick Creek wetland sediments, more accurate degradation rates could be calculated. A flow velocity of 1.8 m/yr for Lauderick Creek sediments, rather than the estimated 0.9 m/yr, would result in the same TeCA degradation rates as those calculated from field data for the West Branch Canal Creek.

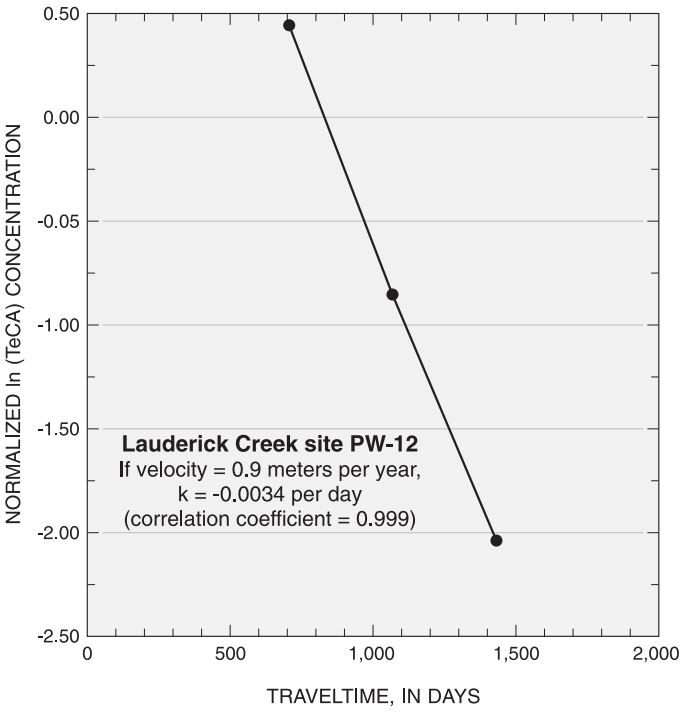

Figure 8. Estimated first-order 1,1,2,2-tetrachloroethane (TeCA) degradation rate calculated using the natural log of TeCA concentrations (normalized by chloride concentrations) at site PW-12, Lauderick Creek area, and estimated traveltime in wetland porewater (calculated assuming a vertical ground-water flow velocity of 0.9 meters per year).

Summary and Conclusions

The U.S. Geological Survey conducted a preliminary evaluation of the microbial communities and biodegradation processes in the wetland sediments at the Cluster 13, Lauderick Creek area, Aberdeen Proving Ground, Maryland, where ground water contaminated with chlorinated volatile organic compounds (VOCs) discharges from a sand aquifer to the wetland. Preliminary data from the Cluster 13, Lauderick Creek wetland area were compared to the more extensive data available from ongoing U.S. Geological Survey (USGS) studies in the West Branch Canal Creek wetland area, Aberdeen Proving Ground, to evaluate the technical feasibility of using natural attenuation or enhanced bioremediation methods for the ground-water plumes at the Lauderick Creek area. At both the Lauderick Creek and West Branch Canal Creek sites, 1,1,2,2-tetrachloroethane (TeCA) is the major VOC present in the aquifer.

Microbial communities in the Cluster 13 wetland area were compared to available USGS data from the West Branch wetland study area to evaluate the presence or absence of critical microbial species involved in TeCA degradation. The microbial community analyses showed that

similar bacterial and methanogen groups are present in the wetland sediments of the Cluster 13, Lauderick Creek area and the West Branch Canal Creek area. The bacterial and methanogen groups that have been identified as potentially important in the degradation reactions at West Branch Canal Creek are present in the Lauderick Creek wetland sediments and show similar seasonal patterns. These results indicate that similar TeCA biodegradation reactions would occur in sediments in both areas, and examination of the vertical distribution of TeCA and its anaerobic degradation products in the wetland porewater at one site (PW-12) supported this hypothesis. The TeCA daughter compounds produced and their relative proportions are similar in wetland porewater at Lauderick Creek and West Branch Canal Creek. Flow velocity in the wetland porewater at Lauderick Creek is unknown. However, if the flow velocity is assumed to be the same as at West Branch Canal Creek, a TeCA degradation rate constant of 0.0034 per day is calculated using the porewater concentrations at site PW-12. This rate constant corresponds to a half-life of about 200 days, whereas the field-calculated half-life of TeCA in the West Branch Creek wetland porewater is about 100 days.

The similar microbial communities and biodegradation processes and rates at Lauderick Creek and West Branch Canal Creek indicate that natural attenuation could be a remediation method for VOCs discharging to the wetland and creek-bottom sediments. Additional data are required to confirm the technical feasibility of natural attenuation, however. TeCA concentrations discharging to the Lauderick Creek wetlands are about a factor of 10 higher than those at West Branch Canal Creek, and substantial concentrations of TeCA, 1,2-dichloroethene, and vinyl chloride remained at the shallowest depth sampled (0.9 meters) at the one site for which contaminant distributions were available along a vertical flowpath (PW-12). Additional porewater data on VOC distributions in the upper 1 meter of the wetland sediments are needed to confirm attenuation of TeCA and its daughter compounds. Seasonal data on VOC distributions in the wetland porewater are critical because the West Branch Canal Creek study has shown that biodegradation rates are slower and VOC concentrations are higher in the wetland porewater in the summer than in the winter/early spring. To accurately determine degradation rates, nested piezometers in the wetland sediments are needed to obtain hydrologic measurements for calculation of flow velocities.

In addition to natural attenuation, enhanced bioremediation and constructed wetlands are possible remediation methods for the contaminants discharging in the Lauderick Creek area. As part of an enhanced bioremediation study in the West Branch Canal Creek area, a mixed culture capable of rapid biodegradation of TeCA and its anaerobic daughter compounds has been derived from the natural microbial community in the wetland sediments. The similarity of the natural microbial communities at Lauderick Creek and West Branch Canal Creek indicates that the mixed culture derived from West Branch Canal Creek wetland sediments could be applied to the Lauderick Creek area. Enhanced bioremediation techniques may be applicable in “hotspots” such as seep areas in wetland areas and Lauderick Creek. Wetlands are not present, however, immediately downgradient from some of the most highly contaminated areas in the aquifer near the south tributary of Lauderick Creek (southeast of sites WLC-84 and WLC-85). Construction of wetlands in this area could allow natural attenuation of the VOCs in wetland sediments before discharge to Lauderick Creek. These possible natural, enhanced, and constructed wetlands remediation methods could be used in conjunction with source removal remediation techniques. If complete source removal is infeasible, the natural and enhanced wetlands could act as a final treatment step for the remaining contaminants flowing from the source areas, and for those contaminants present outside the source treatment areas.

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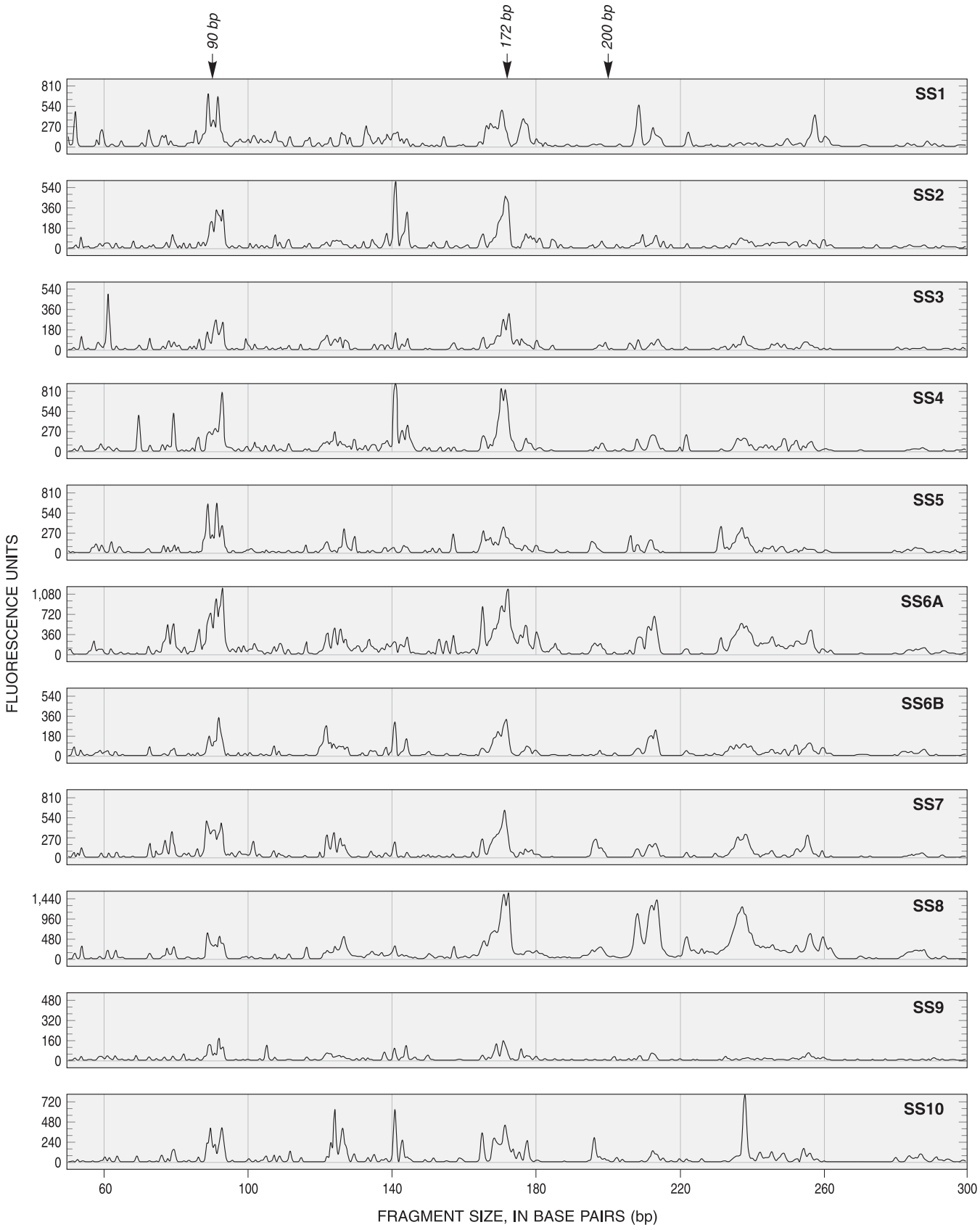
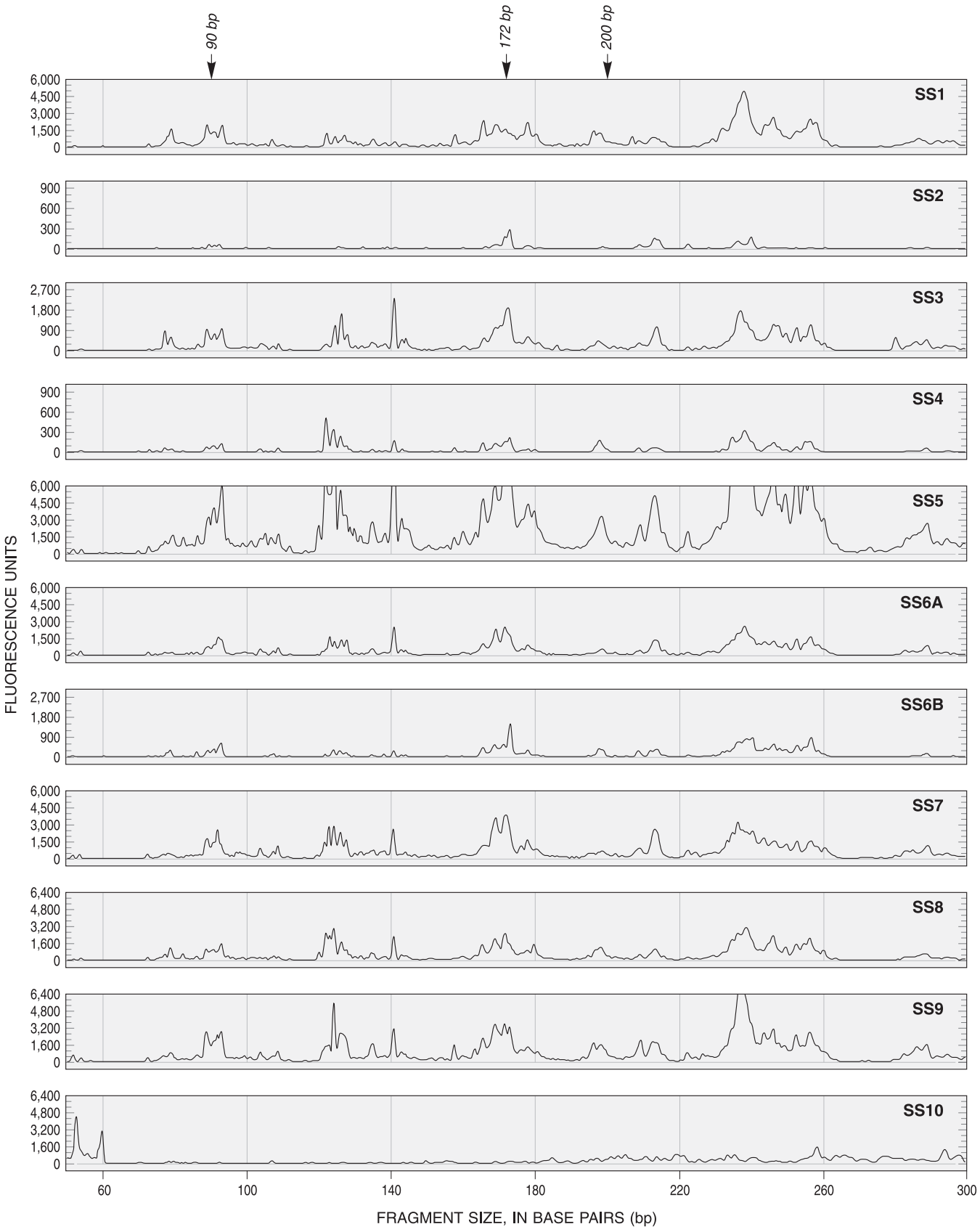
Appendix—Bacterial Profiles

Appendix A1. *Bacteria terminal-restriction fragment length polymorphism (TRFLP) profiles in sediment from Lauderick Creek surficial sediment collection sites, December 18, 2001*



Appendix A2. *Bacteria terminal-restriction fragment length polymorphism (TRFLP) profiles in sediment from Lauderick Creek surficial sediment collection sites, April 9, 2002*



Appendix A3. *Bacteria terminal-restriction fragment length polymorphism (TRFLP) profiles in sediment from Lauderick Creek surficial sediment collection sites, June 18, 2002*