

5.2 SEDIMENT

5.2.1 Perchlorate Occurrence in Sediment Pore Water

The project team sampled sediment pore water constituents to evaluate the presence and distribution of perchlorate in sediment pore water within streams and in delta areas of Lake Waco and Lake Belton. The various studies in which sediment pore water constituents were sampled are discussed in detail in the following sub-sections.

5.2.1.1 *Stream Sediment Pore Water*

5.2.1.1.1 Introduction

Pore water sampling in streams surrounding the NWIRP site is a vital facet of the overall exposure assessment. Pore water concentrations in sediment are both temporal and spatially variable. While bulk stream sampling is a vital means of exposure assessment, it represents a small snapshot of the overall exposure due to its dependence on source flow, dilution, and other variables. Further, it does not represent the exposure to sediment borne organisms or plants that derive their water from the sediment. Sediment pore water data collected can provide both high resolution vertical spatial analysis of perchlorate as well as seasonal variations in exposure. This information benefits the overall effort to determine the potential for exposure and trophic transfer.

5.2.1.1.2 Methodology

Seasonal sediment pore water sampling was conducted at multiple stream sites [Harris Creek at Highway 84 (T19, HW84 Mainstream), the spring on Oglesby Road (T18, HW84 Sidestream), Harris Creek at Highway 317 (T13, HW317), and S Creek at Highway 317 (T15, HW317/MN)] using peepers from May 2001 through October 2002. These locations are shown on **Figure 5-82**, and the sampling plan is summarized in **Table 5-14**. The specifically designed peepers were multi-chambered equilibrium dialysis samplers, each with length of 40-60 cm containing 13-22 chamber cells (**Figure 5-83**). Each chamber cell can hold 9 mL of water, with a center-to-center separation of 2 cm. The design is a modification of the original Hesslein peeper (Hesslein, 1976). Peepers were filled with de-ionized water, then covered with 0.45- μ m pore size Tuffryn membrane (Pall Corporation).

The peepers were inserted into the saturated media by directly driving the peepers using a hammer to the desired depth with minimum disturbance to the sediment layer and allowed to equilibrate for a given period of time (2-4 weeks). In general, samplers were deployed to monitor up to 10 cm above the sediment-water interface and up to 20 cm below the sediment-water interface at 2-cm intervals. After the chambers equilibrated with the surrounding pore water, the peepers were removed and sampled by piercing the membrane with a clean syringe needle and withdrawing the water. Bulk water samples were taken at the time of peeper insertion and retrieval. Water samples were put on ice and then transferred to the laboratory for determination of a variety of anions including ClO_4^- , Cl^- , NO_3^- , NO_2^- , and SO_4^{2-} .

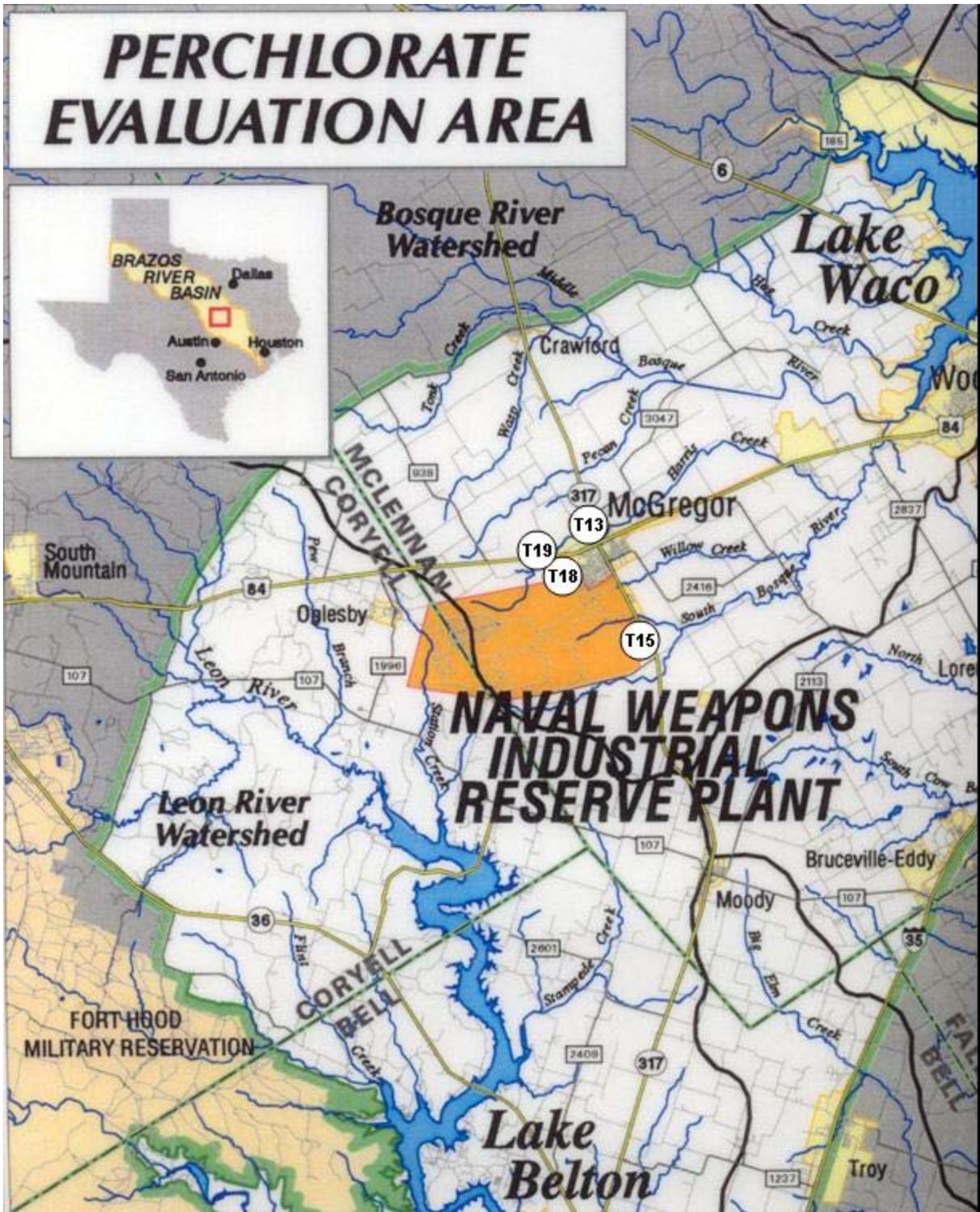
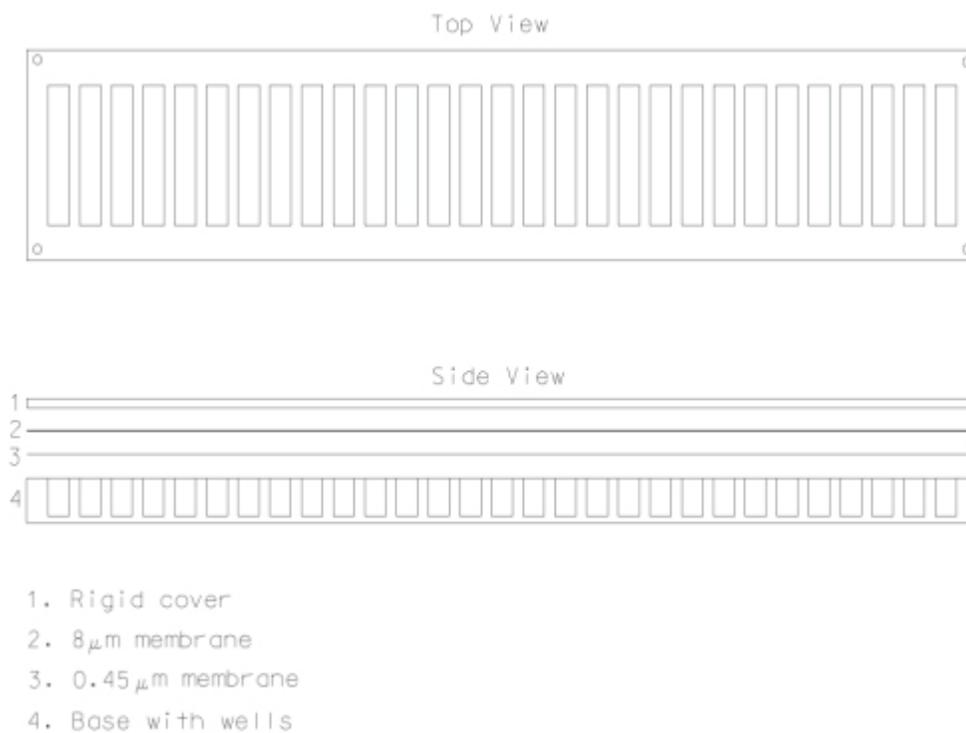


Figure 5-82
Map of Study Area Illustrating the Approximate Locations Where Sediment Pore
Water Samples Were Collected

**Table 5-14
Seasonal Sediment Pore-Water Sampling Plan at Multiple Sites Near NWIRP**

Site	May	Aug.	Oct.		Jan.	April	June	Aug.	Oct.
	2001				2002				
HW84 Sidestream, T18	-	-	+		+	+	+	+	+
HW84 Mainstream, T19	+	-	+		+	*	+	+	+
HW317, T13	-	+	+		+	+	+	+	*
HW317/MN, T15	-	-	+		+	+	*	+	dry stream

+ sampled; - Not sampled; * Peeper was deployed but could not be found.



**Figure 5-83
Schematic of the Peeper (Not to Scale)**

A Dionex DX-500 ion chromatograph was used to determine perchlorate (ClO_4^-) and other anions (Cl^- , NO_3^- , NO_2^- , and SO_4^{2-}) in accordance with EPA Method 314.0 and 300.0, respectively. All samples were filtered with 0.45 μm syringe filter prior to the analysis. The method to determine perchlorate is described in **Appendix X**. The method to determine other anions (Cl^- , NO_2^- , NO_3^- , and SO_4^{2-}) uses IonPac AS14A column (4 x 250 mm), 8 mM Na_2CO_3 / 1mM NaHCO_3 eluent, 1.0 ml/min flow rate, and an anion atlas electrolytic suppressor (AAES) at 57 mA.

5.2.1.1.3 Data

HW84 Sidestream (T18) has a constant flowrate of approximately 1 gal/sec (324 m^3/day) year-around. This sampling location is approximately 200 m downstream from the head of the stream where there is a continuously flowing spring. This stream consistently contains perchlorate in the bulk stream water between 15 ppb and 30 ppb, with a high of 60 ppb observed in October, 2001 (**Figure 5-84**). In case of points not connected by the solid line in the figure, it means there are data missing either due to the broken cells or samples not tested. Results indicate that perchlorate was completely depleted at depths greater than 20 cm below the sediment-water surface for all months studied. Perchlorate and nitrate were degraded simultaneously. Chloride concentration was fairly constant in the sediment pore water, implying biodegradation instead of dilution was a major process resulting in the concentration decrease of other anions in the sediment pore water. Sulfate reduction did not start until preferential electron acceptors nitrate and perchlorate were depleted at depth -15 cm to -20 cm. There were large seasonal variations in profiles of all anions except chloride at this site.

To compare seasonal differences, perchlorate and nitrate concentrations, respectively, were plotted against sampling months in **Figure 5-85**. Bulk water nitrate concentrations in this stream were in the range of about 10 to 20 ppm (reported as NO_3^- -N). Perchlorate penetration into bed sediments ranged from 0-20 cm with minimum penetration in summer (June) and greatest penetration in cold weather-months (October, January, and April). The temporal variation of perchlorate penetration might be linked with the seasonal variation of bacterial activity affected by temperature. Perchlorate distribution closely mirrored nitrate distribution in sediment pore water, and perchlorate degradation and nitrate degradation were observed almost simultaneously in an active degradation zone (with a varying depth of 5-10 cm), from about 10 cm below to 20 cm below the sediment-water interface.

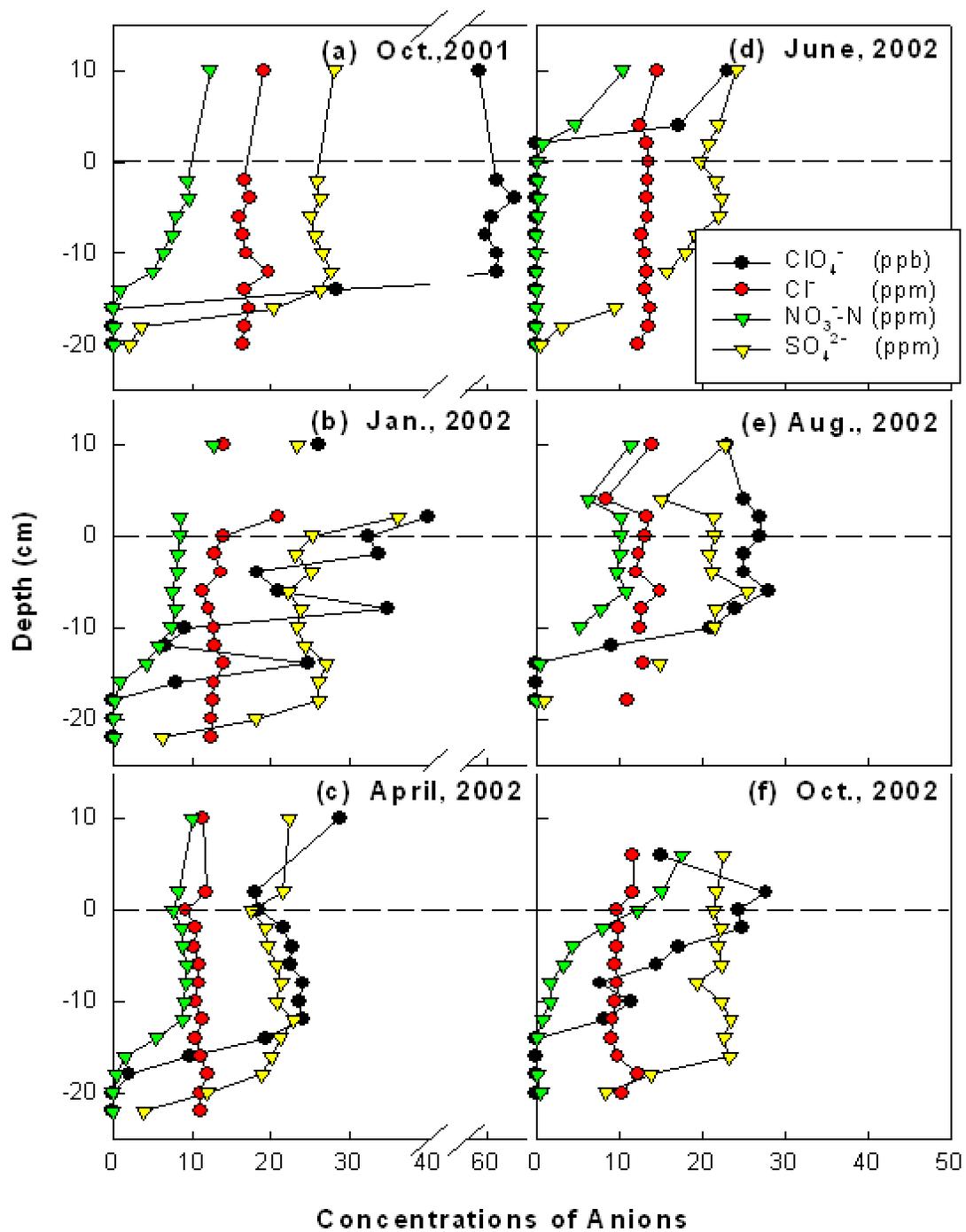


Figure 5-84
Profiles of Anions in Sediment Pore Water at HW84 Sidestream (T18) from October 2001 to October 2002

(Dash lines represent the sediment-water interface. "+" represents depth above the sediment-water interface, and "-" represents depth below the sediment-water interface.)

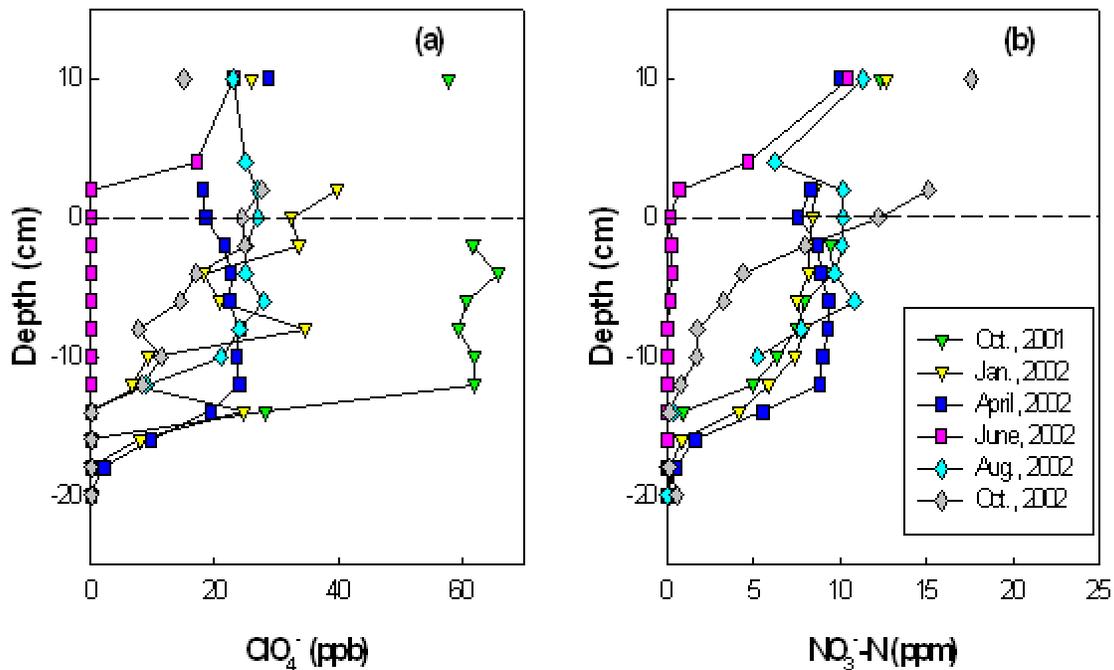


Figure 5-85
Seasonal Variation of Perchlorate and Nitrate Penetration in Sediment Pore Water at HW84 Sidestream (T18): (a) ClO_4^- vs. Depth; (b) NO_3^- -N vs. Depth

(Dash lines represent the sediment-water interface. "+" represents depth above the sediment-water interface, and "-" represents depth below the sediment-water interface.)

The HW84 Mainstream (T19) site receives perchlorate-contaminated water from HW84 Sidestream. The water level in the stream varies dramatically following rainfall events or droughts (fluctuation 0.3-1.5 m yearly). Our results indicate rapid perchlorate degradation occurring in the sediment. Generally, perchlorate penetration in the sediment was not observed for all monitored months with the exception of the May, 2001 and January, 2002 sampling events (**Figure 5-86**). The reason for the perchlorate penetration in May, 2001 is not very clear, but may be linked with high concentration of groundwater discharge. Perchlorate was not degraded until a depth of 10 cm into the sediment in January 2002, which may have been due to slow bacterial degradation rate in cold weather conditions. Perchlorate and nitrate degradation followed similar patterns (**Figure 5-87**).

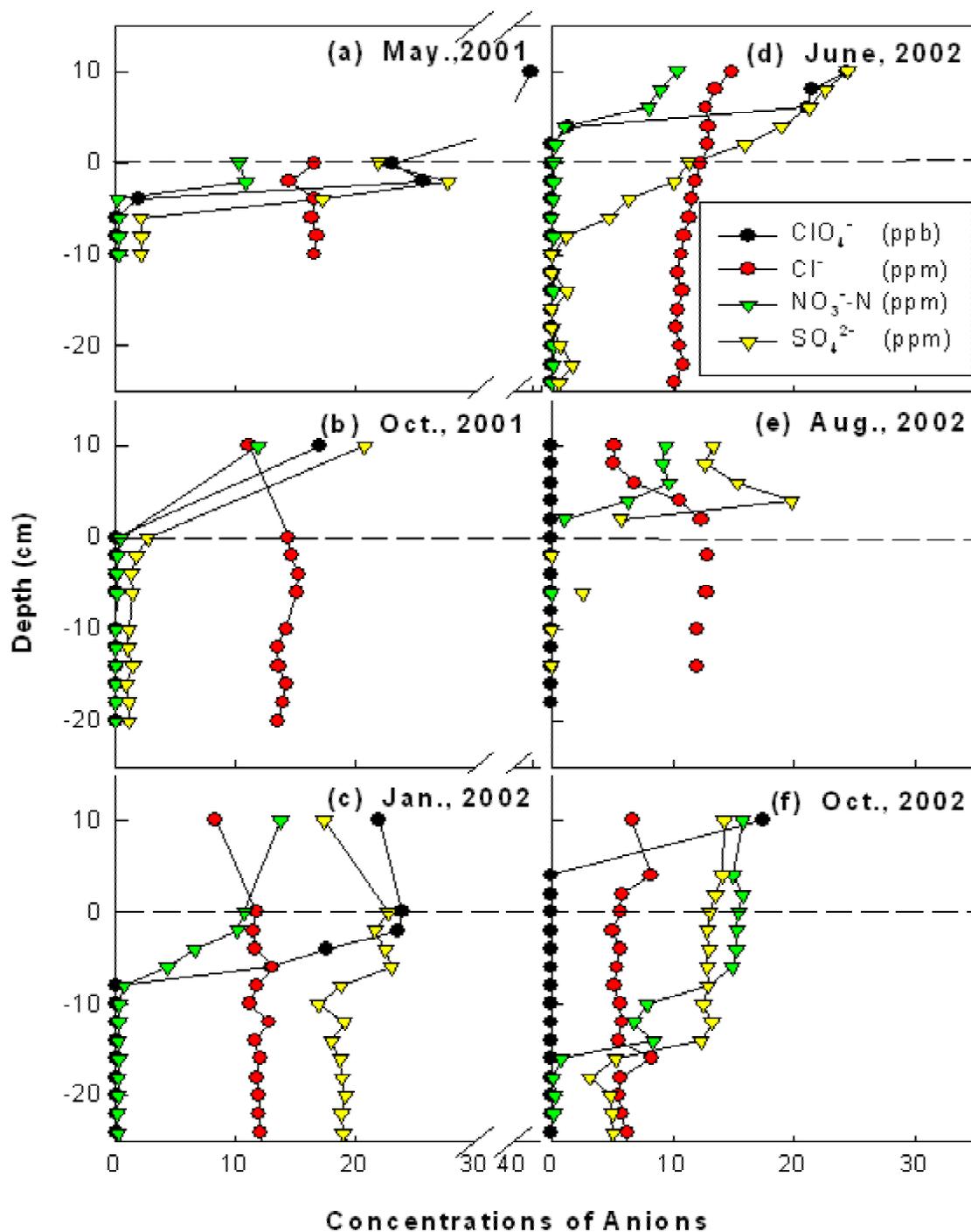


Figure 5-86
Profiles of Anions in Sediment Pore Water at HW84 Mainstream (T19) from May 2001 to October 2002

(Dash lines represent the sediment-water interface. "+" represents depth above the sediment-water interface, and "-" represents depth below the sediment-water interface.)

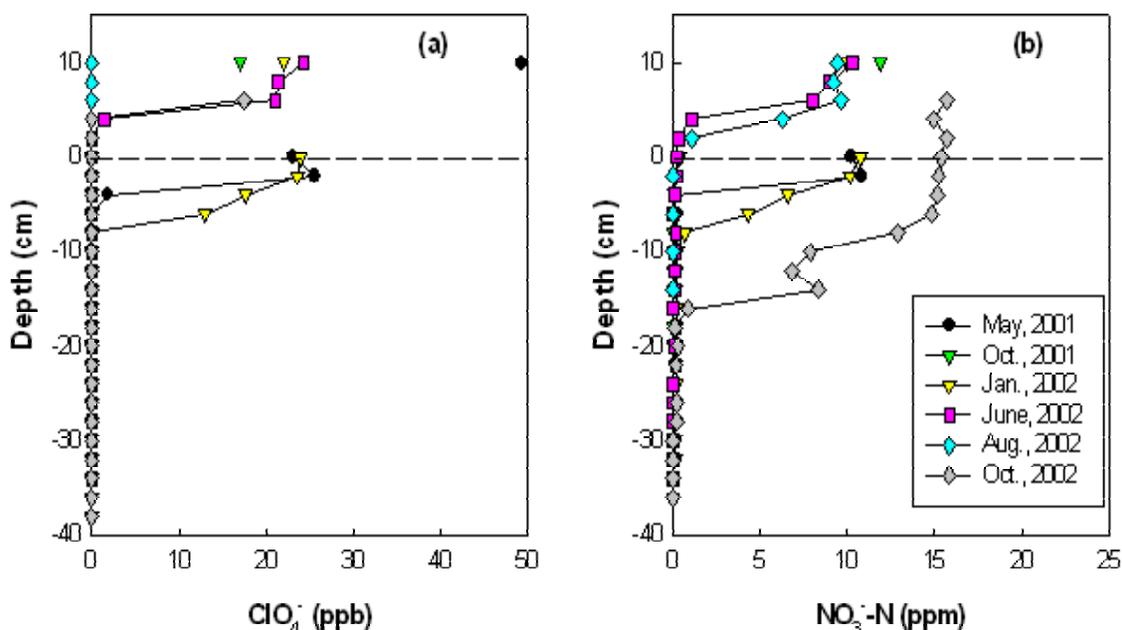


Figure 5-87
Seasonal Variation of Perchlorate and Nitrate Penetration in Sediment Pore Water at HW84 Mainstream (T19): (a) ClO_4^- vs. Depth; (b) NO_3^- -N vs. Depth

(Dash lines represent the sediment-water interface. "+" represents depth above the sediment-water interface, and "-" represents depth below the sediment-water interface.)

The HW317 (T13) site is located downstream of HW84 Mainstream along Harris Creek. This site is a natural wetland habitat with vigorous growth of aquatic plants (smartweed and watercress). This natural wetland habitat is continuously flooded, with a water depth fluctuation of approximately 40-150 cm. Rapid perchlorate degradation occurred in all months monitored, probably resulting from enhanced rhizodegradation due to the availability of organic material in bottom sediments as well as uptake by aquatic plants.

Perchlorate bulk water concentration in the stream ranged from 0 to 15 ppb, which was slightly lower than that of HW84 Mainstream. Perchlorate penetration in sediment was not observed (**Figure 5-88**). Sulfate degradation occurred at depths of (2 to -2 cm), implying that this site had relatively higher organic substrate in the sediments compared to previous two sites, possibly due to rhizosphere exudates from the intensive plant growth at this site. Perchlorate degradation was also found to mirror nitrate degradation (**Figure 5-89**). Both perchlorate and nitrate degradation occurred at an active degradation zone (from 5 cm to 0 cm).

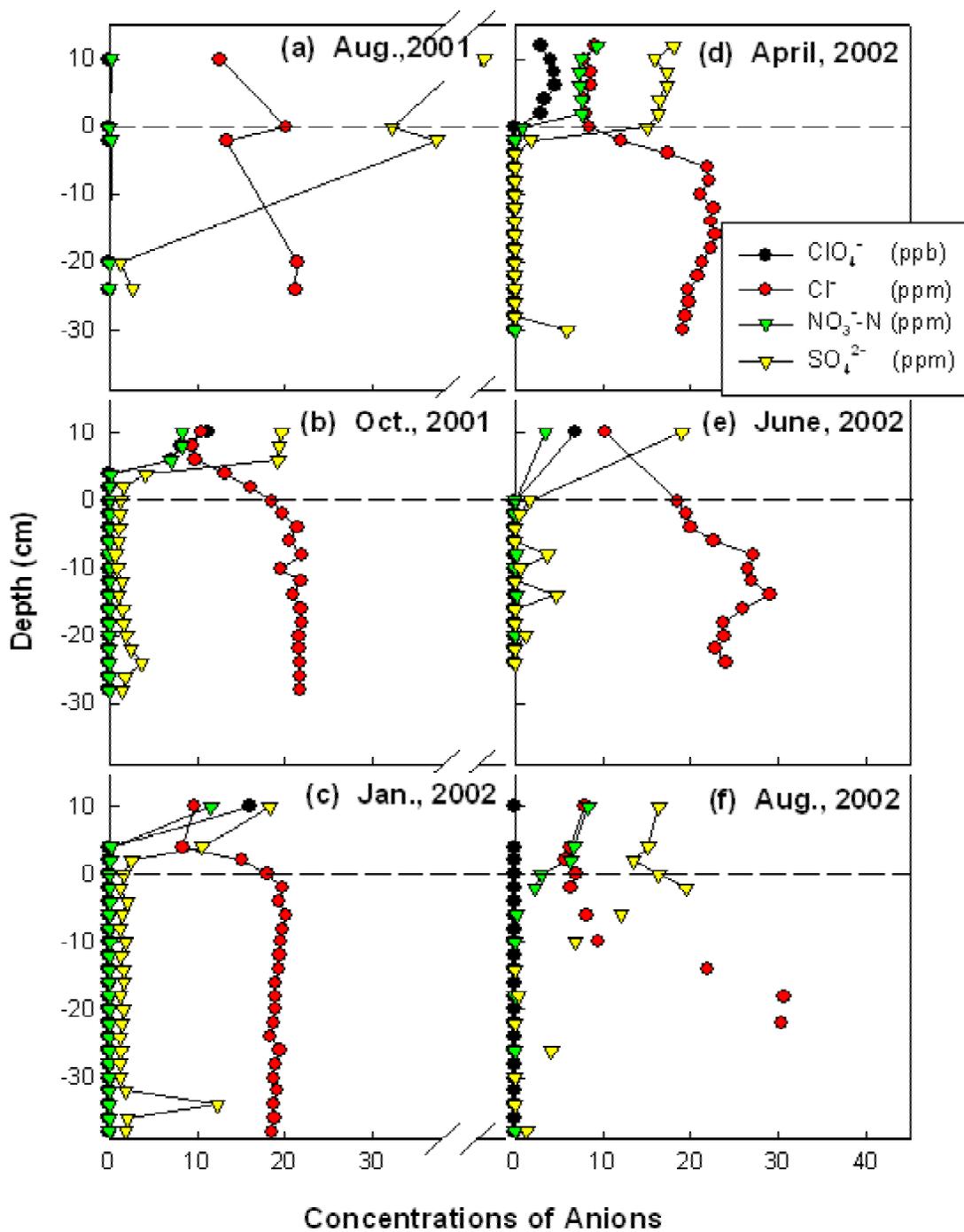


Figure 5-88
Profiles of Anions in Sediment Pore Water at HW317 (T13) from August 2001 to August 2002

(Dash lines represent the sediment-water interface. "+" represents depth above the sediment-water interface, and "-" represents depth below the sediment-water interface.)

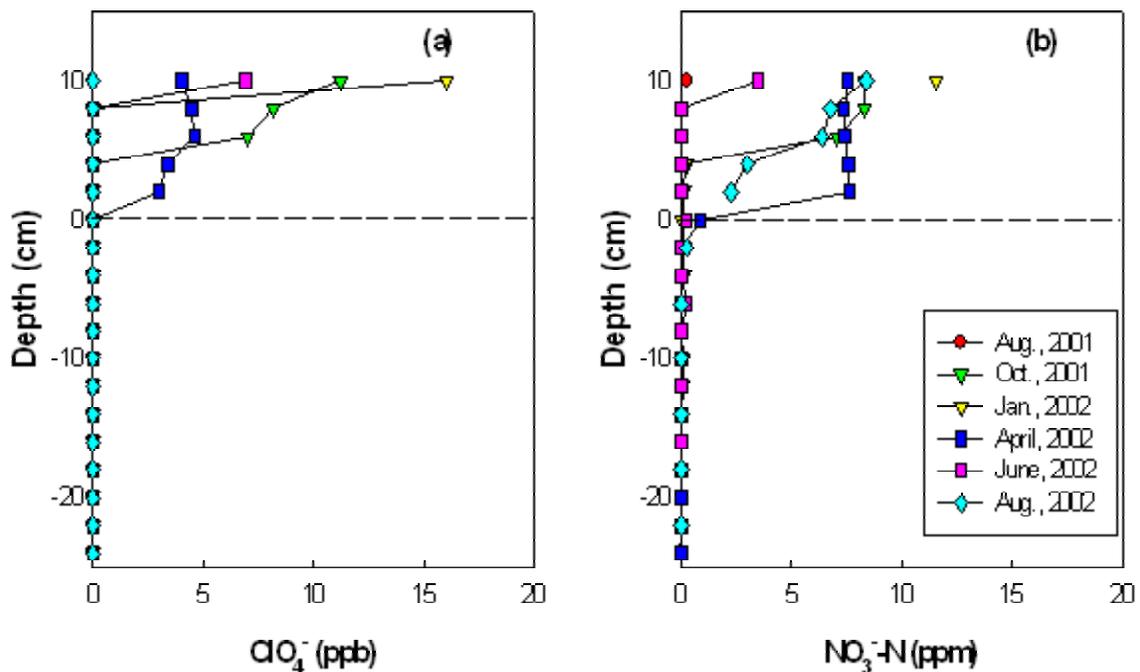


Figure 5-89
Seasonal Variation of Perchlorate and Nitrate Penetration in Sediment Pore Water at HW317 (T13): (a) ClO_4^- vs. Depth; (b) NO_3^- -N vs. Depth

(Dash lines represent the sediment-water interface. "+" represents depth above the sediment-water interface, and "-" represents depth below the sediment-water interface.)

The HW317/MN (T15) site has the highest perchlorate bulk water concentration, with a range of 100 to 400 ppb. Perchlorate penetrated 10 cm below the sediment-water surface in most months monitored except in October 2001 (**Figure 5-90** and **Figure 5-91**). Perchlorate and nitrate degradation followed similar patterns as the HW84 Sidestream site (**Figure 5-91**). At depths from 10 cm above to 5 cm below the sediment-water interface, there was almost no obvious perchlorate degradation. When nitrate-N concentration was degraded to a level below approximately 10 mg/L (at about -10 cm), rapid perchlorate degradation occurred at an active zone of 1-10 cm, although the depth of this zone changed seasonally.

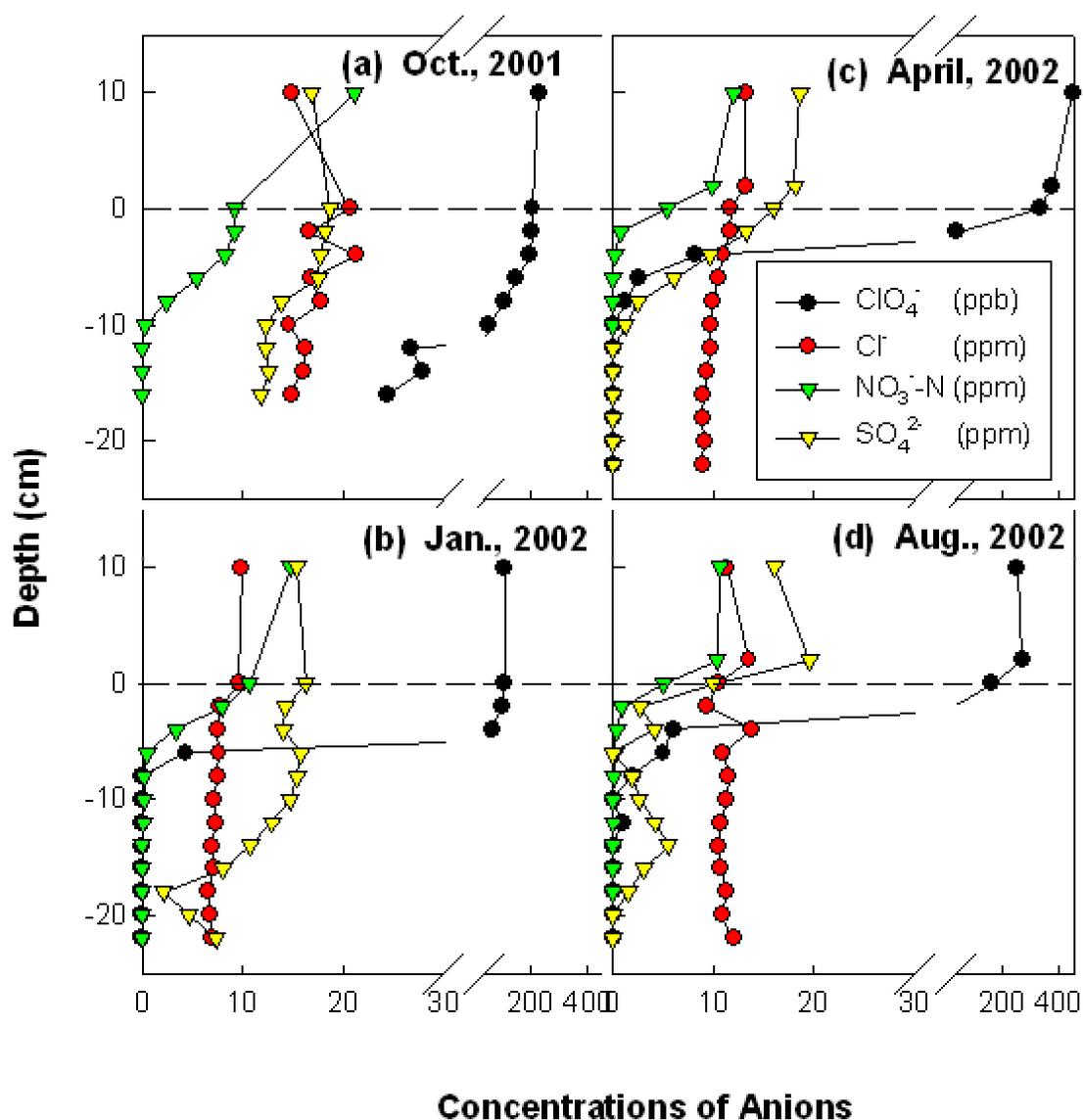


Figure 5-90
Profiles of Anions in Sediment Pore Water at HW317/MN (T15) from October 2001 to August 2002

(Dash lines represent the sediment-water interface. "+" represents depth above the sediment-water interface, and "-" represents depth below the sediment-water interface.)

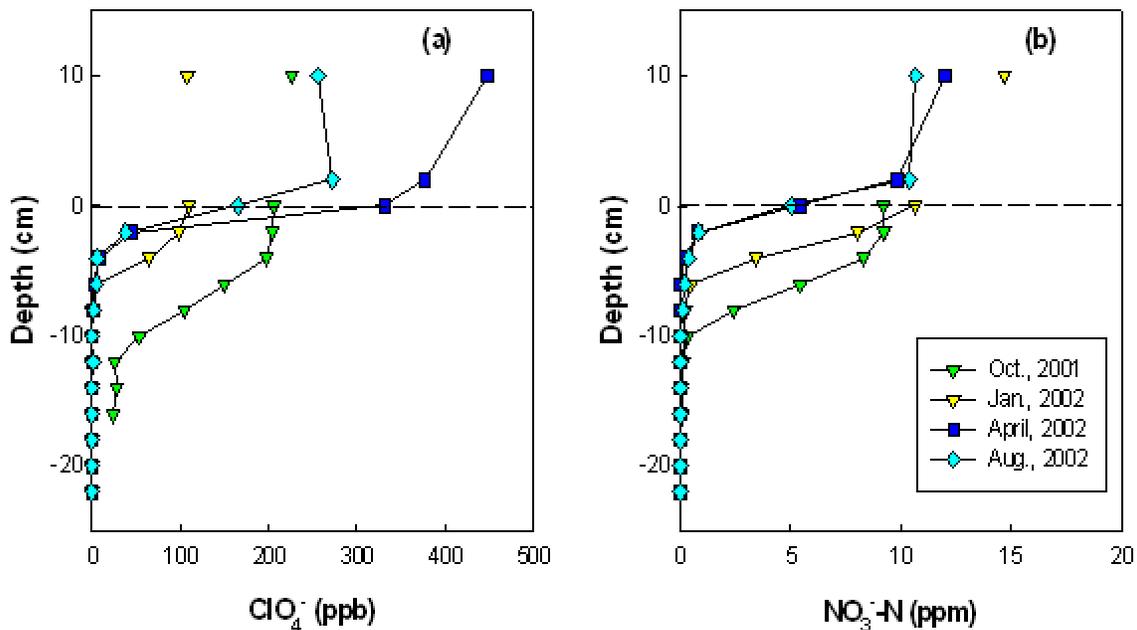


Figure 5-91
Seasonal Variation of Perchlorate and Nitrate Penetration in Sediment Pore Water
at HW317/MN (T15): (a) ClO_4^- vs. Depth; (b) NO_3^- -N vs. Depth.

(Dash lines represent the sediment-water interface. "+" represents depth above the sediment-water interface, and "-" represents depth below the sediment-water interface.)

5.2.1.1.4 Discussion

Perchlorate distribution and persistence in sediment pore water was spatially and temporally dependent, with variations for different streams. Generally, throughout the period of the one and half years of investigation, bulk water ClO_4^- concentration ranged from 0 $\mu\text{g/L}$ to 30 $\mu\text{g/L}$ for most streams, with up to approximately 400 $\mu\text{g/L}$ in one of the streams. More fluctuation was observed for perchlorate concentration in bulk water than in sediment pore water. Significant seasonal variation was observed for those streams with fluctuating flowrate. Biodegradation of perchlorate can occur over a sediment depth of only 1-10 centimeters, although this active depth changes seasonally.

Perchlorate penetrated into sediments to a depth corresponding to the gradual depletion of nitrate, and faster perchlorate degradation was observed at sites with a deeper sediment layer and with vigorous wetland habitat. This study demonstrated a large capacity for natural attenuation in sediment pore water and the importance of nitrate as an indication of perchlorate degradation. It also highlights the need for spatial and seasonal studies involving exposure assessment. In general, perchlorate did not penetrate into bed sediments below 30 cm of the sediment-water surface due to the rapid microbial

degradation. However, perchlorate was generally present in stream water for most seasons. It suggests that exposure assessment should focus on the ecological receptors that may be exposed to stream water and sediment at a shallower depth. If proper environmental conditions exist for the proliferation of perchlorate-reducing bacteria, such as temperature, anaerobic condition, organic substrate, moisture, and nitrate levels, sediments (30 cm below the water surface) would not become a major concern for perchlorate contamination.

5.2.1.2 Delta Areas Sediment Pore Water

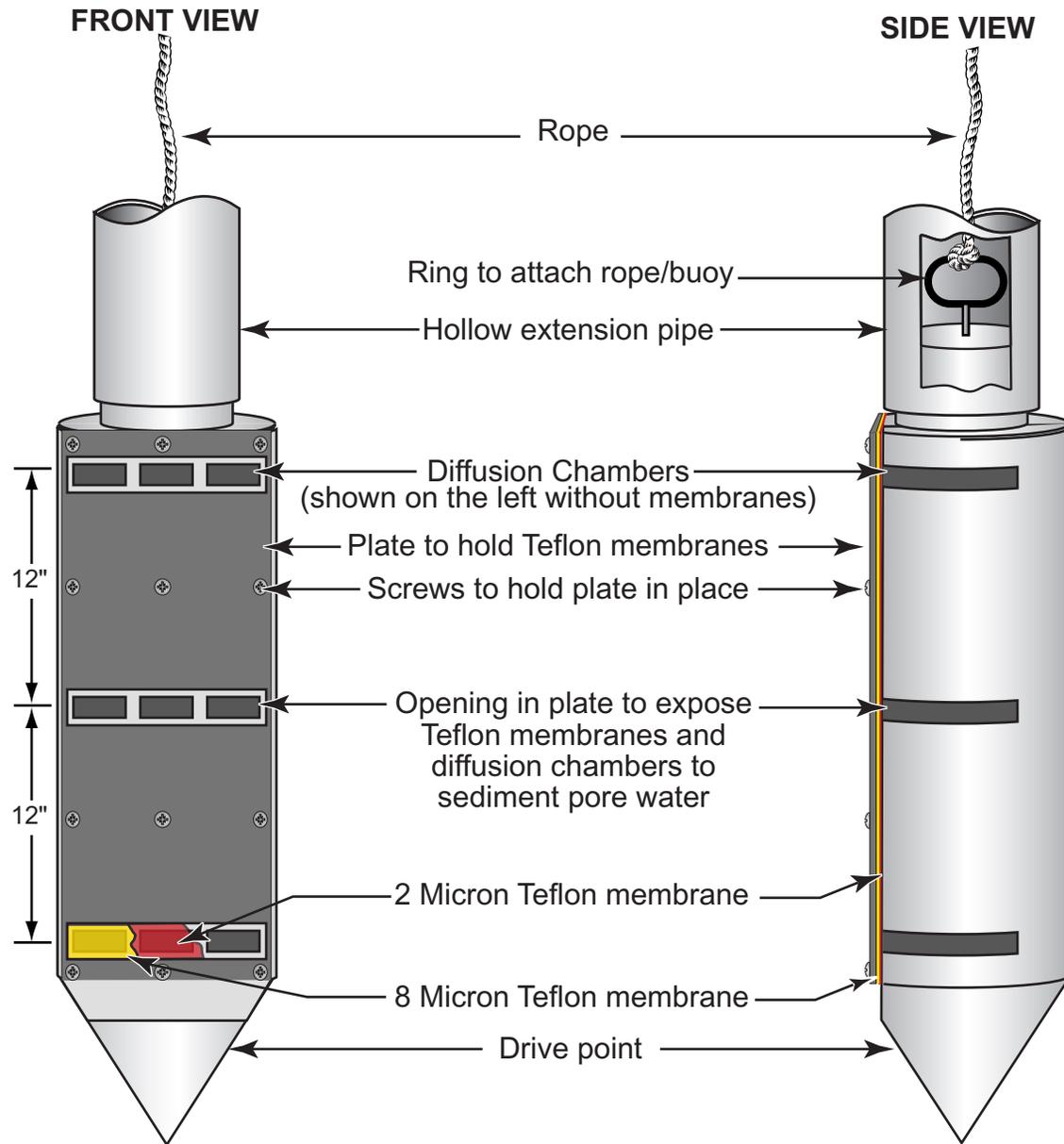
5.2.1.2.1 Introduction

Sediment pore water samples were collected from within the delta areas of Lake Waco and Lake Belton to evaluate the presence and distribution of perchlorate in sediment pore water. This sampling effort was conducted in conjunction with surface water sampling activities performed in these areas as previously described in Section 5.1.2.1, and sediment pore water samples were collected from the same delta area grid sampling locations established for the surface water sampling. The actual GPS coordinates of the sampling locations are documented in **Table 5-3** and actual sampling locations are shown in **Figure 5-18** and **Figure 5-19** in Section 5.1.2.1. This portion of the study was conducted as part of the Delta Areas Study. All of the methodologies followed are detailed in the *Final Lake Belton and Lake Waco Delta Areas Field Sampling Plan* (MWH, 2002c). Any deviations from this plan are discussed further below.

5.2.1.2.2 Methodology

Sediment pore water samples were collected using diffusion samplers (also known as peepers). TIEHH constructed the peepers that were used for this portion of the study. The peepers consisted of a stainless steel or Teflon drive stake with three columns and three rows of diffusion chambers or sample ports bored into the stake. The diffusion chambers were filled with de-ionized water and covered with two Teflon membranes (one 8 micrometers [μm] thick and the other 0.2 μm thick) to allow sediment pore water dissolved constituents to diffuse into the three chambers while keeping the sediments out. The Teflon membranes were held in place with a perforated plate that screws into the drive stake. A drawing showing a typical diffusion sampler is shown in **Figure 5-92**.

The sediment pore water samples were obtained by pushing the peepers into the lake bottom. The peepers were installed by attaching a rope to the top of the peeper and threading the rope through hollow extension pipes that have a diameter slightly smaller than the drive stake of the peeper. For the purposes of this study, the diffusion chambers were spaced along the drive stake such that samples were obtained at three different depths for each sample location: 1) just below the lake water/ sediment interface, 2) one foot below the lake water/sediment interface, and 3) two feet below the lake water/sediment interface. Tension was maintained on the rope to keep the peeper in contact with the lead extension pipe. The extension pipes were approximately 5 feet in length and either had threaded ends or quick-connects to allow additional lengths of pipe to be attached as the sampler was lowered through the water column. Once resistance was felt as a result of the peeper contacting the lake bottom, the peeper was pushed or driven with a slide hammer until the bottom diffusion



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PROJECT:	
BOSQUE AND LEON RIVER WATERSHEDS STUDY	
DRAWING TITLE:	
SEDIMENT PORE WATER SAMPLER (PEEPER)	
Sheet 1 of 1 sheets	
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chamber reached the desired sampling depth (i.e., 2 feet below the sediment/lake water interface). The field team tested the depth to sediment at each sample location with the extension pipes or a weighted probe prior to actually setting the peeper to assure that the samplers were properly installed.

After the peepers were pushed or driven to the desired depth, the extension pipes were retracted, leaving the peeper in place. The rope extending from the top of the drive stake was attached to a buoy to allow for locating and retrieving the peeper. The de-ionized water in the peepers was allowed to equilibrate with the adjacent sediment pore water for a period of two weeks. After the peepers were retrieved, the samples were extracted from the diffusion chambers with a clean syringe and transferred into an appropriate sample container. Each diffusion chamber was capable of holding a minimum of 20 milliliters of de-ionized water, which provided an adequate volume of water for the laboratory to perform the perchlorate analyses. Photographs of installation of peepers and extraction of the sample using a syringe are shown in **Figure 5-93**, **Figure 5-94**, and **Figure 5-95**.



Figure 5-93
Installation of Sediment Pore Water Sampler



Figure 5-94
Installation of Sediment Pore Water Sampler



Figure 5-95
Retrieval of a Sediment Pore Water Sample

Duplicate Samples. Blind duplicate and equipment blank samples were collected at a frequency to represent 10% of the environmental samples collected, and MS/MSD samples were collected at a frequency to represent 5% of the environmental samples collected. The samples were labeled, handled, and shipped according to the procedures described in the QAPP (MWH, 2002e).

The locations of the sampling grid points were documented using a Garmin GPS 76 instrument. The GPS information recorded at each grid point included latitude (degrees and minutes) and longitude (degrees and minutes).

Sample Designation. The sediment pore water samples were designated similarly to the surface water samples collected at the delta area grid locations except that the lake name abbreviation and grid point number was followed by SPW to indicate a sediment pore water sample, and finally the depth from which the sample was obtained. For example the sediment pore water sample collected from just below the lake water/sediment interface at Lake Waco grid point number 1 was designated “LW1-SPW-0’ ”.

Blind duplicate sediment pore water samples were designated with a fictitious number so the laboratory would not know where the sample was collected. For example, the first sediment pore water blind duplicate sample was designated “SPW-1001”. The field crew kept careful records of the designations given to the blind duplicate samples and their corresponding environmental sample so that the analytical results could be correlated with the field locations. Each MS/MSD sample had the same designation as its associated environmental sample except that “MS” or “MSD” followed the sample designation (e.g., “LW1-SPW-0’ MS” and “LW1-SPW-0’ MSD”). Each equipment blank sample had the same designation as its associated sampling location except that “EB” followed the sample designation (e.g., “LW1-SPW-0’-EB”).

Sample Analysis. All sediment pore water samples were analyzed for perchlorate by USEPA Method 314.0 at the USACE Engineer Research and Development Center Environmental Laboratory at the Environmental Chemistry Branch in Omaha, Nebraska (See **Appendix V**). The USACE laboratory conformed to the analytical method requirements, analytical quality control requirements, instrument calibration frequency, and the laboratory quality control requirements presented in the QAPP (MWH, 2002e). The data verification report for samples analyzed by the USACE laboratory is included in **Appendix W**.

5.2.1.2.3 Data

A total of 63 sediment pore water samples including six duplicate samples were collected from 19 sample locations within Lake Belton. Sixty-five sediment pore water samples including six duplicate samples were collected from 20 sample locations within Lake Waco. All of the water quality and temperature data collected from the lake delta areas are included for reference in **Appendix H**. Perchlorate was not detected in any of the sediment pore water samples collected from the lake delta areas (method detection limit

of 1 µg/L). The sediment pore water perchlorate sampling results for each lake are included in **Appendix S**.

Perchlorate was not detected in the equipment blank samples analyzed for either lake. Perchlorate was also not detected in the investigations derived waste (IDW) samples collected from Lake Belton. No IDW was generated from the sediment pore water sampling in Lake Waco, since the sediment pore water samplers were not reused. These results are also included in **Appendix S**.

5.2.1.3 Historical Data

5.2.1.3.1 Introduction

As part of the Phase II Groundwater Investigation at NWIRP McGregor, the U.S. Navy assessed water quality within Lake Belton and Lake Waco as previously described in Section 5.1.2.4. The lake assessment approach is presented in the *Lake Water Quality Assessment Work Plan* (EnSafe, 2000a). This investigation was designed to complete a thorough, yet expedited, environmental assessment of perchlorate occurrence in the lakes and produce data that could be used to assess risk to human health and the environment. EnSafe conducted two sampling events, one in spring 2000 and the other in summer 2000. The spring 2000 sampling event was conducted to assess the lakes under cool weather conditions, while the summer 2000 sampling event was conducted to assess the lakes under warm weather conditions. It should be noted that U.S. Navy sampled bulk sediment, not sediment pore water directly. Therefore, historical results from the U.S. Navy are not necessarily comparable to the sediment pore water samples discussed above.

5.2.1.3.2 Methodology

The overall approach, sampling techniques, and methods used for lake assessments are described in detail in the *NWIRP McGregor Final Groundwater Investigation Work Plan* (EnSafe, 1998b). Both the spring and summer sampling events utilized the same field protocols.

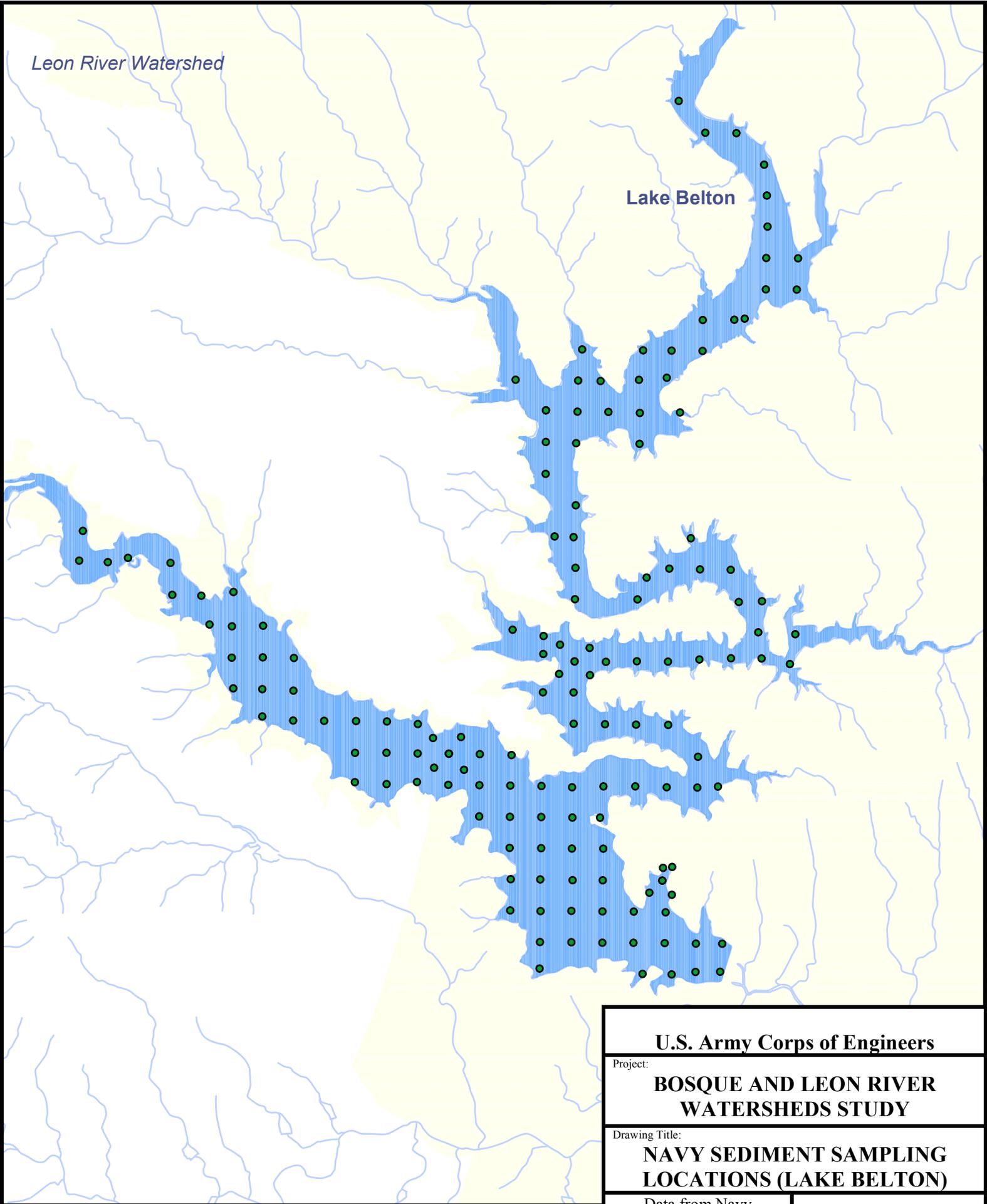
A 2,000-foot by 2,000-foot sampling grid was established in each lake as presented in **Figure 5-96** and **Figure 5-97**.

During the spring 2000 sampling event, one sediment sample was collected at each of the grid sampling locations. TCEQ authorized the reduction of lake-wide sediment sampling during the Summer 2000 sampling event due to the absence of perchlorate in nearly all cool water sediment samples, and sampling was conducted only at locations where perchlorate was previously detected.

All the sediment samples were collected using a petite ponar clam-shell grab sampler developed by a deck-mounted davit, pulley, and power windlass winch system, following the methodology outline in American Society for Testing and Materials (ASTM) D 4342-84:1993 (ASTM, 1997).

Leon River Watershed

Lake Belton



U.S. Army Corps of Engineers

Project:

**BOSQUE AND LEON RIVER
WATERSHEDS STUDY**

Drawing Title:

**NAVY SEDIMENT SAMPLING
LOCATIONS (LAKE BELTON)**

Data from Navy
Environmental
Investigations
(November 6, 2001)

Sheet 1 of 1 Sheets

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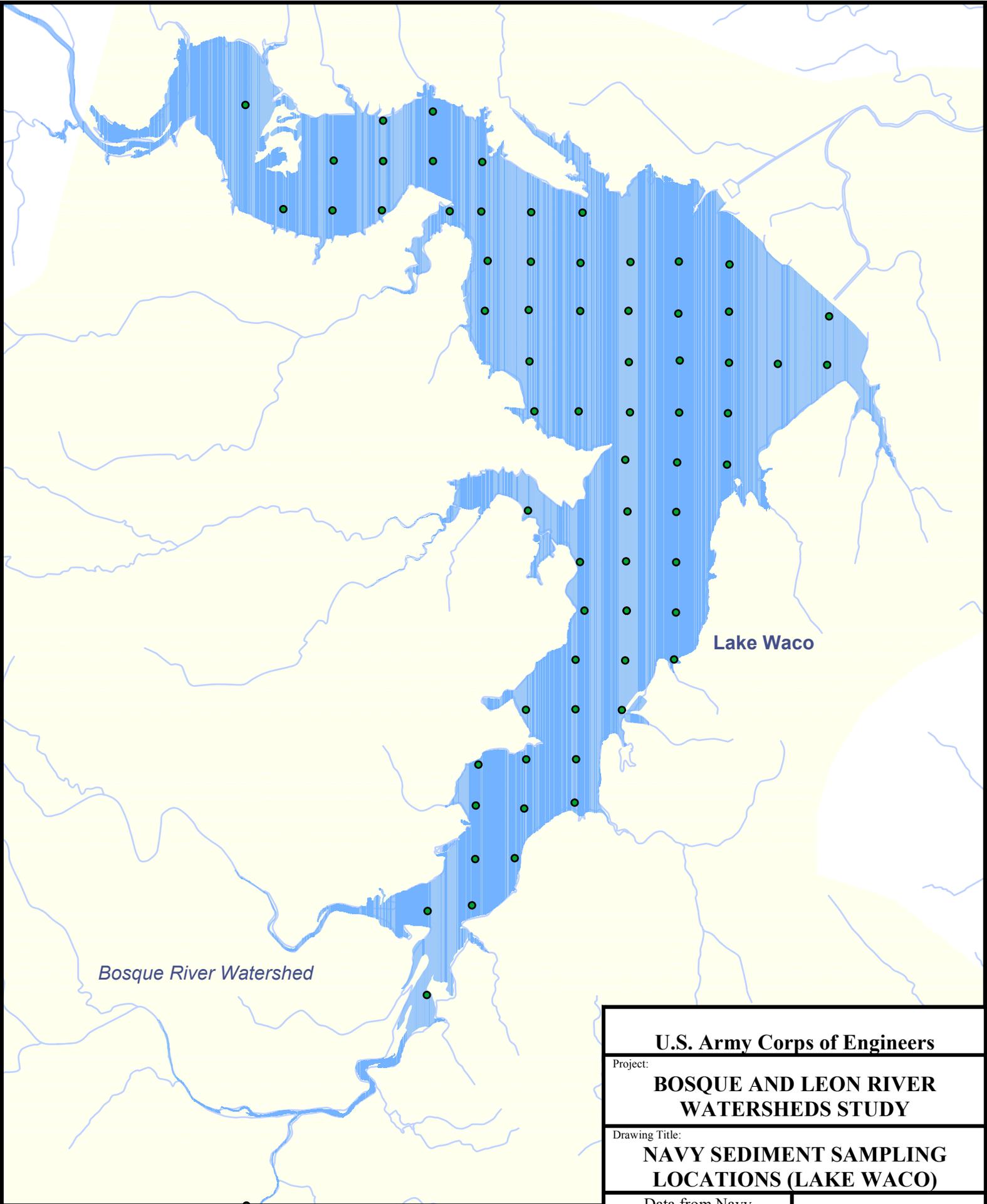
Study Area



Navy Sediment Sampling Locations

0 0.5 1 Miles





U.S. Army Corps of Engineers
 Project:
**BOSQUE AND LEON RIVER
 WATERSHEDS STUDY**
 Drawing Title:
**NAVY SEDIMENT SAMPLING
 LOCATIONS (LAKE WACO)**

Data from Navy
 Environmental
 Investigations
 (November 6, 2001)

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 Study Area

 Navy Sediment Sampling Locations

0 0.5 1 Miles

5.2.1.3.3 Data

Detailed observations and findings made during both the sampling events are provided in the *Final Draft Report Lake Water Quality Assessment* (EnSafe, 2000b). A summary discussion of these findings is presented below.

A total of 138 sediment samples were collected from 141 sample locations in Lake Belton during the spring 2000 sample collection event. In the summer event, a total of 15 sediment samples were submitted from 15 locations. A total of 69 sediment samples were collected from 78 sample locations in Lake Waco during the spring 2000 sample collection event. No sediments were collected from Lake Waco during the summer event.

The following briefly describes the laboratory analytical results for both the lakes.

- **Spring 2000 (cool water sediment)** – No perchlorate was detected in any of the 69 sediment samples collected from Lake Waco. The laboratory detection limits for sediment samples ranged from 0.097 milligrams/kilogram (mg/kg) to 0.28 mg/kg, averaging 0.20 mg/kg. Perchlorate was detected in three sediment samples in Lake Belton (See **Figure 5-98**). Concentrations ranged from 0.13J mg/kg to 0.54 mg/kg (J = laboratory code from estimated concentration).
- **Summer 2000 (warm water sediment)** – No perchlorate was detected in the 15 confirmatory sediment samples from Lake Belton. Laboratory detection limits for the sediments ranged from 0.10 mg/kg to 0.34 mg/kg, averaging 0.20 mg/kg.

Of all the 222 lake sediment samples collected during both the events, only three had very low detects, which represents 1.3% of the samples. They were non-detect on re-sampling. The analytical data produced by this investigation are included in the *Final Draft Report Lake Water Quality Assessment* (EnSafe, 2000b).

The U.S. Navy concluded that there was no apparent distribution pattern for either the detected perchlorate concentrations in Lake Belton sediment during the spring sampling event or the Station Creek/NWIRP McGregor area of the Leon River. Spatially, the perchlorate detects were separated by miles with numerous non-detect samples between them. There was one 0.54 mg/kg perchlorate detect one-half mile east of the City of Gatesville raw water intake.

The U.S. Navy also conducted sediment sampling for perchlorate within NWIRP McGregor in 2000 and in tributaries adjacent to the facility. Locations of sediment sample collection are shown in **Figure 5-99**. Concentrations of perchlorate below 1 mg/kg were detected in one sediment sample collected from Tributary M (SDST-3), in two sediment samples collected from the South Bosque River (SDSB-2 and SDSB-3) and within NWIRP (SD8PAS-5). The locations of these samples are shown in **Figure 5-100**. Reporting limits were below 0.25 mg/kg in the majority of sediment samples collected within the streams.

Leon River Watershed

Lake Belton

BEL-072

BEL-049

BEL-023

U.S. Army Corps of Engineers

Project:

**BOSQUE AND LEON RIVER
WATERSHEDS STUDY**

Drawing Title:

**NAVY SEDIMENT PERCHLORATE
DETECTIONS
(LAKE BELTON)**

Data from Navy
Environmental
Investigations
(November 6, 2001)

Sheet 1 of 1 Sheets

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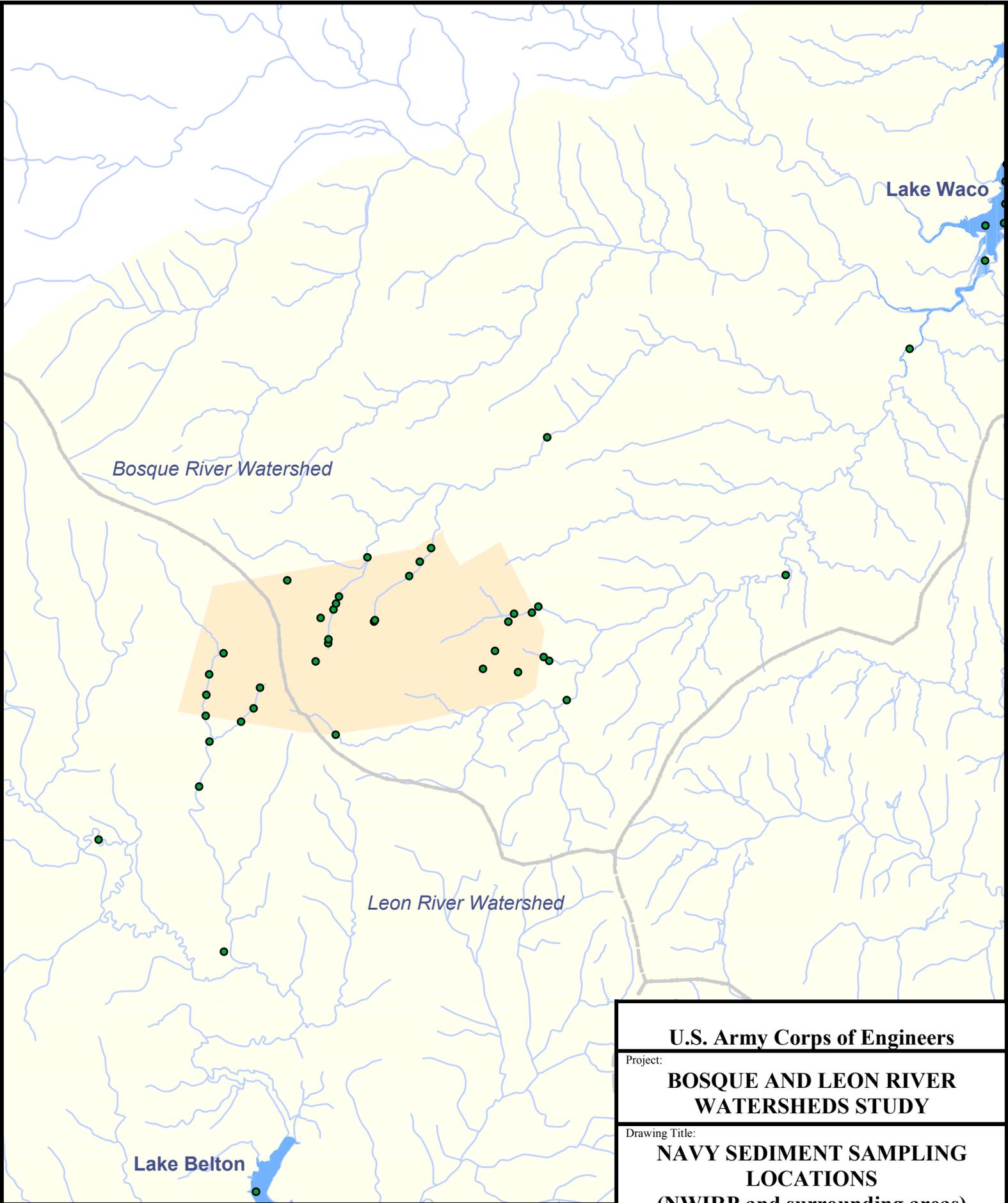
Study Area



Navy Sediment Perchlorate detections

0 0.5 1 Miles





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NWIRP McGregor
 Study Area
 Watershed Boundaries
 Navy Sediment Sampling Locations

0 0.5 1 Miles

U.S. Army Corps of Engineers

Project:
BOSQUE AND LEON RIVER WATERSHEDS STUDY

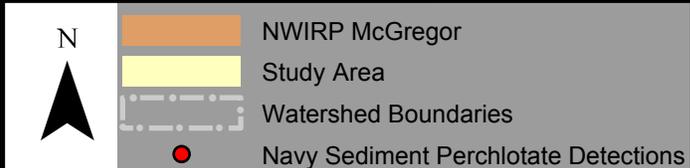
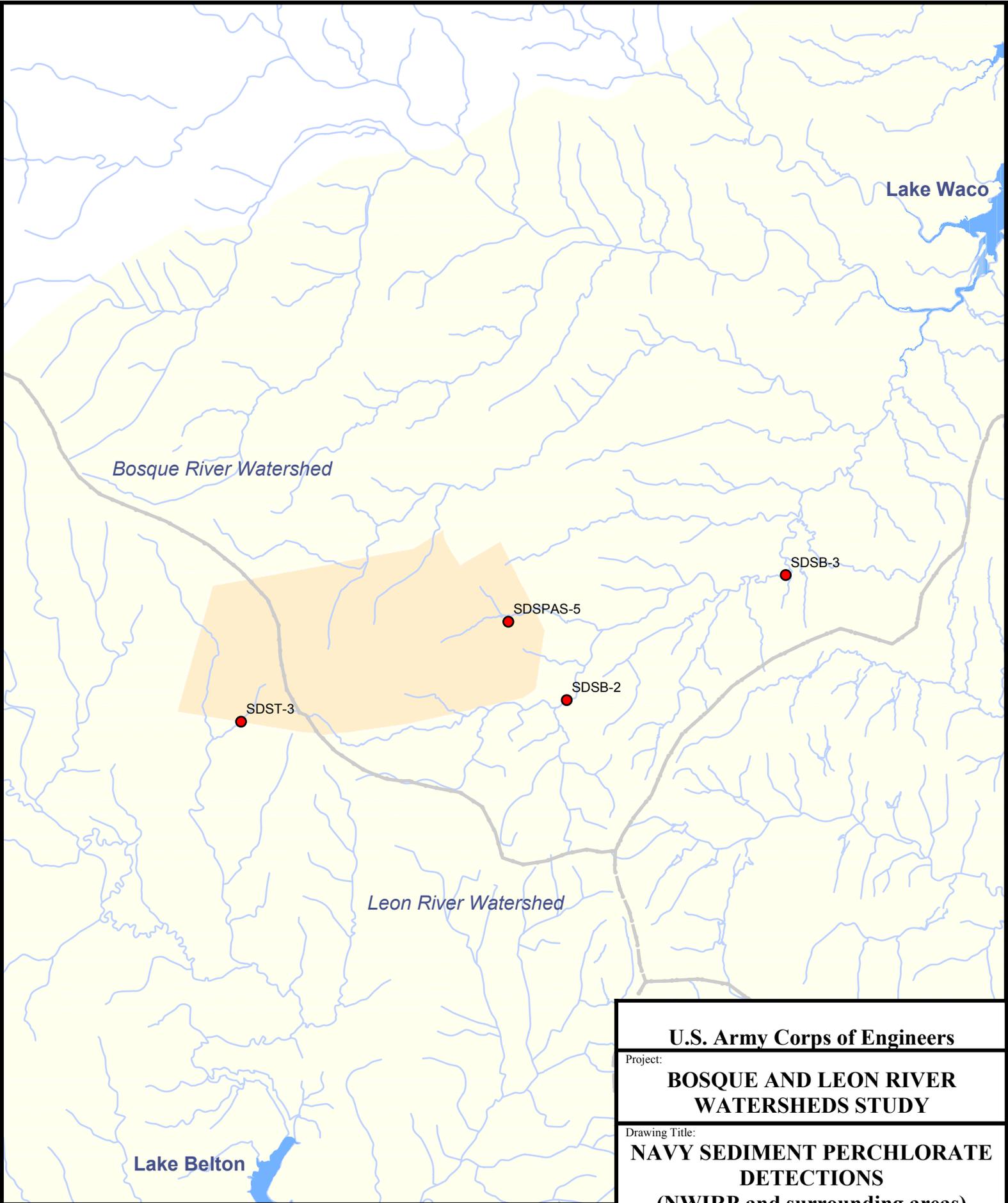
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NAVY SEDIMENT SAMPLING LOCATIONS (NWIRP and surrounding areas)

Data from Navy Environmental Investigations (November 6, 2001)

Sheet 1 of 1 Sheets

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NAVY SEDIMENT PERCHLORATE DETECTIONS (NWIRP and surrounding areas)	
Data from Navy Environmental Investigations (November 6, 2001)	Sheet 1 of 1 Sheets
SCALE: 1:141,015	FIGURE: 5-100

5.2.1.4 Discussion

Perchlorate, if it were reaching the lakes, would likely be found in the delta areas, as these areas receive direct discharge from the Bosque and Leon River watersheds and have the greatest sediment deposition in each lake. However, no perchlorate was detected in the delta area sediment pore water sampling conducted by the project team. In addition to all the results discussed above, the U.S. Navy had a detailed 2,000-foot by 2,000-foot sample grid. Three sediment samples collected by the U.S. Navy on the Lake Belton grid had perchlorate detections, but according to the U.S. Navy, these three samples had no apparent distribution pattern. It should also be noted that the U.S. Navy collected and analyzed bulk sediment samples, as opposed to sediment pore water samples. Although the collection of bulk sediment samples is valid, pore water concentrations are generally more sensitive for highly water-soluble chemicals (USEPA, 1999). Sediment criteria or screening benchmarks are not currently available for perchlorate.

5.2.2 Anoxic Study

5.2.2.1 Introduction

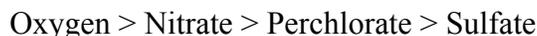
In an effort to assist the USACE in developing a better understanding of the possibility of natural perchlorate reduction within Lake Belton, the project team performed a series of microecology experiments to determine the potential for natural perchlorate bioreduction within the lake. These tests explored the potential for the microorganisms (i.e., bacteria) naturally present in both the water column and sediment at the water/sediment interface to metabolize perchlorate.

The ability for dissimilatory perchlorate and chlorate reduction is widespread among bacteria in the natural environment. Bacteria that practice dissimilatory perchlorate reduction do so as part of the process to gain energy that they can use for growth and maintenance. In this process, oxygen ions are successively stripped from perchlorate until only chloride remains in solution. By-products of this process include the development of biomass and the production of carbon dioxide. A simplified conceptual equation for the biological reduction of perchlorate using acetic acid as the electron donor is:



In the above reaction, perchlorate is the electron acceptor, gaining eight electrons, as it is reduced to chlorate (ClO_3^-), chlorite (ClO_2^-), and finally to chloride (Cl^-). In this same reaction, acetic acid is the electron donor, supplying eight electrons, as it is oxidized to carbon dioxide.

Oxygen, nitrate and sulfate may also be present in natural waters that contain perchlorate. If all these electron acceptors are present, the prevailing order of biological preference is:



In other words, if oxygen is present, the rates of biological reduction of nitrate and perchlorate are usually reduced. Once oxygen is depleted, the biological reductions of nitrate (i.e., denitrification) and perchlorate are greatly improved. However, the reduction of perchlorate to chloride is typically impaired by the presence of nitrate. Once these electron acceptors have been depleted, biological reduction of sulfate is generally initiated.

The actual order that the electron acceptors are reduced and the rate at which this reduction occurs depends on a number of variables. Some of these variables include:

- Biologically active microorganisms (presence/quantity and type)
- Electron acceptors (relative concentrations)
- Electron donors (quantity and type)
- Other biological nutrients (quantity and type, i.e. trace metals)
- Environmental conditions (i.e. temperature, pH, redox potential, etc.)
- Micro-environments (e.g. while both oxygen and nitrate might be present in a bulk fluid, they may be absent or low in portions of a large biological community enabling perchlorate reduction to occur in the micro-environment while in the bulk fluid perchlorate reduction is inhibited)

This portion of the Study was conducted as part of the Anoxic Study. All the methodologies and protocol followed are detailed in the *Final Lake Belton Perchlorate Bioreduction Bench-Scale Study Field Sampling Plan* (MWH, 2002d). Any deviations from the Field Sampling Plan are discussed further below.

5.2.2.2 Methodology

5.2.2.2.1 Field Activities

Sediment samples were collected from the deepest portions of Lake Belton, which were likely to have been under anoxic (oxygen deficient) conditions for the longest period of time, and from three shallower locations where the thermocline was close (1 meter [± 0.5 m] above) to the water/sediment interface. The deep and shallow sampling locations are shown on **Figure 5-101**. The deeper locations were expected to exhibit anoxic conditions because this lake is over 90 feet deep in this area and thermally stratifies. The survey coordinates of the sampling locations and a summary of the samples collected at each location are presented in **Table 5-15**. Samples were collected during the summer (August 2003) when the lake was thermally stratified and when anoxic conditions were expected to occur near the lake bottom and below the thermocline. The field team altered the locations of the three shallow sampling locations based on the thermal profile of the lake at the time of sampling. For example, if a thermocline was not detected at a proposed shallow sampling location, the field team moved to progressively deeper water until the thermocline existed 1 meter (± 0.5 meter) above the lake bottom. Likewise, if the lake bottom was greater than 1.5 meters below the thermocline at a proposed shallow sampling location, the field team moved to progressively shallower water until it was established that a thermocline existed 1 meter (± 0.5 meter) above the lake bottom.

Leon River Watershed

Lake Belton

Shallow 1
Shallow 2
Shallow 3
Deep 1
Deep 2
Deep 3

U.S. Army Corps of Engineers

Project:

**BOSQUE AND LEON RIVER
WATERSHEDS STUDY**

Drawing Title:

**ANOXIC STUDY SAMPLE
LOCATIONS
(LAKE BELTON)**

Data from Navy
Environmental
Investigations
(November 6, 2001)

Sheet 1 of 1 Sheets

SCALE:
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FIGURE:
5-101

N



Legend



Study Area



Anoxic Study Sample Locations

0 0.5 1 Miles



Table 5-15
Anoxic Study Sample Collection Points

Location Identifier	Sample(s)	Latitude	Longitude
Deep [†] 1	Sediment, Water near bottom, Water close to (but below) thermocline, Water close to surface (above thermocline)	31.11070	-97.47860
Deep 2	Sediment	31.12770	-97.51448
Deep 3	Sediment	31.14068	-97.47661
Shallow 1 (Close to Thermocline [‡])	Sediment, Water near bottom	31.14891	-97.53251
Shallow 2 (Close to Thermocline)	Sediment	31.19272	-97.50623
Shallow 3 (Close to Thermocline)	Sediment	31.18243	-97.50602

[†] - The deepest locations of Lake Belton that have presumably been anoxic the longest at the time of sampling.

[‡] - These locations are selected such that the water/sediment interface is close to (but below) the thermocline.

The following field observations and water quality measurements collected at each sampling location were recorded in the field logbook:

Field Observations:

- Air temperature
- Wind speed and direction
- Water color
- Aquatic vegetation in percent cover (qualitative)
- Cloud cover (qualitative)

Water Quality Measurements:

- Secchi Disk transparency
- Temperature (measured every 5 feet to the lake bottom)
- Dissolved oxygen (measured every 5 feet to the bottom of the thermocline)
- pH (measured every 5 feet to the bottom of the thermocline)

Identification of Thermocline

Sample depths were determined based on the location of the thermocline. At each sampling location, the temperature profile was established prior to collecting the samples. A thermometer or multi-parameter instrument (Hydrolab Mini Sonde 4a) was lowered through the water column in order to develop the vertical temperature, dissolved oxygen (DO), and pH profiles for each sampling point. A representative temperature, dissolved oxygen, and pH profile for sample “Deep 1”, which is similar for all sampled locations is shown in **Figure 5-102**. Based on these profiles, it can be seen that the lake temperature varied from 30 to 14 °C. Additionally, DO was not detected below approximately 10 meters below the surface at any of the locations. Data tables summarizing these results and water quality measurements for each location are provided in **Appendix T**. The

selected water quality meter was calibrated daily according to the manufacturer's instructions. The temperature data were collected at 5-foot increments from the lake surface to the lake bottom and recorded.

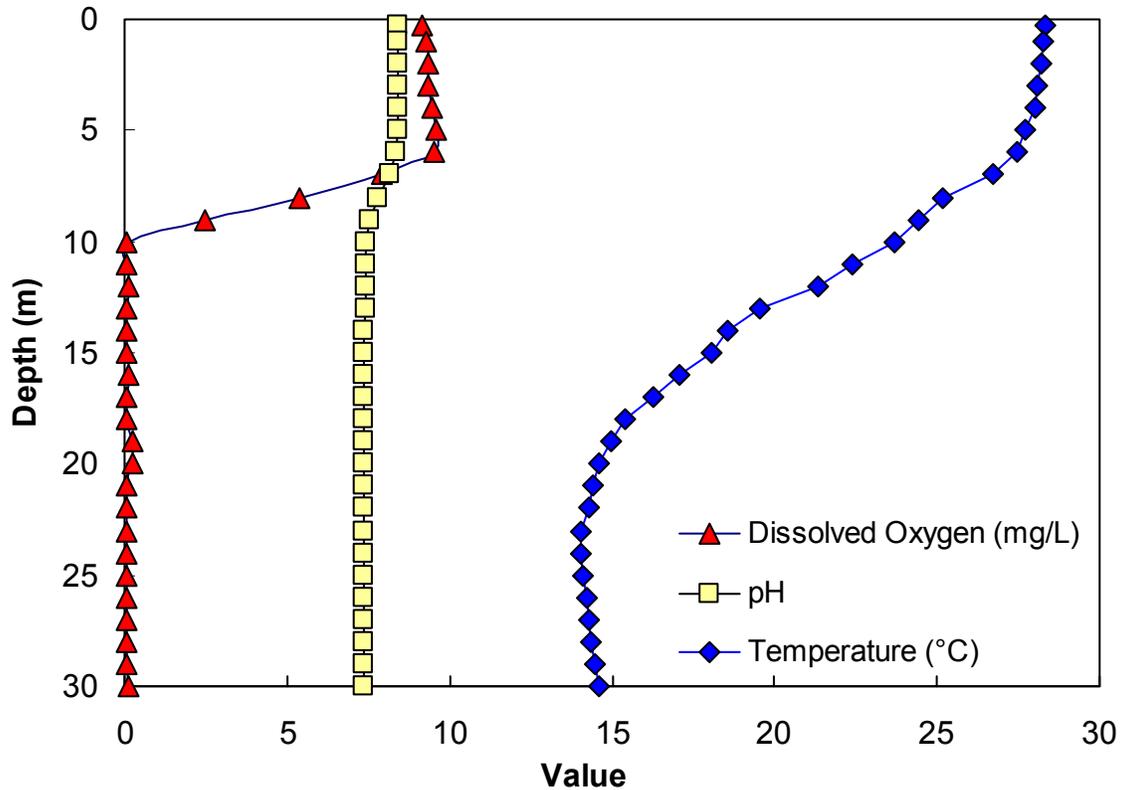


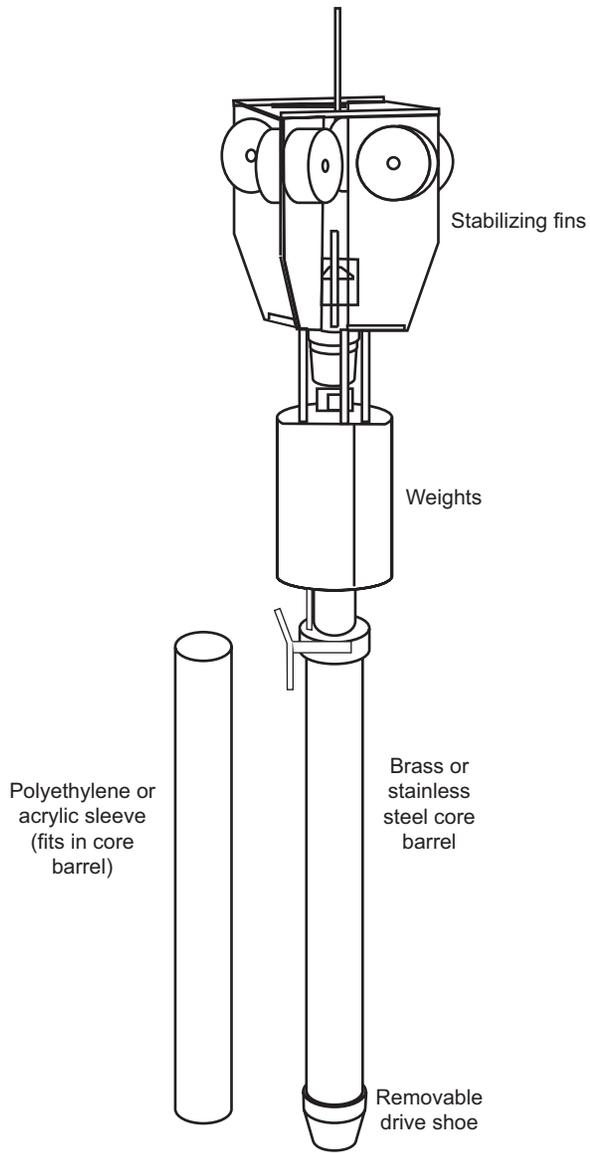
Figure 5-102
Temperature, pH, and Dissolved Oxygen Profile of Lake Belton (*Deep 1* Location)

Lake Water Sampling Equipment and Procedures

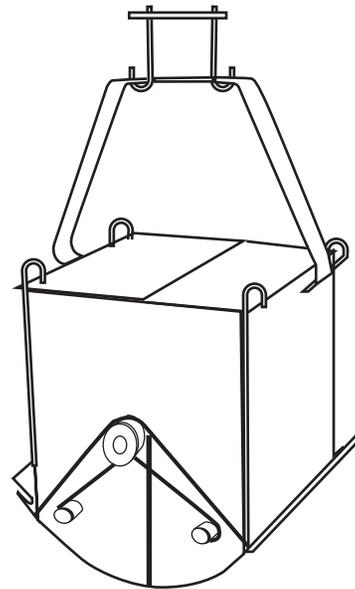
Once the appropriate sample depths were established based on the location of the thermocline, the water samples were collected with an Alpha thief sampler previously described in Section 5.1.2.1.2. A summary of the water samples collected is presented above in **Table 5-15**.

Sediment Sampling Equipment and Procedures

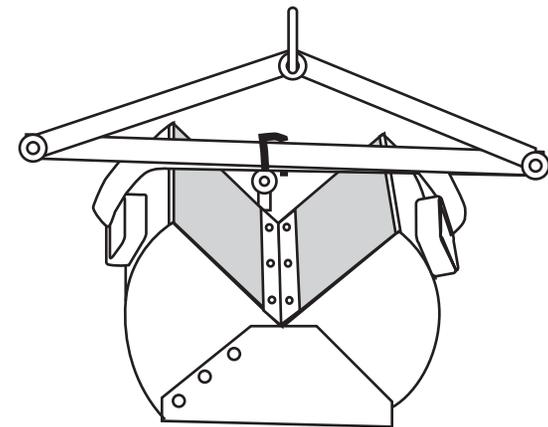
Sediment samples were collected using a gravity corer constructed of an outer rigid metal tube (core barrel) into which a polyethylene or acrylic liner fits with minimal clearance (refer to **Figure 5-103**, diagram A). Samples were obtained by allowing the sampler, which was attached to sufficient length of stainless-steel cable, to drop to the bottom. An opening exists above the liner to allow free flow of water through the corer as it moves vertically through the water and into the sediment. The weight of the sampler drives the core barrel into the sediment to varying depths depending on the characteristics of the sediments.



A. Gravity Corer



B. Ekman Dredge (shown closed)



C. Ponar Dredge (shown open)

U.S. Army Corps of Engineers	
PROJECT:	
BOSQUE AND LEON RIVER WATERSHEDS STUDY	
DRAWING TITLE:	
GRAVITY CORER	
Sheet 1 of 1 sheets	
SCALE	FIGURE
No Scale	5-103

The sampler has a messenger-activated valve assembly that seals the opening above the liner following sediment penetration, which creates a partial vacuum to assist in sample retention during retrieval. Upon retrieval, the liner was removed, trimmed such that the sediments inside the liner are flush with the ends of the liner, and capped with a sheet of Teflon held in place by a plastic cap. The caps were secured with tape on the outside of the liner. The gravity corer was raised and lowered with a stainless-steel cable attached to a boat-mounted winch. A summary of the sediment samples collected is presented in **Table 5-15**. At locations where both sediment and lake water samples had to be collected, the lake water samples were collected prior to collecting the sediment samples to prevent disturbed sediments from contaminating the lake water samples.

Photographs of field sampling activities are provided in **Figure 5-104**, **Figure 5-105**, and **Figure 5-106**.



Figure 5-104
Gravity Corer Photograph



Figure 5-105
Gravity Corer Dropped to Collect Sediment Sample



Figure 5-106
Sediment Sample Retrieval

Sample Frequency

One sediment sample was collected from each of the shallow and deep sampling locations for a total of six sediment samples. At sampling location “Shallow 1”, one lake water sample was collected from just above the lake bottom, below the thermocline. At sampling location “Deep 1”, three lake water samples were collected: 1) from just below the lake surface, 2) from just below the thermocline, and 3) from just above the lake bottom. The sampling locations were documented using a Garmin GPS 76 instrument, which recorded the latitude and longitude (degrees and minutes) at each grid point.

Sample Designation

Each sample was designated with an alphanumeric character string set apart by hyphens. The designation began with the lake name abbreviation (e.g., LB for Lake Belton), followed by LW to indicate a lake water sample or SED to indicate a sediment sample, “Deep” or “Shallow” to indicate the deep or shallow sampling location, and finally by the depth of collection. For example, a lake water sample collected from Lake Belton sampling location “Deep 1” at 1 foot depth (just below the lake surface) would be designated “LB-LW-Deep1-1.” A sediment sample collected from Lake Belton sampling location “Shallow 3” from 60 feet deep would be designated “LB-SED-Shallow3-60.”

5.2.2.2.2 Laboratory Activities

Sample Analysis

Aliquots of all lake water and sediment samples were analyzed for perchlorate by USEPA Method 314.0 at the USACE Engineer Research and Development Center Environmental Laboratory at the Environmental Chemistry Branch in Omaha, Nebraska (See **Appendix V**). The USACE laboratory conformed to the analytical method requirements, analytical quality control requirements, instrument calibration frequency, and the laboratory quality control requirements presented in the QAPP (MWH, 2002e). The data verification report for sample analysis is included in **Appendix W**.

Bioreduction Experiments

Bioreduction experiments on these field samples were designed to address several aspects of the potential for perchlorate biodegradation in Lake Belton. These aspects include:

- *Reductive Medium.* It was anticipated that biological reduction of perchlorate could occur in both the sediment and in the water column. Consequently, tests were performed separately on each of these mediums. Sterile controls were prepared for each of these mediums by autoclaving both the sediment and water column samples. These controls were used to establish that any measured reduction of perchlorate was a result of microbial metabolism and not chemical reduction or physical adsorption.
- *Spatial Variability.* Due to the inherent variability in nature, it could not be expected that the microbiological quality, quantity and composition would be homogeneous throughout the water column, sediment and in different locations in the lake. In order to evaluate how some of this natural variability might impact the perchlorate biodegradation potential; six locations in Lake Belton were selected. Three of the locations were selected in the deeper portions of the lake that were likely to have been under anoxic conditions for

the longest period of time at the time of sampling. The remaining three locations were chosen in shallower water where the thermocline was close to the water/sediment interface. Sediment was collected from each of these locations. Water samples were collected from one of the deep locations near the bottom, close to (but below) the thermocline, and close to the surface (above the thermocline). One additional water sample was collected near the bottom of one of the shallower water locations.

- *Nutrients.* While it is not possible to easily determine the limiting nutrient of these biological systems, experiments were performed to determine if perchlorate degradation in the water column would be enhanced by the addition of an electron donor (acetate) and/or minerals. The minerals were obtained from two sources: 1) sterilized sediment; and 2) laboratory prepared solution. For example, if an enhancement was observed, then the water column had the appropriate microorganisms, but was limited or lacking some nutrient.
- *Oxygen.* Oxygen has been shown to inhibit perchlorate reduction in the facultative reducers. In order to characterize how the oxic/anoxic conditions of the lake can impact perchlorate reduction, an anoxic water sample was oxygenated and provided sufficient electron donor to reduce the oxygen. This was to show if the culture could recover in the time allotted. This was then compared to an unoxygenated system. Additionally, a water sample from above the thermocline was tested for its ability to reduce perchlorate.
- *Temperature.* The temperature in the anoxic region of the lake can be substantially colder than close to the surface. It is likely that these colder temperatures will reduce the rate of any perchlorate biodegradation. To assess the impact of temperature, a set of microecology experiments was carried out at both room temperature (~20°C) and at 6°C.
- *Kinetics.* Determining the rate biodegradation *a priori*, based on the microecology of environmental mixed cultures and available micronutrients is not possible given the current state of knowledge. Consequently, samples were collected at regular intervals over the course of 14 days to establish how rapidly biodegradation took place for selected conditions and establish how different environmental factors may impact this observed rate.

A summary of the experimental matrix is presented in **Appendix U**. This summary outlines general experimental conditions, matrix modifications, incubation periods, number of samples, and quality control samples.

5.2.2.3 *Data*

Spatial Variability

To test the influence of spatial variability within the water column and around the lake on the perchlorate bioreduction potential, samples were collected at each of the locations previously discussed above using the protocols set forth in the Field Sampling Plan (April 2003):

Sediment Variability

Under temperature controlled conditions in an anaerobic chamber, a series of autoclaved 100 mL Amber Pyrex Media bottles were filled with 90 mL of a laboratory prepared stock solution consisting of sterilized water collected from Lake Belton and spiked with 50 $\mu\text{g/L}$ of perchlorate. These bottles were inoculated with 10-mL aliquots of the six different sediments, purged with nitrogen to strip any dissolved oxygen and ensure the headspace in the bottle was oxygen-free, capped, and agitated (in triplicate). All six core sediment samples were processed in triplicate bottles to insure representative sampling of the core plug as a whole. Control samples were prepared, in triplicate, by autoclaving sample bottles inoculated with sediment. The samples were allowed to react for a 14-day period under constant, low-level agitation, provided by an orbital shaker, in a low-temperature incubator set at ambient conditions (20°C). During processing and incubation, all sample exposure to light was minimized to reduce the chance of algal growth that could result in undesirable oxygen generation.

As shown in **Figure 5-107**, significant perchlorate reduction was observed within a 14-day incubation period for all six sediment samples. With the exception of the sediment sample from the *Deep 2* location, which achieved approximately 80% reduction, the initial 50 $\mu\text{g/L}$ of perchlorate was reduced to below the reporting limit (<4 $\mu\text{g/L}$). Since the sediment samples were collected from six widely dispersed locations in the lake, these results indicate that indigenous perchlorate-reducing bacteria are likely to be present within sediment throughout Lake Belton. As complete reduction was observed in most of the samples, it was not possible to characterize the variability in microecologies.

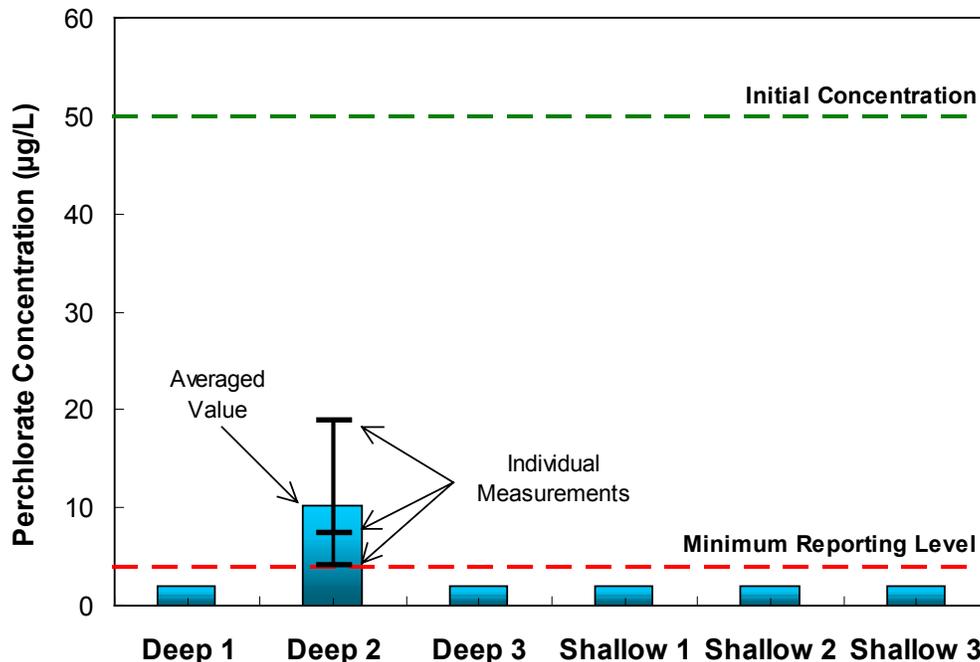


Figure 5-107
Spatial Variability of Perchlorate Reduction for Sediment Sample Cultures[†]

[†] Bars represent average values of the three replicates. “Error bars” represent individual values.

Water Column Variability

Under temperature controlled conditions in an anaerobic chamber, 50 mL aliquots of the three samples from the water column at the *Deep 1* location were volumetrically transferred into amber, 50 mL serum bottles and spiked with 50 µg/L perchlorate. The samples were then nitrogen purged, capped, agitated, and allowed to react for a 14-day period in a low-temperature incubator set at ambient conditions. All sample processes were carried out in triplicate, and exposure to light was minimized. Control samples also were prepared in triplicate by autoclaving sample bottles inoculated with an aliquot of the water column samples. At the end of the contact time, a portion of each sample was filter-sterilized (0.45 µm) and submitted to the USACE laboratory for perchlorate analysis.

Of the three aqueous samples incubated, only the *Deep 1 Bottom* sample achieved removals of perchlorate to below the reporting limit (< 4 µg/L) within 14 days (**Figure 5-108**). The *Deep 1 Thermocline* and *Surface* samples, however, did achieve 50% and 16% reduction, respectively. The difference in perchlorate reduction observed at the three different depths at the *Deep 1* location may be attributed to the anticipated inhibitory effect of dissolved oxygen on populations of perchlorate-reducing microorganisms. At the *Surface* (0.3 m) and *Thermocline* (10 m) depths, dissolved oxygen concentrations were 9.13 mg/L and 0.06 mg/L, whereas the *Bottom* (30 m) sample had 0 mg/L. This phenomenon was further investigated in the Impact of Oxygen experiments (see Oxygen section below). Results of this Water Column Variability experiment demonstrated that the perchlorate reducers are present throughout the water column. However, their activity or numbers appeared to be increasing with depth.

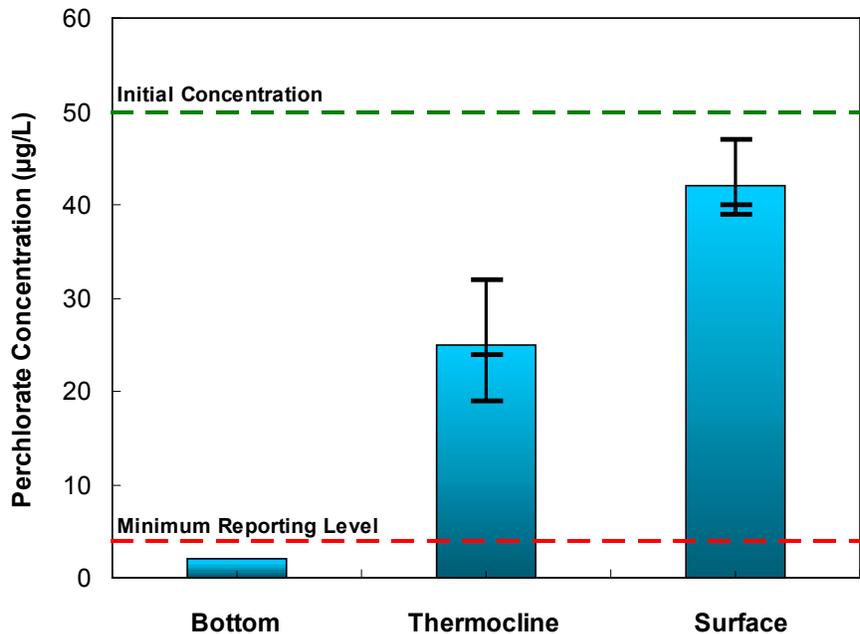


Figure 5-108
Spatial Variability of Perchlorate Reduction for *Deep 1* Aqueous Sample Cultures[†]

[†] Bars represent average values of the three replicates. “Error bars” represent individual values.

Kinetics

As the rate of biodegradation was not known, cultures were prepared using the aqueous *Deep 1* sample and the sediments from the *Deep 1* and *Shallow 1* locations. Samples were prepared so that they could be individually sacrificed at 1, 3, 5, 7, 10 and 14 days to characterize the rate of perchlorate degradation. **Figure 5-109** and **Figure 5-110** compare the rate of perchlorate degradation for both aqueous and sediment samples. For sediment samples, the rate of perchlorate reduction was more rapid for the *Shallow 1* location as compared to the *Deep 1* location with perchlorate reaching non-detect levels ($< 4 \mu\text{g/L}$) in 10 versus 14 days, respectively. It is hypothesized that the *Shallow 1* culture may have a greater diversity, have a higher density, or have a higher activity of microorganisms in this region.

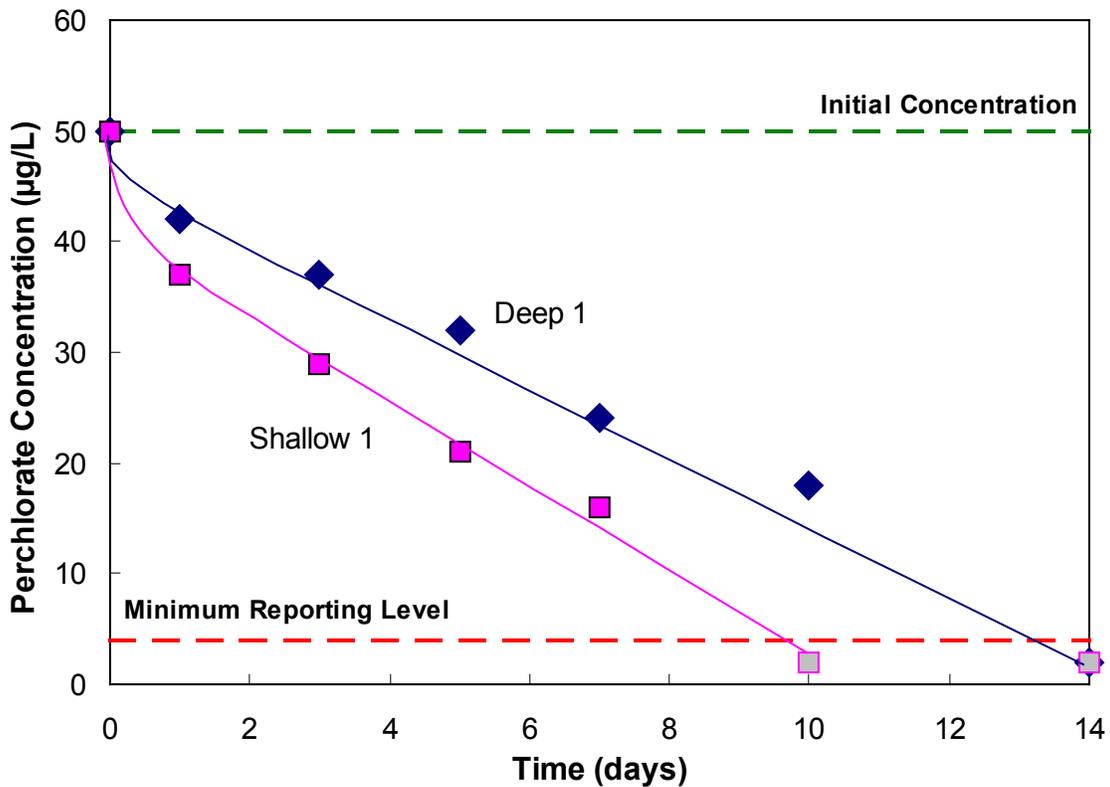


Figure 5-109
Rates of Perchlorate Degradation for Sediment Sample Cultures

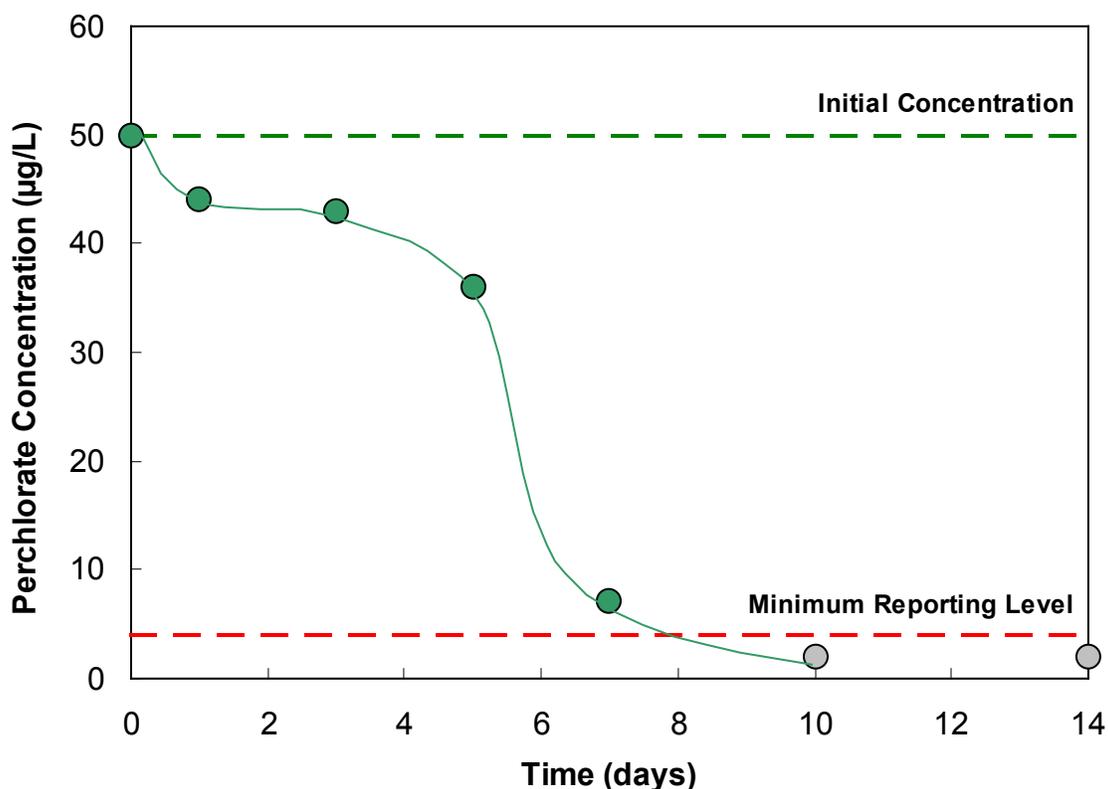


Figure 5-110
Rates of Perchlorate Degradation for Aqueous Sample Cultures (Deep 1)

The aqueous *Deep 1* samples reduced perchlorate to non-detect levels ($< 4 \mu\text{g/L}$) within 10 days. Perchlorate reduction, however, was slow for the first four days and then accelerated quickly thereafter. This slow initial response represented the lag time required for the perchlorate-reducing bacteria to begin the expression of (per)chlorate-reductase, whereas little to no lag was observed in the samples containing sediment-borne bacteria. The lag observed in aqueous cultures may be associated with (1) the possible exposure to dissolved oxygen during sampling, transportation, or handling of the sample which temporarily inhibited the expression of (per)chlorate-reductase, (2) temporary inhibition due to the presence of nitrate, (3) differing initial microbial composition – type of organisms and quantities of each organism and/or (4) the limited surface area available for attached growth (i.e., soil particles available in sediment inoculated cultures). However, additional and more detailed microecology experiments would be needed to further develop these hypotheses.

Nutrients

To determine if perchlorate reduction might be inhibited by a nutrient deficiency, samples of the sediment and water column were amended with an electron donor and/or metals.

Sediment Nutrients

Portions of the sediment samples collected were processed as described in the “Sediment Variability” section of this document. These samples were then spiked to 50-mg/L acetate; nitrogen purged, agitated and allowed to react for a 14-day period. At the end of the contact time, filter-sterilized (0.45 μm) samples were collected and submitted to the USACE laboratory for perchlorate analysis.

Comparing results of the amended and unamended cultures, it can be seen in **Figure 5-111** that perchlorate reduction to below reporting limits was observed in all acetate-amended cultures. Specifically for the *Deep 2* sediment culture, perchlorate reduction improved from approximately 80% to greater than 92% when amended with acetate. Although this marked improvement was noticeable for the *Deep 2* sediment culture, based on the range of cultures tested from Deep and Shallow sediments, it would appear that sufficient electron donor and carbon source exists for the indigenous perchlorate-reducing microorganisms to subsist. Additional perchlorate reduction tests were performed to evaluate if the addition of acetate improved the rate at which perchlorate was degraded.

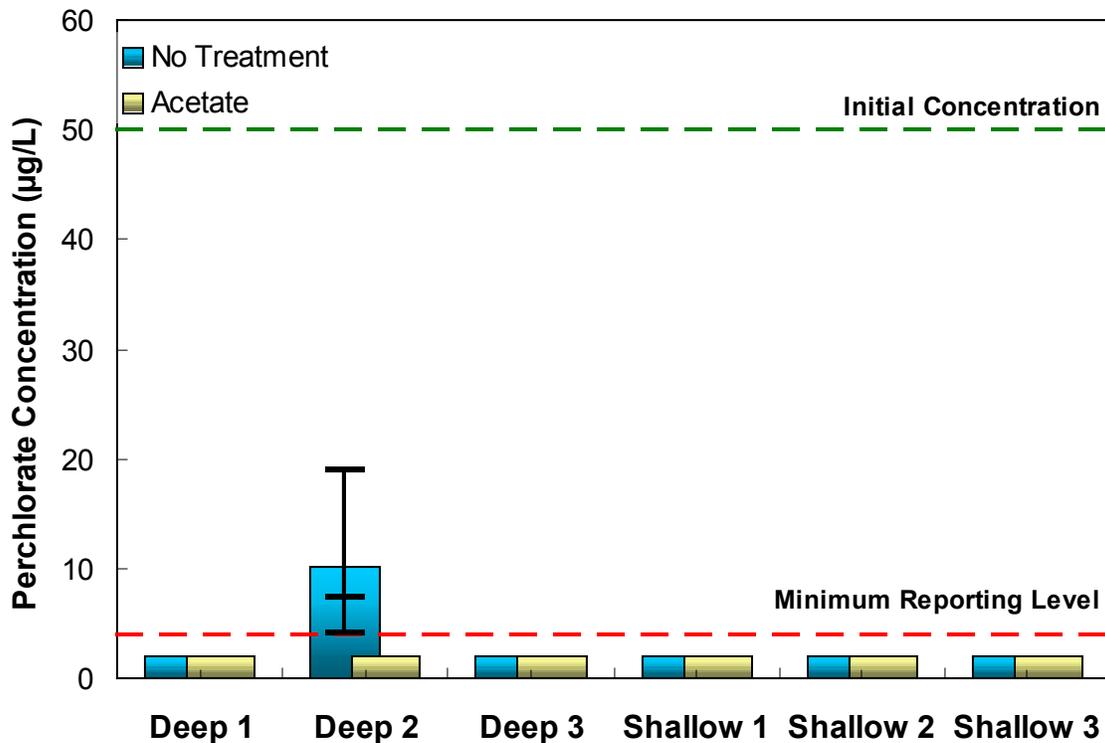


Figure 5-111

Effect of Acetate Addition on Perchlorate Reduction for Sediment Sample Cultures[†]

To determine if the addition of a readily accessible electron donor would alter the rate of biodegradation, additional cultures were prepared for two of the sediments. These

[†] Bars represent average values of the three replicates. “Error bars” represent individual values.

cultures were individually sacrificed at 1, 3, 5, 7, 10 and 14 days to characterize any potential impacts on perchlorate degradation.

As shown in **Figure 5-112** and **Figure 5-113**, the addition of acetate improved the rate of perchlorate biodegradation in both the *Deep 1* and *Shallow 1* sediment cultures. A significant improvement was observed with the *Deep 1* culture as perchlorate was reduced to below detection limits ($< 4 \mu\text{g/L}$) within 5 days, compared to the 14 days required for unamended cultures. A similar but slight improvement was noticed for *Shallow 1* cultures as perchlorate reduction improved from 10 to 7 days with the addition of acetate. These results revealed that while neither sediment sample was stoichiometrically limited by the electron donor, readily accessible excess electron donor could accelerate perchlorate reduction.

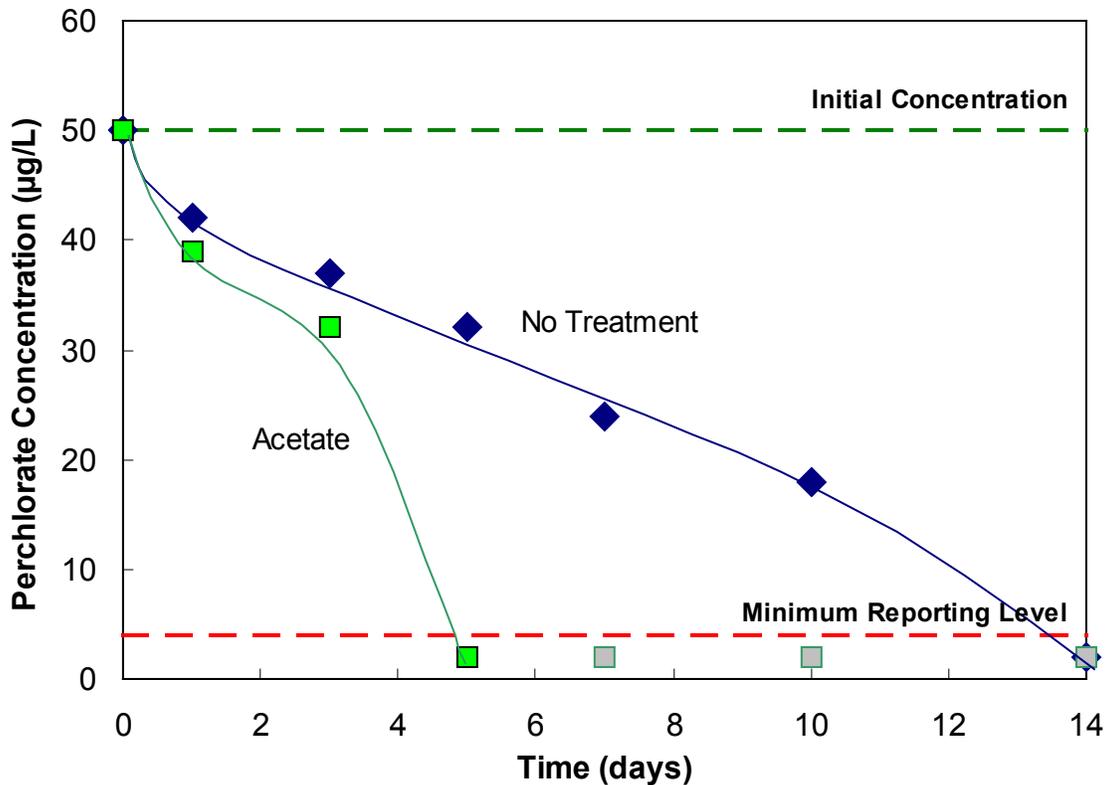


Figure 5-112
Impact of Acetate Addition on Perchlorate Reduction Rates for *Deep 1* Sediment Cultures

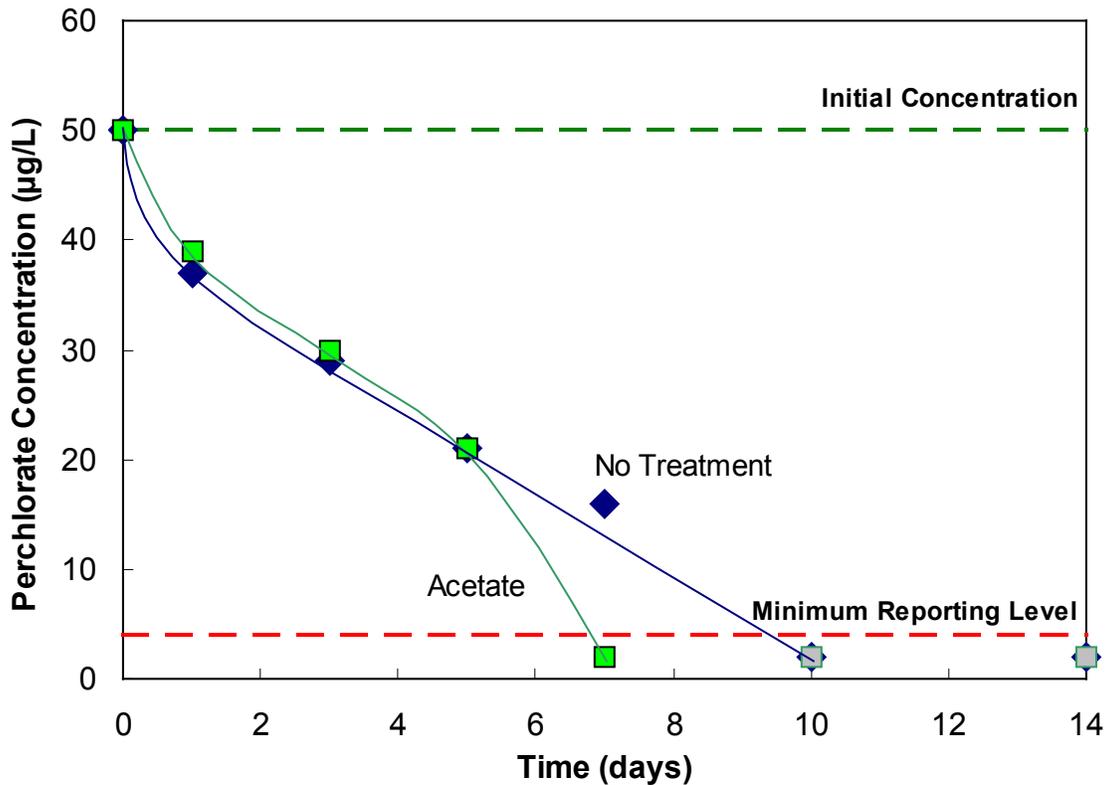


Figure 5-113
Impact of Acetate Addition on Perchlorate Reduction Rates for *Shallow 1* Sediment Cultures

Water Column Nutrients

A portion of the aqueous samples collected was processed as described in the “Water Column Variability” section of this document. Each of these samples (*Deep 1 Bottom*, *Thermocline*, *Surface*, and *Shallow 1 Bottom*) was amended with the acetate, metal and/or sediment combinations identified in the **Table 5-16**. Once amended, triplicate samples were agitated and allowed to react for a 14-day period. At the end of the contact time, filter-sterilized (0.45 µm) samples were collected and submitted to the USACE laboratory for perchlorate analysis.

Table 5-16
Nutrient Amendments for Water Column Cultures

Series	Nutrient Amendment	
1	50 mg/L acetate	
2	50 mg/L acetate and 50 mg/L autoclaved and dried “Deep 1” sediment	
3	50 mg/L acetate and 1 mL/L of mineral solution [†]	
[†] Mineral Solution Composition in Deionized Water (g/L)		
	(NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O	10
	ZnCl ₂	0.05
	H ₃ BO ₃	0.3
	FeCl ₂ ·4H ₂ O	1.5
	CoCl ₂ ·6H ₂ O	10
	MnCl ₂ ·6H ₂ O	0.03
	NiCl ₂ ·6H ₂ O	0.03

Results of the nutrient-amended aqueous cultures were compared to the corresponding unamended culture from the “Water Column Variability” tests. As shown in **Figure 5-114**, the addition of minerals to the *Deep 1 Bottom* culture caused the perchlorate-reducing ability of the indigenous microorganisms to completely cease. Results from the *Shallow 1 Bottom* culture were nearly identical. Interestingly, while each of the minerals has been identified as necessary micro-nutrients for many biological systems, some component(s) in the mineral solution appeared to be inhibitory. Additional detailed experiments would be required to identify the mineral(s) of concern and determine at what concentration the micro-nutrient becomes inhibitory.

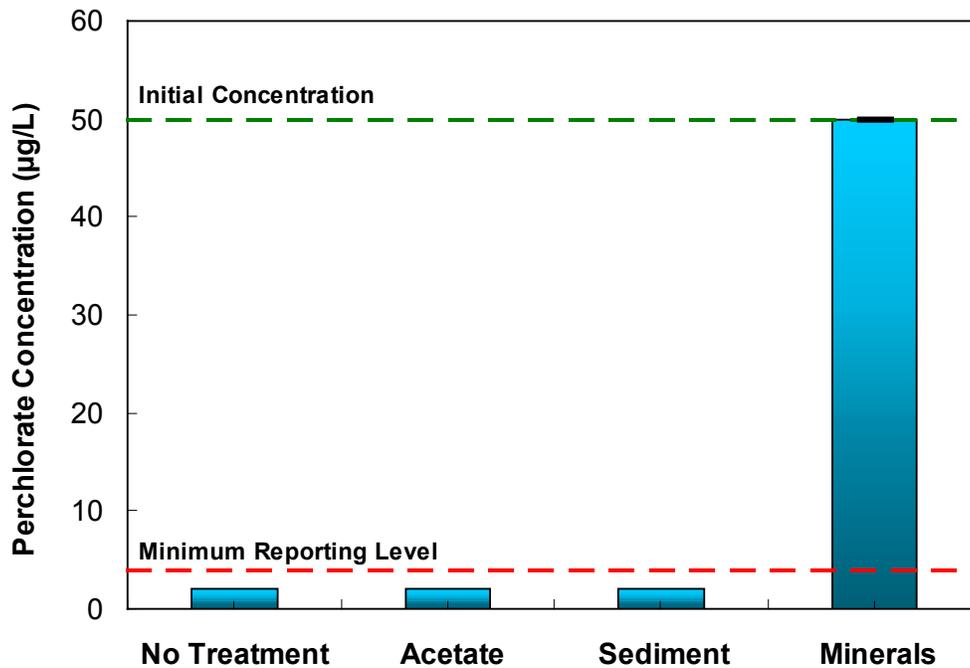


Figure 5-114
Impact of Nutrient Addition on *Deep 1 Bottom* Aqueous Cultures

In the “Water Column Variability” tests, the unamended *Deep 1 Thermocline* and *Surface* cultures were only able to reduce the initial 50- $\mu\text{g/L}$ perchlorate by 50 and 16%, respectively. **Figure 5-115** shows the impact of adding acetate, sediment, or minerals to the *Deep 1 Surface* culture. Similar results were observed in the *Deep 1 Thermocline* culture. The addition of acetate did not improve perchlorate reduction and the addition of the mineral solution resulted in a complete inhibition of perchlorate reduction. However, sediment addition resulted in complete reduction of the perchlorate. Several possibilities may explain this improved reduction: 1) an increase in biomass; 2) scavenging of oxygen by reduced metals in the sediment; 3) increase in electron donor concentration; 4) perchlorate adsorption on/in to the sediment; or 5) addition of critical nutrients. However, the first four are not considered to be likely. Since the sediment was sterilized, any additional biomass added would be inactive, and through the sterilization and drying process, it is likely that any oxygen-scavenging metals would have been oxidized. As the addition of acetate did not result in a significant improvement in the reduction, it is unlikely that this alone could cause the observed response. An autoclaved control bottle of the entire system revealed that perchlorate adsorption on/in to the sediment is not an issue. This leaves the addition of some critical nutrient(s) not included in the mineral solution or the addition of a different concentration of the essential nutrient.

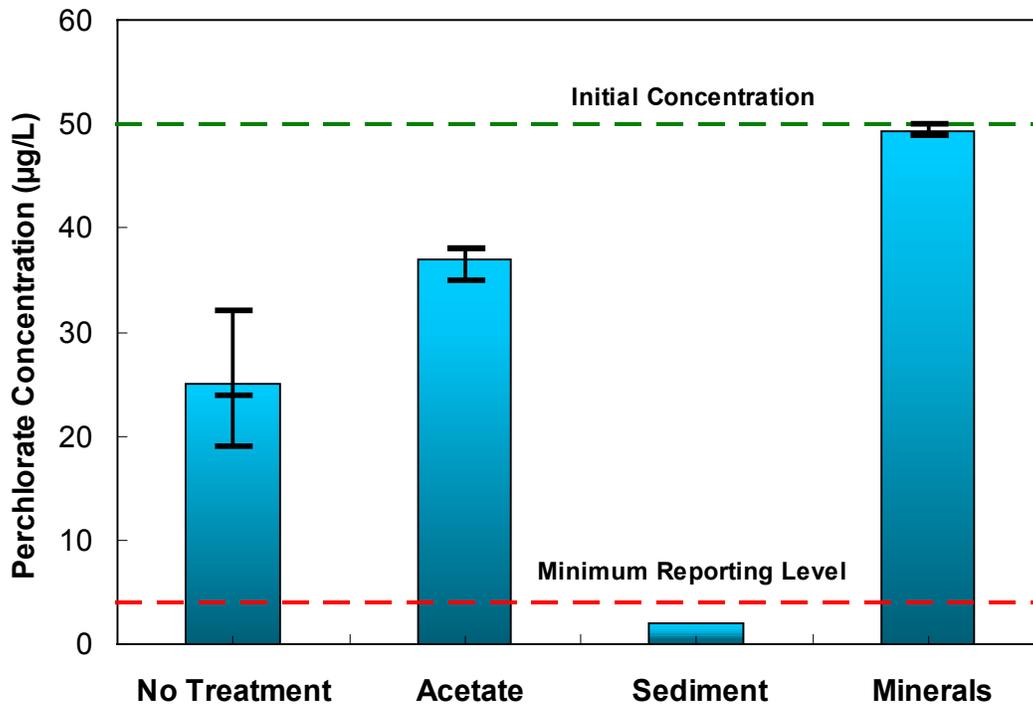


Figure 5-115
Impact of Nutrient Addition on *Deep 1 Surface* Aqueous Cultures[†]

This demonstrated that the perchlorate-reducing bacteria, that were likely to have been disrupted by the presence of dissolved oxygen at the thermocline or surface, could be induced to reduce the perchlorate.

[†] Bars represent average values of the three replicates. “Error bars” represent individual values.

Kinetic studies were performed for the *Deep 1 Bottom* sample location to characterize any potential impacts of various nutrient amendments on the measured rate of perchlorate degradation. Five additional cultures were prepared for each of the nutrient addition conditions (acetate, sediment, and minerals) and were individually sacrificed at 1, 3, 5, 7, 10 and 14 days. As previously observed, there was little to no difference in the rate of perchlorate degradation between the unamended and acetate-amended cultures. As seen in **Figure 5-116**, however, when nutrients were introduced through the addition of sterilized sediment, perchlorate reduction to below reporting limits ($< 4 \mu\text{g/L}$) improved from approximately 10 to 6 days.

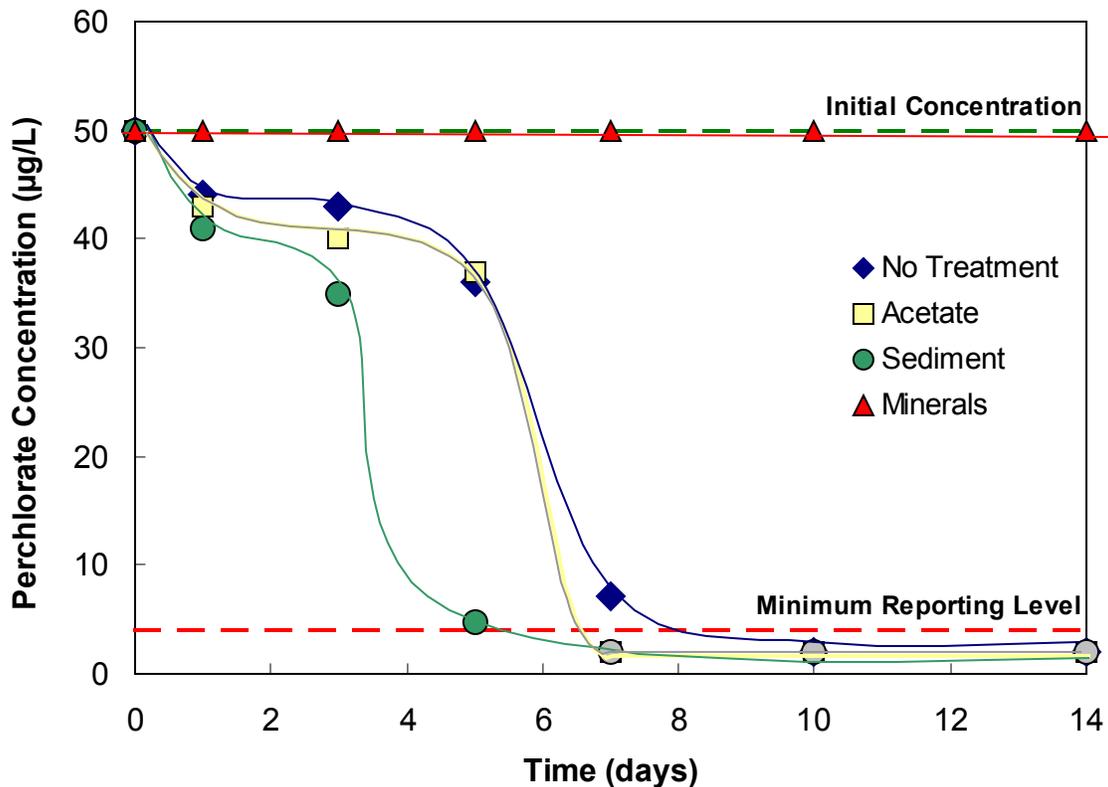


Figure 5-116
Impact of Nutrient Addition on Perchlorate Reduction Kinetics for *Deep 1 Bottom* Aqueous Cultures

These results confirmed the ability of the unknown nutrients in the sediment matrix to stimulate the activity of perchlorate-reducing bacteria present in the aqueous sample. However, additional ecology experiments would need to be performed to characterize the effect and activity of the many different microbial populations that contribute to perchlorate reduction and rate of biodegradation.

Oxygen

The inhibitory effect of oxygen was only evaluated in the water column. In addition to the effects of oxygen that can be observed in selected cultures from the “Spatial

Variability” tests, the oxygen tests were used to differentiate between the possible impacts of brief oxygen exposure to elevated dissolved oxygen levels for the *Deep 1 Bottom*, *Deep 1 Thermocline*, *Deep 1 Surface* and *Shallow 1 Bottom* samples.

Two aliquots were prepared from each water sample by either: 1) oxygenating the sample only or 2) oxygenation with a subsequent nitrogen purge and the addition of a small amount of sulfide to remove the available dissolved oxygen and lower the redox potential. Once prepared, all of the aliquots were then dosed with 50-µg/L perchlorate and 50-mg/L acetate, sealed, agitated, and returned to the inert environment. The cultures were allowed to react for a 14-day period before samples were filter-sterilized (0.45 µm) and submitted to the USACE laboratory for perchlorate analysis.

From **Figure 5-117** it can be seen that for the *Deep 1 Bottom* culture, the exposure of the organisms to high concentrations of oxygen had a significant negative impact on the ability of the indigenous microorganisms to effectively reduce perchlorate. Even when the oxygenated samples were sparged with nitrogen and dosed with sulfide to remove dissolved oxygen; the perchlorate-reducing capability of the culture was inhibited and could not recover within the 14-day incubation period. Similar results were observed for the additional samples prepared for the *Deep 1 Thermocline*, *Deep 1 Surface*, and *Shallow 1 Bottom* locations.

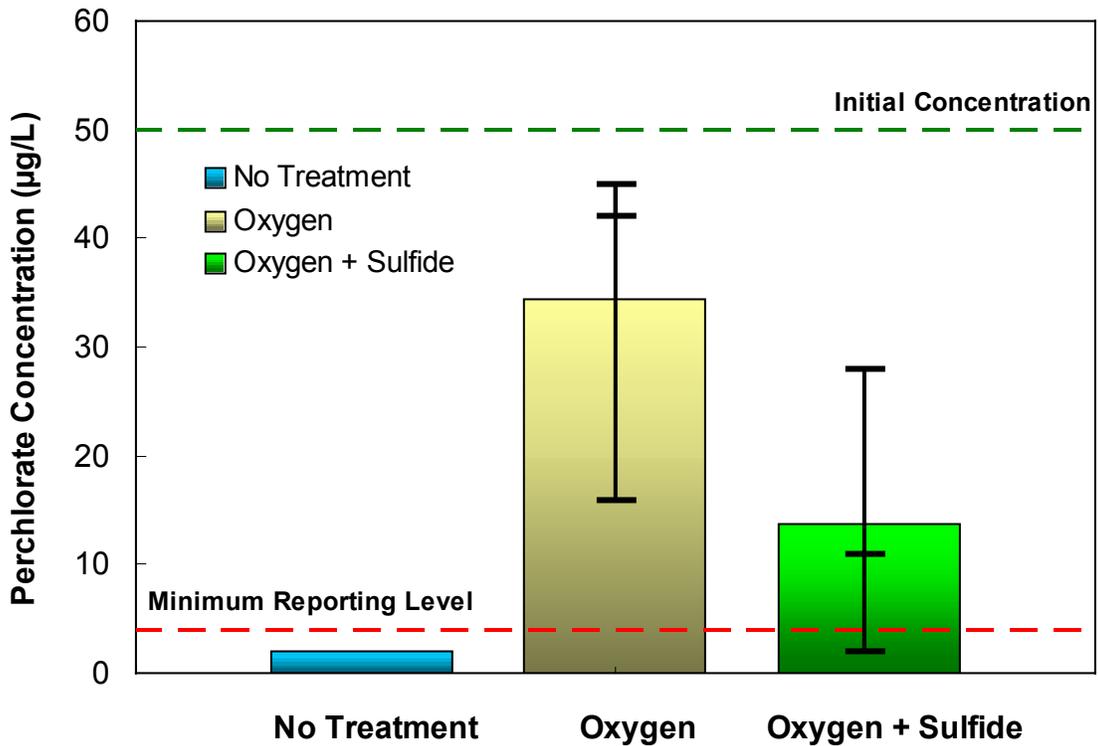


Figure 5-117
Impact of Oxygen on Perchlorate Reduction for *Deep 1 Bottom* Aqueous Cultures[†]

[†] Bars represent average values of the three replicates. “Error bars” represent individual values.

Temperature

It is expected that the lower temperatures at the bottom of the lake will slow the rate of perchlorate reduction. To measure this variability, a portion of the water and sediment samples collected was processed as described in the “Water Column Variability” and “Sediment Variability” sections of this document, respectively. The processed samples were agitated, nitrogen purged and stored in a low temperature incubator set at 6°C. After the respective incubation periods, filter-sterilized (0.45 µm) samples were collected and submitted to the USACE laboratory for perchlorate analysis.

Figure 5-118 compares the results of aqueous samples run at ambient and low temperatures. As expected, the rate of perchlorate reduction suffered for samples incubated at 6 °C. This is best represented by the *Deep 1 Bottom* and *Shallow 1 Bottom* samples, in which the perchlorate-reducing bacteria (sampled from expected anoxic environments) performed the best at ambient temperature. When incubated at a low temperature, however, only 20% of the perchlorate was reduced indicating that the perchlorate-reducing bacteria located in the aqueous phase were inhibited by the low temperature environment. Since these bacteria were highly active at an ambient temperature (20 °C), it is likely that the mixed culture in the aqueous matrix was comprised of primary mesophilic bacteria (capable of surviving between 20 – 40 °C).

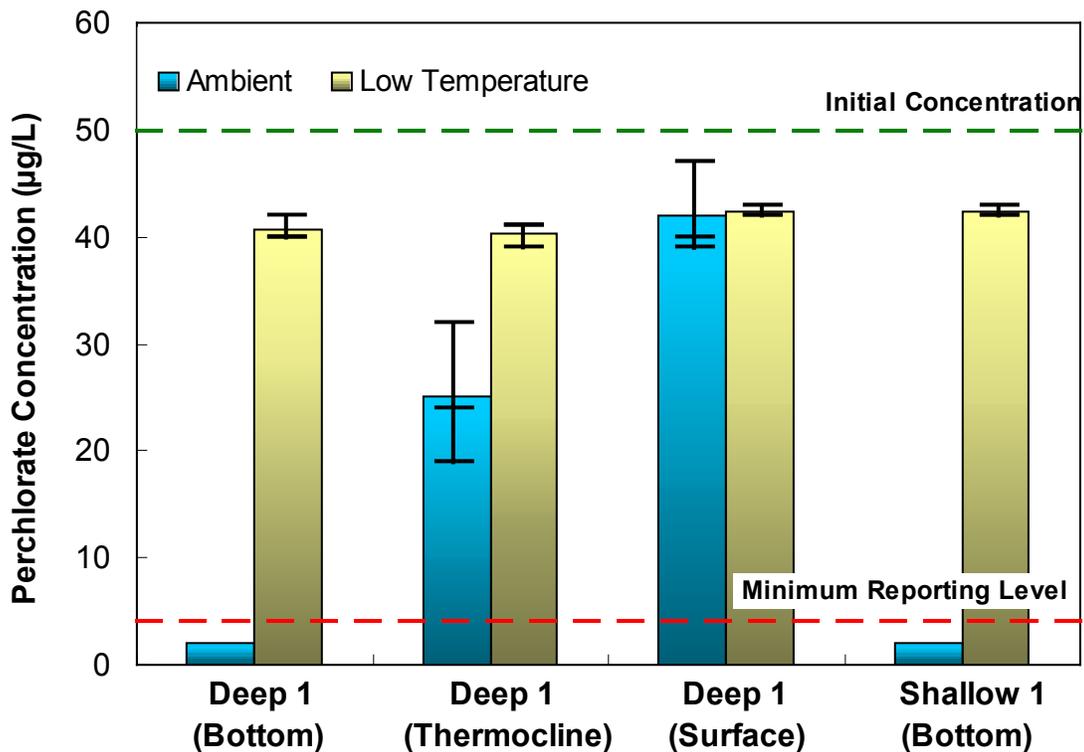


Figure 5-118

Effect of Temperature on Perchlorate Degradation for Aqueous Sample Cultures[†]

[†] Bars represent average values of the three replicates. “Error bars” represent individual values.

Figure 5-119 compares the results of sediment samples run at ambient and low temperatures. As expected, *Deep* sediment samples incubated at 6 °C experienced similar diminished performance compared to the samples incubated at ambient temperature. Comparing the perchlorate reduction of aqueous and sediment samples from the *Deep 1* location incubated at 6 °C, it is interesting to note that more perchlorate reduction was observed in sediment samples (60% compared to 20%). The perchlorate-reducing capability of the sediment-borne bacteria was possibly related to potentially larger numbers and/or different types of microorganisms in the sediment cultures.

Interestingly, results from the shallow sediment samples indicated that highly active populations of perchlorate-reducing bacteria existed in the *Shallow 2* and *Shallow 3* locations, as perchlorate reduction was observed below reporting limits (< 4 µg/L) for both ambient and low temperature conditions. The improved performance compared to *Shallow* aqueous samples is likely due to the fact that a larger population of perchlorate-reducing microorganisms and/or more diverse microorganisms are available in the sediment matrix (i.e., perchlorate-, nitrate-, perchlorate/nitrate- reducing bacteria, mesophiles, psychrophiles, etc.). Additional microecology experiments would be required to characterize the quantity and types of these microorganisms that are present in sediment compare to aqueous matrices.

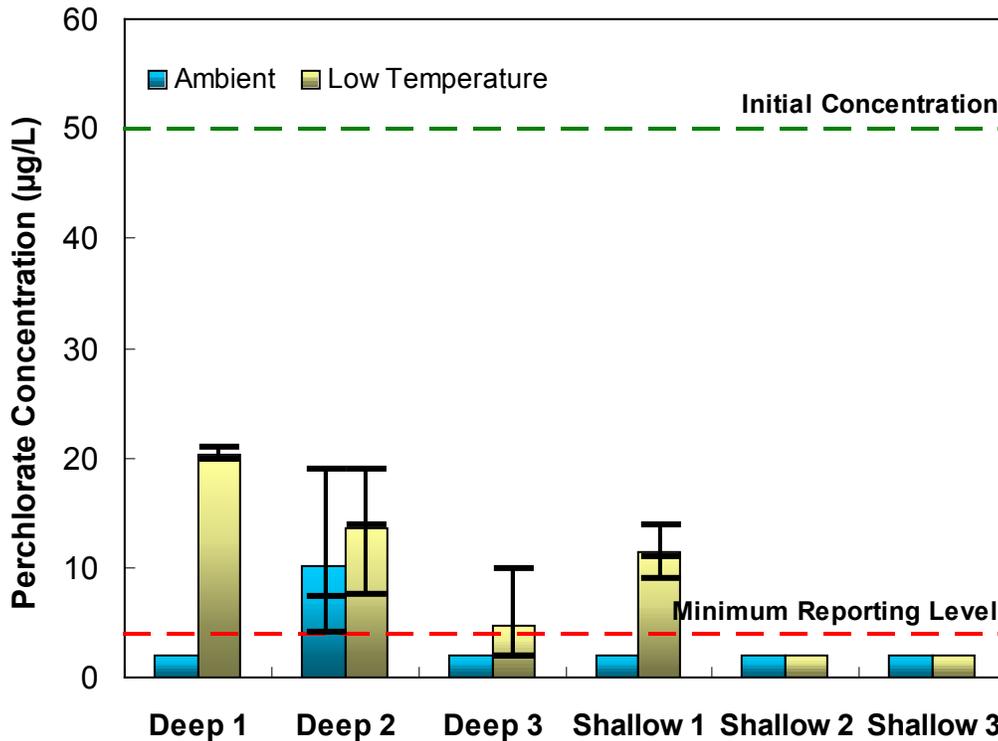


Figure 5-119
Effect of Temperature on Perchlorate Reduction for Sediment Sample Cultures[†]

[†] Bars represent average values of the three replicates. “Error bars” represent individual values.

5.2.2.4 Discussion

The overall results of this testing indicated that Lake Belton has the potential for the natural attenuation, or more specifically, intrinsic bioremediation of perchlorate. Based on the results of specific experiments performed, the following conclusions can be made:

- Indigenous perchlorate-reducing microorganisms are present throughout the water column and sediment of Lake Belton. All experiments performed with aqueous and sediments cultures demonstrated the capability to reduce 50 µg/L of perchlorate spiked to the samples. Several cultures, which were not amended with additional electron donor or nutrients, reduced perchlorate to non-detect levels (< 4 µg/L) in less than 14 days.
- The presence of oxygen impacts the rate of perchlorate biodegradation. Comparing results of samples collected at the bottom, thermocline, and near the surface of the lake, is apparent that the presence of dissolved oxygen has an inhibitory effect on the perchlorate-reducing ability of the microorganisms. Cultures present in the bottom or sediments of both deep and shallow portions of the lake were consistently able to reduce 50 µg/L perchlorate to below the 4 µg/L reporting limit within a 14-day incubation period. As a result, the majority of perchlorate bioreduction is expected to occur in the deeper areas of Lake Belton.
- Sufficient electron donor is available to the perchlorate-reducing bacteria for natural attenuation. Sufficient electron donor was present for complete reduction of perchlorate. Additional tests, performed by augmenting the sample with 50 mg/L acetate indicated that a further elevation of the electron donor did not consistently result in a significant improvement in the rate of perchlorate degradation.
- The amendment of sediment nutrients to water column cultures improved perchlorate reduction. Aqueous cultures, found to be impacted by the inhibitory effect of oxygen, were tested with the amendment of sterilized sediment. Results revealed that the perchlorate reduction was improved by approximately 90%. While several possibilities existed, it is likely that a critical nutrient(s) in the sediment was responsible for the observed increase in biological activity.
- Biological reduction of perchlorate is inhibited at lower temperatures. Results showed that mesophilic (moderate temperature tolerant) bacteria are dominant in the water column samples. In several samples, perchlorate reduction slowed from nearly 95 to 10% when incubated at 6 °C. Compared to the aqueous cultures, sediment-borne cultures achieved improved perchlorate-reducing capability in a cold environment. Complete perchlorate reduction was observed in several sediment cultures at 6 °C . These results indicated that the sediment contained more robust perchlorate-reducing bacteria (i.e., mesophiles and psychrophiles) and larger populations of these microorganisms.

5.2.3 Perchlorate Transformation Study

5.2.3.1 Introduction

The fate of perchlorate in the environment can be influenced by any transformation (mainly microbial) that occurs. As plants senesce vegetative parts can be returned to the soil/sediment. The return of perchlorate to the soil/sediment may be a beneficial process due to the movement of perchlorate from stable environments (aerated stream water or low carbon deeper sediments) to areas where rapid transformation can occur (top organic rich soil layers). One of the most important aspects of the fate of perchlorate in these systems is the rate of transformation. Understanding the magnitude and environmental influences on these microbial degradation rates is imperative to understand the long term stability in the environment.

5.2.3.2 Methodology

5.2.3.2.1 Site Description and Sampling

Sediment and soil samples were obtained from two sites historically associated with ClO_4^- discharge in Texas. One site was the Naval Weapons Industrial Reserve Plant (NWIRP) in McGregor, Texas. The other site was the Longhorn Army Ammunition Plant (LHAAP) in Karnack, Texas, which was another primary manufacturer of solid propellant rocket motors. The locations of these sites are shown on **Figure 5-120**. The LHAAP, listed on the USEPA National Priorities List, is located in the Caddo Lake watershed. This site covers an area of about 8,500 acres. Perchlorate contamination in water, soil, sediment, vegetation, and animal tissue samples has been reported at this site (Smith et al., 2001).

Four sediment samples were collected from the top 10 cm of bottom sediments at four selected creek locations surrounding NWIRP, namely, Harris Creek at Highway 84 west of McGregor (T19), a branch of Harris Creek at Highway 84 (T18), Harris Creek at Highway 317 (T13), and S Creek at Highway 317 (T15). These multiple stream sites were hereafter referred as HW84 Mainstream, HW84 Sidestream, HW317, and HW317/MN, respectively (**Figure 5-120**). HW84 Sidestream is a groundwater discharge zone, with a spring identified at the head of the stream.

At the LHAAP site, several kilograms of relative dry soil were collected from the top 15 cm near Building 25C (a perchlorate grinding facility). The specific soil sampling location layout at LHAAP site has been described in detail elsewhere (Smith et al., 2001). The soil had prior exposure to ClO_4^- . Descriptions of sediments and soil are summarized in **Table 5-17**.

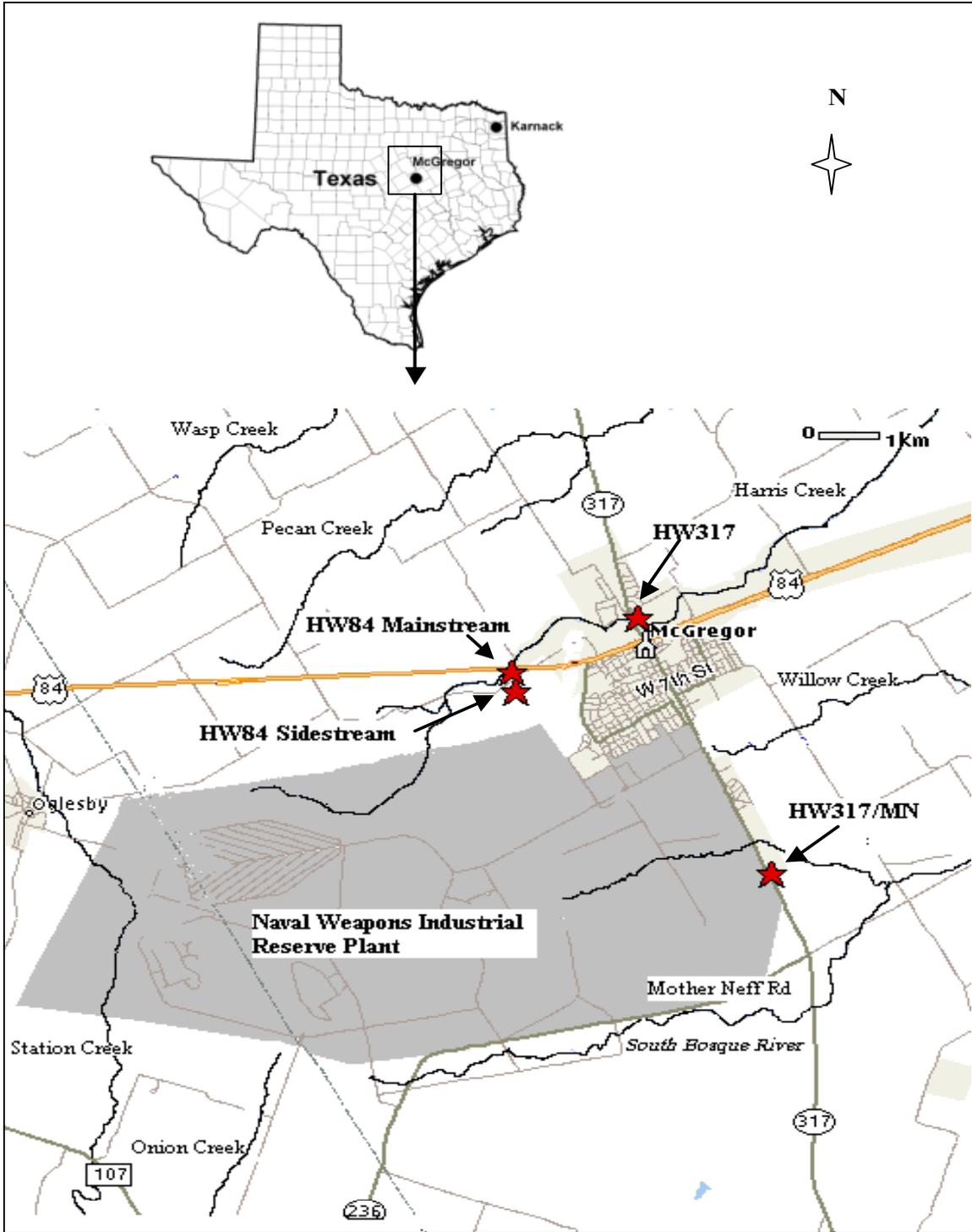


Figure 5-120
Locations of the NWIRP and LHAAP Sites

Table 5-17
Description of Sediments and Soils Used in Microcosm Studies on Perchlorate Transformation

Site	Location	Matrix	Description
HW84 Sidestream (T18)	NWIRP	Sediment	Continuously exposed to ClO_4^-
HW84 Mainstream (T19)	NWIRP	Sediment	Intermittently exposed to ClO_4^-
HW317 (T13)	NWIRP	Sediment	Intermittently exposed to ClO_4^-
HW317/MN (T15)	NWIRP	Sediment	Continuously exposed to ClO_4^-
Longhorn	LHAAP	Soil	Prior exposure to ClO_4^-

Water samples were taken simultaneously with sediments at each location. Water samples were not taken at the LHAAP site, because surface water was not available near Building 25C at the time of sampling. Sediment samples were maintained under anaerobic conditions by a layer of water over the surface. Water, sediment, and soil samples were stored on ice and then transported immediately to the lab for microcosm studies.

5.2.3.2.2 Laboratory Microcosm Studies

The sediment and water from individual locations were used to construct each microcosm. The microcosms consisted of 120 ml of a 1:3 sediment/water (by weight) slurry. Care was taken to avoid the introduction of oxygen. Bottles were sealed with septa and aluminum crimp caps, then incubated anaerobically at room temperature. Five ml of slurry were taken from each bottle with a clean bone marrow needle at a frequency of twice per week during the first few weeks, and once per week during the remaining period. All samples were analyzed for ClO_4^- , Cl^- , NO_3^- , NO_2^- , and SO_4^{2-} .

Generally, five treatments were conducted using HW84 Mainstream, HW84 Sidestream sediments, and Longhorn soil as representative samples to explore the influence of terminal electron acceptors. Treatments consisted of autoclaved (abiotic condition), control (no amendment), 5 ppm nitrate (corresponding to 1.1 ppm NO_3^- -N) spiked, 50 ppm nitrate (corresponding to 11.3 ppm NO_3^- -N) spiked, and 300 ppm sulfate spiked. Only the control treatment (no amendment) was conducted for HW317 and HW317/MN sediments. All microcosm treatments are summarized in **Table 5-18**. The autoclaved treatments were prepared by autoclaving the sediments and soils for 20 minutes at 15 psig and 121 °C on 3 successive days, in order to assess the possible abiotic loss of perchlorate. Three replicates were run for each treatment. The initial ClO_4^- concentration for each treatment was 5 ppm (mg/L). Another treatment was conducted by using HW84 Sidestream sediment and DI water instead of site water to study the kinetics under lower levels of nitrate (i.e., 1 ppm nitrate-N spiked), because there were relatively high nitrate background levels in site water. One additional treatment was conducted for HW84 Sidestream to study the ClO_4^- degradation rate under a lower initial ClO_4^- concentration. The initial ClO_4^- concentration was spiked at 0.5 ppm for this treatment. The sampling interval was every two or three days.

Table 5-18
Summary of Treatment Conditions Used in Microcosm Studies on Perchlorate Transformation

Site	ClO ₄ ⁻ ppm	Autoclaved	Control	Site Water			DI Water
				NO ₃ ⁻ -spiked 1.1 ppm	NO ₃ ⁻ -spiked 11.3 ppm	SO ₄ ⁻ -spiked 300 ppm	NO ₃ ⁻ -spiked 1 ppm
HW84 Sidestream (T18)	0.5	-	+	-	-	-	-
	5.0	+	+	+	+	+	+
HW84 Mainstream (T19)	5.0	-	+	+	+	+	-
HW317 (T13)	5.0	+	+	-	-	-	-
HW317/MN (T15)	5.0	-	+	-	-	-	-
Longhorn	5.0	+	+	+	+	+	+

“+” treatment conducted

“-” treatment not conducted

5.2.3.2.3 Analytical Methods

Perchlorate analysis procedures are described in **Appendix X**.

Total volatile solids (TVS) content offers a rough approximation of the amount of organic matter content in wetland sediments. The standard method 2540 G (APHA et al., 1998) was followed to determine volatile solids in semisolid samples.

5.2.3.2.4 Kinetic Data Analysis

Microbial degradation rates were calculated by assuming first-order kinetics. Microcosm degradation curves were fitted using non-linear regression by Sigmaplot software (SPSS Inc.) to the first-order kinetic equation:

$$C = C_0 \exp(-kt)$$

where C = perchlorate concentration (ppm or ppb), t = time (day), C₀ = initial perchlorate concentration (ppm or ppb), and k = kinetic rate (day⁻¹).

5.2.3.3 Data

5.2.3.3.1 Degradation Curves of Perchlorate and Nitrate

The microcosm degradation curves of perchlorate and nitrate under different initial NO₃⁻-N for sediments and soil from three locations are presented in **Figure 5-121**. The results of three treatments are compared, including control (no amendment), 1.1 ppm NO₃⁻-N spiked, and 11.3 ppm NO₃⁻-N spiked. All treatments were at the same initial ClO₄⁻ concentration of 5 ppm. These treatments were chosen to cover NO₃⁻-N ranges from approximately 5 ppm to 25 ppm, corresponding to the nitrate concentration at a typical perchlorate-contaminated site.

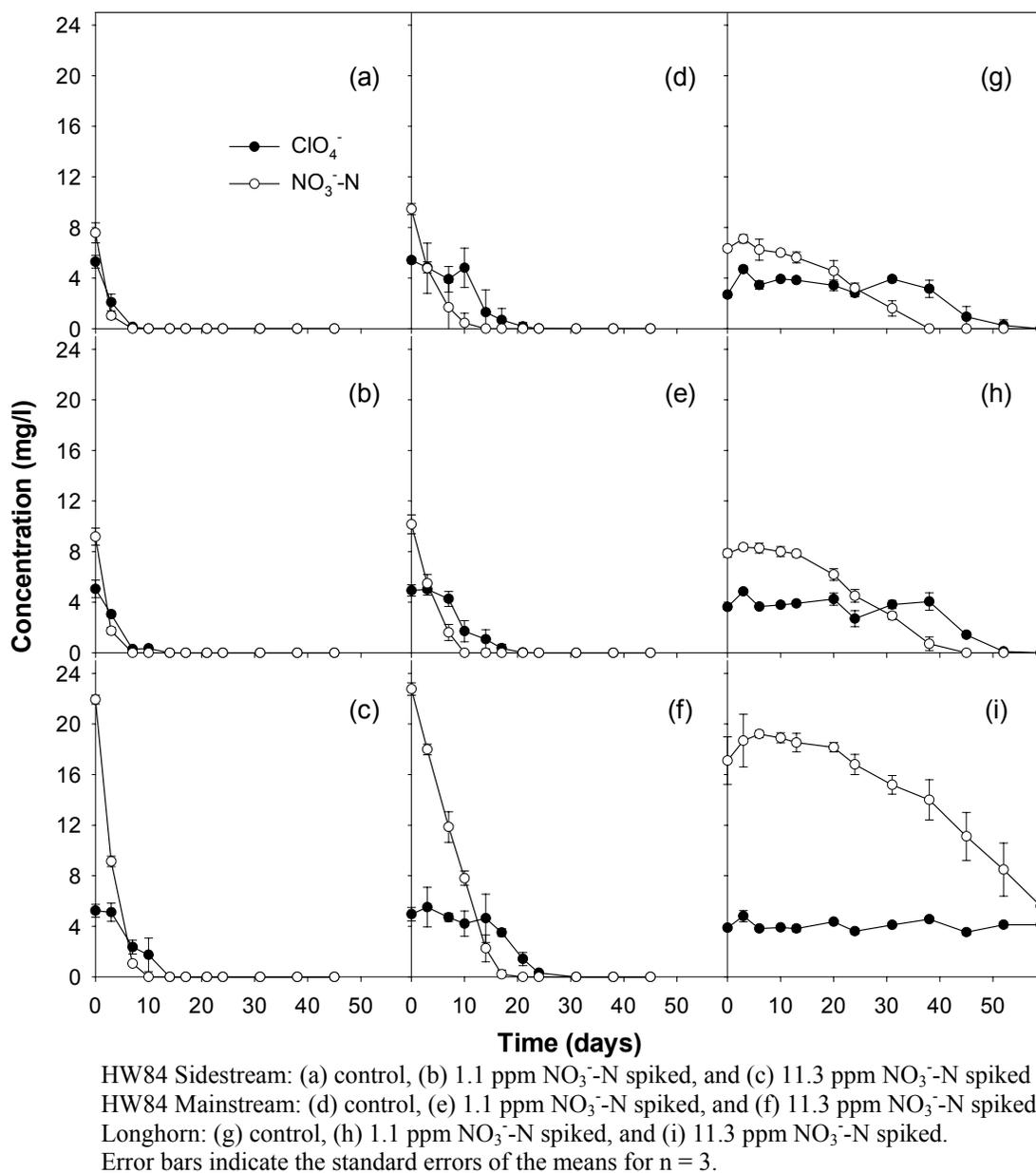


Figure 5-121
Degradation of Perchlorate and Nitrate Spiked at Different Nitrate Levels for Sediments and Soils from Three Locations

For all three treatments, perchlorate and nitrate were degraded to below the reporting limit (4 ppb) during the experimental period for HW84 Sidestream (**Figure 5-121** a, b, and c) and HW84 Mainstream (**Figure 5-121** d, e, and f). For Longhorn soil, perchlorate and nitrate were degraded to below the reporting limit within 60 days with up to 1.1 ppm NO_3^- -N spiked (**Figure 5-121**). However, in the case of 10 ppm NO_3^- -N spiked in Longhorn soil, ClO_4^- degradation did not occur during the 60 days of the experimental period and NO_3^- -N (about 6 ppm) was still above the ClO_4^- concentration (approximately

4 ppm) on day 60 (**Figure 5-121**). There was less than 5% ClO_4^- degradation observed for all the autoclaved control treatments during the 60 days of the experimental period (data not shown).

No major difference was observed between the degradation curves with 1.1 ppm NO_3^- -N spiked (**Figure 5-121** b, e, and h) and those of control treatments (**Figure 5-121** a, d, and g), which was probably due to the elevated initial nitrate background level in sediments and soil (6.3 mg/L to 9.4 mg/L as N), such that the small amount of NO_3^- -N addition could not exert an obvious effect. Significant differences were observed for the degradation curves in treatments spiked with 11.3 ppm NO_3^- -N (**Figure 5-121** c, f, and i).

For HW84 Sidestream (**Figure 5-121** a, b, and c), simultaneous perchlorate reduction and nitrate reduction were observed during the experimental period. For HW84 Mainstream and Longhorn soil, it was observed that rapid ClO_4^- degradation did not happen until NO_3^- -N was degraded to a relatively low level (normally below the ClO_4^- concentration) (**Figure 5-121** d, e, f, g, h, and i). After the beginning of ClO_4^- degradation, simultaneous perchlorate reduction and nitrate reduction were observed. This suggested an interference effect of nitrate on perchlorate degradation. On the other hand, by examining all the control treatments (**Figure 5-121** a, d, and g), it was obvious that perchlorate and nitrate degradation curves followed different patterns for different sediments and/or soil. This significant difference of degradation behavior might be linked with the variation of organic substrate availability, bacterial population, and initial nitrate concentration in individual sediments and/or soils.

5.2.3.3.2 Degradation Kinetics and Lag Times

Reported half-saturation constants (K_s) for heterotrophic perchlorate reducers ranged from 45 mg/L to 470 mg/L (Logan et al., 2001), and K_s with respect to NO_3^- -N was estimated to be 1.4 mg/L (Aesoy and Odegaard, 1994). With respect to the initial concentration of ClO_4^- (5 mg/L) and NO_3^- -N (above 5 mg/L) in our study, ClO_4^- and NO_3^- -N degradation would be expected to follow first-order and zero-order kinetics, respectively. The shape of degradation curves (**Figure 5-121**) supports this hypothesis. It appears that NO_3^- -N degradation normally complied with zero-order kinetics during the initial rapid degradation period when the NO_3^- -N concentration was still relatively high, then was followed by a transition to first-order kinetics when the NO_3^- -N concentration was degraded to a lower level (below 2 mg/L). Both first-order and zero-order kinetics fit well for NO_3^- -N degradation in terms of r^2 (> 0.86). However, first-order rates were selected to use for both ClO_4^- and NO_3^- -N degradation for comparison.

Each microcosm treatment was run in triplicate. Data in the exponential decay period of each run were first fitted using nonlinear regression analysis to obtain individual first-order rate constants, which were then averaged to obtain kinetic rate constants of each treatment including standard deviation. Lag time (acclimation period without degradation or with less than 5% degradation loss) of each run was obtained from the degradation curves, and then averaged to obtain the lag time for each treatment. First-order rate constants and lag times determined for perchlorate and nitrate degradation from each degradation curve under different treatment conditions are summarized in **Table 5-19**. In

general, different sites showed variations in perchlorate and nitrate degradation rates and lag times, which were affected by numerous factors, such as organic substrate availability (represented by TVS), initial nitrate concentration, and prior exposure. The effects of these factors are discussed in detail individually in the following section.

Table 5-19
Perchlorate and Nitrate Degradation Kinetic Data for Sediments/Soils

Site	TVS ^a mg/g	Treatment	Initial NO ₃ ⁻ -N mg/L	Rate K _{ClO₄⁻} d ⁻¹	Rate K _{NO₃⁻-N} d ⁻¹	ClO ₄ ⁻ Lag d	NO ₃ ⁻ -N Lag d
HW84 Sidestream (T18)	115.9 ± 12.2	low nitrate	1	0.36 ± 0.01	0.66 ± 0.04	0.0 ± 0.0	0.0 ± 0.0
		control	7.6	0.37 ± 0.07	0.68 ± 0.05	0.0 ± 0.0	0.0 ± 0.0
		1.1 ppm NO ₃ ⁻ -N	9.2	0.25 ± 0.01	0.57 ± 0.03	0.0 ± 0.0	0.0 ± 0.0
		11.3 ppm NO ₃ ⁻ -N	21.9	0.21 ± 0.05	0.33 ± 0.01	3.0 ± 0.0	0.0 ± 0.0
HW84 Mainstream (T19)	84.5 ± 3.7	control	9.4	0.14 ± 0.02	0.30 ± 0.11	3.3 ± 3.5	0.0 ± 0.0
		1.1 ppm NO ₃ ⁻ -N	10.2	0.16 ± 0.05	0.26 ± 0.02	4.3 ± 2.3	0.0 ± 0.0
		11.3 ppm NO ₃ ⁻ -N	22.8	0.18 ± 0.03	0.13 ± 0.01	14.0 ± 0.0	0.0 ± 0.0
HW317 (T13)	160.5 ± 0.5	control	6.3	0.46 ± 0.04	1.42 ± 0.67	0.0 ± 0.0	0.0 ± 0.0
HW317/MN (T15)	70.6 ± 0.2	control	16.6	0.13 ± 0.05	0.11 ± 0.03	12.0 ± 1.7	0.0 ± 0.0
Longhorn	43.3 ± 5.7	control	6.3	0.16 ± 0.08	0.06 ± 0.01	35.7 ± 4.0	8.0 ± 5.6
		1.1 ppm NO ₃ ⁻ -N	7.9	0.14 ± 0.02	0.07 ± 0.00	35.7 ± 4.0	14.0 ± 0.0
		11.3 ppm NO ₃ ⁻ -N	17.1	NA	0.03 ± 0.01	60.0 ± 0.0	29.7 ± 9.1

Data are average ± standard deviation of triplicate run

^abased on dry weight

^bClO₄⁻ degradation not observed during 60 days of study

NA – not available.

5.2.3.3.3 Effect of Organic Substrate Availability

The organic substrate availability, represented here by TVS, was found to be one of the crucial factors affecting microbial degradation rates and lag times. Control treatments for HW317 and HW84 Sidestream had a higher TVS content (160.5 mg/g and 115.9 mg/g, respectively), so both the ClO₄⁻ and NO₃⁻-N degradation rate (K_{ClO₄⁻} ranged from 0.37 d⁻¹ to 0.46 d⁻¹; K_{NO₃⁻-N} ranged from 0.68 d⁻¹ to 1.42 d⁻¹) were significantly higher than those of other sediments and soil (K_{ClO₄⁻} ranged from 0.13 d⁻¹ to 0.16 d⁻¹; K_{NO₃⁻-N} ranged from 0.06 d⁻¹ to 0.30 d⁻¹) (Table 5-19). Additionally, lag times of ClO₄⁻ and NO₃⁻-N degradation generally increased with a decrease in TVS content of a similar magnitude as initial NO₃⁻-N concentration. No lag time was observed for both ClO₄⁻ and NO₃⁻-N degradation of control treatments for sediments of HW317 and HW84 Sidestream, but lag times of 35.7 days (ClO₄⁻ degradation) and 8.0 days (NO₃⁻-N degradation) were observed for Longhorn soil which had the lowest TVS content (43.3 mg/g) (Table 5-19). Compared to organic substrate availability, prior exposure does not appear to be a major factor affecting degradation rates and lag times. Sediment from HW317, which was only intermittently exposed to ClO₄⁻ (Table 5-19), had the highest perchlorate degradation rate (0.46 day⁻¹), while HW317/MN and HW84 Sidestream (which are continuously exposed to ClO₄⁻) had relatively lower rates (0.13 and 0.36 day⁻¹). Likewise, lag times appear to

be more related to TVS than prior exposure as decreasing TVS is directly related to increasing lag times.

5.2.3.3.4 Effect of Initial Nitrate Concentration

Results indicated that nitrate was another important factor affecting ClO_4^- degradation. When comparisons were made among different treatments (control, 1.1 ppm NO_3^- -N spiked, and 11.3 ppm NO_3^- -N spiked) for a specific sediment (i.e. HW84 Sidestream, HW84 Mainstream, Longhorn, respectively), any variation in degradation rates and lag times would be due to the different nitrate concentration present since the organic availability (TVS) did not vary for different treatments of each sediment. Lag times of ClO_4^- degradation increased considerably with the increase in NO_3^- -N concentration (HW84 Mainstream (3.3 ~14.0 days), HW84 Sidestream 0 ~3.0 days, and Longhorn soil (35.7 ~ 60.0 days)) (**Table 5-19**). In addition, with the increase in NO_3^- -N concentration, ClO_4^- degradation rates for a specific sediment or soil under different treatment conditions were generally constant in terms of magnitude (e.g. HW84 Mainstream (0.14 ~ 0.18 day^{-1}), and Longhorn soil (0.14 ~ 0.16 day^{-1})), but in some cases the ClO_4^- rates declined slightly (HW84 Sidestream (0.37 ~ 0.21 day^{-1})). The slight decrease of $K_{\text{ClO}_4^-}$ for HW84 Sidestream sediments with the increase in nitrate concentrations is probably caused by substrate availability due to simultaneous perchlorate and nitrate degradation throughout the experimental period. Only sediments from this site exhibited simultaneous perchlorate degradation when NO_3^- -N concentration was greater than ClO_4^- . As the substrate supply would be relatively constant, it would be shared by populations using each electron acceptor and thus would be reduced in both cases. For all other sites, nitrate was essentially depleted before ClO_4^- degradation commenced and thus ClO_4^- degradation rates remained relatively constant. This implies that HW84 Sidestream sediments contained populations with as high an affinity for ClO_4^- as NO_3^- , or populations that were capable of using ClO_4^- but not NO_3^- . However, for most sediments examined, NO_3^- appears to prevent the use of ClO_4^- as an electron acceptor.

Generally, there was no observed lag time for NO_3^- -N degradation in sediments except for the Longhorn soil. The lag phase was absent because acclimated denitrifying microorganisms existed in sediments. Nitrate degradation rates remained almost constant at lower NO_3^- -N concentration (control treatment and treatment with 1.1 ppm NO_3^- -N spiked), but decreased with 11.3 ppm NO_3^- -N spiked (**Table 5-19**). Under the assumption of first-order kinetics, the rates should remain constant. However, the decline of NO_3^- -N degradation rates under highly elevated NO_3^- -N concentration may be linked with the limiting effect of organic substrate availability. The long lag time observed for Longhorn soil was probably associated with a low initial denitrifier population in the dry soil than that in sediments, and the lowest organic substrate content (TVS) compared to other sediments. Furthermore, NO_3^- -N degradation rate constants were higher than ClO_4^- rate constant under the same treatment (i.e. $K_{\text{NO}_3^-} = 0.68 \text{ d}^{-1}$, and $K_{\text{ClO}_4^-} = 0.37 \text{ d}^{-1}$ for control treatment of HW84 Sidestream), this might be caused by higher bacterial affinity for nitrate than that of perchlorate (because half saturation constant K_s for nitrate is much smaller than that for perchlorate).

5.2.3.3.5 Effect of Initial Perchlorate Concentration

Since the typical ClO_4^- concentration in the environment is at ppb levels, another microcosm control treatment (no amendment) with ClO_4^- of 0.5 ppm and the same initial NO_3^- -N of 7.6 mg/L was conducted for sediment from HW84 Sidestream. Perchlorate degradation rate constants remained the same ($K_{\text{ClO}_4^-} = 0.37 \text{ d}^{-1}$) for both lower and higher initial ClO_4^- concentration. One interesting aspect was that there was a 1 day lag time for ClO_4^- degradation at 0.5 ppm, compared to no lag time for ClO_4^- degradation at 5 ppm. This supports the assumption that the lag time of ClO_4^- degradation was dependent on the relative ratio of a competitive electron acceptor (NO_3^-). A previous study by Attaway and Smith (1993) found that nitrate did not inhibit ClO_4^- reduction (no lag time), perhaps because the ClO_4^- concentration (9 mM, or 896 mg/L) used in the experiment was much higher than the nitrate concentration (9 mM, corresponding to NO_3^- -N of 126 mg/L).

5.2.3.3.6 Effect of Sulfate Concentration

The effect of sulfate on ClO_4^- degradation was examined using microcosm treatment of 300 ppm SO_4^{2-} . It was found that sulfate did not exert an obvious effect on ClO_4^- degradation, which was consistent with previous findings (Attaway and Smith, 1993; Herman and Frankenberger, 1998; 1999). Compared to control treatments, ClO_4^- degradation rate constants at 300 ppm SO_4^{2-} remained almost constant, and no significant variation in lag time was observed. For example, $K_{\text{ClO}_4^-}$ was 0.14 d^{-1} and 0.15 d^{-1} ; lag time of ClO_4^- degradation for sediment HW84 Mainstream was 3.3 day and 4.3 day, for the control treatment and treatment of 300 ppm SO_4^{2-} , respectively.

5.2.3.4 Discussion

Perchlorate reducing bacteria are believed to be distributed ubiquitously in the environment (Coates et al., 1998; 1999; Wu et al., 2001; Logan, 1998; 2001). Microcosm studies, using site sediment and water collected from four natural habitats near NWIRP, McGregor, have indicated that rapid intrinsic bio-remediation is possible in the stream sediments which are continuously or intermittently exposed to perchlorate. Microcosm treatments using soil from another perchlorate-contaminated site, LHAAP also were capable of ClO_4^- degradation although a long lag period (up to 60 days) may be necessary, depending on the environmental conditions. Intrinsic ClO_4^- degradation rates ranged from 0.13 day^{-1} to 0.37 day^{-1} for four sediments, corresponding to a half-life ($t_{1/2} = 0.693/k$) range of 1.9 days to 5.0 days, with variation of rates depending mainly on the organic substrate availability.

Although it is clear that nitrate does interfere with ClO_4^- degradation, to date the pathway and mechanism involved is poorly understood. Most, but not all, perchlorate reducers could use nitrate as electron acceptor (Coates et al., 1999), and some denitrifying bacteria are capable of ClO_4^- degradation (Logan et al., 2001). Recently, Giblin and Frankenberger (2001) held the view that separate terminal reductases capable of reducing other electron acceptors were responsible for ClO_4^- and nitrate degradation in an isolated bacterium strain. However, the strain grew more rapidly with nitrate. Our results supported this hypothesis. Based on our results, we could extrapolate that the microbes in the sediments and soil from our study can use both ClO_4^- and nitrate as alternative

electron acceptor, depending on the relative electron equivalence ratio. In the presence of relatively high nitrate concentration, the bacteria will preferentially use nitrate as electron acceptor because the growth on nitrate is much faster. After nitrate has been depleted, the ClO_4^- reductase will function to use ClO_4^- as electron acceptor. Thus, the presence of nitrate only affects the lag time of ClO_4^- degradation under the assumption that organic substrate availability is not a limiting factor. The fact that ClO_4^- degradation rate ($K_{\text{ClO}_4^-}$) of HW84 Sidestream remained almost constant even if the NO_3^- -N was lowered to 1.0 mg/L (**Table 5-19**), compared to 7.6 mg/L in the control treatment, also supported this assumption. If only one enzyme was involved, perchlorate and nitrate would become competitive inhibitors, and we would see a significant increase of ClO_4^- degradation rate because of the depletion of the competitive electron acceptor nitrate.

From the point view of thermodynamics in terms of energy yield of electron acceptors, perchlorate's energy yield (Gibb's free energy per electron equivalent $\Delta G_0' = -112.1 \text{ kJ/e}^-$) as an electron acceptor is similar to that of nitrate ($\Delta G_0' = -112.2 \text{ kJ/e}^-$), when hydrogen is used as the electron donor (Nerenberg et al., 2002). This implies that denitrification is not more energy-favorable than perchlorate degradation. The preference of ClO_4^- to NO_3^- as electron acceptor should be associated with different enzyme involved which lowered the activation energy. Further research should be conducted to fully understand the metabolism involved. The presence of nitrate may explain the persistence of ClO_4^- in the environment, especially when ClO_4^- concentration is considerably lower than that of nitrate concentration (i.e. in the most case of groundwater), which may require a relatively longer lag time for ClO_4^- degradation to happen.

Our studies also indicate that higher organic substrate availability can shorten the lag time of both ClO_4^- and nitrate degradation. Sediment HW317 was not continuously exposed to ClO_4^- , but it had the highest ClO_4^- and nitrate degradation rates (**Table 5-19**). This might imply one of the effective enhancement strategies of ClO_4^- natural attenuation by substrate amendment.

An attempt was made to correlate environmental conditions (i.e. organic substrate availability (represented by TVS), and/or nitrate concentration) with degradation rates and lag times. In general, no correlations were found, probably due to limited data as well as the complexity of the environmental system. Perchlorate degradation in the sediments and soil is affected by numerous environmental conditions (i.e. substrate, perchlorate concentration, population of perchlorate reducers, nitrate, physics of sediments and soil, temporal and spatial etc.) and other factors.

This study explores the intrinsic kinetics of ClO_4^- degradation in the natural sediments and soil, including the role of nitrate. More intensive *in situ* studies may be necessary to understand the effects of numerous other environmental conditions on the degradation of perchlorate in the natural system.