Intrinsic Bioremediation of Landfills Interim Report

by

R. L. Brigmon

Westinghouse Savannah River Company
Savannah River Site
Aiken, South Carolina 29808

C. B. Fliermans

DOE Contract No. **DE-AC09-96SR18500**

DISCLAIMER

Intrinsic Bioremediation of Landfills Interim Report

Prepared for the U.S. Department of Energy under Contract No. DE-AC09-96SR-18500

Intrinsic Bioremediation of Landfills Interim Report

Derivative Classifier _____
T.C. Hazen, Section Manager
Authorized Derivative Classifier

Westinghouse Savannah River Company
Savannah River Site
Aiken, SC 29808

Prepared for the U.S. Department of Energy under Contract No. DE-AC09-96SR-18500

Intrinsic Bioremediation of Landfills Interim Report

Robin L. Brigmon and Carl B. Fliermans

Authentication: _____
Terry C. Hazen

Prepared for the U.S. Department of Energy under Contract No. DE-AC09-96SR-18500

Intrinsic Bioremediation of Landfills

Robin L. Brigmon, Carl Fliermans

Executive Summary

The purpose of this Project was to document the microbial and geochemical activities necessary to establish the extent and effectiveness of intrinsic bioremediation occurring at the Sanitary Landfill site. Intrinsic bioremediation has proven to be an efficient, cost effective, and safe method for restoration of select contaminated sites. The research was conducted at the same site where bioremediation optimization demonstrations have been completed, thereby capitalizing on the characterization and monitoring done at the site over the last 10 years. The previous demonstrations provide outstanding controls, baseline data, and comparisons for the current evaluations.

The Savannah River Site's (SRS) Sanitary Landfill (SLF), a Resource Conservation and Recovery Act (RCRA) Solid Waste Management Unit site, was evaluated for intrinsic bioremediation. The SLF operated for 20 years at the Department of Energy (DOE) facility. Specific contaminants of concern detected in groundwater from the SLF include vinyl chloride (VC) and trichloroethylene (TCE). Other contaminants of interest in the groundwater at the SLF include chlorobenzene (CB), tritium (H^3), dichloroethylene (DCE) and perchloroethylene (PCE). A previous study using a particle flow model predicted greater TCE, VC, and CB plume advancement over the past 5 years than was actually observed.

In this project microbiological, physical, and chemical data were combined to establish that intrinsic bioremediation was occurring at the SLF. These data, in combination with historical site data, aquifer geochemistry and soil properties, provide a detailed characterization of contaminant plumes at the SLF. Using this information, a more accurate approach for predicting plume behavior and managing subsurface contamination using intrinsic bioremediation can be instituted.

Increases or decreases in TCE levels in specific monitoring wells were correlated with concomitant decreases or increases in chloride (Cl) levels respectively. There is a VC plume on the Southwestern side or older portion of the SLF. A TCE plume is evident in the Southeastern section or most recently filled portion of the SLF. In situ respiration tests one year apart at two sites on the SLF demonstrated differences in oxygen demand in the subsurface. Geochemical evidence from the SLF site suggests that aerobic respiration is a major contributor to bioremediation in the most recently filled section of the landfill while anaerobic processes dominate the older sections. This project demonstrates the ongoing intrinsic bioremediation processes in the subsurface of the SLF by the following evidence:

1. documented reduction of contaminants at the site faster than rates of contaminant migration,
2. evidence that indigenous bacteria in sediment and groundwater are capable of degrading CB and TCE at the SLF,
3. greater microbial activity inside contaminant plumes than outside,
4. geochemical evidence that daughter products of TCE and CB biodegradation including DCE, VC, and Cl are being produced.

Key Words: Intrinsic Bioremediation, Trichloroethylene, Tritium, Landfills, Vinyl Chloride, Chloride, Chlorobenzene, BIOLOG, FAME, Microorganisms, Respiration

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS AND ACRONYMS

ACL	Alternate Concentration Limit
AIW	Air Injection Well
ASW	Air Sampling Well
BTEX	Benzene, Toluene, Ethylbenzene, and Xylene
CAP	Corrective Action Plan
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act
CB	Chlorobenzene
COC	Contaminant of Concern
DO	Dissolved Oxygen
DFA	Direct Fluorescent Antibody
DOC	U.S. Department of Commerce
DOE	U.S. Department of Energy
ECD	Electron Capture Detector
EFE	Ecofunctional Enzyme
ELISA	Enzyme Linked Immunosorbent Assay
EPA	U.S. Environmental Protection Agency
FFA	Federal Facilities Agreement
FID	Flame Ionizing Detector
gal	gallon
GC	Gas Chromatograph
GWPS	Groundwater Protection Standard
IC	Ion Chromatography
l/min	liter per minute
lb	pound
LRCC	Land Resource Conservation Commission
LSM	Laser Scanning Confocal Microscope
m	meter
mg/kg	milligram per kilogram
mg/l	milligram per liter
ml	milliliter
ml/min	milliliter per minute
MPN	Most Probable Number
ND	Non-detects
NPL	National Priority List
ORP	Oxidation-reduction Potential
PCE	Perchloroethylene
pH	negative log of hydrogen ion concentration
PID	Photo Ionization Detector
POC	Point of Compliance
POE	Point of Exposure
PPE	Personal Protective Equipment
PTYG	Peptone-Trypticase-Yeast-extract-Glucose
QA/QC	Quality Assurance/Quality Control
RCRA	Resource Conservation and Recovery Act
ROI	Radius of Influence
RPD	Relative Percent Difference
SCDHEC	South Carolina Department of Health and Environmental Control
SWMU	Solid Waste Management Unit
TCE	Trichloroethyene
UTRC	Upper Three Runs Creek
VC	Vinyl Chloride

1.0 Introduction

Intrinsic bioremediation is a risk management option that relies on natural biological and physical processes to contain the spread of contamination from a source. The option is most appropriate when the concentrations of contaminants are reduced to regulatory limits before ground water discharges to surface water or is collected by a monitoring well (Wilson et al., 1994). This requires that a projection of themovement and concentration of the contaminant plume in time and space be made. The projection is based of historical variations in the contaminant plume as well as the measured rates of contaminant attenuation. The amount of contamination removal over time by intrinsic bioremediation can be estimated from microcosm studies. It is incumbent on the proponent of the technology to provide sufficient evidence to demonstrate that the mechanisms of intrinsic remediation will reduce contaminant concentrations to acceptable regulatory levels before potential receptors are reached.

The efficiency of intrinsic bioremediation to contain and/or reduce contaminant migration in ground water systems can be quantitatively measured by comparing rates of contaminant transport with rates of biodegradation. When contaminant transport rates are faster than biodegradation rates, contaminants can migrate freely with ground water flow and possibly reach a point of contact with human or wildlife populations (Godsey, 1994). Conversely, if transport rates are slow relative to biodegradation rates, contaminant migration will be more confined and less likely to reach a point of contact. In either case, the efficiency of intrinsic bioremediation can be determined by evaluating the presence or absence of contaminant transport to predetermined points of contact. Thus, this assessment includes geochemical, hydrologic (rates of ground water flow), microbiologic (rates of biodegradation), and sociopolitical (points of contact) components (Chapelle, 1994; Kao and Borden, 1994). Evidence of biodegradation includes production of breakdown products of the contaminants of concern.

The benefits of intrinsic bioremediation are that it's low risk due to minimal handling, cost effectiveness compared to other technologies (i.e. pump and treat), aesthetically appealing to the public perception as a natural process, and proven track record on specific sites (Wiedemeier et al., 1995). Evidence is presented in this report that intrinsic bioremediation is occurring at the SLF is fundamental to support incorporation into a Corrective Action Plan (CAP).

1.1 Site Description

The Savannah River Site (SRS) is owned by the U.S. Department of Energy (DOE) and operated by Westinghouse Savannah River Company (WSRC), with the mission to produce nuclear materials for national defense. The SRS Sanitary Landfill is approximately a 70-acre site within the 310 square mile facility located in a rural area along the Savannah River, principally in Aiken and Barnwell Counties, South Carolina. The approximately 70 acre three parcel (original, Northern Expansion and Southern Expansion) Sanitary Landfill at the Savannah River Site began operations in 1974. The original parcel reached capacity in 1987 at which time the Northern and Southern Expansions were added.

1.2 Site Characteristics

In 1988, evidence of elevated concentrations of RCRA hazardous waste constituents were found in groundwater at the SLF. Consequently, the SLF was designated a RCRA Solid Waste Management Unit (SWMU). A renewal for a RCRA Part b permit application was prepared for the South Carolina Department of Health and Environmental Control (SCDHEC). As part of the Part b application, WSRC performed a risked-based alternative concentration limit (ACL) demonstration for all SLF contaminants of concern (COCs). In order to complete an ACL demonstration for the SLF COCs, transport of the contaminants from the SLF through the groundwater regime was quantitatively evaluated (WSRC, 1993). The COCs of interest at the SLF found to be in significant concentrations are TCE and VC. In order to evaluate the potential importance of groundwater movement in the SLF relative to COCs, a vertical cross-section and transport model was developed between the SLF and surface receptors. Surface receptors in the groundwater flowpath include Upper Three Runs Creek (UTRC) approximately 1000 meters from the SLF and a large bordering wetland about 300 meters from the SLF. A modeling study was developed to quantify the transport of the COCs between the SLF and the point of exposure (POE). The model was conservative in that it modeled maximum COC concentrations along the centerline of the plume neglecting lateral transverse dispersion. Relative and absolute concentrations were calculated for each COC and POC, the POE (nearest wetland discharge point), and UTRC. Both capped and non-capped conditions for the SLF were simulated for

two different source loading scenarios. The relationship between concentrations at the point of compliance (POC) and the POE were quantified with attenuation factors.

In 1993 the operations at the southern expansion were ceased. The Northern Expansion continues to accept non-organic wastes on a limited basis. According the previous report (WSRC, 1993), COCs, VC and TCE, were estimated to have impacted the waters of UTRC by 1996. The concentrations of the COCs in the SLF are not well defined since the predominate sources are rags soaked with solvents from industrial degreasing applications. The report took into consideration estimates of biodegradation half lives of the contaminants present. These included 70 days for PCE, 120 days for TCE, 200 days for dichloroethylene (DCE), and 1000 days for VC. All biodegradation kinetics were assumed to be first order. For CB no biodegradation was assumed in the model. A significant source of Chloride (Cl) in the SLF groundwater is microbial dechlorination of TCE, VC, DCE, PCE, and CB. The H^3 present in groundwater of the SLF is at low concentrations (3.69-8.34 x $10^{-7}\mu Ci/ml$). The source of the H^3 is not clear as it was not officially disposed of at the SLF. While material disposed of at the SLF was scanned for radioactivity, it is presumed that enough low levels of H^3 were present in buried material to cause the contamination.

1.3 Technology Evaluation and Selection

The goal of this project was to evaluate the effectiveness of intrinsic bioremediation as a viable addition and/or alternative to enhanced bioremediation. Intrinsic bioremediation could be incorporated as part of a Corrective Action Plan (CAP) at the SRS Sanitary Landfill.

Such a goal was addressed in a two-fold manner. Innovative monitoring techniques have been developed that allow the measurement of subtle changes in the subsurface microbial physiology and populations before contamination can be measured by chromatographic techniques (Fliermans et al., 1997; Phelps et al., 1988a; Phelps et al., 1988b). Such monitoring tools have been used to define groundwater contaminants, their movements, as well as assessing the bioremediation ability of the subsurface environments (Brockman et al., 1994;

Fliermans and Balkwill, 1989). Secondly, the targeted landfill site has key monitoring wells complete with historic COC data so that the extent and direction of the contaminant plumes have been defined.

There are three primary criteria by which the overall success of this demonstration was evaluated:

1. Retardation of the contaminant plumes greater than can be explained by physical processes including chemical half-lives, advection, absorption, and dilution by ground water transport.

2. Geochemical evidence of intrinsic bioremediation including documented loss of chlorinated solvents and presence of chemical breakdown products at the field scale correlated with increases in chloride in the groundwater.

3. The presence of notable densities of specific contaminant degrading bacteria including CB biodegraders and methanotrophic bacteria in groundwater from plumes.

2.0 Intrinsic Bioremediation Methods

2.1 Site Geochemistry

Statistical analysis and visualization comparisons of SLF physical and chemical data over the past 5 years from SRS environmental monitoring is ongoing. The data for the SLF COCs was obtained from the SRS quarterly groundwater monitoring data. Wells were examined over time individually and collectively. Visualization and numerical analysis of data collected from subsurface environments is being processed to develop appropriate models for intrinsic bioremediation at this site. For statistical analysis JMP® is being used to analyze the data (SAS, 1996). Three-dimensional digital imaging is being utilized whereby scattered environmental data is transformed into a realistic image of actual property values throughout the subsurface. This information is used to give a clear picture of geochemical interactions pertaining to bioremediation processes in the subsurface. Particular

emphasis is placed on groundwater concentrations of TCE breakdown products including DCE, VC, and Cl.

2.2 *In situ* respiration tests.

As part of the technology selection and verification process for the SRS Sanitary Landfill Remediation system, two test sites were installed at the SLF (WSRC, 1996). One site was installed at the edge of the landfill within the TCE plume (Site 1) generated in the Southern Expansion section. The other site was installed at the western edge within the VC plume (Site 2) generated in the original section. Two separate test areas were selected to verify that the *in situ* bioremediation technique selected for the landfill was viable for both major contaminant plumes. In addition to the installation of the Bioremediation System, the landfill was capped in 1996.

Aside from the difference in locations, the layout and installation of the two test sites were nearly identical (WSRC, 1996). At each site, three, two inch diameter stainless steel vertical gaseous injection wells were installed into the saturated zone using a hollow core auger. Each injection well contained a 10 foot screened section which was placed within the fluid sands of the unconfined aquifer (55 foot below the surface at Site 1 and 40 foot below the surface at Site 2) and therefore did not require the installation of a filter pack. A Bentonite seal (approximately 2 foot thick) was placed directly above the screened section and the remaining bore hole sealed with Bentonite grout to grade. These wells were installed in a triangular pattern approximately 35 foot from each other. At the center of this array, a 4 inch diameter PVC extraction well was installed, using a hollow core auger, into the overlaying vadose zone to provide control over the radius of influence (ROI) of the injection wells during the injection campaigns. Each extraction well contained a 10 foot screened section. The bottom of the screen at Site 1 was installed approximately 23 foot below grade and the bottom of the screen at Site 2 was installed approximately 19 foot below grade. A filter pack was installed opposite each screened section and sealed with an approximately 2 foot section of Bentonite. The remaining bore holes were filled with Bentonite.

In addition to the injection and extraction wells Saturated Zone and Vadose Zone Piezometers were installed in 14 locations throughout each test site to provide a well defined sampling area as previously described (WSRC, 1996). At each location,

two Vadose Zone Piezometers (VZPs) were installed within the same hollow core augered bore hole. Two feet long, 1 inch diameter well screens were placed at depths of 16 and 10 foot below grade to provide gaseous sample locations. Filter pack was installed directly opposite the well screen and a Bentonite seal was installed between each screened level and to grade. Saturated Zone Piezometers (SZPs) were installed to provide aqueous sampling points. Two SZPs were installed at each location at Site 1 and one SZP was installed at each location at Site 2. Ten foot sections of 2 inch diameter PVC well screen were placed at depths of 55 and 40 foot below grade at Site 1 and 40 foot below grade at Site 2. Each piezometer was installed within a hollow core augered bore hole drilled into the fluid sands of the unconfined aquifer, as with the injection wells, filter pack was not required and all of the SZPs were sealed with Bentonite directly above the well screen.

A portable diesel air compressor, receiver tank, and the gaseous injection system were located at a central location and connected to the individual sites via temporary steel piping routed on the ground. The three injection wells were manifolded to the injection line at each site and the flow were evenly distributed using inline valves. Vacuum and blowers were locally connected to each extraction well and the flow adjusted to create a controllable radius of influence for the injection wells.

Various injection campaigns were performed, during a 12 month period from June 1995 to September 1996, to validate the selected remediation technique and to determine the injection parameters required for the final full scale remediation system. The in situ respiration test campaigns injected air alone. During all of the injection campaigns approximately 15 standard cubic feet per minute (SCFM) of air was injected into each site (approximately 5 SCFM into each injection well). Approximately 15 to 20 SCFM of air was removed via the centralized extraction well. The *in situ* respiration tests were performed at the two sites twice at one year intervals.

Data was collected before (i.e. Baseline), during, and after injection campaigns to determine the impact these campaigns had on the; contaminant levels, microbial populations, groundwater parameters, etc. To determine the change in oxygen demand and its corresponding effect on microbial activity, in situ respiration tests were performed at each site, before and after the injection campaigns.

Groundwater dissolved oxygen (DO), pH, temperature, conductivity, and redox levels were monitored using a Hydrolab Data Sonde3 and collected via a Hydrolab Surveyor 3 Water Quality Logging system (Hydrolab Inc., Austin, TX). The monitoring of these parameters provides information concerning the effect of the injection campaign and microbial activity. Submersible pumps installed in SZPs supplied a constant flow of groundwater to the Hydrolab throughout the respiration test. Data collection began during an injection period and continued after injection had ceased.

2.3 Well Sampling.

Thirty-nine preexisting groundwater monitoring wells were sampled twice from the SLF. Monitoring wells were made of 2" or 4" PVC piping and screened on the bottom. Fifteen wells reached the water table surface (9-13 m below the surface), 12 wells were 7.6 to 9.1 m below the water table, and 14 wells were set just above the Meyers Branch Confining System (19-23 m below the surface).

Wells in groups of 4 to 9, were sampled between 8 July and 14 August 1996. Groundwater sampling was performed by RCS, Inc., Athens, GA, a subcontractor. Samples could not be obtained directly due to low groundwater concentrations of tritium and mercury. Wells were purged until pH readings stabilized after which groundwater was collected in sterile 3-gallon polyethylene jugs and five 25 ml glass gas chromatography vials with teflon-lined crimpable seals, and kept on ice until processing at the laboratory.

2.4 Microbiological Methods

2.4.1 BIOLOG

We incorporated BIOLOG GN 96-well microplates to evaluate the metabolic activity of the groundwater bacterial community (BIOLOG, Inc., Hayward, CA). Each microplate contained 95 pre-dried substrates and one substrate blank well. Each well contained tetrazolium redox dye, a clear compound that becomes purple when reduced, resulting in a change in absorbance (Haack et al., 1995).

Triplicate BIOLOG plates were inoculated with 150µl groundwater per microwell within 2 hours of well sampling. Plates were sealed with Fisher tape, and incubated in the dark at 15°C. After 3 weeks, absorbance changes were measured

at 590 nm using a BIOLOG Microplate Reader, connected to a computer with Microlog 3.5N software (BIOLOG, Inc., Hayward, CA). The raw optical density was recorded and the substrate blank well was subtracted from all readings as previously described (Garland and Mills, 1991).

2.4.2 Fatty Acid Methyl Esters

Whole groundwater community fatty acid methyl ester (FAME) were analyzed by vacuum filtering 3 liters of groundwater through a 47 mm 0.22 µm Nucleopore polycarbonate filter. Trapped organisms and the filter were extracted as follows: (i) the sample was placed in 1 ml of methanolic NaOH, vortexed for 5 seconds, saponified at 100°C for 5 min. in a water bath, vortexed again, and returned to water bath for another 25 min., after which the samples were cooled rapidly in cold water; (ii) the fatty acids were esterified with 2 ml of 3.25 N HCl in 46% (vol/vol) methanol, vortexed for 5 sec., placed in an 80°C water bath for 10 min., and then cooled rapidly; (iii) fatty acid methyl esters were extracted into 1.25 ml of 1:1 methyl-*tert*-butyl ether-hexane, and inverted for 10 min after which the bottom phase was discarded; and (iv) FAMEs were washed with 3 ml of 1.2% NaOH, and inverted for 5 min. Two-thirds of the organic phase (top layer) was transferred by pipette to a 2 ml GC vial and sealed immediately with a teflon-lined crimpable cap. This vial was stored at -20 °C for no longer than 2 weeks before analysis. Negative and positive controls were a reagent blank and 40 mg of *Stenotrophomonas maltophilia.*, respectively.

FAME samples were placed on an HP 7673A 5890 (Hewlett Packard, Rolling Meadows, IL.) water-cooled autosampler and were measured by gas chromatography using an HP 5890 equipped with an HP 25 m x 0.2 µm cross-linked methyl-silicone capillary column. The initial oven temperature was 170 °C, and increased 5 °C/min. until 260 °C, at which point it increased at 30 °C/min. until 300 °C and held constant for 2 min. Helium was used as the carrier gas. Methyl esters were identified using an a HP 486 computer with Sherlock software (Microbial Identification, Inc., Newark, DE.), and based on actual calibration retention times run prior to sample analysis.

Fatty acid methyl esters are designated by the number of carbon atoms in the aliphatic chain, followed by a colon and the number of double bonds: the position of the double bond nearest the aliphatic (w) end of the molecule is next (Haack et al., 1994). The configuration abbreviated as *c* for cis and *t* for trans follows.

Suffixes *a*, *I*, *br*, and *cy* indicate anteisobranching, isobranching, branching, and cyclopropane rings, respectively, which are present in the molecule (Smith et al., 1986).

2.4.3 Acridine Orange Direct Counts

Acridine orange direct count (AODC) was used to estimate the total number of bacteria from each groundwater well. One ml acridine orange stain (Fisher Scientific, Fairlawn, NJ) was mixed with 2 ml groundwater for 2 min. in the dark. The solution was vacuum filtered through 25 mm 0.22 µm polycarbonate-black filters (Nuclepore). A microscope slide was prepared by placing a drop of immersion oil on the slide, followed by the filter, a second drop of immersion oil, and covering this with a glass cover slip. Slides were viewed with epifluorescent light microscopy (Carl Zeiss Inc., Thornwood, NY). A background and negative control slide were prepared using double distilled water in place of groundwater, and replacing stain with double distilled water, respectively.

2.4.4 Microbial Activity

The stain CTC (5-cyano-2,3-ditolyl tetrazolium chloride, PolySciences, Warrington, PA.) was used to enumerate actively respiring microbial cells in groundwater (Rodriguez et al., 1992). Two ml's of 5 mM CTC were incubated with 20 ml groundwater in sterile 50 ml centrifuge tubes in the dark, for 4 h at 28°C while mixing at 150 rpm. Cells were captured using vacuum filtration on 25 mm, 0.22 µm polycarbonate-black filters. Slides were prepared and viewed with an epifluorescent microscope. In respiring cells, CTC is reduced to a formazan molecule which appears fluorescent green under epifluorescent light; non-reduced CTC remains colorless. Thus, only green cells were scored as positive.

2.4.5 Direct Fluorescent Antibodies

Antibodies in this project were developed against methanotrophic bacteria isolated from the Sanitary Landfill of the Savannah River Site and those obtained from the American Type Culture Collection (ATCC). Polyclonal Antibodies (PABs) were developed in New Zealand White rabbits at the Medical College of Georgia (MCG), Augusta GA. (Fliermans and Schmidt, 1975). Immunoglobins were isolated by ammonium sulfate precipitation from rabbit antisera having agglutination titers >1280 and conjugated with Fluorescein isothiocyanate (FITC) to produce the Direct Fluorescent Antibodies (DFA's) used to label the methanotrophic bacteria found in

an environmental sample, thus rendering them visible (Brigmon et al., 1997). The DFA's were separated from unconjugated FITC on Sephadex G-25 columns and stored at -70 °C. Using 1 ml of this concentrate, a 1:20 dilution was made and filtered through a 0.2 mm syringe filter. This dilution was then used in the preparation of slides. For slides, an initial volume of sample (usually double that used in AODC's) was filtered through a 0.2 mm filter (Nuclepore) in a vacuum manifold. With the vacuum off, 20 µl of the 1:20 antibody dilution were used to flood the filter on the vacuum manifold, the manifold was then covered with aluminum foil and allowed to incubate for 30 min at room temperature. After incubation, the vacuum is turned on and the filter washed with 20 ml of Phosphate buffered saline (PBS) to remove unconjugated antibody (background). The filter was then mounted on a microscope slide using a drop of elvanol with another drop of elvanol on top of the filter and a cover slip applied (Rodriguez, 1992). A control was tested using 25 ml of filter sterilized PBS to assure that the antibody and the PBS were not contaminated. Methanotrophic bacteria were then enumerated using an epifluorescent microscope.

2.4.6 Viable Plate Counts

Viable, culturable, heterotrophic aerobic and facultative anaerobic bacteria were isolated on 1% peptone-trypticase-yeast extract-glucose (PTYG) medium (Balkwill, 1989). Duplicate spread plates were inoculated with 0.1 ml from a 1:10 serial dilution (10^0, 10^{-1}, 10^{-2}) of groundwater in PBS. Plates were incubated at room temperature (approximately 22°C) for 1 week. Plates were set on a darkfield colony counter (Reichert-Jung) and visible colonies were counted.

2.4.7 Chlorobenzene Degrading Bacteria Enumeration

The number of viable aerobic, and facultatively anaerobic chlorobenzene-degrading bacteria in groundwater samples were estimated as described by Spain and Nishino (1987). One-tenth of 1 ml of solution from the dilution tubes were used to inoculate 25-cm petri plates containing minimal salts solidified with 1.8% agar (w/v) supplemented with 10 mg/l yeast extract. The ingredients for the minimal salts medium per liter are as follows: $MgSO_4$-$7H_2O$, 1.6 g; $CaCl_2$-$2H_2O$, 0.16 g; $NaNO_3$, 8.0 g; KCl, 0.32 g; KH_2PO_4, 1.28 g; 80.0 ml of 0.15 g Na_2HPO_4-$7H_2O$ dissolved in 500 ml d. H_2O, $ZnSO_4$-$7H_2O$, 0.007 g; $MnSO_4$-H_2O, 0.0017 g; H_3BO_3, 0.002 g; $CaCl_2$-$6H_2O$, 0.001 g; $CuCl_2$-$2H_2O$, 0.003; $NiCl_2$-$6H_2O$, 0.002; and Na_2MnO_4-$2H_2O$, 0.003 g. Plates were set in a stainless steel desiccating

chamber which also contained a petri dish filled with aqueous chlorobenzene. The chamber was sealed with vinyl tape and stored under a fume hood at room temperature. Chlorobenzene was replenished weekly. Plates were counted after 6 to 8 weeks as described above.

2.5 Chemical Analysis.

2.5.1 Gas Chromatography

Contaminants were analyzed in triplicate from 25 ml groundwater using a HP 5890 Series II Plus gas chromatograph (GC) (Phiffner et al., 1997). The GC was equipped with an Electron Capture Detector (ECD), an HP 694 Headspace sampler, an HP Vectra XM2 4/100i computer, computer-controlled data control and acquisition via Chemstation software, and a 60 m x 0.75 mm ID Supelco VOCOL wide bore capillary column coated with a 1.5 μm film. The minimum detection limit for contaminants was 5 ug/l.

2.5.2 Ion Chromatography

Groundwater was measured for nitrate, nitrite, sulfate, chloride, and phosphate using an ion chromatograph (IC) (Enzien et al., 1994). All samples were measured in duplicate. Samples were analyzed using a Dionex QIC 2 ion chromatograph. A FAST ANION (P/N 39590, 4 x 250 mm) ion exchange column equipped with polymeric packing was used for separation of chloride, nitrite, nitrate, phosphate and sulfate. A conductivity detector was used to measure μS. The ions were eluted with a 200 mM Na_2CO_3 / 75 mM $NaHCO_3$ solution at a flow rate of 2 ml/min.

2.5.3 Field Groundwater Chemical Analysis

RCS, Inc. provided field data from all sampled groundwater. Groundwater was sampled after pH stabilized for Eh (mV), pH, and dissolved oxygen concentration measured with a YSI Model 310 analyzer (Yellow Springs Instruments, Yellow Springs, Ohio).

2.6 Statistical Analysis.

To determine the difference in sampling parameters (e.g., Microbial counts, BIOLOG substrates, FAMEs) based on isoconcentrations of TCE, VC, DO, and CB, a general linear model was defined. The biogeochemical used as parameters were the dependent variables, and the isopleth parameter, well, and days between resampling of a well, were the independent variables. An analysis of variance

(ANOVA) was performed based on this model to determine significant differences (α=0.05, $p<0.05$) of dependent variables above and below an isoconcentration. Least square means (adjusted for well and time effects) were also calculated for each dependent variable above and below each isoconcentration.

Principal components analysis was performed using SAS (Statistical Analysis System; SAS Institute Inc., Cary, NC.) version 6.11 using the SAS Prin Comp statement. BIOLOG data was entered as average optical density of three replicates with the substrate blank well subtracted from each replicate. FAMEs were entered as percent of total FAMEs identified, with a zero amount assigned to FAMEs that were absent from any given sample.

3.0 Results

3.1 Geochemical

Environmental quarterly data from groundwater monitoring wells was analyzed from 1990-1995. Visualization of the TCE, CB, VC, and H^3, Cl plumes through time are shown (Fig. 1-5). Analysis of quarterly groundwater monitoring data from SRS through 1996 have indicated the COCs have not moved as far as predicted by earlier reports (Table 1).

According to the Intera report, the TCE plume was predicted to have reached the wetland area by June, 1997 (WSRC, 1993). However, the plume has advanced from it's original location approximately 150 meters since 1992. There was no forward movement or growth in the TCE plume from 1994 to 1995 (Fig. 1e & f) The concentrations of the TCE within the plume did increase in 1995 indicating there could still be some leaching of TCE from material (i.e. plastic bags) in the SLF.

Vinyl chloride, a daughter product from TCE anaerobic biodegradation, was not detected in the SLF in significant quantities 1990 (Fig. 2a). The VC plume was first detected in 1991 (Fig. 2b). The VC plume grew in concentration and moved with the groundwater flow from 1991-1994 (Fig. 2b-2e) and then stabilized in 1994-1995 (Fig 2e-f).

The CB plume was predicted to have reached UTRC by this time. Results show that the CB plume has moved some 350 meters since 1991 (Fig. 3a-e). There was no forward movement of the CB plume from 1994 to 1995 (Fig. 3d-e). The overall size of the CB plume has decreased in size and concentration.

The H^3 plume appears to follow groundwater movement which is estimated for the SLF area to be 144 feet/year (Fig. 5 a-f). The source of H^3 seems to be more diffuse which may be because low levels of contaminated materials have been buried throughout the SLF (Fig. 5c). As of 1995, the H^3 plume had advanced 1000 feet from the landfill southern edge (Fig. 5f). As stated earlier, with low concentrations at the SLF and a half-life of 12 years, H^3 is not a major concern.

The Cl seems to remain after the COCs have been biodegraded (Fig. 4a-4f). The Cl plume follows the VC, TCE, and CB plumes from 1990-1995 (Fig. 4a-f). Just as the VC, CB and TCE plumes seemed to stop moving significantly from 1994-1995 (Figures 1,2, and 3), so has the Cl plume (Fig. 4e-f).

Several contaminants were found to be significantly elevated in low oxygenated (<0.2mg /L) groundwater in a 1996 survey of 42 wells inside and outside the COC plumes. These include PCE, DCE, toluene, xylene, and propylbenzene.

Table 1 shows a comparison of Intera model estimates for SLF contaminants of concern for wetland outfall concentrations (ppb) (WSRC, 1993) and actual measured well values in this zone from select wells for 1996. It is clearly evident from Table 1 that the initial report over estimated the plume movement. We believe the reason is because the microbiological assessment of the bioremediation potential of the subsurface at this site had not been clearly understood. The Intera report also estimated that PCE and TCE would reach UTRC by 1997 and 1998 respectively. However, at this point in time there is no significant evidence of that occurring.

3.2 Respiration Test

The data from the Site 1 Respiration Tests (Figure 6a) of 1995 and 1996 exhibit similar saturation curves and respiration rates. The elevated oxygen saturation level for the 1996 test represents a value above 100% saturation and is attributed to a

combination of temperature effects, accuracy of oxygen measurements at or near 100%, and pressure differences between the aeration location below grade and the measurement location at grade level. Both tests exhibited identical oxygen declines from saturation to approximately the 4.5 mg/L dissolved oxygen level. The similarity in these levels suggest that oxygen is not a limiting factor at Site 1. Other data from the SL In Situ Optimization Test (WSRC, 1996) supports the finding of carbon source and nutrient limitations are the main microbial needs at Site 1.

The data from the Site 2 Respiration Tests (Figure 6b) exhibit similar saturation curve forms and respiration rates but differ in the oxygen saturation levels obtained and in the final oxygen level. The low saturation levels support the landfill records which suggest anaerobic activity at Site 2. The inability to increase the oxygen level above 2.5 mg/L suggests that the oxygen demand at Site 2 was much greater than the amount supplied (15 SCFM) to the site during the test period. The change in oxygen saturation levels between the 1995 test and 1996 test may be attributed to many factors. Although the amount of oxygen being supplied is somewhat small, this oxygen stimulated aerobic microbial activity which in turn increased oxygen demand and consequently depleted the already low levels of dissolved oxygen.

3.3 Microbiological Parameters

Based on vertical stratification, the average AODC was highest among shallow wells that intercept the water table surface (Table 2). Acridine orange direct counts from wells set approximately 25-30 feet below the water table surface were similar, at 2.22×10^5 bacteria cells per ml groundwater. Corresponding geochemical data is listed in Table 1. AODC from the deepest wells (set just above the Meyers Branch Confining System), contained approximately one order of magnitude fewer bacterial cells than either of the shallower wells. Corresponding geochemical data is in Table 2. However, none of the AODC values were significantly different (alpha=0.05). A general linear model was used to compare means of AODC above and below geochemical isoconcentrations (Table 3A). The total bacterial population did not vary significantly above or below the following isoconcentrations (p-value was greater than 0.05): 2 mg/L chloride ion; 0.2% dissolved oxygen; 5 µg/L vinyl chloride; and 5 µg/l chlorobenzene. The average AODC above 5 µg/l TCE was

significantly higher compared to the adjusted average of AODC from 71 wells below 5 µg/L TCE (p-value less than 0.05) (Table 3A).

Based on isoconcentrations, only TCE had an affect on the number of actively respiring cells (Table 3A). These data reflect the same trend as found for the total number of cells, where more cells were present above 5 µg/L TCE. Above 5 µg/L TCE, CTC was 25X lower than AODC; whereas below 5 µg/L TCE CTC was 51X lower than AODC. Counts of actively respiring cells followed the spatial trend of AODC counts as well, however CTC counts were lower. The highest average number of respiring cells was found in the shallow wells, at 8.35×10^3 bacteria cells per ml. The moderately deep wells contained 5.48×10^3 bacteria cells per ml, while the deepest wells contained one order of magnitude fewer respiring cells than the shallowest wells, at 8.30×10^2 bacteria cells per ml. None of the CTC data were significantly different by depth. Principal components analysis showed CTC or active microbial activity accounted for most of the variation, but only for PC1 (Table 5).

A greater number of methanotrophic bacteria indigenous to the Sanitary Landfill were present compared to the number of actively respiring bacteria at the two lower depths, 6.60 and 2.39×10^3 bacteria cells per ml, respectively for moderate and deep wells. The shallow wells had 1.21×10^3 cells per ml on average. None of the counts were significantly different based on vertical distribution (Table 3A). The correlation results showed DFA counts for methanotrophic bacteria were correlated to TCE above 5 µg/L (Table 4A). The principal component analysis showed DFA counts did not contribute significantly to the total variation (Table 5).

Viable, culturable heterotrophic aerobic or facultatively anaerobic bacteria were least numerous in the shallow well, (6.10×10^2 bacteria colony forming units (CFU)/ml) (Table 2). The number increased with depth, from 1.24×10^3 CFU/ml groundwater in the moderately deep wells to 2.44×10^3 CFU in the deepest wells (Table 2). However, none of the viable counts were significantly different (Table 3A). The GLM showed PTYG counts were not different above or below any geochemical isoconcentration (Table 3A). The correlation results show PTYG was not correlated to any isoconcentration (Table 4A). The principal component

analysis showed PTYG or viable culturable counts explained most of the variability (Table 5). Aerobic, culturable, viable bacteria able to grown in the presence of chlorobenzene vapor were only 5 per ml from the shallow well, and 10 per ml in the moderately deep well. The number increased 25-fold from the deepest wells to 266 CFU/ml. None of the CB counts were significantly different based on depth or any of the isoconcentrations (Table 2).

The correlation results showed chlorobenzene degraders (CB) were highly correlated to TCE above 5 µg/l (0.95) (Table 4A). Principal component analysis showed chlorobenzene degraders to have high loading (Table 5).

A summary of contaminants measured in groundwater from the SLF are shown in Table 6. Only those results are presented where statistical analyses were proven to be significant. Based on vertical distribution, none of the contaminants were significantly different from one another. Based on dissolved oxygen isoconcentration, chloroform, and carbon tetrachloride were significantly higher above 0.2% dissolved oxygen. The contaminants 2-chlorotoluene, 1,2-dichlorobenzene, 1,3-dichlorobenzene, and freon-12 were significantly higher below 0.2% dissolved oxygen. The contaminants chlorobenzene, freon-12, 1,1,1-TCA, toluene, xylene, and benzene were significantly higher in the TCE isoconcentration above 5 ug/l. Carbon tetrachloride was the only contaminant significantly lower when TCE was below 5 ug/l. Eight contaminants were greater in the chloride isoconcentration above 2 mg/l: benzene, chlorobenzene, ethylbenzene, freon-12, PCE, 1,1,1-TCA, TCE, and toluene. The contaminants carbon trichloride and carbon tetrachloride were significantly lower in the vinyl chloride isoconcentration below 5 ug/l. All the significantly different contaminants were higher when chloride was above 2 mg/l (the background chloride ion concentration) include benzene, chlorobenzene ethylbenzene, freon-12, PCE, 1,1,1-TCA, TCE, and toluene. The VC isoconcentration contained two contaminants that were significantly greater when the isoconcentration was below 5 ug/l, carbon trichloride and carbon tetrachloride. None of the contaminants were significantly different based on the chlorobenzene isoconcentrations. Correlation analysis showed several contaminants were highly correlated (> 0.30, p-value > 0.05) to geochemical isoconcentrations (Table 4A).

Based on depth, nitrate was significantly greater at moderate depth than at shallow depth (Table 8). Sulfate was greater in the deep wells as compared to either

shallow or moderate wells (Table 8). Phosphate was greater above 5 ug/l chlorobenzene, nitrate was greater above 0.2% dissolved oxygen, nitrite was greater above 5 ug/l TCE, and chloride was greater below 5 ug/l TCE (Table 3A). Correlation analysis showed chloride and nitrate were correlated to the isoconcentration above 0.2% dissolved oxygen (Table 3A). Nitrate and dissolved oxygen concentrations were significant variables above and below 2 mg/l chloride, while sulfate was significant only below 2 mg/l chloride (Table 3A).

Based on depth, seven BIOLOG substrates were significantly different among the three depths (Table 8). The least significant means from the general linear model are shown in Table 3B. A total of thirty-four substrates were significantly different based on isoconcentrations. Of the nine significantly different substrates separated by dissolved oxygen isoconcentrations, seven were greater when dissolved oxygen was above 0.2%. Four out of five substrates were greater in the TCE isoconcentration above 5 ug/l. Seven out of eight substrates were greater in the vinyl chloride concentration below 5 ug/l. All twelve substrates were greater above 5 ug/l CB. Finally, two substrates were greater when chloride concentration was above 2 mg/l.

Correlation analysis of BIOLOG substrates is shown in Table 4B. Twenty-seven substrates had a correlation greater than 0.3 (alpha=0.5, p-value < 0.05). Correlations ranged from -1.0 (d-gluconic acid) to 0.99 (d-raffinose). Principal components analysis showed the cumulative variation explained by the first three principal components was 0.33, 0.45, and 0.54 (i.e., the first component explained 33% of the cumulative variability). The highest loading (0.28) was D,L-a-glycerol phosphate in PC3. There were no loadings over 0.20 in PC1, four in PC2, and six in PC3 (Table 5).

Principal component analysis of BIOLOG substrate utilization was performed. The first three components explained 54% of the cumulative variation and covariation among 95 BIOLOG substrates. Seventy-nine of the 95 BIOLOG substrates were significant terms in the principal components. The result of the PCA of BIOLOG was similar to the FAME PCA results in that BIOLOG substrate utilization data obtained from whole communities was not able to separate wells based on any of the geochemical isoconcentrations. With BIOLOG the pattern of substrate utilization is examined as opposed to the density of the reaction. Thus with the incubation time the cell concentration is not a critical point.

In contrast to FAME, bacterial communities could be differentiated from three depths by using BIOLOG data (Table 8). However, only seven substrates were significantly different from one another at one of three depths. This raises the question how reproducible are these data since less than 10% of the total number of substrates gave a positive response to depth. Thus, it is difficult to validate whether the difference in substrate utilization was a function of spatial microbial activity variability, or merely normal variation from sample to sample. The fact that the BIOLOG was run on groundwater samples in triplicate reduced the variability.

Based on isoconcentrations, nine out of 95 total substrates were significantly different above or below 0.2% dissolved oxygen. Two substrates were used more extensively above 0.2%, while seven were used below 0.2% (Table 4B). Four out of five substrates were utilized significantly in the isoconcentration above 5 ug/l TCE. Fourteen substrates were significantly different based on the chlorobenzene isoconcentration, and all were used to a greater degree when above 5 ug/l chlorobenzene. Seven out of a total eight significantly different substrates were separated by the vinyl chloride isoconcentration were used more extensively below 5 ug/l vinyl chloride. Finally, two substrates were significantly different and both were higher in the chloride isoconcentration above 2 mg/l.

Principal component analysis of fatty acid methyl esters was performed. The first three principal components explained 53% of the cumulative variation and covariation among the 59 FAME variables. Nineteen of the 59 FAME variables were significant terms in the principal components. (In this study, variables with loadings above 0.20 (Table 3) were considered significant.) However, according to this statistical approach, these significant fatty acids were not sufficient to successfully separate communities from groundwater wells based on any of the geochemical isoconcentrations.

Based on depth none of the individual fatty acids were significantly different from one another. Nine FAMEs were significantly different based on TCE isoconcentrations. Eight of these FAMEs had higher relative amounts when below 5 ug/l TCE. One FAME that was significantly different above 5 ug/l VC. Seven FAMEs were significantly different based on chloride isopleths. Four were lower relative amounts above 2 mg/l Cl. None of the FAMEs were significantly different based on dissolved oxygen isoconcentrations. Principal components analysis showed the cumulative value explained by the first three components was 0.17,

0.32, and 0.47 (Table 5). There were 15 FAMEs in PC1 with loadings above 0.20, 10 FAMEs in PC2, and eight in PC3.

4.0 DISCUSSION

A significant problem encountered during subsurface sampling is to obtain a sample representative of the microbial community as it exists in situ. It is often difficult to determine the amount of sample needed to adequately compare microbiota from different environments *a priori* to sampling. Other challenges include the necessary frequency that samples are taken and the speed by which those samples can be analyzed by a given technique. Determination of the extent to which the groundwater sample microbial analysis does not account for microbes attached to the soil matrix, biofilms etc. is difficult (Prieme et al., 1996). Since the same groundwater samples were used for direct enumeration techniques, plate counts, and FAME and BIOLOG analyses, the limitations presented in each sample of groundwater were felt equally by all the methods. Thus, a fair comparison of the appropriateness or efficacy among methods was possible.

Previous studies have shown that bacteria are able to use the products of chlorinated hydrocarbon biodegradation for energy and growth under laboratory and field conditions (Major et al., 1995). The range of concentrations of TCE and other volatile organic compounds (e.g., benzene, toluene) was much lower in this project than previous intrinsic biodegradation studies (Major et al., 1995; King et al., 1995). In some studies, the background levels reported were orders of magnitude greater than the greatest concentration of contaminant measured in this study (Barker et al., 1995; Lee et al., 1995). However, results from the general linear model indicate that indeed the microbial community was affected by the TCE concentrations observed in this study, albeit comparatively lower.

Fatty acid methyl esters extracted from groundwater provide a range of information about the microbial community, from gross taxonomic designations such as Gram negative or Gram positive groups, to the presence or absence within the community of a given genus (Cavigelli et al., 1995). For example, Gram negative bacteria contain predominantly 16:0, with lesser amounts of 14:0 and 18:0. The major unsaturated fatty acids of gram negative bacteria are 16:1 and 18:1, and many are hydroxylated. Less common to Gram negative bacteria are branched-chain and odd-numbered fatty acids, and cyclopropane or polyunsaturated fatty acids. Gram

positive bacteria contain an abundance of branched-chain fatty acids (Zelles et al., 1994). Based on these physiological properties, it was hypothesized that fatty acids could be used to characterize and compare groundwater communities based on various geochemical isoconcentrations.

Fatty acid methyl esters were useful to identify the Gram-negative methanotrophic population. The relative amount (the relative amount of a fatty acid is the percentage of a particular fatty acid, out of the total fatty acids positively identified by the MIDI system) of nine out of 17-total fatty acids identified were significantly different based on isoconcentrations of TCE. All but one of these hydroxylated, odd- and even-numbered fatty acids were measured in higher amounts in the TCE isoconcentration below 5 ug/l. The fatty acid 18:0, which is less common in gram-negative bacteria, was greater in the TCE isoconcentration above 5 ug/l. As indicated by the 16:0 10 methyl fatty acid, these data suggest fewer methanotrophic bacteria were present when TCE was not being degraded (i.e., when TCE was above 5 ug/l), and the opposite was true when TCE was being degraded. However, these data were not supported by DFA enumeration's for methanotrophic bacteria, and may represent a change in the amount of fatty acids of gram-positive bacteria, rather than a change in gram-negative bacteria.

The major disadvantages of using FAME to demonstrate intrinsic biodegradation are lack of sensitivity and information regarding the function and quantitative composition of groundwater microbial communities. Lack of clear separation by the principal components analysis suggests fatty acid composition was similar in communities above and below 5 ug/l TCE. Additionally, the composition of fatty acid methyl esters did not appear to be significantly altered either by the presence of 0 to 13 ug/l TCE, or by the biodegradative activity of bacteria towards TCE. The lack of clear separation based on differences in fatty acid methyl esters does not signify a concomitant lack of intrinsic biodegradation. On the contrary, the increase in chloride ion concentration and decrease in TCE suggests the occurrence of intrinsic biodegradation; however, the effect of intrinsic biodegradation on the fatty acid composition of microbial community was minimal.

BIOLOG substrate utilization information was obtained to show the ecofunctional enzyme activity of whole groundwater communities. Substrate utilization is a laboratory measure of the potential metabolic capability of bacterial communities from groundwater samples. A BIOLOG substrate may be metabolized, its oxidation linked to the electron transport chain of aerobically respiring bacteria.

Thus, if one site contains a population of bacteria that is not present in a separate community, there should be a difference in the number and extent of substrate utilization making it possible to delineate the communities. A drawback of this method is the potential for a community, that under field conditions is stressed or very small, to proliferate under the controlled incubation conditions. This would result as the appearance of a population that had little environmental significance, yet seemed more important under the laboratory conditions. In addition, the initial cell density inoculated into each substrate well may differ, causing more rapid utilization of substrate(s).

The BIOLOG data suggests communities that utilize a greater number of substrates to a greater extent are metabolically more active than the communities that do not. No more than 35 out of a total 95 different substrates were significantly different above or below any isoconcentrations. The low number of substrates utilized suggests low sensitivity of the communities to the substrates provided in the BIOLOG microtiter assay, and furthermore that separation by depth and isoconcentration may have been an artifact of the normal variability from sample to sample.

The information provided by BIOLOG has the advantage over FAME and direct cell enumeration techniques because BIOLOG describes the potential rate of metabolic function and activity of entire groundwater communities. Such information is useful to better understand the function of microorganisms in the groundwater, and goes further than merely providing the number of a certain population or cell type.

The occurrence of intrinsic biodegradation at the site was verified by evaluating the biogeochemical data based on contaminant and geochemical isoconcentrations. This allowed comparison of biogeochemical data above and below an isoconcentration. Isoconcentrations above and below 1 mg/l dissolved oxygen and 10 ug/l TCE, VC, and sulfate have been used to monitor intrinsic bioremediation (Guest et al., 1995; Major et al., 1995; Lee et al., 1995). The following three contaminant isoconcentrations above and below 5 ug/l were used in this study: (i) trichloroethylene; (ii) chlorobenzene; and (iii) vinyl chloride. Trichloroethylene and chlorobenzene are primary contaminants; the presence of vinyl chloride provides evidence PCE or TCE are reductively dechlorinated because vinyl chloride was not produced at the site. Groundwater below 5 ug/l was considered contaminant-free, because the analyte was not detected at a concentration greater than the associated quantitation limit (Cox et al., 1995). An isoconcentration of dissolved oxygen

above 0.2% was used to indicate conditions which support aerobic or facultatively anaerobic microorganisms, and below 0.2% to indicate conditions suitable to anaerobic and facultatively aerobic microorganisms (M. J. B. Paynter, pers. comm.). An isoconcentration of chloride was used to indicate a change in chloride ion concentration above or below the background concentration of 2 mg/l found *a priori* in SLF groundwater.

Acridine orange direct counts were significantly higher in groundwater above 5 ug/l TCE. Both this trend and the number of total cells from contaminated versus non-contaminated groundwater resemble the values reported for BTEX contaminated groundwater (4.6×10^6 cells/ml) versus non-contaminated control wells (1.5×10^5 cell/ml) (Toze et al., 1995). However, AODC counts were approximately ten times lower than reported for either saturated or non-saturated sediment (Ghiorse and Balkwill, 1983). The significant decrease in total cell counts witnessed below 5 ug/l TCE may result from absence of a labile organic carbon compound below 5 ug/l. However, because dissolved organic carbon was not measured in this study, the latter possibility remains unfounded. The decrease in cells below 5 ug/l TCE is most likely caused by formation of the metabolite TCE-epoxide, which is toxic and can kill bacteria with which it has contact (Henry and Grbic-Galic, 1991). Additionally, increased metabolic activity and greater actively respiring bacteria above 5 ug/l TCE suggests a community less stressed than the community below 5 ug/l TCE. Since oxidation of TCE does not regenerate $NADH^+$, a labile pool of suitable electron donors would be required to regenerate $NADH^+$ *and* sustain degradative activity. Without a donor constantly present, cells eventually would become somnolent, or die. Further, since no laboratory studies have shown use of TCE as a sole carbon source; there is no reason to believe an increase in the methanotrophic population would occur solely as a result of TCE degradation. Had a sufficient source of electron donors been present during TCE oxidation, and if organisms were in close enough proximity to acquire the degradation byproducts, then an increase in bacterial numbers and/or activities would be expected.

Fluorescent antibody counts were not significantly different based on any of the isoconcentrations. It is somewhat surprising the number of methanotrophs was not lower where TCE degradation had occurred. However, it is possible the initial population of methanotrophs was small but active in both TCE isoconcentrations so that any change in number was insignificant using the GLM procedure.

The isoconcentration below 5 ug/l TCE contained significantly more hydroxylated fatty acids, the type most common to Gram-negative bacteria, i.e., *Pseudomonas, Rhizobium*, methanotrophs (methane-oxidizing bacteria). The presence of hydroxylated fatty acids below 5 ug/l TCE suggests a microbial community dominated by Gram-negative bacteria. However, reports of methanotrophic-signature fatty acids do not include hydroxylated fatty acids. Type-I (bundled vesicular disks) methanotrophic bacteria have the signature fatty acids 16:0 and 16:1, 16:1w8c, 16:1w8t, and 16:1w5c; whereas Type-II contain an abundance of 18:1, 18:1w8c, 18:1 w8t, and 18:1w6c (Zelles et al., 1994)). Therefore, methanotrophic bacteria most likely were not significantly stimulated within the microbial community at the time of sampling (Table 2).

In the SLF wells available for testing, 55 out of 78 total wells had more than 0.2% dissolved oxygen. The concentration of nitrite was significantly higher above 5 ug/l TCE. The average concentration of oxygen in those wells was above 0.2% dissolved oxygen. Conditions above 5 ug/l TCE may have had too much oxygen to foster the complete denitrification of nitrite from nitrate, then to 2NO (nitric oxide), then N_2O (nitrous oxide), and then to N_2 (elemental nitrogen). Or, the presence of contaminants may have been toxic to *Nitrobacter* (bacteria that convert NO_2^- to NO_3^- in the presence of oxygen), and thus stopping nitrification (NH_4^+ to NO_2^- to NO_3^-). Although no significant difference was found for the concentration of dissolved oxygen with TCE, the presence of PCE above 5 ug/l TCE indicates primarily aerobic conditions that could not support reductive dechlorination; whereas below 5 ug/l TCE partially anaerobic conditions existed, as evidenced by lesser PCE and TCE concentrations, and increased chloride ion concentration. Other contaminants (Table 5) were present in significantly higher concentrations in the isoconcentration above 5 ug/l TCE, suggesting either preferential oxidation of TCE (due to lower substrate specificity for TCE than other contaminants), or adaptation of TCE-degrading bacteria within the microbial community.

According to the correlation analysis, *cis*-DCE was positively correlated to the TCE isoconcentration below 5 ug/l. The presence of *cis*-DCE, a known byproduct of anaerobic reductive dechlorination of TCE and PCE, suggests anaerobic microsites existed within this isoconcentration. Had a greater number of sites been anaerobic, a larger difference between *cis*-DCE and vinyl chloride concentrations would have been expected, because anaerobic reducing conditions foster the transformation of

vinyl chloride from cis-DCE. Thus, gross aerobic conditions were likely present above and below 5 ug/l TCE.

The contaminants carbon trichloride and carbon tetrachloride were present in lower concentrations below 0.2% dissolved oxygen than above 0.2% dissolved oxygen, suggesting conditions were anaerobic and able to support the reductive dechlorination of these contaminants in the former isoconcentration. Carbon tetra- and tri-chloride have been observed to undergo reductive microbial dechlorination at concentrations of up to 59 mg/l (Lee et al., 1995). In the isoconcentration below 5 ug/l vinyl chloride, the concentration of carbon trichloride and carbon tetrachloride was higher than in the isoconcentration above 5 ug/l vinyl chloride. However, it would have been expected to see a decrease of carbon tri- and tetra-chloride below 5 ug/l vinyl chloride, because conditions that support reductive dechlorination of vinyl chloride should also support the transformation of carbon tri- and tetra-chloride. The concentration of both contaminants is below 5 ug/l in either dissolved oxygen isoconcentration, which makes interpretation of these results difficult. The most probable explanations may be given as the presence of anaerobic microsites within gross aerobic sites, since dissolved oxygen was greater than 0.2% and not significantly different in either vinyl chloride isoconcentration.

In summary, only AODC and CTC were able to detect changes in microbial communities that seemed to be associated with the intrinsic biodegradation of chlorinated hydrocarbons. The significant decrease in TCE concentration and concomitant increase in chloride ion concentration suggests the intrinsic biodegradation of TCE. Thus, AODC and CTC are satisfactory direct enumeration techniques for obtaining microbiological data as evidence for proving intrinsic TCE biodegradation.

The presence of VC in the SLF (Fig. 2b-f) is indicative of anaerobic dechlorination of PCE and TCE. Results from the *in situ* respiration test confirm the high oxygen demand and low redox of site 2 which was in the VC plume. While there appears there is little overlap of the TCE and VC plumes this is due to the fact that TCE has been degraded to VC in the Southern side of the SLF.

Several physical changes occurred at the SLF over recent years which can have significant effects on the groundwater movement and associated COC plumes. These include the addition of several drainage basins on the Southwest and

Southeast sides which are the lower elevations. Another more important factor, and much harder to quantify, is the fact that approximately half way through the first series of injection tests landfill capping activities began. These activities began at the northern edge of the original section and continued southward toward the southern edge of the southern expansion. And although the original oxygen levels at Site 2 were depressed, the impact of capping the area directly above the VC plume source can not be ignored.

Installation of the permanent cap over the landfill was in progress during this study and is now complete. The cap is designed to inhibit further infiltration of contaminants into the groundwater. The cap will influence groundwater flow and plume movements. A number of monitoring wells have been removed while others were modified. This activity will affect sampling strategies.

It has been recommended that a remediation system should be installed incorporating 2 injection zones along the south and west sides of the landfill. Since groundwater consistently flows parallel to the long axis of the SRS landfill two horizontal wells, one running along the south side of the southern expansion and the other along the west side should be able to bioremediate any solvents coming from the site. Based on the optimization test and probable future leaching changes, both injection systems should inject at a depth of 20-30 ft below the water table. This will provide a sparge zone that will biotreat all current and future leachate. Cost analysis will determine if horizontal wells or a series of vertical injection wells are most appropriate. Keeping in mind that the shallow depth of the wells, the length of the west side, and the need to remediate groundwater associated with the Interim Sanitary Landfill may make a sequential series of vertical injection wells the most appropriate. The remediation system should be able to handle any future leaching from the original, southern expansion and the northern expansion since the proposed configuration and prevailing groundwater flow would contain any leachate from these areas. The injection system will consist of a compressor with the ability to add nitrous oxide, triethylphosphate, and methane. The south side injection will need to be controlled separately from the west side injection, since different strategies will be necessary for the most cost effective in situ bioremediation. However, both wells will need all capabilities since conditions may change as the landfill ages. The results of the Bioremediation Optimization Test have shown that the use of bioremediation via *in situ* stimulation of

indigenous microorganisms is an effective long-term of obtaining ultimate groundwater restoration at the SRS Sanitary Landfill (WSRC, 1996).

Note: In light of some of the unexpected findings of this study some short term additional studies are recommended. These new studies would determine the most cost effective strategy or operations at the two sites. The questions to be answered would be: l) what is the minimum interval of injection on and off that would insure bioremediation of both sites, and 2) what is the minimum sampling interval for both areas to monitor bioremediation.

5.0 CONCLUSIONS

1. The evidence of intrinsic bioremediation is presented here by the documented loss of the contaminants TCE and CB.
2. The geochemical evidence of intrinsic bioremediation include daughter products of anaerobic TCE biodegradation including DCE and VC.
3. Chloride concentrations in the groundwater were correlated with TCE, CB, and VC indicating microbial dechlorination.
4. The presence of microorganisms that can specifically degrade these compounds proves the bioremediation potential of the system.
5. Total cells/ml and viable cells/ml were found to be significantly increased by concentrations of at least 5 ug/l TCE in groundwater.
6. BIOLOG and FAME analyses show variability due to factors including depth other than COC contamination.
7. Evaluation of BIOLOG and FAME showed BIOLOG to be more sensitive to lower concentrations of contaminants, as a greater number of substrates were separated by isoconcentrations than fatty acid methyl esters.

The heterogeneity of the material buried in the SLF at different times contributes to the diverse activity and geochemistry described here. Intrinsic bioremediation of TCE and CB is evident from the geochemical data and the documented loss of contaminants at the field scale. While statistical analysis of microbiological data is ongoing, preliminary results indicate a significantly higher number of bacteria within the COC plumes than outside. Whether this greater biomass is from other the carbon from the COCs or other material in the landfill is not clear. However,

greater microbial biomass has proven to be a major factor in bioremediation. The reason the Intera report overestimated the movement of the COC plumes is that there was not a good understanding of the microbiology at the SLF.

While H^3 concentrations were low in groundwater at the SLF such that it was not a COC, there was enough to make it a good tracer (Figure 5). The H^3 plume appears to move at the estimated rate of groundwater of the SLF which is 144 ft/year (WSRC, 1996). From the location and shape of the H^3 plume, it is evident that it must have been placed in the SLF with materials containing TCE. It is of interest that as the plume of TCE did not progress from 1994-1995 (Figures 1E and 1F), the H^3 plume continued moving at the rate of SLF groundwater (Figures 5E and 5F).

The final report from the Sanitary Landfill In Situ Optimization Test has been completed and related information (microbiology, contaminant analysis, hydrology) for implementation of intrinsic bioremediation technology at the site is being evaluated (WSRC, 1996). In situ respiration tests one year apart at two sites on the landfill indicated differences in oxygen demand in the subsurface during that time period. Site 1 was installed within the TCE plume and Site 2 was installed within the VC and CB plume. Site 2 had a much greater difference in metabolic activity (five orders of magnitude over the 1 year) as compared to site 1 over that time period (Fig. 6). It is not clear if the change was in part due in part to the installation of the CAP during this time period, changes in subsurface microbiology, geochemistry, activities or a combination of the above. Dissolved oxygen content of groundwater appears to the most significant physical parameter influencing subsurface bioremediation of contaminants of at this site. This oxygen in combination with methane from the landfill contributes to the intrinsic bioremediation. The fact that oxygen is not a limiting factor in the Southern part of the SLF could be the main contributing factor to halt of COC plume advancement. Results from Acridine Orange Direct Counts (AODC) in groundwater from select wells inside and outside the COC plumes indicated total bacteria numbers or biomass are significantly greater within the COC plume than outside. This is probably due to the increased carbon source within the landfill and the associated materials.

Complete mineralization of the COCs in the groundwater by indigenous microbes was demonstrated, thereby effecting the complete destruction of these compounds in situ. As densities of contaminant-degraders increased in the groundwater in response to stimulation, the COC concentrations decreased, and the chloride concentrations increased. Complete mineralization of chlorinated solvents results in the production of chloride.

6.0 Recommendations

Future analyses will be used to monitor contaminants, ground water chemistry, microbiological components, respirometry and ecofunctional enzyme patterns of metabolic activity in the ecotone zones of bioremediation. Computer models will be developed to measure parameters for tracer dispersion, TCE/VC loss, microrespirometry and ecofunctional enzyme patters of metabolic activity in monitoring and background wells. Additional piezometric and groundwater monitoring wells need to be strategically placed based on current monitoring data to better characterize the contaminant plume movements. A better analysis of the SLF would be possible through more monitoring data from wells placed on the front edge of the plumes. Injection of inherent conservative tracers to the subsurface environment should be conducted in order to compare their movement to that of chlorinated solvents. This work would enable the determination of rates of intrinsic bioremediation at the geochemically different regions of the SRS Landfill site.

7.0 Key Contacts and Participants

Key personnel contacts for each organization are shown below:

U. S. Department of Energy (SRO)
Karen Adams, Program Officer

WSRC Environmental Restoration Department
Issac Rucker, Task STR/Project Manager

WSRC Savannah River Technology Center
Carl Fliermans, Fellow Scientist
Robin L. Brigmon, Senior Scientist

Participants in the field project included the following:

U. S. Department of Energy (SRO)
Karen Adams, Program Officer
Steve Wright, Program Officer
Gene Turner, Program Officer

WSRC Environmental Restoration Department
Joseph Shappell, Task STR/Project Manager
Ronald P. Socha, Task STR/Project Manager
Issac Rucker, Task STR/Project Manager
Chris Bergren, Program Manager
Neal O'Halloran, Project Manager
Ahmet Suer, Project Manager

WSRC Savannah River Technology Center
Terry C. Hazen, Fellow Scientist/Environmental Biotechnology Section Manager
Ken H. Lombard, Principal Engineer
Denis J. Altman, Principal Engineer
Christopher J. Berry, Senior Engineer
Fatina A. Washburn, Engineer
Marilyn M. Franck, Senior Scientist
Eugene P. Shine, Applied Statistics

Post Doctoral Fellows and Students
Jill D. Young, Post-Doctoral Fellow
Geralyne Lopez-deVictoria, Post-Doctoral Fellow
Jorge Santo-Domingo, Post-Doctoral Fellow
Edward Becker, Cornell University
Demetrius Jones, University of South Carolina
Andrew Glucksman, Clemson University
Pendella Schmidt, University of South Carolina
Barbara Richardson, University of South Carolina

Clemson University
Horace Skipper, Professor, Agronomy Department
William Bridges, Associate Professor, Statistics Department

8.0 REFERENCES

Balkwill, D. L. (1989) Numbers, diversity, and morphological characteristics of aerobic, chemoheterotrophic bacteria in deep subsurface sediments from a site in South Carolina, Geomicrobiol. J. 7:33-52.

BIOLOG Inc. 1991. Microstation System, BIOLOG Inc., 3447 Investment Blvd., Hayward, CA.

Bowman, J.P., Jimenez, L., Rosario, I., Hazen, T.C. and Sayler, G.S. (1993) Characterization of the methanotrophic bacterial community present in a trichloroethylene-contaminated subsurface groundwater site. Appl. Environ. Microbiol. 59:2380-2387.

Brigmon, R.L., M.M. Franck, J.S. Bray, D.F. Scott, K.D. Lanclos, and C.B. Fliermans. 1997. Direct immunofluorescence and enzyme-linked immunosorbent assays for evaluating organic contaminant degrading bacteria. (In Review). WSRC-MS-97-0300.

Brockman, F.J., Fredrickson , J.K., Sun, W., and Kieft, T.L. (1994) Microbial response to remediation and other perturbations of subsurface environments. In: In-Situ Remediation : Scientific Basis for Current and Future Technologies. (Gee, G.W. and Wing, N. R. eds), pp. 77-84. Battelle Press, Columbus, OH.

Cavigelli, M. A., G. P. Robertson, and M. J. Klug. 1995. Fatty acid methyl ester (FAME) profiles as measures of soil microbial community structure. Plant and Soil. 170:99-113.

Chapelle, Francis H. 1994. Assessing the Efficiency of Intrinsic Bioremediation. In EPA Symposium on Intrinsic Bioremediation of Ground Water, EPA/540/R-94/515. August 1994. Denver, CO.

Enzien, M. V., F. Picardal, T. C. Hazen, R. G. Arnold, and C. B. Fliermans. 1994. Reductive dechlorination of trichloroethylene under aerobic conditions in a sediment column. Appl. Environ. Microbiol. 60:220-2204.

Fliermans, C. B. and D. L. Balkwill. 1989. Life in the terrestrial deep subsurface. BioScience 39: 370-377.

Fliermans, C.B., Dougherty, J.M., Franck, M.M., McKinsey, P.C., and Hazen, T.C. (1994) Immunological techniques as tools to characterize the subsurface microbial community at a trichloroethylene contaminated site. In: Applied Biotechnology for Site Remediation (Hinchee R. E. ed.), pp. 186-203. Lewis Publishers. Boca Raton, FL.

Fliermans, C.B., M.M. Franck, T.C. Hazen and R.W. Gorden. 1997. Ecofunctional Enzymes of Microbial Communities in Groundwater. FEMS Microbiological Reviews (accepted).

Fliermans, C. B. and T. C. Hazen (eds.). 1990. Proceedings of the First International Symposium on Microbiology of the Deep Subsurface. January 15-19, 1990, Orlando, Florida, WSRC Information Services, Aiken, SC

Fliermans, C. B., T. J. Phelps, D. Ringelberg, A. T. Mikell, and D. C. White. 1988. Mineralization of trichloroethylene by heterotrophic enrichment cultures. Appl. Environ. Microbiol. 54: 1709-1714.

Fogel, M.M., Taddeo, A.R., and Fogel, S. 1986. Biodegradation of chlorinated ethylenes by a methane-utilizing mixed culture. Appl. Environ. Microbiol. 51, 7220-724.

Garland, J. L., and A. L. Mills. 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Appl. Environ. Microbiol. 57:2351-2359.

Godsey, E. Michael. 1994. Microbiological and Geochemical Degradation Processes. In EPA Symposium on Intrinsic Bioremediation of Ground Water, EPA/540/R-94/515. August 1994. Denver, CO.

Gorden, R.W., Hazen, T.C., and Fliermans, C.B. (1993) Rapid screening for bacteria capable of degrading toxic organic compounds. J. Microbiol. Meth. 18, 339-347.

Guest, P. R., L. A. Benson, and T. J. Rainsberger. 1995. Inferring biodegradation processes for trichloroethene from geochemical data, pp. 233-243. *In* R. Hinchee, J. Wilson, and D. Downey (eds.), Intrinsic Bioremediation. Battelle Press, OH.

Haack, S. K., H. Garchow, D. A. Odelson, L. J. Forney, and M. J. Klug. 1994. Accuracy, reproducibility, and interpretation of fatty acid methyl ester profiles of model bacterial communities. Appl. Environ. Microbiol. 60:2483-2493.

Haack, S. K., H. Garchow, M. J. Klug, and L. J. Forney. 1995. Analysis of factors affecting the accuracy, reproducibility, and interpretation of microbial community carbon source utilization patterns. Appl. Environ. Microbiol. 61:1458-1468.

Hazen, T. C., K. H. Lombard, B. B. Looney, M. V. Enzien, J. M. Dougherty, C. B. Fliermans, J. Wear, and C. A. Eddy-Dilek. 1994. Summary of in-situ bioremediation demonstration (methane biostimulation) via horizontal wells at the Savannah River Site integrated demonstration project. *In* G. W. Gee, and N. R. Wing (eds.) In-situ remediation: scientific basis for current and future technologies. Part 1. Battelle Press, Columbus. p. 137-150.

Henry, S., and D. Grbic-Galic. 1991. Influence of exogenous and exogenous electron donors and trichloroethylene oxidation toxicity on trichloroethylene oxidation by methanotrophic cultures from a groundwater aquifer. Appl. Environ. Microbiol. 57:236-244.

Kao, C. M. and R.C. Borden. 1994. Enhanced aerobic bioremediation of a gasoline contaminated aquifer by oxygen-releasing barriers. In: Hydrocarbon bioremediation. Boca Raton, Fl: Lewis Publishers.

King, J. D., D. C. White, and C. W. Taylor. 1977. Use of lipid composition and metabolism to examine structure and activity of estuarine detrital microflora. Appl. Environ. Microbiol. 33:1177-1183.

Little, C. D., A. V. Palumbo, S. E. Herbes, M. E. Lidstrom, R. L. Tyndall, and P. J. Gilmer. 1988. Trichloroethylene biodegradation by a methane-oxidizing bacterium. Appl. Environ. Microbiol. 54:951-956.

Lee, M. D., P. F. Mazierski, R. L. Buchanan, Jr., D. E. Ellis, and L. S. Sehayek. 1995. Intrinsic in situ anaerobic biodegradation of chlorinated solvents at an industrial landfill, pp. 205-222. *In* R. Hinchee, J. Wilson, and D. Downey (eds.), Intrinsic Bioremediation. Battelle Press, OH.

Major, D., E. Cox, E. Edwards, and P. Hare. 1995. Intrinsic dechlorination of trichloroethene to ethene in a bedrock aquifer, pp. 197-203. *In* R. Hinchee, J. Wilson, and D. Downey (eds.), Intrinsic Bioremediation. Battelle Press, OH.

National Research Council. 1993. In Situ Bioremediation, When does it Work? National Academy Press, Washington, DC.

Nishino, S.F., Spain, J.C., Belcher, L. A., and Litchfield, L. A. (1992) Chlorobenzene degradation by bacteria isolated from contaminated groundwater. Appl. Environ. Microbiol. 58:1719-1726.

Phelps, T.J., E. G. Raione, D.C. White, and C. B. Fliermans. 1988a. Microbial activities in deep subsurface environments. Geomicrobiology J. 7: 79-91.

Phelps, T.J., D. Ringelberg, D. Hedrick, J. Davis, C. B. Fliermans, and D.C. White. 1988b. Microbial biomass and activities associated with subsurface

environments contaminated with chlorinated hydrocarbons. Geomicrobiology J. 6:157-170.

Pfiffner, S.M., Palumbo, A.V., Phelps, T.J., Hazen, T.C. Effects of nutrient dosing on subsurface methanotrophic populations and trichloroethylene degradation. 1997. J. Industrial Microbiology & Biotechnology. 18:204-212.

Prieme, A., Sitaula, J. L. B., Klemedtsson, A.K., and Bakken, L.R. (1996) Extraction of methane-oxidizing bacteria from soil particles. FEMS Microbiol. Ecol. 21, 59-68

Rodriguez,G.G., D. Phipps, K. Ishiguro, and H.F. Ridgway. 1992. Use of a fluorescent redox probe for direct visualization of actively respiring bacteria. Appl. Environ. Microbiol. 58:1801-1808.

SAS Institute Inc. 1996, Cary NC.

Smith, G. A., J. S. Nickels, B. D. Kerger, J. D. Davis, S. P. Collins, J. T. Wilson, J. F. McNabb, and D. C. White. 1985. Quantitative characterization of microbial biomass and community structure in subsurface material: a prokaryotic consortium responsive to organic contamination. 32:104-111.

White, D. C., R. J. Bobbie, J. D. King, J. Nickels, and P. Amoe. 1979. Lipid analysis of sediments for microbial biomass and community structure, pp. 87-103. *In* C. D. Litchfield and P. L. Seyfried (eds.). Methodology for biomass determinations and microbial activities in sediments, ASTM STP 673. ASTM, PA.

White, D. C., G. A. Smith, M. J. Gehron, J. H. Parker, R. H. Findlay, R. F. Martz, and H. L. Fredrickson. 1982. The groundwater aquifer microbiota: biomass, community structure, and nutritional status. Dev. Ind. Microbiol. 24:201-211.

Wilson, J.T., J. F. McNabb, J. Cochran, T.H. Wang, M.B. Tomson, and P.B. Bedient. 1985. Influence of microbial adaptation on the fate of organic pollutants in ground water. Environ. Toxicol. Chem. 4:721-726.

Wilson, J. T., F. M. Pfeffer, J. W. Weaver, and D. H. Kampbell. 1994. Intrinsic bioremediation of JP-4 jet fuel. *In* Symposium on intrinsic bioremediation in groundwater. EPA/540/R-94/515.

Winter, R. B., K. Yen, and B. D. Ensley. 1989. Efficient degradation of trichloroethylene by a recombinant *Escherichia coli*. Bio/technol. 7:282-285.

WSRC. 1993. Sanitary Landfill Local Scale Flow and Transport Modeling in Support of Alternative Concentration Limit Demonstrations, Savannah River Site. WSRC-TR-93-093. Westinghouse Savannah River Company Environmental Restoration Department. Aiken, SC.

WSRC. 1996. Sanitary Landfill In Situ Bioremediation Optimization Test Final Report, Savannah River Site. WSRC-TR-96-0065. Westinghouse Savannah River Company. Aiken, SC

Wünsche, L., L. Brüggemann, and W. Babel. 1995. Determination of substrate utilization patterns of soil microbial communities: an approach to assess population changes after hydrocarbon pollution. FEMS Microbiol. Ecol. 17:295-306.

Zak, J. C., M. R. Willig, D. L. Moorhead, and H. G. Wildman. 1994. Functional diversity of microbial communities. Soil. Biol. Biochem. 26:1101-1108.

Zelles, L, Q. Y. Bai, T. Beck, and F. Beese. 1992. Signature fatty acids in phospholipids and lipopolysaccharides as indicators of microbial biomass and community structure in agricultural soils. Soil Biol. Biochem. 24:317-323.

Zelles, L., Q. Y. Bai, R. X. Ma, R. Rackwitz, K. Winter, and F. Beese. 1994. Microbial biomass, metabolic activity and nutritional status determined from fatty acid patterns and poly-hydroxybutyrate in agriculturally-managed soil. Soil Biol. Biochem. 26:439-446.

Figures

Figure 1A. Trichloroethylene Map Fourth Quarter 1990.

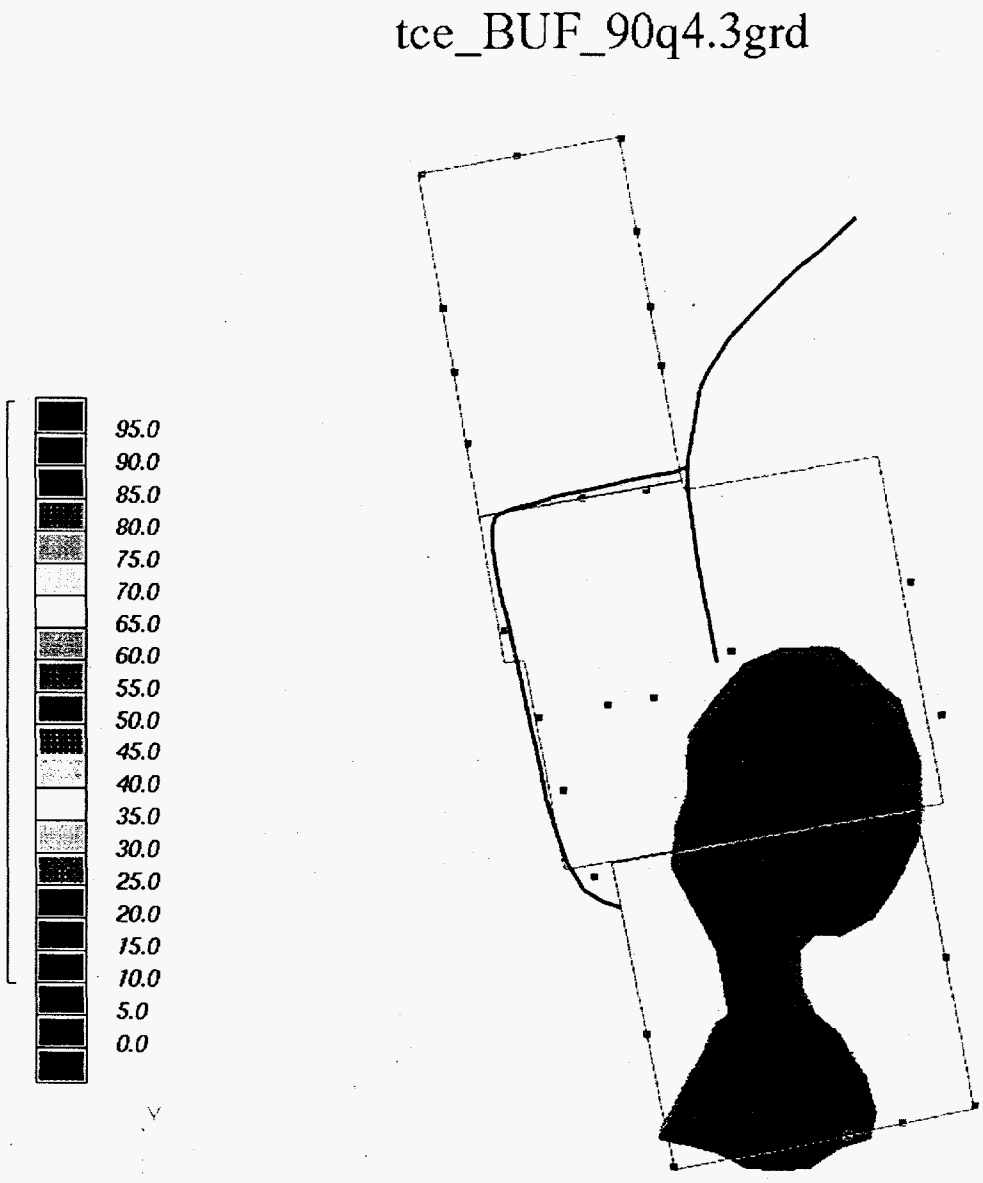

Figure 1B. Trichloroethylene Map Fourth Quarter 1991.

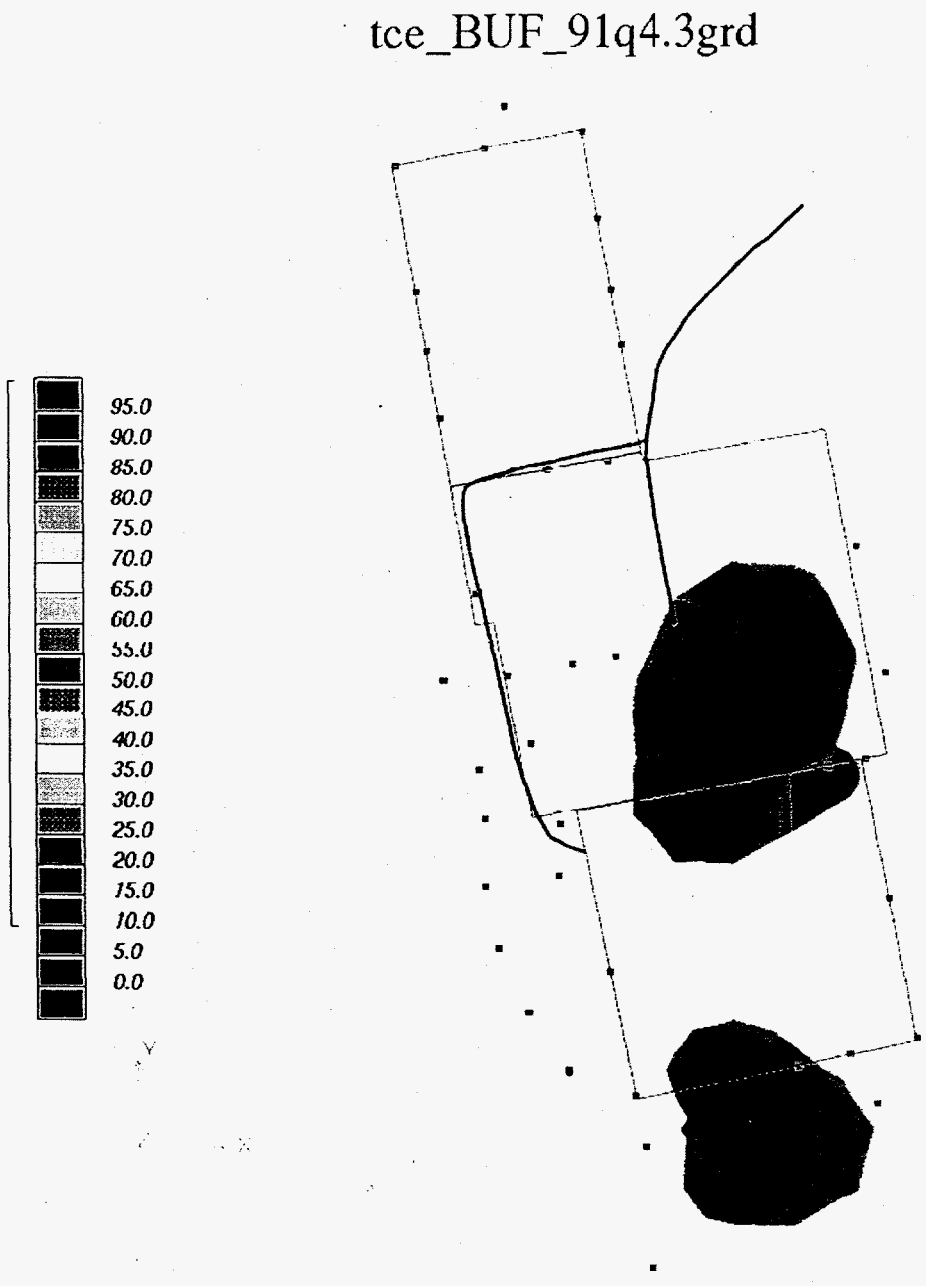

Figure 1C. Trichloroethylene Map Fourth Quarter 1992.

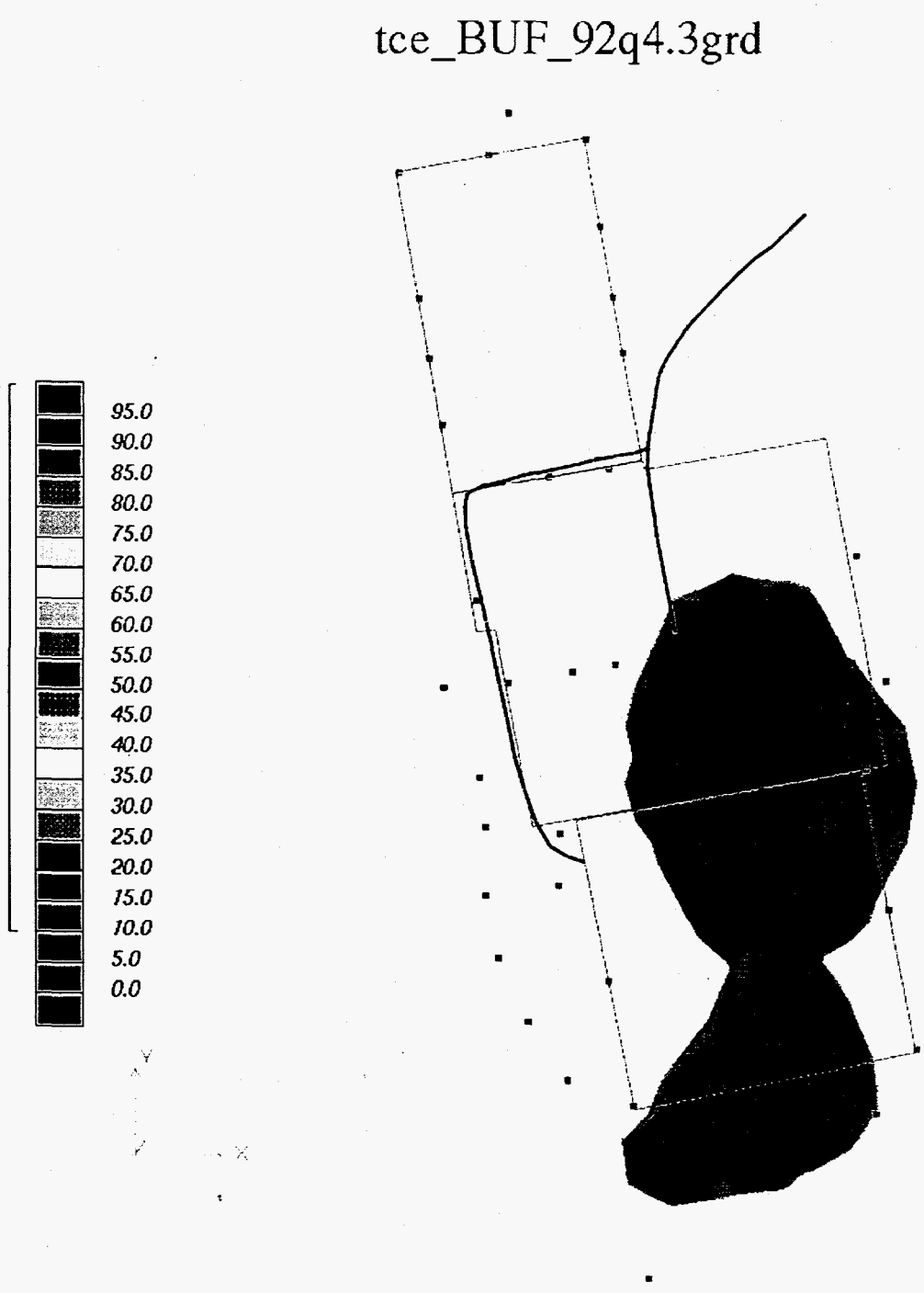

Figure 1D. Trichloroethylene Map Fourth Quarter 1993.

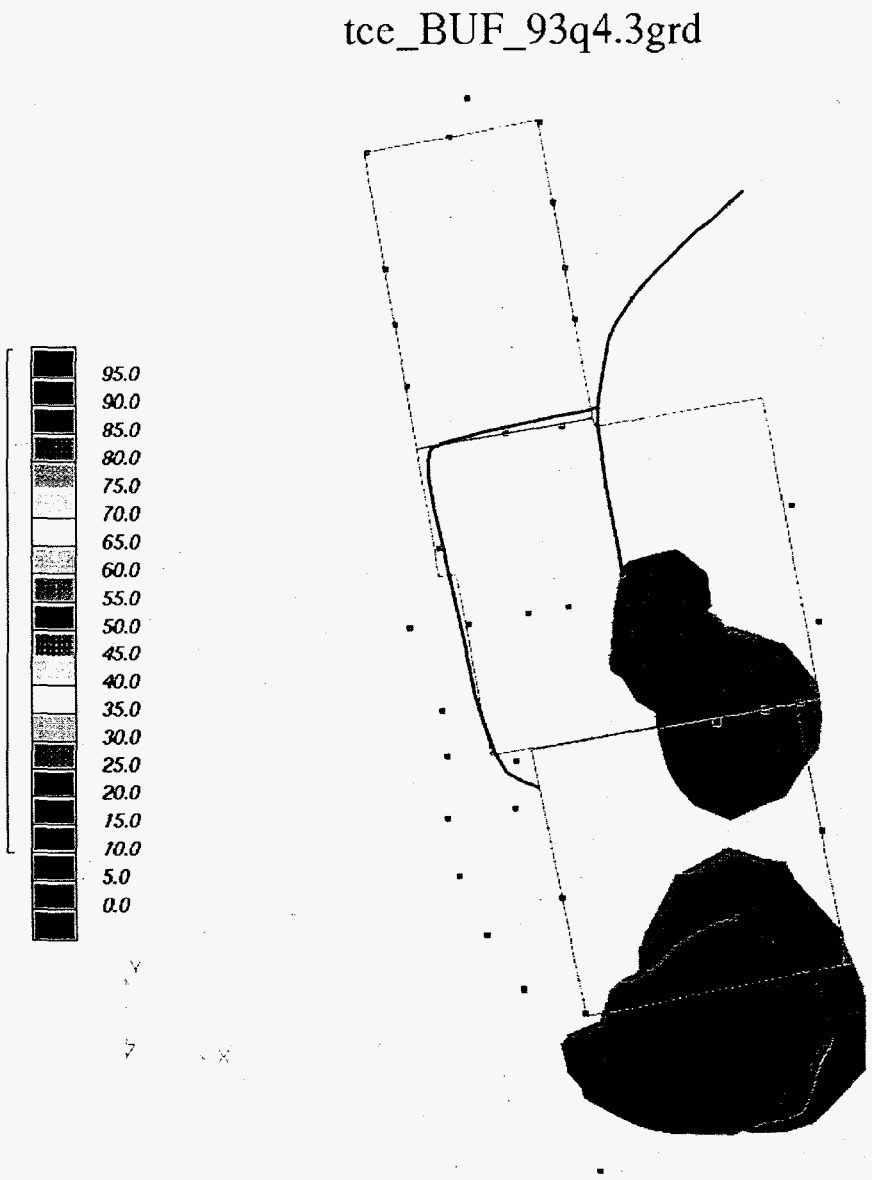

Figure 1E. Trichloroethylene Map Fourth Quarter 1994.

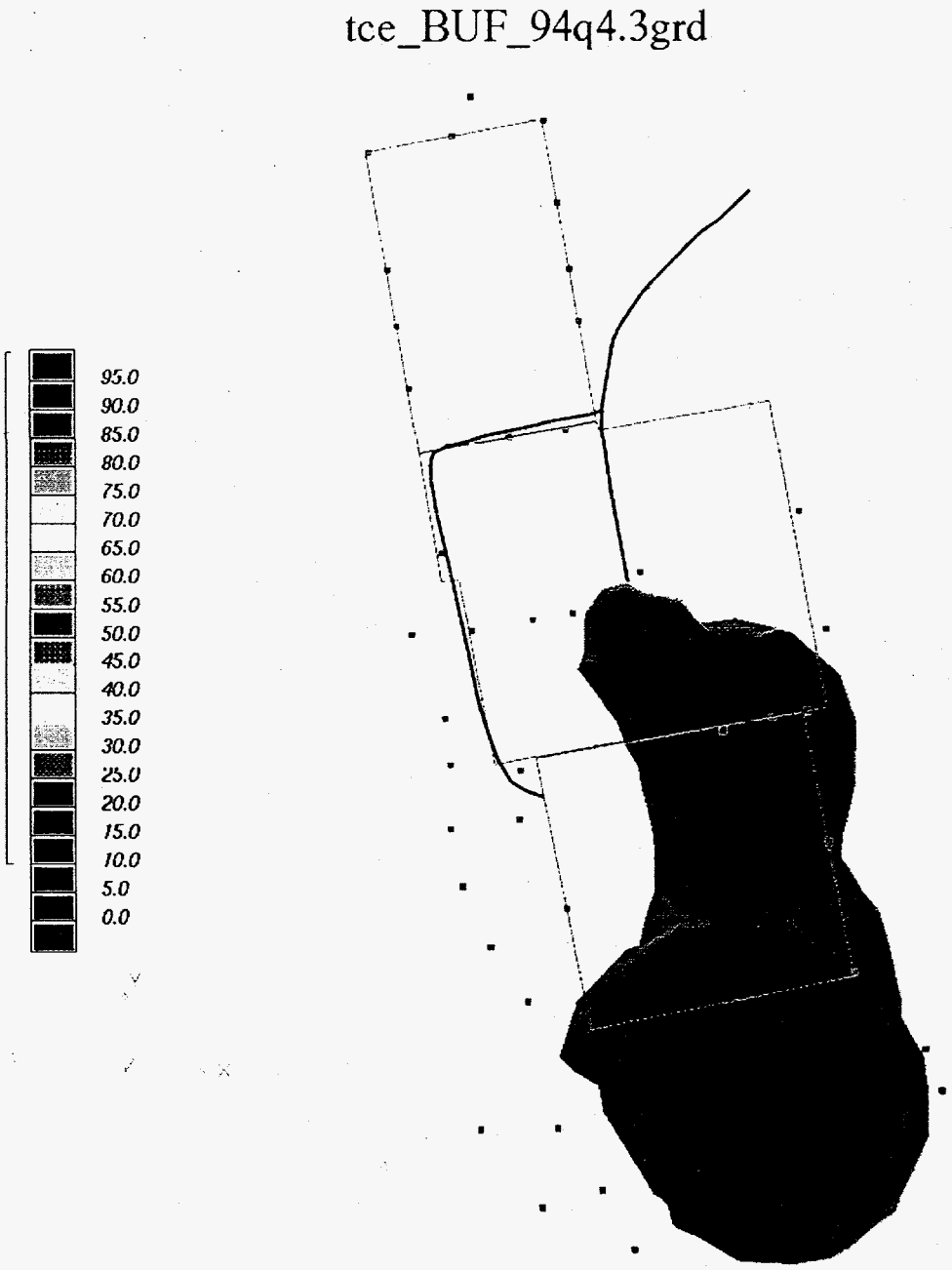

Figure 1F. Trichloroethylene Map Fourth Quarter 1995.

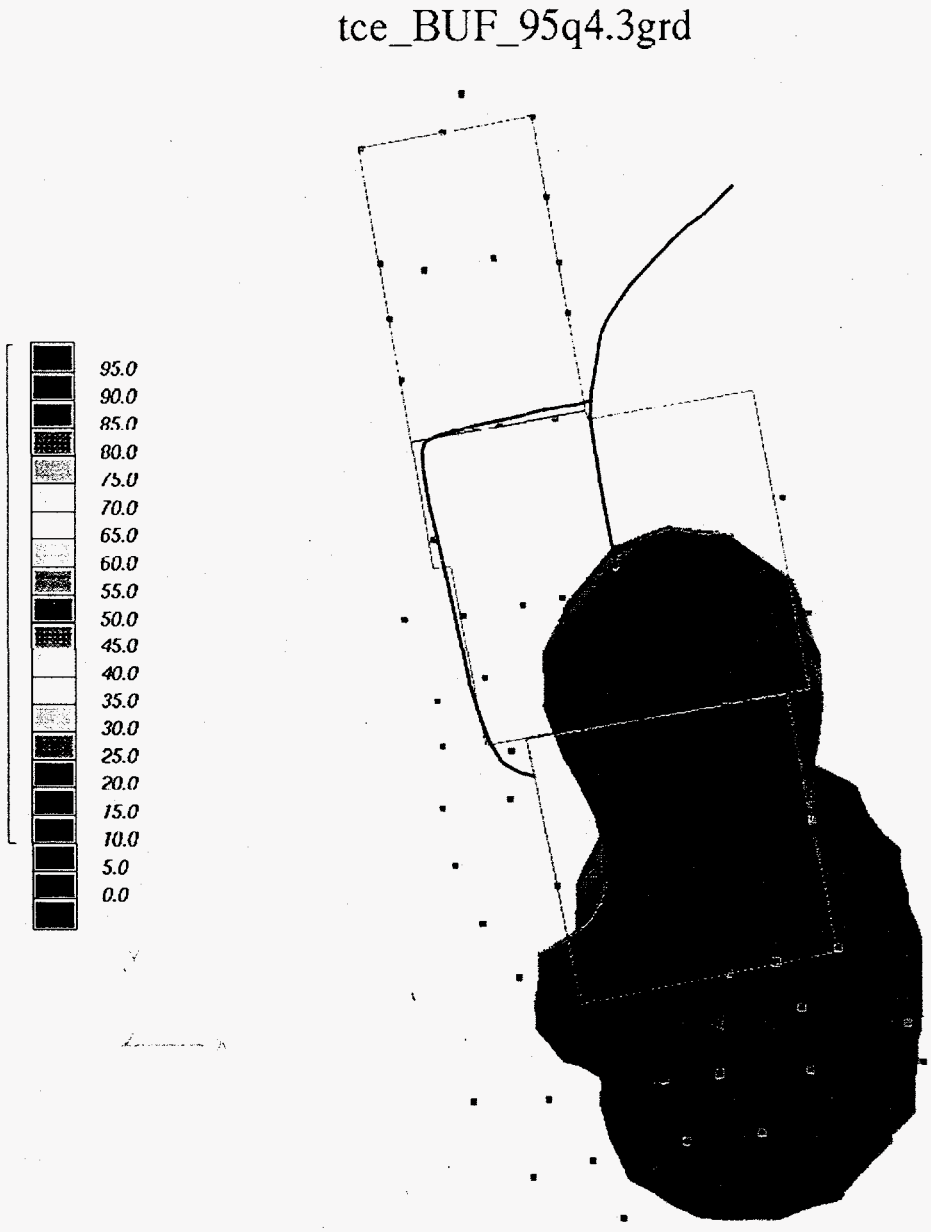

Figure 2A. Vinyl Chloride Map First Quarter 1990.

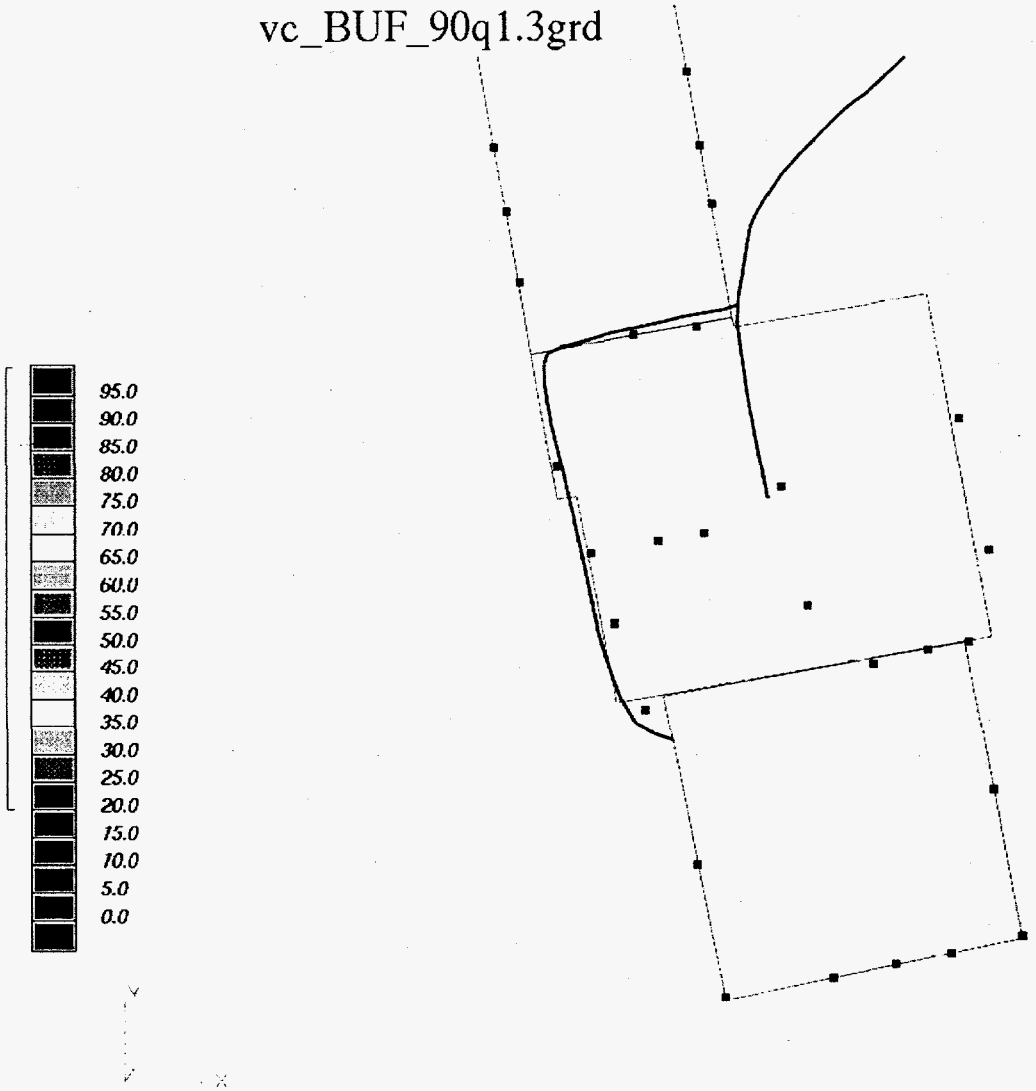

Figure 2B. Vinyl Chloride Map First Quarter 1991.

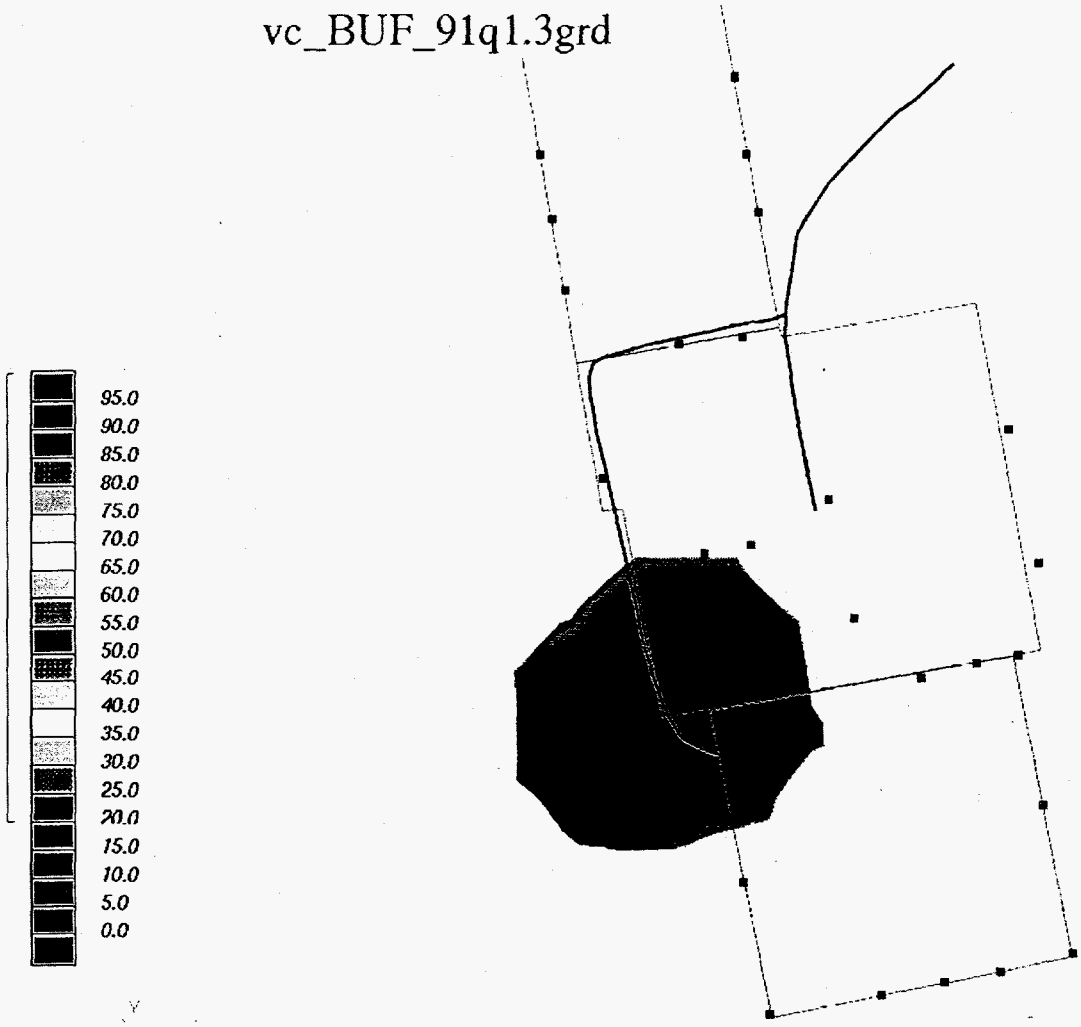

Figure 2C. Vinyl Chloride Map First Quarter 1992.

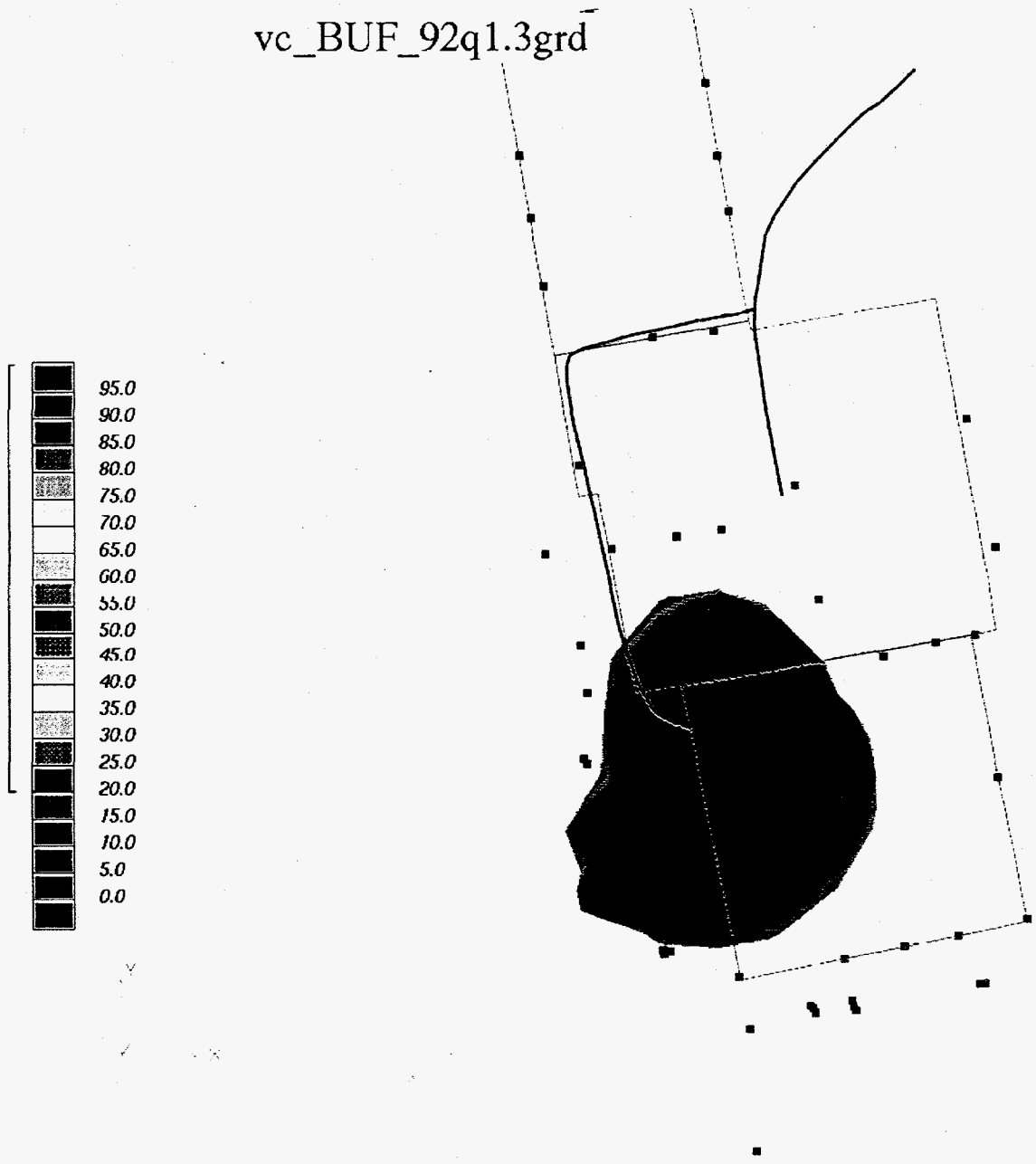

Figure 2D. Vinyl Chloride Map First Quarter 1993.

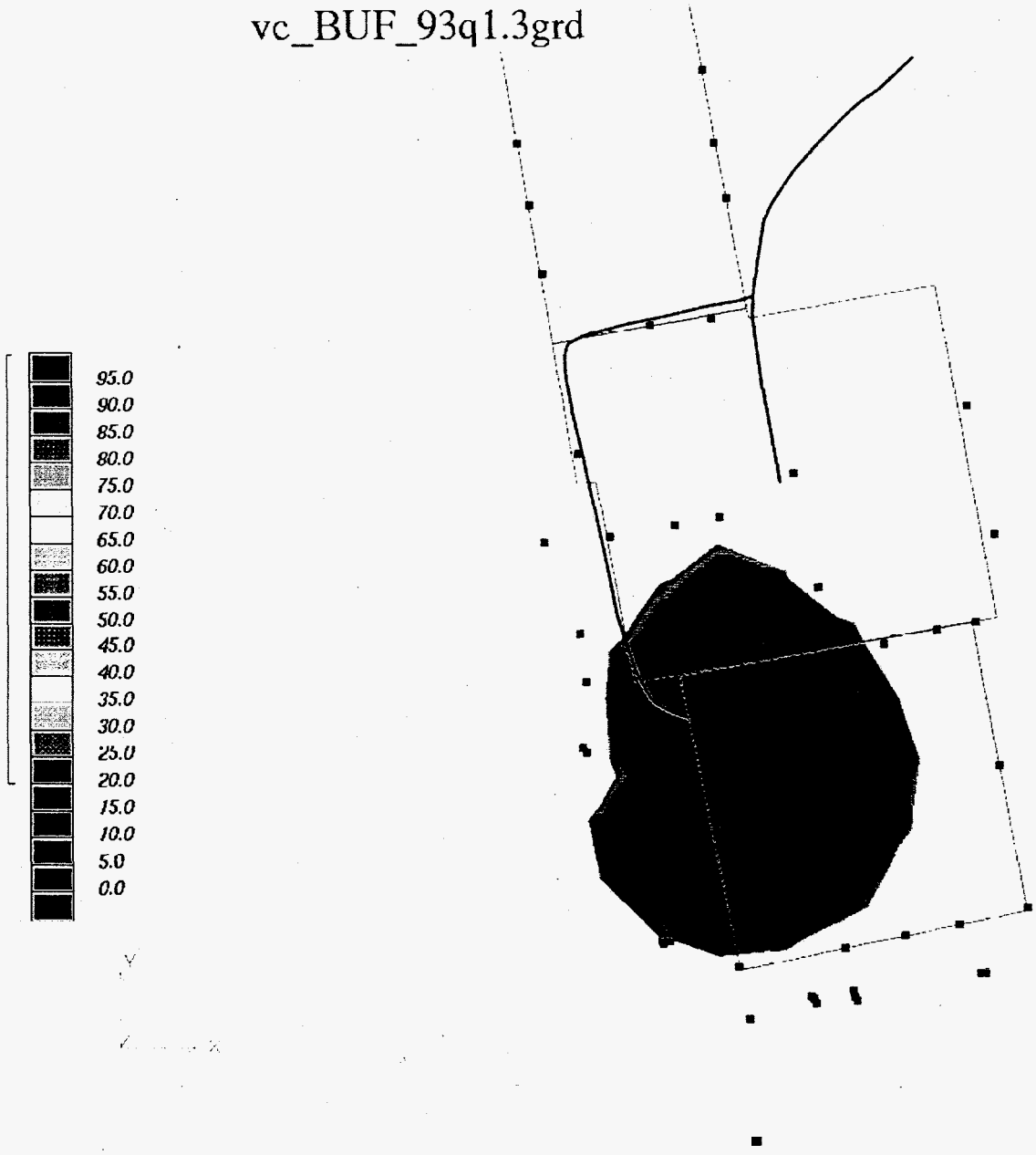

Figure 2E. Vinyl Chloride Map First Quarter 1994.

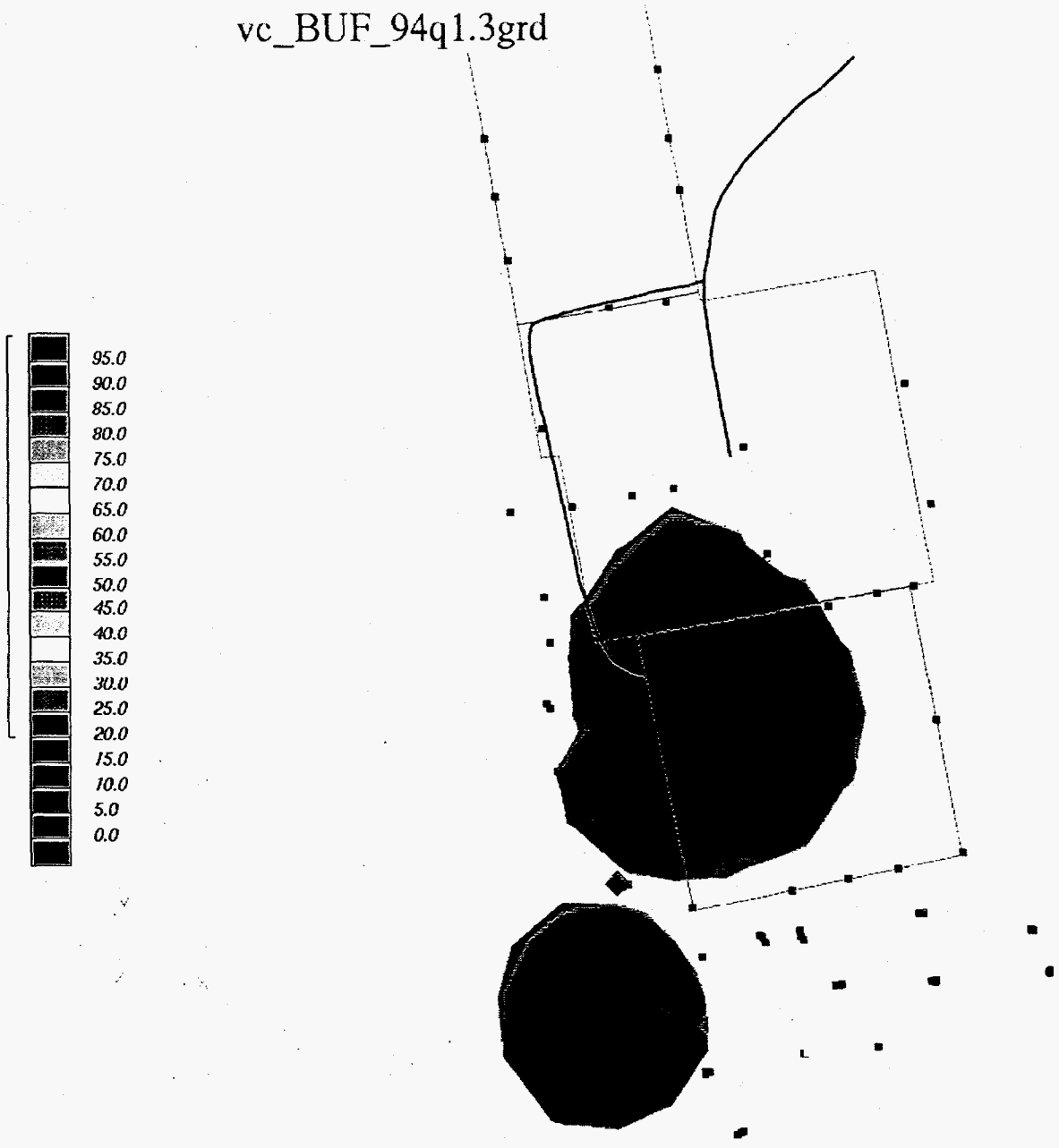

Figure 2F. Vinyl Chloride Map First Quarter 1995.

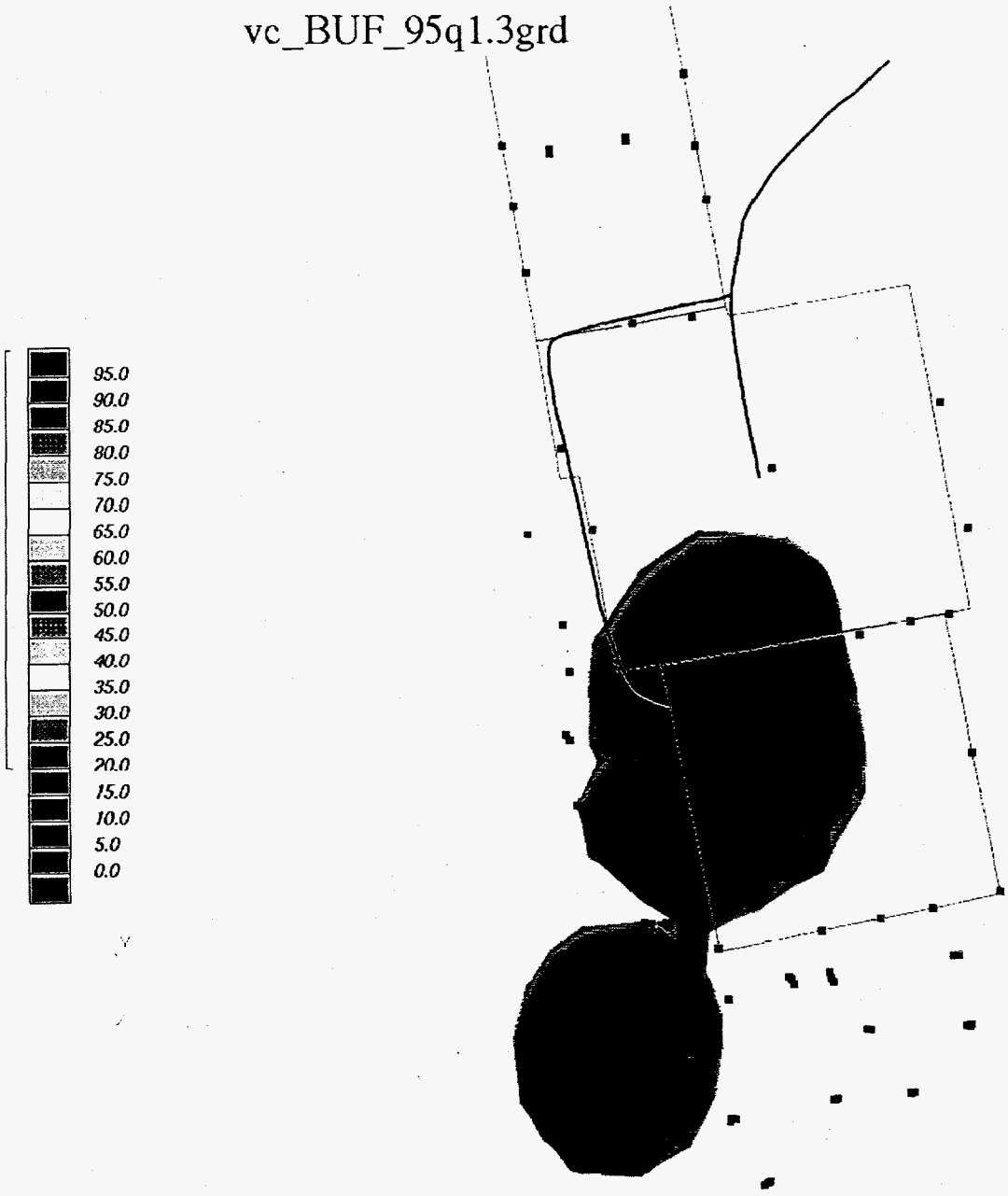

Figure 3A. Chlorobenzene Map Fourth Quarter 1991

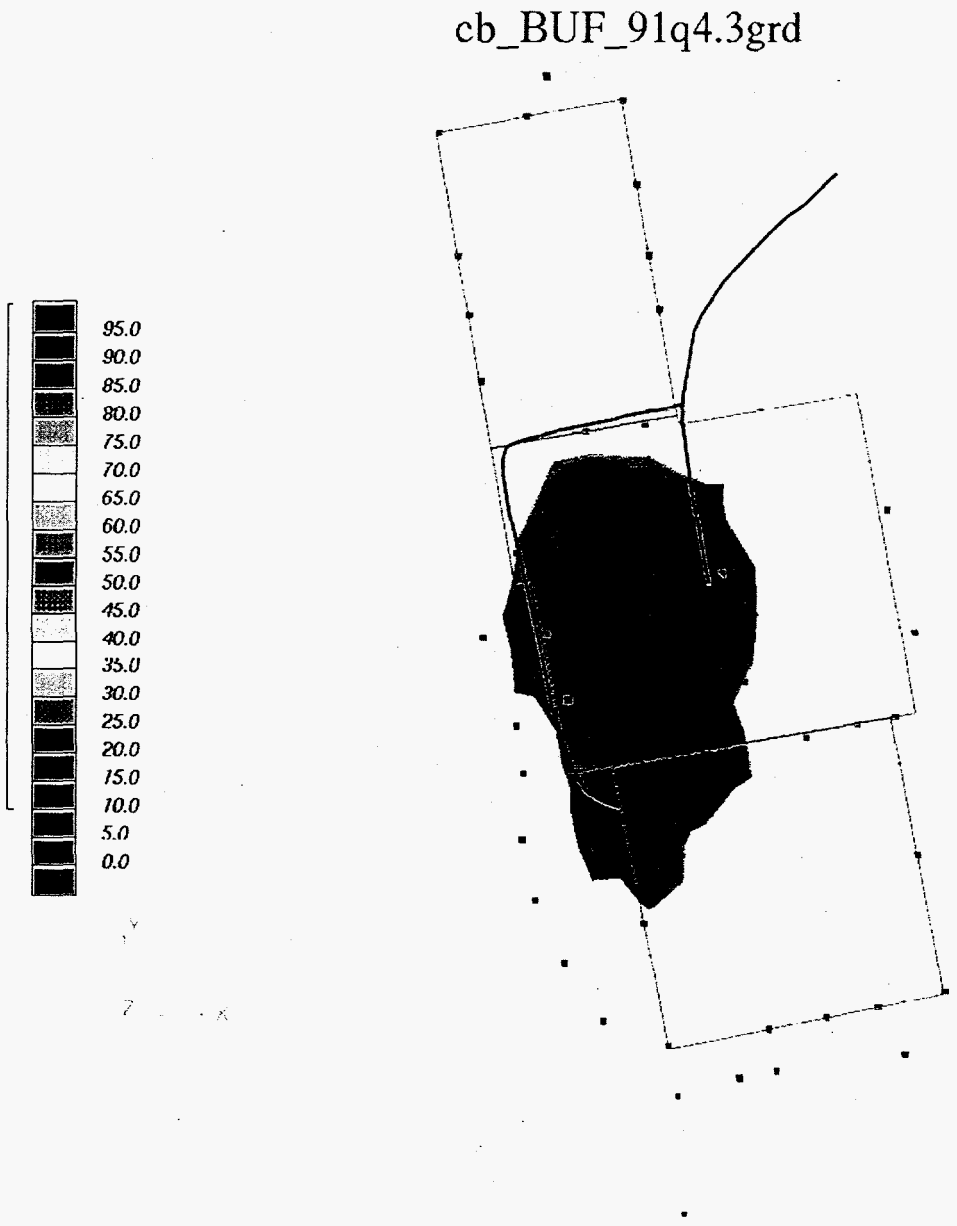

Figure 3B. Chlorobenzene Map Fourth Quarter 1992

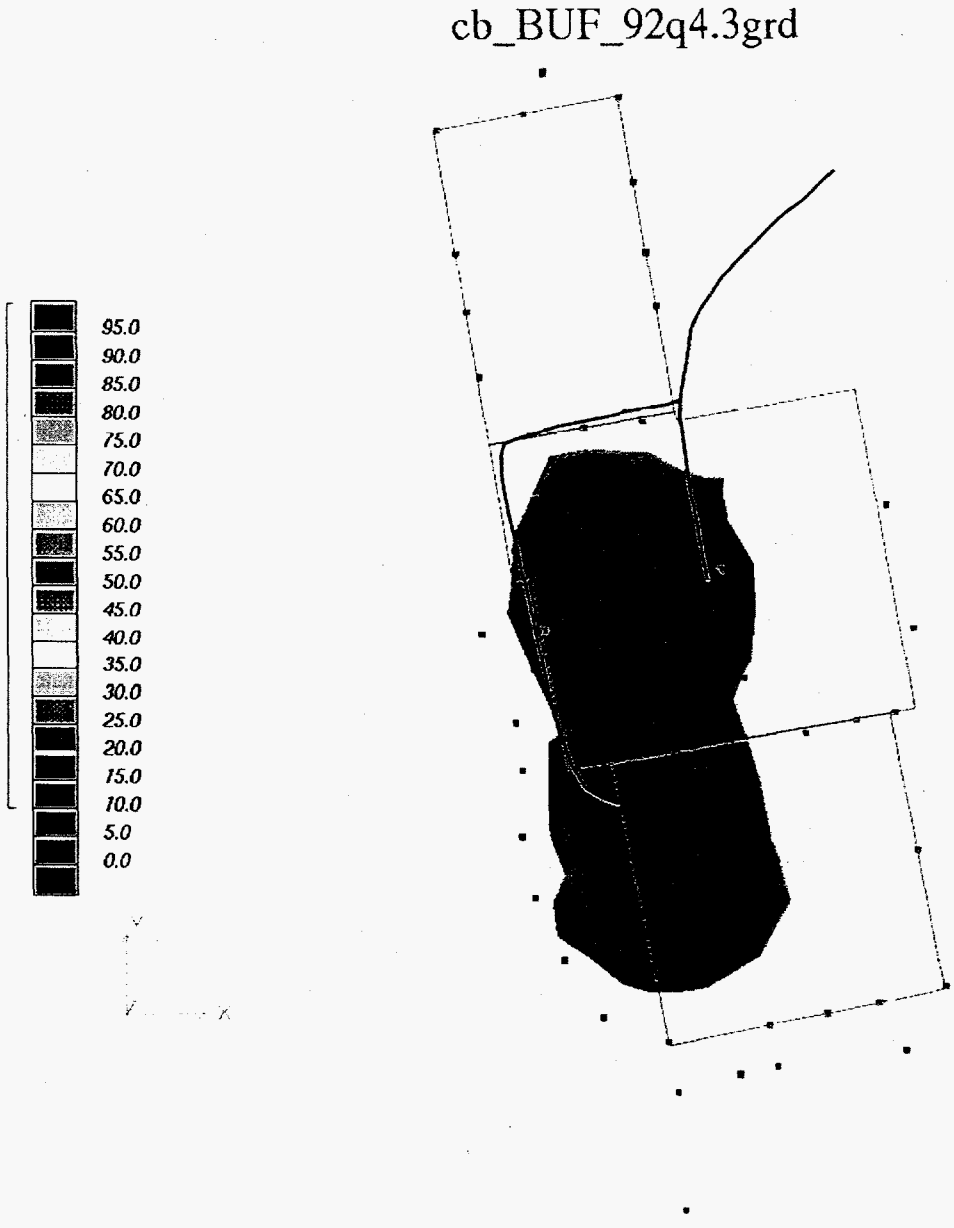

Figure 3C. Chlorobenzene Map Fourth Quarter 1993

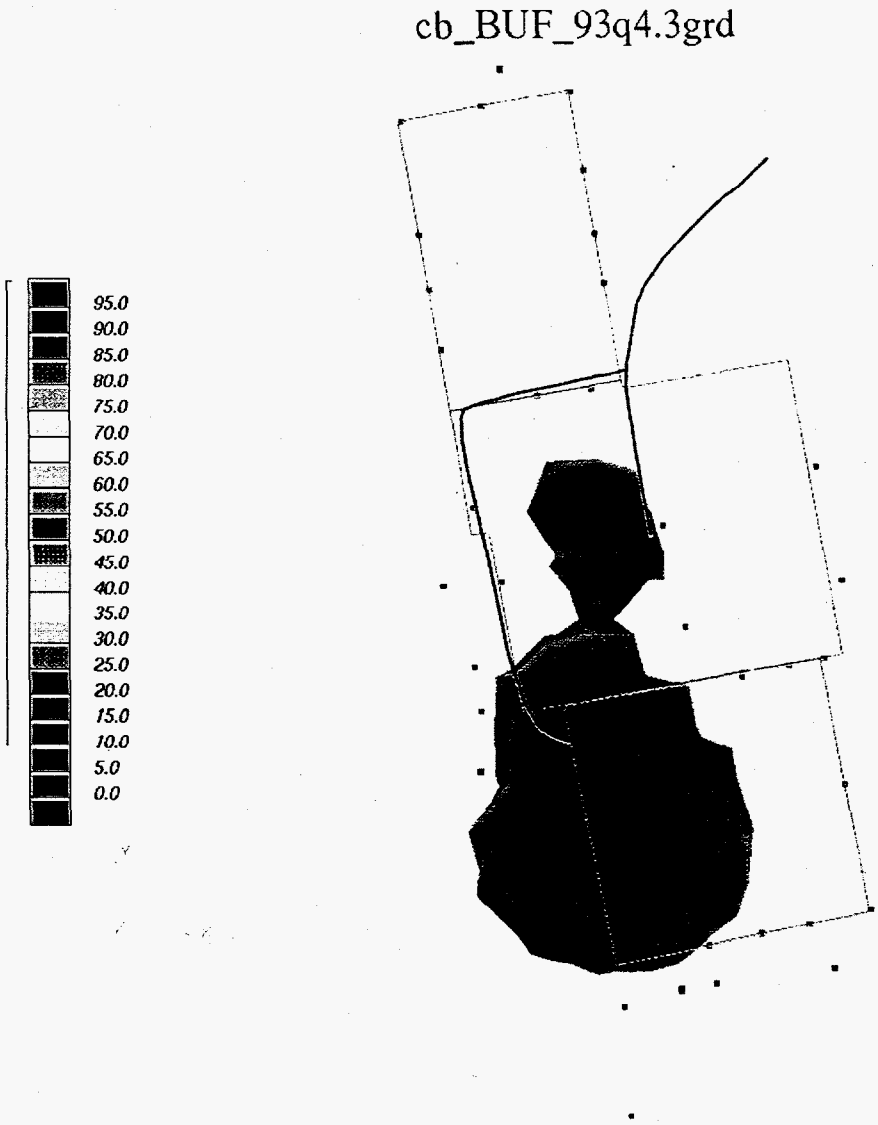

Figure 3D. Chlorobenzene Map Fourth Quarter 1994

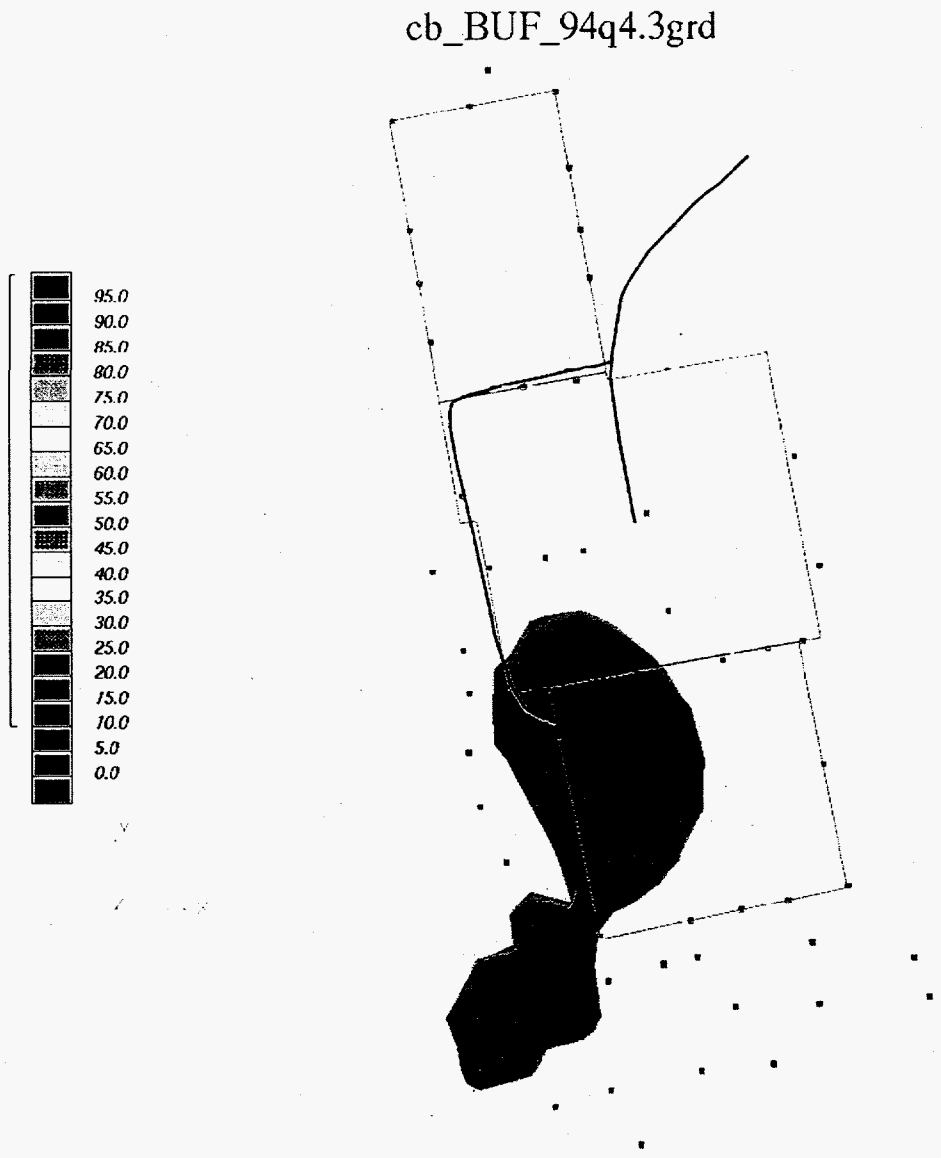

Figure 3E. Chlorobenzene Map Fourth Quarter 1995

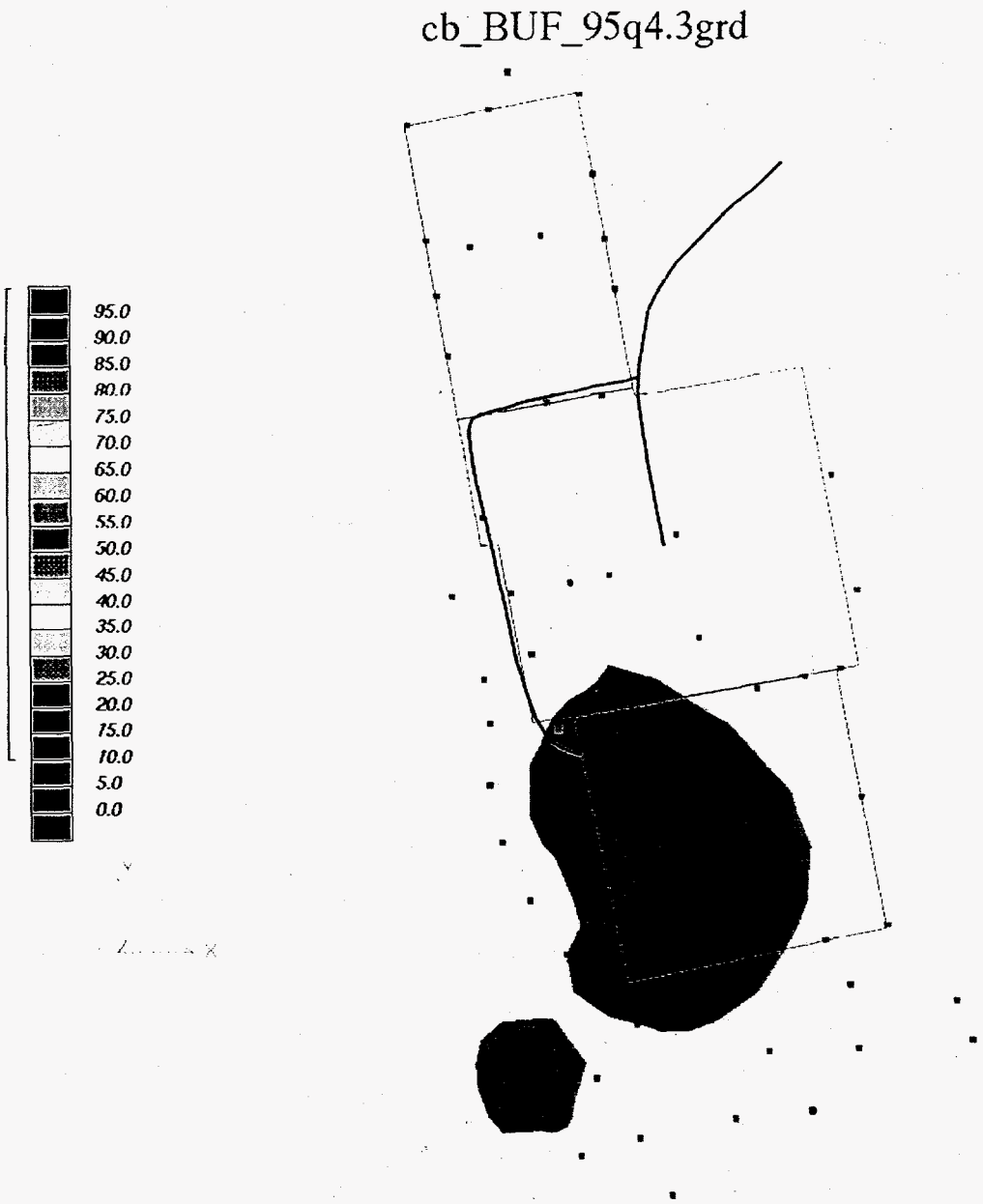

Figure 4A. Chloride Map Fourth Quarter 1990

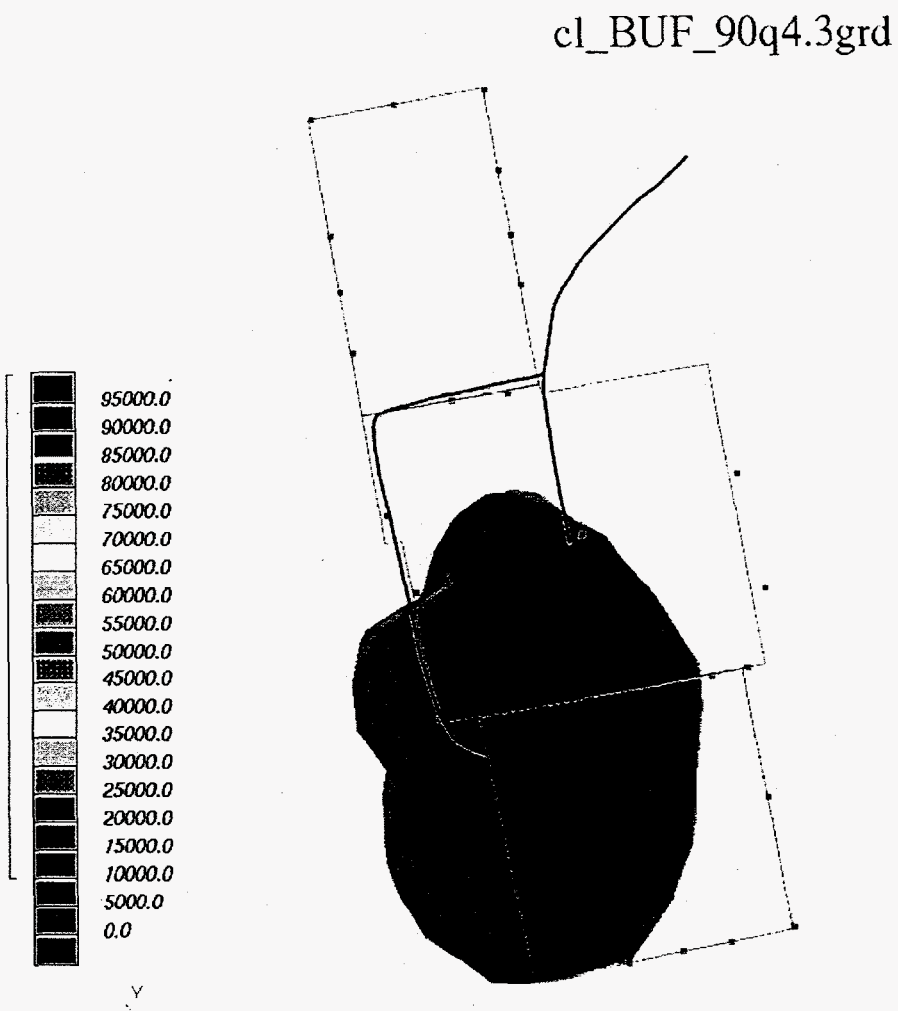

Figure 4B. Chloride Map Fourth Quarter 1991

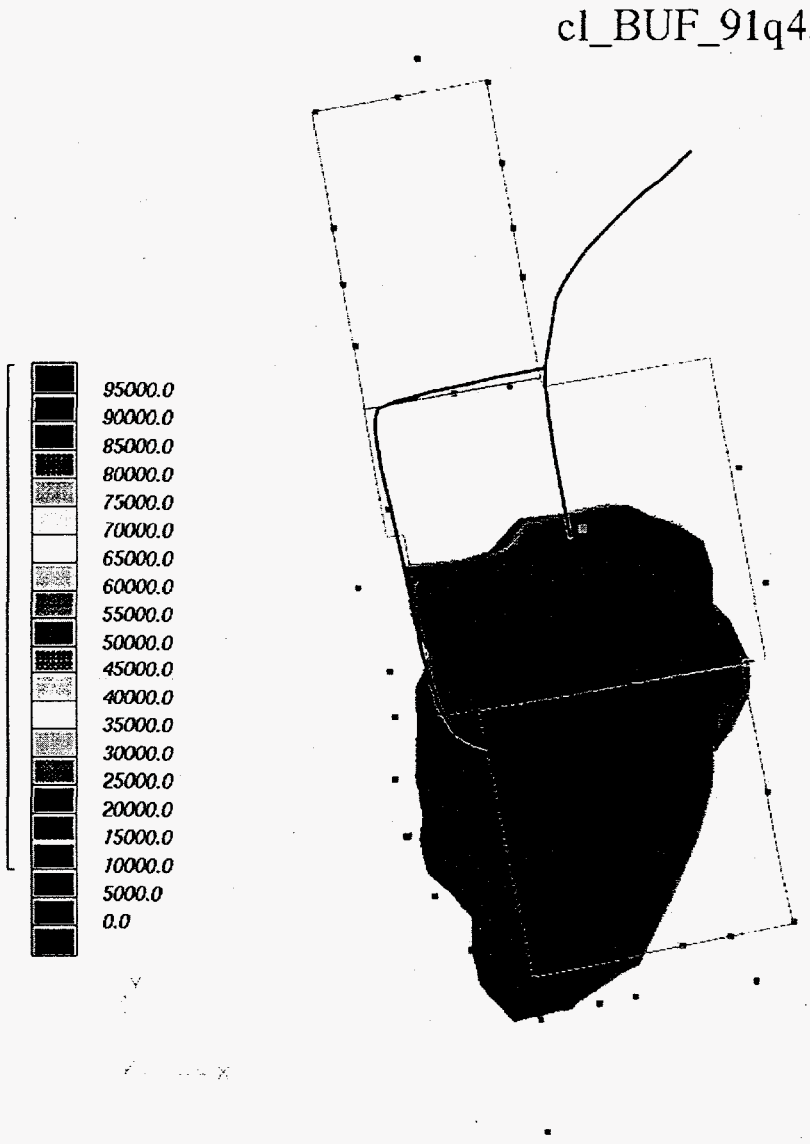

Figure 4C. Chloride Map Fourth Quarter 1992

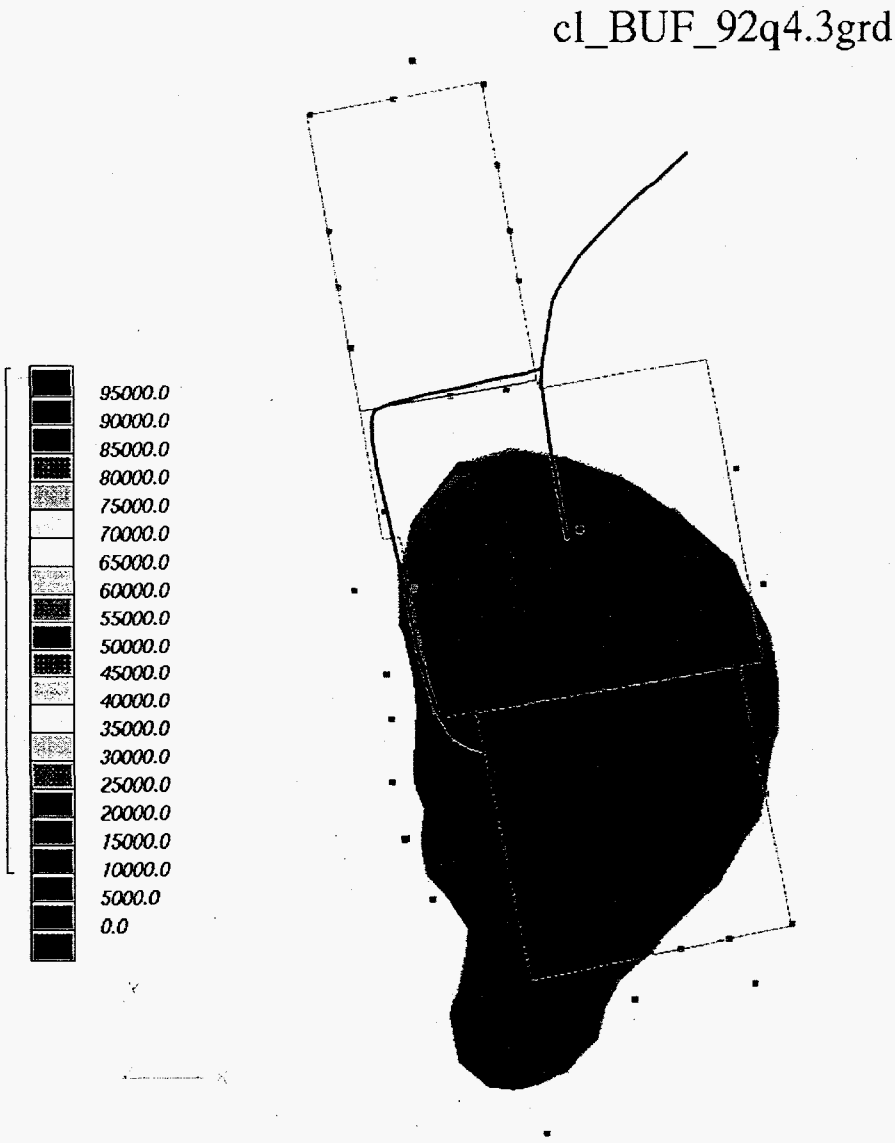

Figure 4D. Chloride Map Fourth Quarter 1993

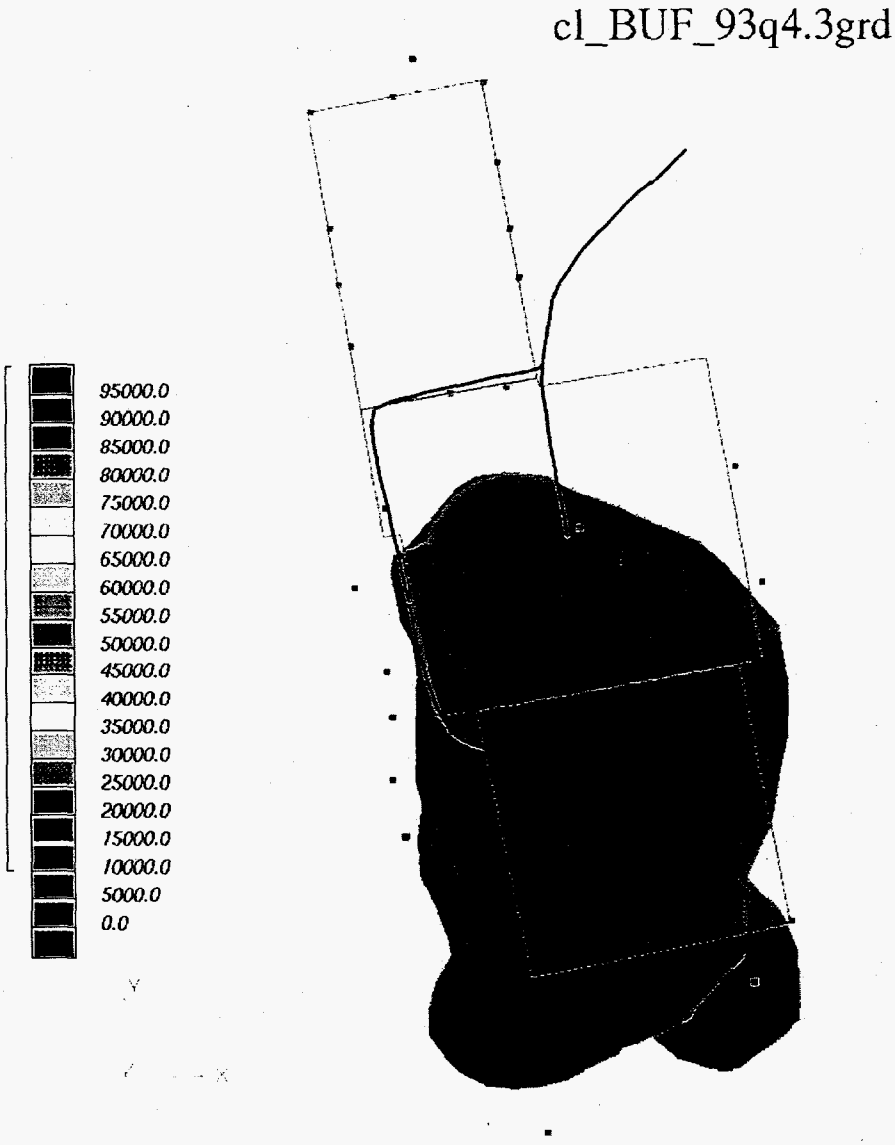

Figure 4E. Chloride Map Fourth Quarter 1994

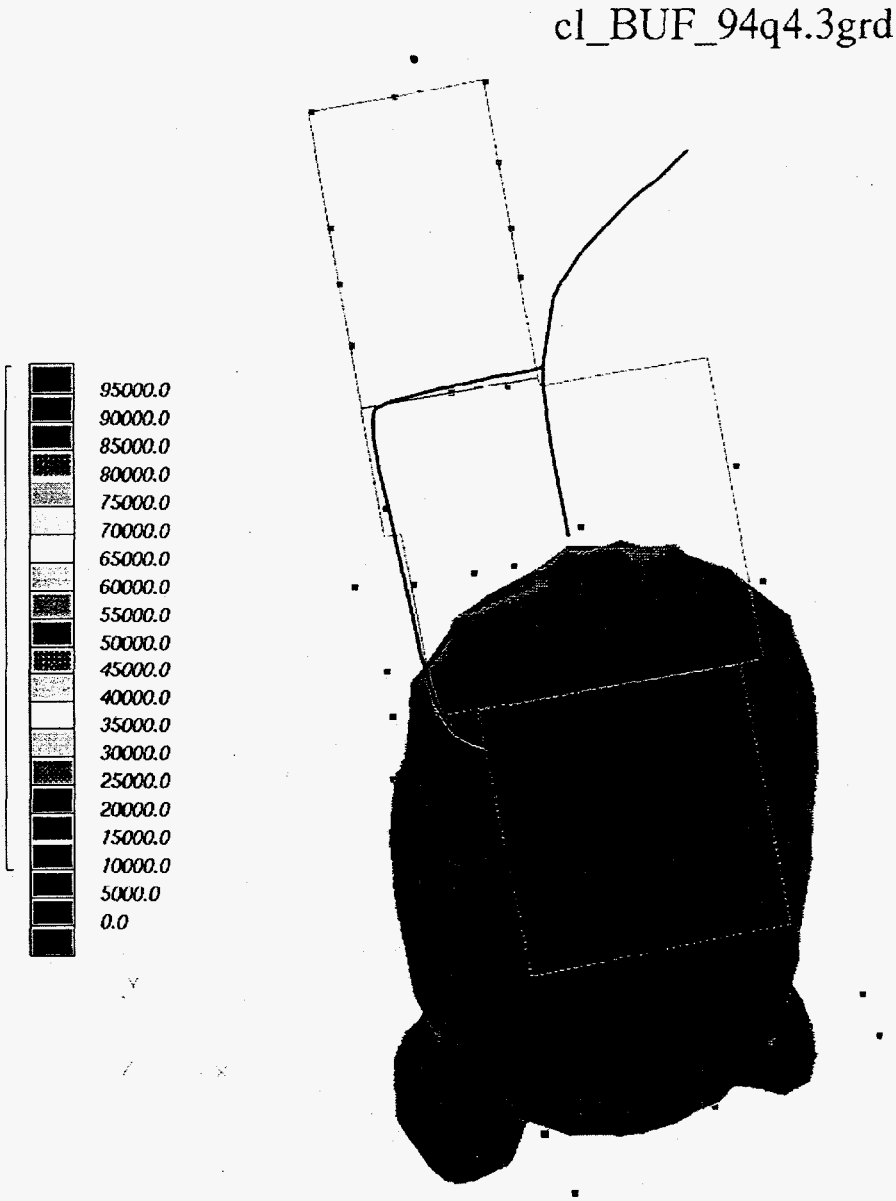

Figure 4F. Chloride Map Fourth Quarter 1995

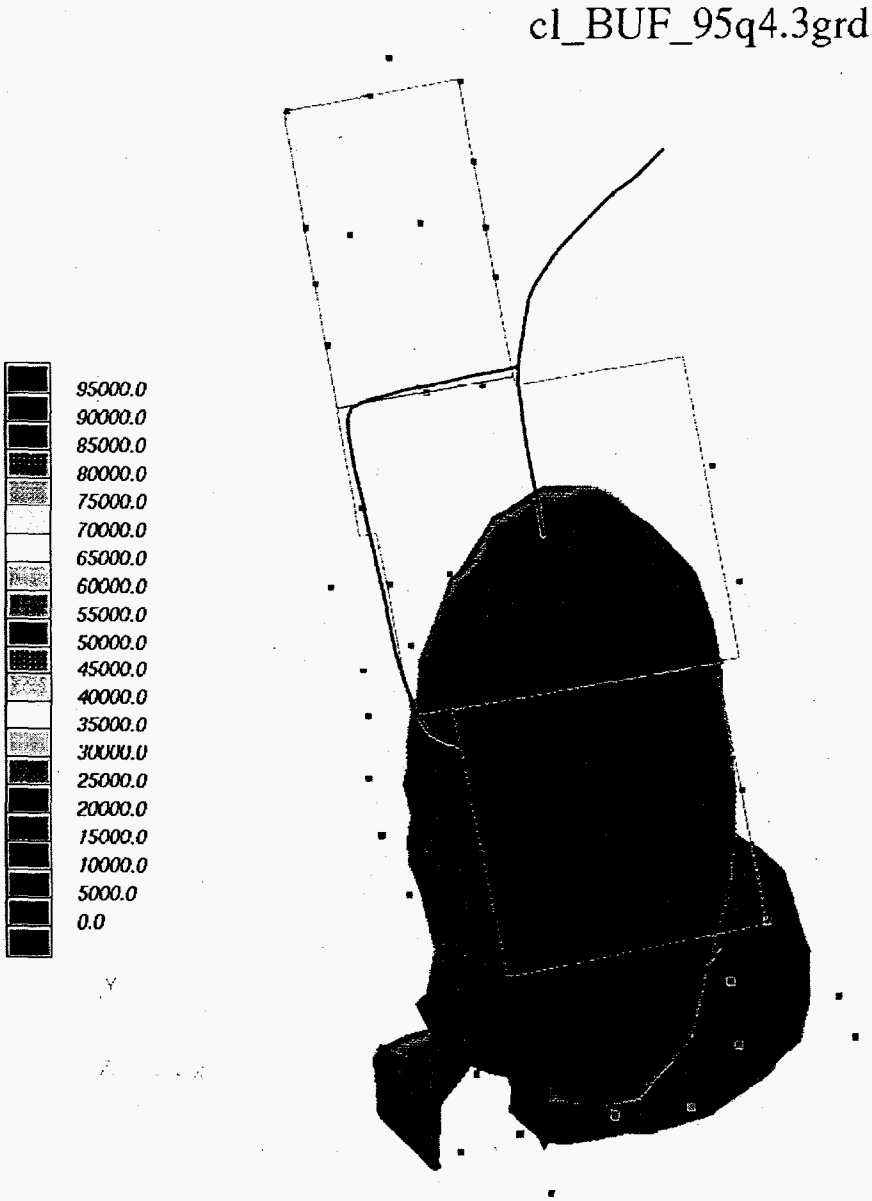

Figure 5A. Tritium Map First Quarter 1990

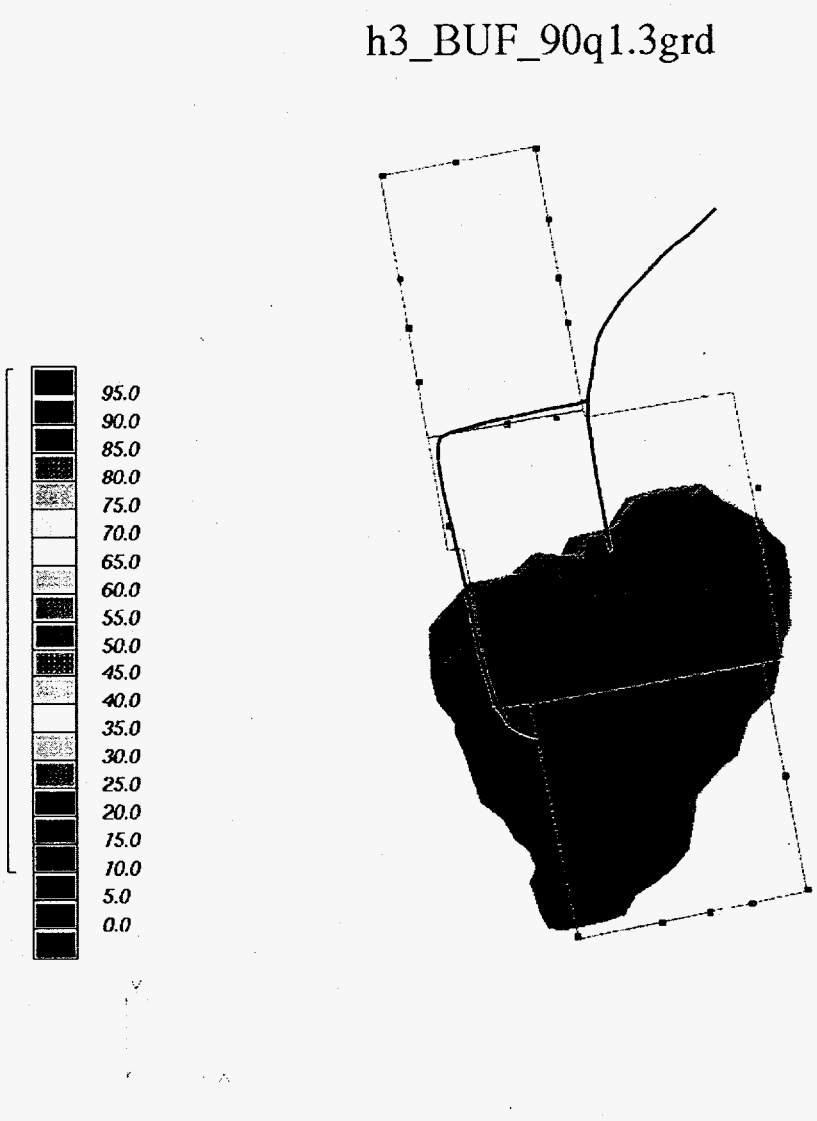

Figure 5B. Tritium Map First Quarter 1991

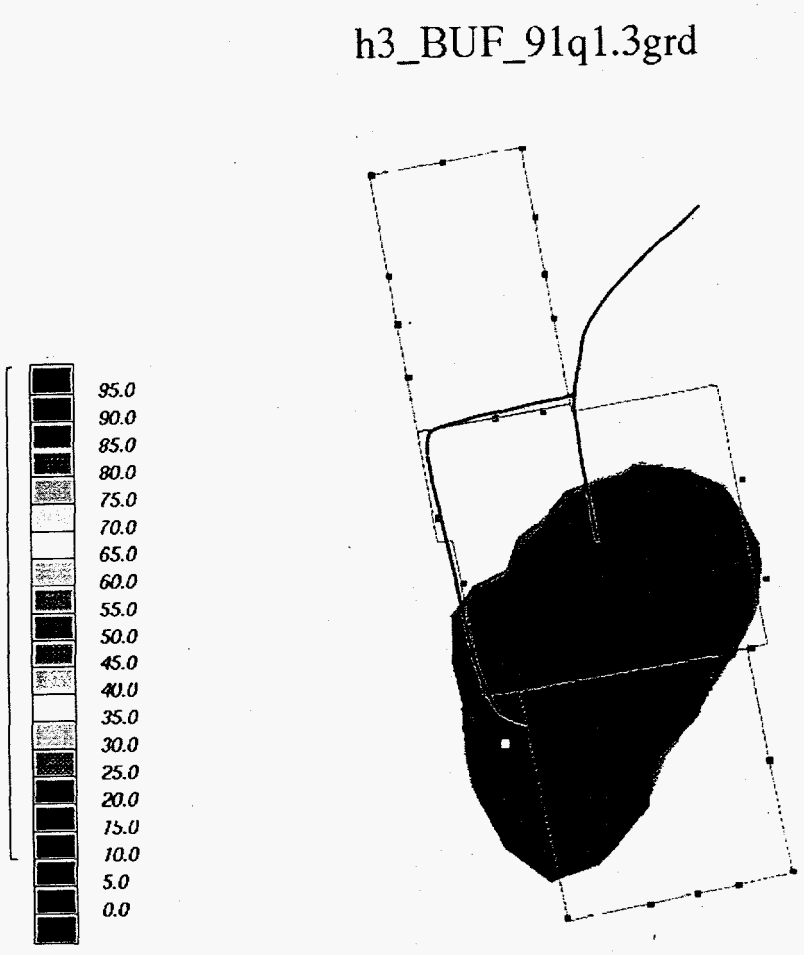

Figure 5C. Tritium Map First Quarter 1992

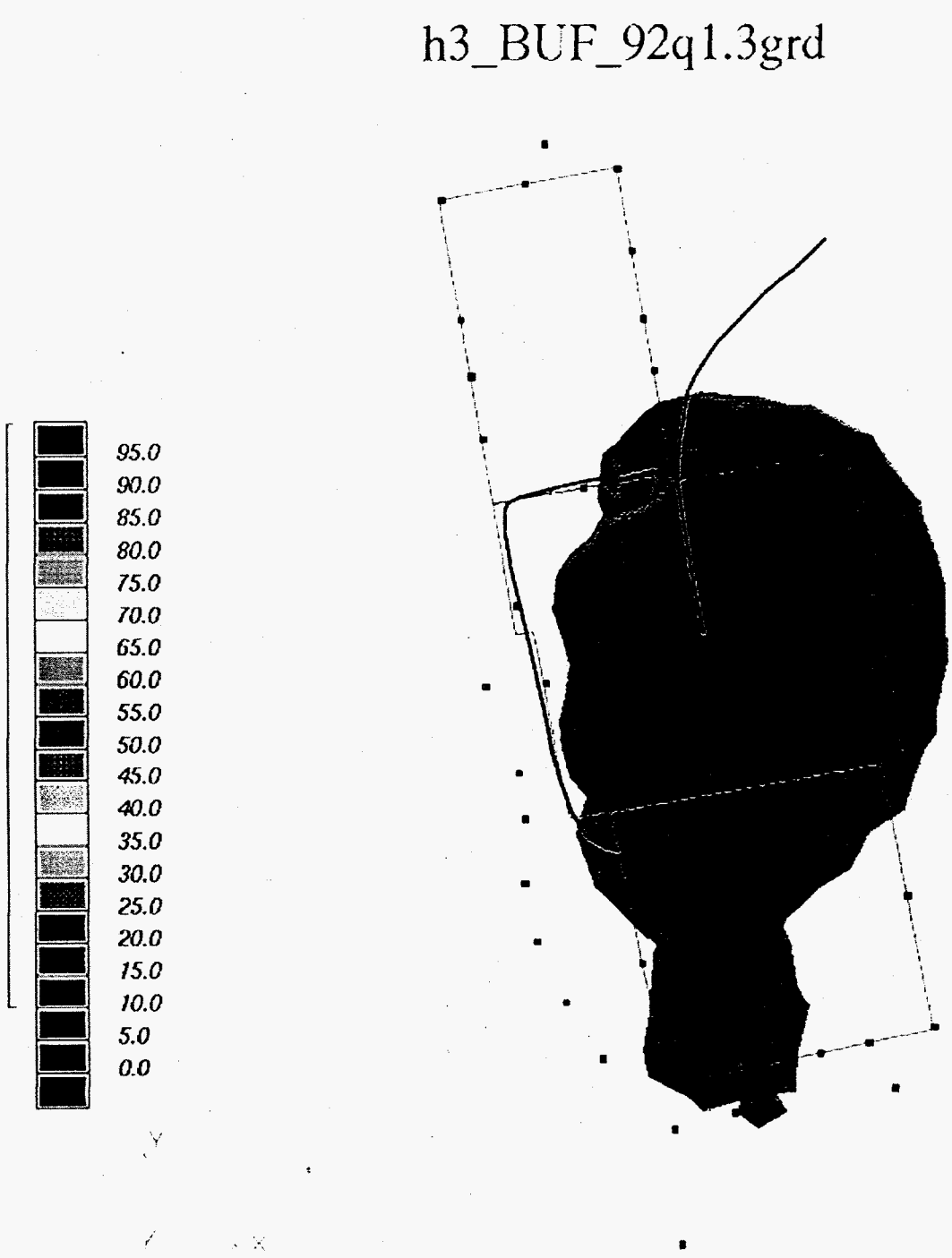

Figure 5D. Tritium Map First Quarter 1993

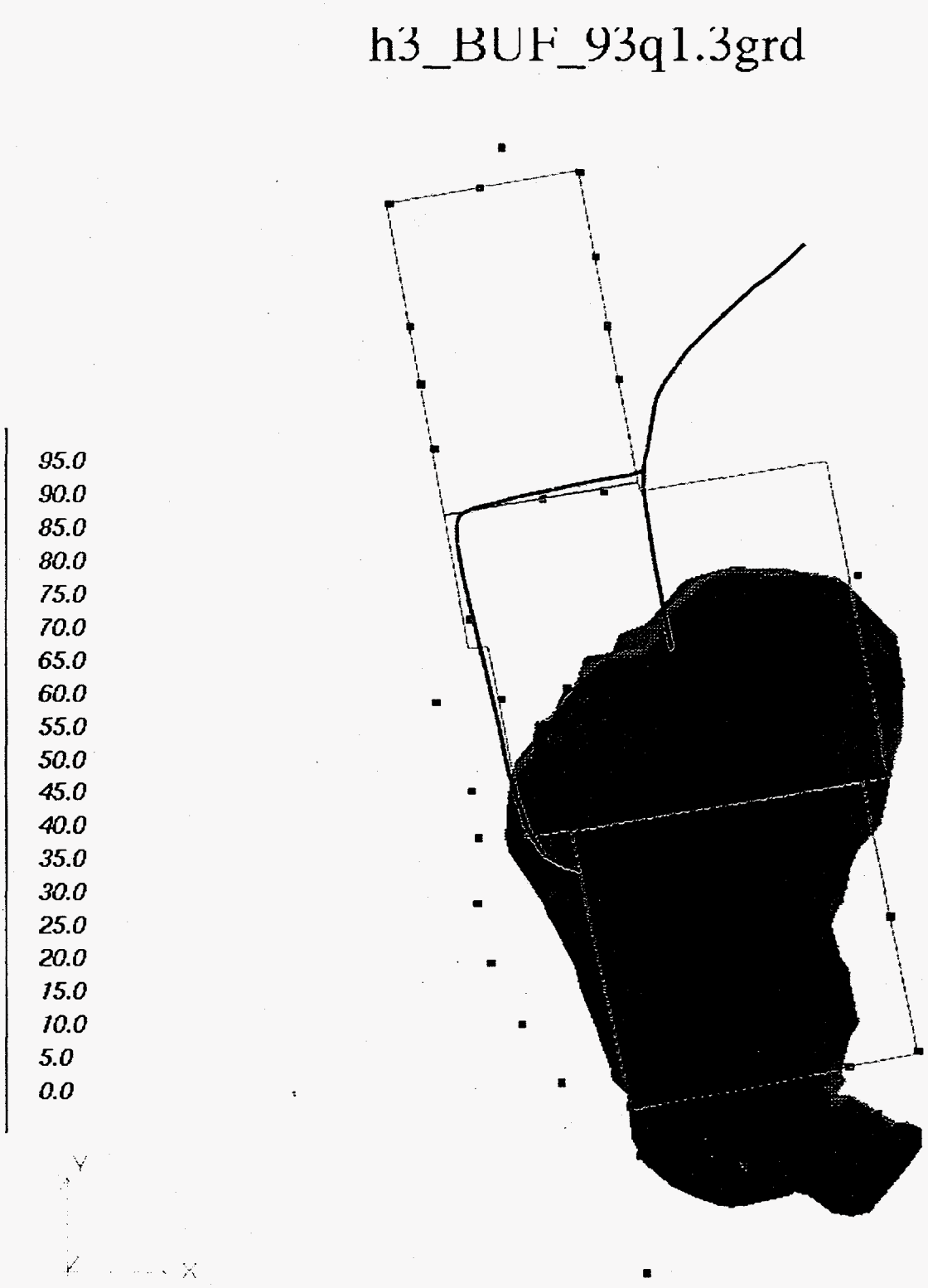

Figure 5E. Tritium Map First Quarter 1994

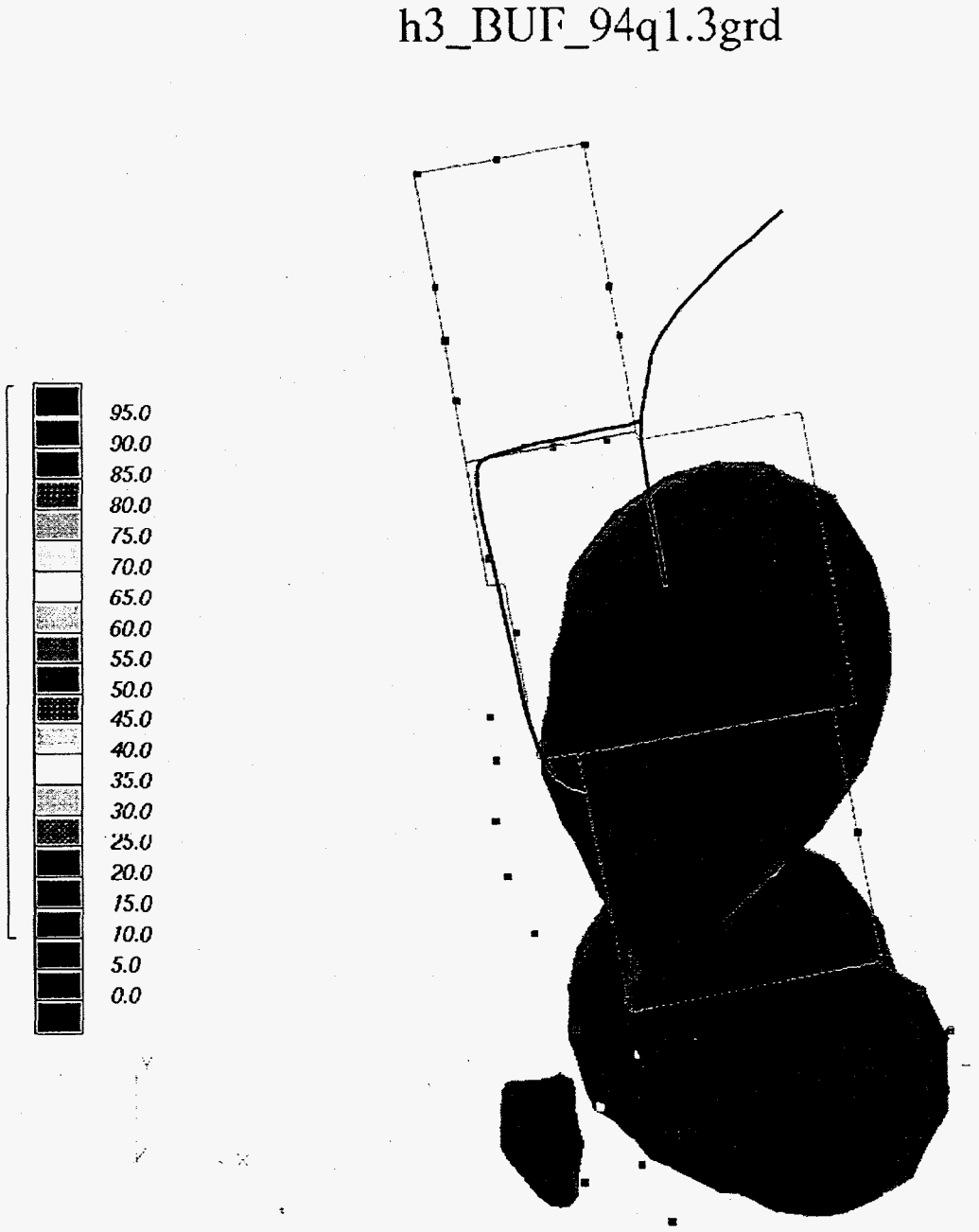

Figure 5F. Tritium Map First Quarter 1995

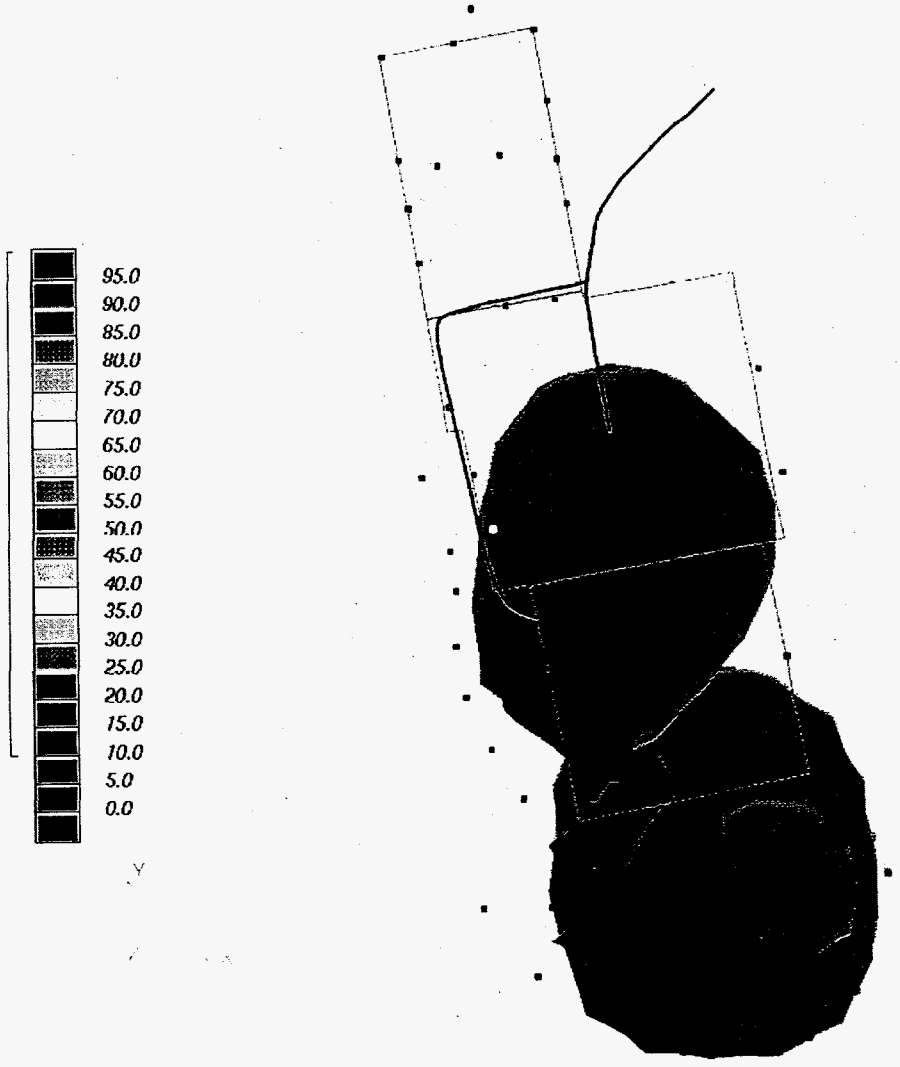

Table 1. Comparison of Intera Model Estimates for Sanitary Landfill Contaminants of Concern Wetland Outfall Concentrations (PPB) and Actual Well Values for 1996.

Constituent	Intera Model 1996 Prediction (Wells 69B,70C,72B)	Well 69B 1996 Measured	Well 70 C 1996 Measured	Well 72B 1996 Measured
Trichloroethane	7.8	0	0	0
Tetrachoroethylene	0.3	0	0	0
Trichloroethylene	0.1	0	0	0
Dichloroethylene	4.9	0	0	0.1
Vinyl Chloride	20.0	13.0	0	12.0
Dichlorobenzene	2.8	0	0	0
Chlorobenzene	27.7	0	0	0

Table 2. Average geochemical and microbiological data collected from three depths at the SLF.

Depth	PCE ppb	VC ppb	Cl ppm	TCE ppb	AODC cells/ml	CTC cells/ml	DFA cells/ml	PTYG CFU/gdw	CB ppb	DO ppm
Shallow (9-13 m)	2.1	22.8	8.8	1.9	2.58×10^5	8.35×10^3	1.21×10^3	6.09×10^3	5	0.37
Moderate (19-23 m)	3.7	5.8	6.4	2.3	2.22×10^5	5.48×10^3	6.60×10^3	1.24×10^3	10	0.49
Deep (29-34 m)	1.8	8.8	1.8	1.3	2.43×10^4	8.30×10^2	2.39×10^3	2.44×10^3	266	0.38

Table 3A. General linear model results of microbiological and chemical results separated by isoconcentrations of various geochemicals. Blank space represents LSM that is not significantly different at $p < 0.05$. Blank=no significant contribution.

Variable	DO > 0.2% n=55	DO < 0.2% n=23	TCE > 5 ug/l	TCE < 5 ug/l	VC > 5 ug/l n=47	VC < 5 ug/l n=31	CB > 5 ug/l	CB < 5 ug/l	Cl > 2 mg/l n=36	Cl < 2 mg/l n=42
AODC			1.19×10^6	9.97×10^4						
DFA										
CTC			4.18×10^4	1.94×10^3						
PTYG										
CB										
phosphate							8.13	-0.75		
nitrate	1.46	1.36								
chloride			-0.30	5.93						
nitrite			9.03	-0.32						
10:0 3OH			-0.18	0.02						
11:0 2OH			-4.85	0.54						
13:1			-0.33	0.04						
13.566									-0.06	0.06
15:0 iso 2OH/16:1 w7c									8.14	-5.17
15:0 iso			-5.97	1.99						
16:0 10 methyl			-1.04	0.25						
17:0 anteiso									0.03	0.09
17:0 cyclo			-0.64	0.14						
17:1 w8c									0.03	0.01
17:0									-0.09	0.09
17:0 ISO 3OH			-0.47	0.05						
16:0 3OH			-0.59	0.33					0.70	-0.09
18:0			33.7	16.9						
19:0									-0.09	0.09
19:1 w12t					2.98	-1.18				

Table 3B. General linear model for BIOLOG results separated by isoconcentrations of various geochemicals. Blank space represents LSM that is not significantly different at ($p < 0.05$). Blank=no significant contribution

BIOLOG SUBSTRATE	DO > 0.2% n=55	DO < 0.2% n=23	TCE > 5 ug/l	TCE < 5 ug/l	VC > 5 ug/l n=47	VC < 5 ug/l n=31	CB > 5 ug/l	CB < 5 ug/l	Cl > 2 mg/l n=36	Cl < 2 mg/l n=42
α-D-glucose	1.81	2.37								
b-methyl d-glucoside	0.57	0.13								
d-melibiose	0.68	0.17								
d-raffinose	0.70	0.15								
d-saccharic	0.83	1.32								
d-sorbitol	1.13	0.54								
i-erythritol	0.43	0.02								
psicose	0.47	-0.08								
turanose	0.71	0.17								
L-pyroglutamic			2.92	1.26						
Putrescine			0.93	0.27						
quinic acid			2.34	1.1						
d-arbitol			2.11	0.92						
d-galacturonic			-0.42	1.34						
L-proline					1.34	1.93				
L-threonine					0.92	1.48				
m-inositol					0.93	1.48				
phenethylamine					0.39	0.85				
sebacic acid					1.13	1.62				
sucrose					1.11	1.68				
turanose					0.46	0.85				
cyclodextrin					0.80	0.04				
alaninamide							1.17	0.70		
d-glucuronic acid							1.3	0.90		
d-mannitol							1.32	0.90		
g-amibutyric							1.41	0.97		
glycerol							1.38	0.87		
glylaspa							0.49	0.22		
hydroxybutyric							1.30	0.86		
ketovalenic							0.49	0.22		
malenic acid							0.70	0.40		
mono-methylsuc							1.35	0.89		
thymidine							0.25	0.00		
uridine							0.37	0.11		
phydphac									1.80	0.71
xylitol									0.85	0.01

Table 4A. Correlation results of microbiological and chemical results separated by isoconcentrations of various geochemicals. Blank space represents no significantly difference at $p < 0.05$. Blank=no significant contribution

	DO_2 > 0.2%	DO_2 < 0.2%	TCE > 5 ug/l	TCE < 5 ug/l	VC > 5 ug/l	VC < 5 ug/l	CB > 5 ug/l	CB < 5 ug/l	Cl > 2 mg/l	Cl < 2 mg/l
AODC	-0.26								0.47	
DFA			0.96							
CTC										
PTYG										
CB			0.95							
freon-12	-0.28									
benzene									0.68	
cis-DCE				0.35					0.57	
1,2-DCB									0.61	
1,3-DCB									0.52	
1,1-DCE									0.36	
ethylbenzene									0.44	
freon-12									0.36	
freon-13									0.47	
methylchloride				0.34					0.78	
O-xylene				0.53					0.34	
PCE				0.54					0.34	
TCE									0.57	
chlorobenzene			0.94							0.37
xylene			0.99	0.56						
1,1,1-TCA				0.33						
toluene				0.57						
chloride	-0.28									
nitrate	0.65								-0.46	0.56
DO_2									-0.43	0.49
sulfate										-0.36

Table 4B. Correlation results of BIOLOG separated by isoconcentrations of various geochemicals. Blank space represents LSM that is not significantly different at $p < 0.05$. Blank=no significant contribution

BIOLOG Substrate	DO_2 > 0.2%	DO_2 < 0.2%	TCE > 5 ug/l	TCE < 5 ug/l	VC > 5 ug/l	VC < 5 ug/l	CB > 5 ug/l	CB < 5 ug/l	Cl > 2 mg/l	Cl < 2 mg/l
d-melibiose	-0.28									
gentiobiose	-0.30									
NAD-galactose	-0.27									
D-sacchar		-0.83								
L-leucine		0.71								
L-pyroglutamic		-0.79								
malonic		-0.77								
betahbut									0.46	
bromsuccinic									0.42	
d-gluconic									0.42	
d-l-lactic acid				0.27					0.61	
d-sorbitol									0.42	
itaconic acid									0.37	
ketoglutamic									0.35	
ketovalenic									0.37	
l-aspartic acid									0.53	
succinic									0.38	
succinic				0.33					0.62	
tween 40				0.27					0.40	-0.59
cis-aconitic										-0.40
d-galuron										-0.35
g-hydroxybutyric										0.37
sucrose										-0.36
d-glucuronic			-1.0							
d-raffinose			0.99							
glycogen				-0.25						
l-aspartic				0.27						

Table 5. Principal components analysis. Blank=no significant contribution.

Microbiology variable	Prin 1	Prin 2	Prin 3
Cumulative	0.37	0.70	
AODC	0.70		
DFA			
CTC	0.69		
PTYG		0.70	
CB		0.70	
BIOLOG variables > 0.20			
Cumulative	0.33	0.45	0.54
a-lactose		-0.24	
butandiol			0.25
d,l; a-glycerol phosphate			0.28
d,l,carnitine			0.20
d-melibiose		-0.23	
d-raffinose		-0.21	
gentiobiose		-0.20	
glucose-1-P			0.27
glucose-6-P			0.22
thymidine			0.23
FAME variables > 0.20			
10:0 3OH	0.24		-0.26
12:0 iso 3OH		0.28	0.26
13:0 anteiso		0.28	0.28
13.566	0.22	-0.26	
14:0 anteiso		0.25	0.25
13:1	0.24		-0.26
15:0 iso	0.23		
16:1 w7c/15 iso 2OH	0.23		
16:0	-0.20		
15:0 2OH		0.28	
Iso 17:1	0.24		-0.26
Iso 17:1 w5c	0.20	0.30	
16:0 10 methyl	0.23	-0.27	
17:0 iso	0.22	-0.26	
17:0 anteiso	0.25		-0.27
17:1 w8c	0.22	-0.26	
17:0 cyclo	-0.21		
17:0	0.24		-0.26
18:0	0.22	-0.26	

TABLE 6. The contaminants of concern measured in SLF groundwater.

Compound	No. wells >5ppb	Range [ug/l]	Mean [ug/l]
1,2-dichlorobenzene	0		
1,3-dichlorobenzene	0		
2-chlorotoluene	0		
bromo-benzene	1	5.85	5.85
ethyl-benzene	2	6.55-8.6	7.57
tetrachloroethylene	0		
trichloroethylene	3	6.45-8.3	7.21
carbon tetrachloride	0		
trichloroethane	0		
trans-dichloroethylene	0		
1,1-dichloroethylene	16	5.05-9.41	6.39
freon-13	11	5.6-87.25	28.69
1,4-dichlorobenzene	4	5.15-20.4	13.28
1,3,5-trichlorobenzene	0		
n-propylbenzene	0		
O-xylene	3	5.85-28.55	14.28
chlorobenzene	4	5.65-11.6	8.47
toluene	2	8.9-14.9	11.9
benzene	1	6.65	--
1,1,1-TCA	3	5.1-12.1	7.46
cis-dichloroethylene	16	5.05-9.41	6.39
methyl-chloride	5	5.34-70.75	24.44
freon-12	10	5.37-141	49.79
vinyl chloride	30	5.1-67.4	15.15

Table 7. GLM significantly different contaminants for various isoconcentrations.

Contaminant	DO Above 0.2%	DO Below 0.2%
chloroform	2.02	1.06
carbon tetrachloride	0.97	0.36
2-chlorotoluene	0.00	0.10
1,2-dichlorobenzene	0.01	0.21
1,3-dichlorobenzene	0.00	0.14
freon-12	6.5	19.6
	TCE Above 5 ug/l (n=5)	TCE Below 5 ug/l (n=67)
chlorobenzene	6.21	1.68
carbon tetrachloride	-0.83	0.90
freon-12	104	4.02
PCE	7.44	2.1
1,1,1-TCA	9.55	2.3
toluene	7.36	0.91
xylene	14.1	0.22
TCE	7.47	1.39
	Chloride Above 2 mg/l	Chloride Below 2 mg/l
benzene	1.66	0.58
chlorobenzene	2.96	1.19
ethylbenzene	1.19	0.01
freon-12	20.2	2.63
PCE	3.41	1.66
1,1,1-TCA	3.45	2.14
TCE	2.41	1.27
toluene	2.08	0.72
(n=48, 30, respectively)	Vinyl chloride Above 5 ug/l	Vinyl chloride Below 5 ug/l
carbon trichloride	1.39	2.34
carbon tetrachloride	0.59	1.14
(n=15, 63, respectively)	Chlorobenzene > 5 ug/l	Chlorobenzene < 5 ug/l

Table 8. Significantly different geochemicals and BIOLOG substrates based on depth (alpha=0.05, p-value > 0.05).

Depth	Nitrate	Sulfate	Alaninamide	Bromosuccinic	itaconic	ketobutyric	ketovaleric	l-leucine	d-trehalo
Shallow 9-13 m	0.77b	0.92b	1.03a	0.23ab	0.92a	0.47b	0.35a	1.31a	1.41a
Moderate 19-23 m	2.22a	4.29b	0.59b	0.35a	0.71ab	0.77a	0.31ab	1.18ab	0.83b
Deep 30-34 m	1.98ab	10.85a	0.51b	0.19b	0.56b	0.44b	0.15b	0.82b	1.1ab