ENGINEERING SERVICE CENTER
Port Hueneme, California 93043-4370

CONTRACT REPORT
CR-05-002-ENV

FINAL REPORT
FIELD DEMONSTRATION AND VALIDATION OF A BIOAVAILABLE FERRIC IRON ASSAY

by

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January 2005

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0811

The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing the burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information, it if does not display a currently valid OMB control number.

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1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From – To)*
January 2005	Final	January 2001 to October 2002

4. TITLE AND SUBTITLE

FINAL REPORT - FIELD DEMONSTRATION AND VALIDATION OF A BIOAVAILABLE FERRIC IRON ASSAY

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESSES

Commanding Officer
Naval Facilities Engineering Service Center
1100 23rd Avenue
Port Hueneme, CA 93043

8. PERFORMING ORGANIZATION REPORT NUMBER

CR-05-002-ENV

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

ESTCP
901 N. Stuart St., Suite 303
Arlington, VA 22203-1853

10. SPONSOR/MONITORS ACRONYM(S)

ESTCP

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT

13. SUPPLEMENTARY NOTES

14. ABSTRACT

This report describes the demonstration and validation of a novel analytical technology: a bioavailable ferric iron (BAFeIII) assay. Demonstration and validation of the BAFeIII assay was conducted at four Department of Defense (DoD) installations. Specific objectives were to: (1) Evaluate the bioavailable ferric iron assay method using a combination of geochemical and microbiological techniques and assessment of available site data and (2) Quantify costs associated with the technology.

This report presents the results of the demonstration and validation of the BAFeIII assay technology at DOD installations.

15. SUBJECT TERMS

Bioavailable Ferric Iron Assay (BAFeIII), ESTCP, geochemical,

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	U	149	

Environmental Security Technology Certification Program (ESTCP)

Final Report
Field Demonstration and Validation of a Bioavailable Ferric Iron Assay
ESTCP Project Number 200009

November 2004

Final

Table of Contents

Preface 1
Executive Summary 2
1.0 Introduction 7
1.1 Background 7
1.2 Objectives of the Demonstration 8
1.3 Regulatory Drivers 11
1.4 Stakeholder/End-User Issues 11
2.0 Technology Description 13
2.1 Technology Development and Application 13
2.2 Previous Testing of the Technology 18
2.3 Factors Affecting Cost and Performance 19
2.4 Advantages and Limitations of the Technology 19
3.0 Demonstration Design 22
3.1 Performance Objectives 22
3.2 Selection of Test Sites 24
3.3 Test Site Description 25
3.4 Pre-Demonstration Testing and Analysis 35
3.5 Testing and Evaluation Plans 35
3.5.1 Demonstration Installation and Startup 35
3.5.2 Period of Operation 38
3.5.3 Amount/Treatment Rate of Material to Be Treated 38
3.5.4 Residuals Handling 38
3.5.5 Operating Parameters for the Technology 38
3.5.6 Experimental Design 38
3.5.7 Sampling Plan 39
3.5.7.1 Sample Collection 39
3.5.7.2 Sample Analysis 40
3.5.7.3 Experimental Control 41
3.5.7.4 Quality Assurance & Quality Control 42
3.5.8 Demobilization 45
3.6 Selection of Analytical/Testing Methods 45
3.7 Selection of Analytical/Testing Laboratory 45
4.0 Performance Assessment 47
4.1 Performance Criteria 47
4.2 Performance Confirmation Methods 48
4.3 Data Analysis, Interpretation and Evaluation 51
4.3.1 Intra-Laboratory Precision 51
4.3.2 Inter-Laboratory Precision 53
4.3.3 Range Comparison 56
4.3.4 Association with Bioavailable Fe III Factors 57
4.3.5 Association with Microcosm Reducible Fe III 61
4.3.6 Association with 0.5N and 6.0N HCl Extractable Fe III 62

4.3.7 Association with Citrate Dithionite Bicarbonate Extractable Fe, Ammonium Oxalate Extractable Fe, and Total Fe 64
4.3.8 Relationship with Fe III Mineral Crystallinity and Surface Area 66
4.3.9 Mass Balance 72
5.0 Cost Assessment 78
5.1 Cost Reporting 78
5.2 Cost Analysis 80
6.0 Implementation Issues 81
6.1 Environmental Checklist 81
6.2 Other Regulatory Issues 81
6.3 End-User Issues 81
7.0 References 82
8.0 Points of Contact 85
8.1 ESTCP Program Office 85
8.2 Project Management 85
8.3 CDM Staff 85
8.4 Partners 86

Appendices

Appendix A BAFeIII Assay Protocol and Confirmatory Analyses Methods
Appendix B Quality Assurance Plan
Appendix C Health and Safety Plan
Appendix D Field Notes
Appendix E Analytical Data

List of Figures

2-1 BAFeIII Assay Kit. 13
2-2 BAFeIII Assay Kit Procedure 15
3-1 Vicinity Map, SUBASE Bangor 26
3-2 Sample Collection Points, SUBASE Bangor 27
3-3 Vicinity Map, Ft. Lewis 29
3-4 Sample Collection Points, Ft. Lewis 30
3-5 Vicinity Map, NAS Pensacola 31
3-6 Sample Collection Points, NAS Pensacola 32
3-7 Vicinity Map, USCG Support Center, Elizabeth City, NC 34
3-8 Fuel Farm Sample Collection Points, USCG Support Center, Elizabeth City, NC 36
3-9 North Beach Sample Collection Points, USCG Support Center, Elizabeth City, NC 37
3-10 Soil Sample Allocation 46
4-1 Intra-Laboratory Replicate Precision for BAFeIII Assay 53
4-2 Inter-Laboratory Replicate Precision for BAFeIII Assay 55
4-3 Analytical Range Comparison. 57
4-4 Percent Variance Explained by PCA Components 59
4-5 Loadings for PCA Component 1 60
4-6 Loadings for PCA Component 2 61
4-7 Association Between BAFeIII and Microcosm Reducible FeIII 61
4-8 Association Between BAFeIII and Microcosm Reducible FeIII with BrY 62
4-9 Association Between BAFeIII and 0.5N HCl Extractable FeIII 63
4-10 Association Between BAFeIII and 6.0N HCl Extractable FeIII 63
4-11 Association Between BAFeIII and Citrate Dithionite Bicarbonate Extractable Fe 64
4-12 Association Between BAFeIII and Ammonium Oxalate Extractable Fe 65
4-13 Association Between BAFeIII and Total Fe 65
4-14 BAFeIII Assay versus Surface Area for Iron Oxide Standards 67
4-15 Microcosm Reducible Fe III with BrY versus Surface Area for Iron Oxide Standards 68
4-16 0.5N HCl Extractable Fe III versus Surface Area for Iron Oxide Standards 69
4-17 6.0N HCl Extractable Fe III versus Surface Area for Iron Oxide Standards. 69
4-18 Citrate Dithionite Bicarbonate Extractable Fe versus Surface Area for Iron Oxide Standards 70
4-19 Ammonium Oxalate Extractable Fe versus Surface Area for Iron Oxide Standards 70
4-20 Total Fe versus Surface Area for Iron Oxide Standards 71
4-21 Summary of Iron Oxide Results 72
4-22 Consumed BAFeIII versus Depth at Elizabeth City – Fuel Farm 75
4-23 Consumed BAFeIII versus TPH + VOC at Elizabeth City – Fuel Farm 75
4-24 Consumed BAFeIII versus Methane at Elizabeth City – Fuel Farm 76
4-25 Consumed BAFeIII versus Fe II at Elizabeth City – Fuel Farm 76
4-26 BAFeIII Mass Balance for Elizabeth City – Fuel Farm 77

List of Tables

1-1 Iron Oxide Standards 11
2-1 Bioavailable Ferric Iron Assay Composition....14
3-1 Performance Objectives for BAFeIII Assay 23
3-2 Sample Summary for BAFeIII Demonstration and Validation 39
3-3 Analyses for BAFeIII Demonstration and Validation 41
4-1 Performance Criteria for BAFeIII Assay 47
4-2 Expected Performance and Performance Confirmation Methods for BAFeIII Assay 49
4-3 Intra-laboratory Precision for the BAFeIII Assay Conducted on Multiple Samples by EPA/Ada....53
4-4 Double-Blind Ferrous Iron Analysis Results....56
4-5 Range of the BAFeIII Assay....57
4-6 Principle Component Analysis Variable Set....58
4-7 Component Loadings 59
4-8 Iron Mass Balance for Elizabeth City Fuel Farm 73
4-9 Consumed BAFeIII and Dissolved CH_4, TPH + VOC, and FE II at Elizabeth City Fuel Farm 74
5-1 BAFeIII Assay Costs 79

Acronyms

%D	Percent difference
%R	Percent recovery
%RSD	Percent relative standard deviation
05FEII	0.5N HCl extractable ferrous iron
05FEIII	0.5N HCl extractable ferric iron
05FETOT	0.5N HCl extractable total iron
6FEII	6N HCl extractable ferrous iron
6FEIII	6N HCl extractable ferric iron
6FETOT	6N HCl extractable total iron
AFB	Air Force Base
ASTM	American Society for Testing Materials
AOFE	Ammonium oxalate extractable iron
AVSFEII	Acid volatile sulfide bound ferrous iron
BAFeIII	Bioavailable ferric iron
BET	Brunauer-Emmett-Teller
BETSA	BET Surface Area
bgs	Below ground surface
BTEX	Benzene-toluene-ethylbenzene-xylenes
BrY	*Shewanella alga* BrY
CDBFE	Citrate dithionite bicarbonate extractable iron
cDCE	cis-dichloroethene
CDM	Camp Dresser & McKee Inc.
CDMAFEII	Ambient ferrous iron measured by CDM
CDMBAFEIII	Bioavailable ferric iron measured by CDM
CRSFEII	Chromium reducible sulfide bound ferrous iron
CV	Coefficient of variation
DCA	Dichloroethane
DCE	Dichloroethene
DFEII	Dissolved ferrous iron
DH	Dissolved hydrogen
DMETH	Dissolved methane
DO	Dissolved oxygen
DoD	U.S. Department of Defense
DS	Dissolved sulfide
EAB	Enhanced anaerobic biodegradation
EMPA	Electron microprobe analyses
EPA	U.S. Environmental Protection Agency
EPAAFEII	Ambient ferrous iron measured by EPA/Ada
EPABAFEIII	Bioavailable ferric iron measured by EPA/Ada
ESTCP	Environmental Security Technology Certification Program
Fe II	Ferrous iron
Fe III	Ferric iron

FEAS	Ferrous ethylene ammonium sulfate
FeRB	Iron-reducing bacteria
g/L	Grams per liter
GIT	Georgia Institute of Technology
HRC	Hydrogen Release Compound
IDW	Investigative derived waste
LAC	Lactate consumption
LCS/LCSD	Laboratory control sample/laboratory control sample duplicate
mL/L	Milliliters per liter
MFE	Microcosm reducible ferric iron
MFEBRY	Microcosm reducible ferric iron with BrY
MFEBRYFEOOH	Microcosm reducible ferric iron with BrY/FeOOH
MFEFEOOH	Microcosm reducible ferric iron with FeOOH
MNA	Monitored natural attenuation
MPNA	Most probable number of aerobes
MPNFeIIIR	Most probable number of ferric iron reducers
MPNMnIVR	Most probable number of MnIV reducers
MPNNR	Most probable number of nitrate reducers
MPNSR	Most probable number of sulfate reducers
MS/MSD	Matrix spike/matrix spike duplicate
msl	Mean sea level
MTBE	Methyl tertiary butyl ether
N	Number of samples
NAPL	Nonaqueous-phase liquid
NAS	Naval Air Station
NATS	Natural Attenuation Test Site
NFESC	Naval Facilities Engineering Services Center
NHD	New Horizons Diagnostics Corporation
O&M	Operations and maintenance
ORP	Oxidation-reduction potential
OU	Operable Unit
PCA	Principal component analysis
PH	pH – negative log of hydrogen ion concentration
ppm	Parts per million
PRBs	Permeable reactive barriers
PWIA	Public Works Industrial Area
QA	Quality assurance
QA/QC	Quality assurance/quality control
RABITT	Reductive Anaerobic *In Situ* Treatment Technology
RL	Reporting limit
RMHEM	Electron microprobe analysis, relative mass percent hematite
RMGEO	Electron microprobe analysis, relative mass percent goethite
RMLIM	Electron microprobe analysis, relative mass percent limonite
RMFES	Electron microprobe analysis, relative mass percent FeSiO4

RMMAG	Electron microprobe analysis, relative mass percent magnetite
RMILM	Electron microprobe analysis, relative mass percent ilmenite
RMPYR	Electron microprobe analysis, relative mass percent pyrite
RMJAR	Electron microprobe analysis, relative mass percent jarosite
RMFEM	Electron microprobe analysis, relative mass percent Fe-metal
RMTIS	Electron microprobe analysis, relative mass percent Ti-Mn Spinel
RMSTA	Electron microprobe analysis, relative mass percent stainless steel
RMMNF	Electron microprobe analysis, relative mass percent Mn-FeOOH
RMCRS	Electron microprobe analysis, relative mass percent Cr-Spinel
RMBRO	Electron microprobe analysis, relative mass percent brookite
RMORG	Electron microprobe analysis, relative mass percent organics
RPD	Relative percent difference
SBIR	Small Business Innovative Research
SC	Specific conductance
SD	Standard deviation
SUBASE	Submarine Base
SCEC	Support Center, Elizabeth City
SVOC	Semivolatile organic compound
TCAAP	Twin Cities Army Ammunition Plant
TCE	Trichloroethene
TDP	Technology Demonstration Plan
TEAPs	Terminal electron accepting processes
TFE	Total iron
TPH	Total petroleum hydrocarbons
UC	University of Colorado
USAF	U.S. Air Force
USCG	U.S. Coast Guard
USCS	Unified Soil Classification System
USGS	U.S. Geological Survey
UST	Underground storage tank
VC	Vinyl chloride
VOC	Volatile organic compound

Preface

This report describes the demonstration and validation of a novel analytical technology: a bioavailable ferric iron (BAFeIII) assay. Demonstration and validation of the BAFeIII assay was conducted at four Department of Defense (DoD) installations.

CDM in cooperation with the Naval Facilities Engineering Services Center (NFESC) was the principal investigator. Several organizations assisted in the validation of the BAFeIII assay, including the U.S. Environmental Protection Agency (EPA), U.S. Geological Survey (USGS), Georgia Institute of Technology (GIT), and University of Colorado (UC). Individuals contributing to completion of this project are listed below:

Carmen Lebron (PI)	NFESC
Barbara Sugiyama	NFESC
Patrick Evans, Ph.D. (Co-PI)	CDM
Mary Trute	CDM
Roger Olsen, Ph.D.	CDM
Rick Chappell, Ph.D.	CDM
John Eisenbeis, Ph.D.	CDM
John Wilson, Ph.D.	EPA
Eric Weber, Ph.D.	EPA
John Kenneke, Ph.D.	EPA
Tom DiChristina, Ph.D.	GIT
John Drexler, Ph.D.	UC
Frank Chapelle, Ph.D.	USGS

This work also would not have been possible without the access to and help from the following DoD installations:

SUBASE Bangor
Ft. Lewis
NAS Pensacola
US Coast Guard Support Center, Elizabeth City

Points of contact for this project are provided in Section 8.

Executive Summary

Monitored natural attenuation (MNA) is a cost-effective remediation approach that is applicable to many sites and, when appropriate, has been advocated by the U.S. Department of Defense (DoD). Natural attenuation is used to mitigate petroleum hydrocarbon, chlorinated hydrocarbon, and metal-contaminated sites as an alternative to pump and treat methods.

Enhanced anaerobic biodegradation (EAB) is another cost-effective remediation approach that is applicable for sites deemed inappropriate for MNA. EAB involves addition of electron donors that stimulate reductive dechlorination of contaminants such as chlorinated hydrocarbons.

Ferric iron (Fe III) is an important terminal electron acceptor with significant assimilative capacity in many natural environments. Dissolved ferrous iron (Fe II) in groundwater is typically measured to assess Fe III reduction and calculate assimilative capacity, but this measurement underestimates this terminal electron accepting process because most Fe II remains bound to the soil. Dissolved Fe II also gives no indication of the amount of Fe III present in aquifer soil that is bioavailable. Bioavailable Fe III (BAFeIII) in the soil must be measured in order to quantify the true assimilative capacity of an aquifer.

Iron-reducing bacteria (FeRB) are known to oxidize or mineralize various organic compounds, such as benzene, toluene, vinyl chloride (VC), and methyl tertiary butyl ether (MTBE), and continued activity over a period of years is dependent on the presence of sufficient BAFeIII. BAFeIII is defined as follows:

> *Ferric iron (Fe III) that is capable of being reduced by microorganisms that oxidize another chemical species and derive energy from the electron transfer.*

Generally several questions arise during demonstration of MNA. Key questions are:

- How much contaminant mass has been consumed historically?
- Is there enough BAFeIII left to sustain MNA?
- Is BAFeIII a sink for electrons produced by oxidation of natural or anthropogenic organic carbon and does it therefore limit elevation of dissolved hydrogen concentrations and in turn prevent complete reductive dechlorination?
- Can iron-reducing bacteria limit migration of vinyl chloride or other incomplete degradation products of reductive dechlorination through promotion of contaminant oxidation?

Additionally, EAB involves the addition of electron donors that can be consumed by iron-reducing bacteria. Key questions with respect to EAB are:

- What is the electron donor demand of BAFeIII and how much electron donor must be added to overcome this demand?

- What limitations are imposed on EAB by iron-reducing bacteria and their effect on establishing of steady-state dissolved hydrogen concentrations sufficient to promote complete reductive dechlorination?

- Can FeRB limit migration of vinyl chloride or other incomplete degradation products of reductive dechlorination commonly observed during EAB?

BAFeIII in the soil must be measured in order to quantify the true assimilative capacity of an aquifer and answer these questions. Not all Fe III present on aquifer soil particles is bioavailable. Weak acid extraction using 0.5N HCl has been used to attempt to quantify BAFeIII, but is not a direct measurement of bioavailability does not necessarily correlate to the concentration of BAFeIII. Other chemical extraction methods including 0.5N HCl-hydroxylamine, 6N HCl, citrate dithionite bicarbonate, and ammonium oxalate have also been considered but are indirect methods as well. Therefore, an alternative, direct method is required to estimate BAFeIII concentrations in soil.

A direct BAFeIII assay was invented and developed by CDM with funding from the US Air Force. This assay is a standardized microcosm that directly measures the concentration of BAFeIII in soil or sediment. Such a test kit for this analyte does not exist elsewhere. The only other method of directly measuring BAFeIII involves setting up microcosms in a fixed-based laboratory with anaerobic microbiology capabilities. This approach is expensive and standardization is virtually impossible since accepted protocols for this analysis do not exist.

A BAFeIII test kit based on the assay is manufactured by New Horizons Diagnostics Corporation (NHD) of Columbia, Maryland. The BAFeIII assay involves addition of a soil sample to a test tube that contains the lyophilized iron-reducing bacterium *Shewanella alga* BrY, lactate as an electron donor, and a mineral salts medium supplemented with reagents that accelerate the assay. The tube is incubated for one month and the ferrous iron that is reductively produced by the iron-reducing bacteria is extracted and measured using a Hach test kit. The ambient concentration of ferrous iron initially present in the soil is measured by similarly extracting ferrous iron from un-incubated soil. The ambient ferrous iron concentration is subtracted from the incubated ferrous iron concentration to obtain the BAFeIII concentration.

The overall objective of this project was to demonstrate and evaluate the BAFeIII assay as an analytical technology for use in supporting MNA and EAB. Specific objectives were to:

- Evaluate the bioavailable ferric iron assay method using a combination of geochemical and microbiological techniques and assessment of available site data.

- Quantify costs associated with the technology.

The objectives were further refined to be:

- Determine the relationship between BAFeIII and 6N HCl extractable Fe III (i.e., total Fe III) in samples from aquifer zones having widely different reducing conditions.

- Quantify the precision of the BAFeIII assay using site samples and iron oxide standards.

- Characterize the relationship between the BAFeIII assay and confirmatory analyses including: 0.5N HCl extractable Fe III, 6N HCl extractable Fe III, ammonium oxalate extractable iron, citrate dithionite bicarbonate extractable iron, microcosm-reducible Fe III in the absence and presence of *Shewanella alga* BrY (the bacteria utilized in the BAFeIII assay kit), and the relative iron oxide composition determined to the extent possible using electron microprobe analysis.

The following initial specific criteria for BAFeIII assay validation were developed based on the project objectives:

- Relationship between results of the BAFeIII assay, the degree of iron oxide crystallinity/surface area, and results measured using microcosms.

- Range as demonstrated by a relative response that is consistent with other analytical techniques used to quantify different iron oxide species.

- Precision as demonstrated by a low relative percent deviation between duplicate analyses.

- Sample throughput as measured by labor time required for assay setup and final analysis.

- Versatility as demonstrated by consistent performance at all test sites.

- Relationship between the BAFeIII assay and 6 N HCl extractable Fe III (i.e., total Fe III) in samples from aquifer zones having widely different reducing conditions.

- Using site samples and iron oxide standards, the relationship between the BAFeIII assay and confirmatory analyses including: 0.5N HCl extractable Fe III, 6N HCl extractable Fe III, citrate dithionite bicarbonate extractable Fe III, ammonium oxalate extractable Fe III, microcosm-reducible Fe III in the absence and presence of *Shewanella alga* BrY, and the relative iron oxide composition determined to the extent possible using electron microprobe analysis.

The following sites were utilized for soil sample collection and demonstration/validation of the BAFeIII assay:

- Bangor Naval Submarine Base in Kitsap County, Washington (SUBASE Bangor) – dissolved petroleum hydrocarbons and chlorinated VOCs.

- Fort Lewis Logistics Center near Tillicum, Washington (Fort Lewis) – chlorinated VOCs.

- Naval Air Station (NAS) in Pensacola, Florida (NAS Pensacola) – chlorobenzene and TCE plumes.

- US Coast Guard Support Center in Elizabeth City, North Carolina – North Beach site containing TCE and Fuel Farm site containing fuel hydrocarbons and MTBE.

CDM conducted BAFeIII assays on all soil samples. The following laboratories conducted confirmatory analyses on the soil samples:

- U.S. EPA, Subsurface Protection and Remediation Division, National Risk Management Research Laboratory, Ada, Oklahoma (EPA/Ada), Dr. John Wilson – BAFeIII assay, citrate dithionite bicarbonate extraction, ammonium oxalate extraction, and total iron analysis of Elizabeth City samples.

- U.S. EPA, Ecosystems Research Division, National Exposure Research Laboratory, Athens, Georgia (EPA/Athens), Dr. Eric Weber – HCl extractions and iron sulfide analyses of all samples.

- Georgia Institute of Technology (GIT), School of Biology, Dr. Thomas DiChristina – Microcosms and most probable number analyses.

- University of Colorado (UC), Geology Department, Dr. John Drexler – Electron microprobe analysis.

The following table presents validation results and indicates that the BAFeIII assay is a precise, easy-to-use analytical method that is capable of direct BAFeIII quantification.

Performance Objectives for BAFeIII Assay

Type of Performance Objective	Primary Performance Criteria	Expected Performance (Metric)	Actual Performance Objective Met?
Qualitative	Relationship between BAFeIII assay and degree of iron oxide crystallinity/surface area.	Positive association	Yes
	Relationship between BAFeIII assay and confirmatory analyses and other BAFeIII factors (total iron, groundwater chemistry, microbial composition).	Positive association	Yes

Performance Objectives for BAFeIII Assay (cont.)

Type of Performance Objective	Primary Performance Criteria	Expected Performance (Metric)	Actual Performance Objective Met?
Qualitative (cont.)	Range of BAFeIII assay relative to other analytical techniques.	Similar or better range	Yes
	Sample throughput of BAFeIII assay.	Labor time ≤ similar methods	Yes
	Versatility of BAFeIII assay.	Consistent performance	Yes
Quantitative	Intra-laboratory precision of BAFeIII assay based on soil and laboratory replicates.	Absolute RPD ≤ 35	Yes
	Inter-laboratory precision of BAFeIII assay based on replicates analyzed by both CDM and EPA/Ada.	-35 ≥ RPD ≤ 35	Yes

A mass balance on iron was conducted for the Elizabeth City Fuel Farm site to further validate the BAFeIII assay and illustrate the use of BAFeIII data. The balance was conducted by calculating the mass of BAFeIII originally present in the area impacted by hydrocarbons, calculating the mass of ferrous iron advectively removed, and then comparing the two values. Results of the mass balance indicated that the 0.5 N HCl, ammonium oxalate, and citrate dithionite bicarbonate extractions all underestimated the mass of BAFeIII that is present in background site soil. The BAFeIII assay did not underestimate this mass and therefore gave a more realistic representation of actual BAFeIII present in soil.

The BAFe III assay purchase cost ranges from $50 to $75 each depending on the quantity purchased. Additional equipment, supplies, and labor are required and the estimated unit analysis cost was calculated to be $212 based on analysis of 6 samples. This cost is about 30 percent greater than the cost for doing a chemical extraction using the synthetic precipitation leaching procedure (SPLP) or toxicity characteristic leaching procedure (TCLP) with zero headspace extraction (ZHE) to minimize ferrous iron oxidation. Thus the BAFeIII assay cost is reasonable.

Use of the direct BAFeIII assay is recommended as a replacement for indirect chemical extraction methods. Additionally, it is recommended that BAFeIII analysis of soil be conducted in addition to ferrous iron analysis in groundwater.

1.0 Introduction

1.1 Background

Monitored natural attenuation (MNA) is a cost-effective remediation approach that is applicable to many sites and has been embraced by the U.S. Department of Defense (DoD). Natural attenuation is used to mitigate petroleum hydrocarbon, chlorinated hydrocarbon, and metal-contaminated sites as an alternative to pump and treat methods.

Determination of the technical applicability of MNA for a given site is based on sampling and analysis, data evaluation, and long-term monitoring. Parameters that are evaluated include concentrations of contaminants, electron acceptors, and electron donors. These concentrations in combination with hydrogeological, soil, and microbiological characteristics are used to assess the fate and transport of contaminants and the potential for MNA.

Concentrations of electron acceptors or their reduced products are typically used to: (1) identify terminal electron accepting processes (TEAPs) responsible for contaminant biodegradation that is occurring in specific areas of a contaminant plume, and (2) quantify assimilative capacity of an aquifer for contaminants of concern. TEAPs affect *in situ* transformation of many pollutants through control of the dissolved hydrogen (DH) concentration in groundwater. Identification and quantification of TEAPs is an important aspect of all site assessments for MNA and bioremediation in general.

Ferric iron (Fe III) is an important terminal electron acceptor with significant assimilative capacity in many natural environments (Lovley, 1991). Dissolved ferrous iron (Fe II) in groundwater is typically measured to assess Fe III reduction and calculate assimilative capacity, but this measurement underestimates this TEAP because most Fe II remains bound to the soil. Dissolved Fe II also gives no indication of the amount of Fe III present in aquifer soil that is bioavailable. Bioavailable Fe III in the soil must be measured in order to quantify the true assimilative capacity of an aquifer.

Bioavailable Fe III is defined as follows:

> *Ferric iron (Fe III) that is capable of being reduced by microorganisms that oxidize another chemical species and derive energy from the electron transfer.*

Iron-reducing bacteria (FeRB) are known to oxidize or mineralize various organic compounds, such as benzene, toluene, vinyl chloride (VC), and methyl tertiary butyl ether (MTBE), and continued activity over a period of years is dependent on the presence of sufficient bioavailable ferric iron (BAFeIII). Bioavailable ferric iron in the soil must be measured in order to quantify the true assimilative capacity of an aquifer. Not all Fe III present on aquifer soil particles is bioavailable. Weak acid extraction using 0.5N HCl has been used to attempt to quantify BAFeIII, but is not a direct measurement of bioavailability and does not necessarily correlate with the total amount of BAFeIII (Evans, 2000; Forstner, 1993; Lovley and Phillips, 1986;

Lovley and Phillips, 1987). Other chemical extraction methods including 0.5N HCl-hydroxylamine, 6N HCl, citrate dithionite bicarbonate, and ammonium oxalate have also been considered but are indirect methods as well. Therefore, an alternative, direct method is required to estimate BAFeIII concentrations in soil.

A direct BAFeIII assay was invented and developed by CDM with funding from the US Air Force. A test kit for this analyte does not exist elsewhere. The only other method of directly measuring BAFeIII involves setting up microcosms in a fixed-based laboratory with anaerobic microbiology capabilities. This approach is expensive and standardization is virtually impossible since accepted protocols for this analysis do not exist.

Standardized and cost-effective analytical technologies to support MNA efforts are necessary. The DoD is responsible for approximately 2,093 characterized chlorinated solvent plumes. MNA is applicable to approximately 29% of chlorinated solvent sites, or 420 of the DoD plumes. Enhanced anaerobic biodegradation (EAB) may also be applicable to many of these sites. BAFeIII analysis is not currently used because of difficulty and cost. This technology will benefit the DoD by making this analysis available, which will promote application of MNA and EAB at these sites. The average cost for a pump and treat operation is $4.2 million per site. If MNA is applied to 25% of the chlorinated plumes (~100 sites) at a cost of $1 million per site, the potential savings could reach $320 million (CDM, 2001).

1.2 Objectives of the Demonstration

The overall objective of this project was to demonstrate and evaluate the BAFeIII assay as an analytical technology for use in supporting MNA and enhanced anaerobic biodegradation (EAB). Specific objectives were to:

- Evaluate the bioavailable ferric iron assay method using a combination of geochemical and microbiological techniques and assessment of available site data.

- Quantify costs associated with the technology.

The initial demonstration plan was to conduct field testing at six DoD sites across the country, including: Laurel Bay Exchange – Marine Corps Air Station in South Carolina; Fort Lewis and Submarine Base (SUBASE) Bangor in Washington; Dover Air Force Base (AFB) in Delaware; Naval Air Station (NAS) Pensacola in Florida; and either Cape Canaveral in Florida or Moody AFB in Georgia. In July, 2002, a revised demonstration plan was initiated based on preliminary findings at the first three of these sites investigated, SUBASE Bangor, Fort Lewis, and NAS Pensacola.

Results of the BAFeIII assay for soil samples collected from SUBASE Bangor and Ft. Lewis were quite similar. Likewise, the acid extraction and microcosm confirmatory analysis results showed little variation among different samples. The reason for this lack of variation was concluded to be due to little variation in TEAPs in the aquifer locations where the samples were

collected. No discernable correlations between various parameters were observed because of sample similarity.

In contrast, preliminary results from NAS Pensacola showed correlations between BAFeIII and other parameters including 0.5N HCl extractable Fe III, 6N HCl extractable Fe III, and microcosm-reducible Fe III. The reason correlations were observable at NAS Pensacola was attributed to a significant variation in the concentration of total iron in collected samples. Many samples were beach sand-like and contained very little iron. Other samples were brown to red and clearly contained greater amounts of iron.

Dr. John Wilson of the EPA had independently conducted BAFeIII analyses using the CDM BAFeIII test kit on archived soils from the Thermochem Superfund site in Muskegon, Michigan. His preliminary results showed correlations between BAFeIII and other parameters including proximity to the water table, concentration of electron donors, and extractable iron (unpublished results). Based on these encouraging results and discussions with ESTCP and Drs. John Wilson and Eric Weber, also of the EPA, a revised validation approach was proposed. This approach was based on validation of the BAFeIII assay using samples collected from the well-characterized Thermochem site which has a variety of TEAPs. In addition, the revised approach included testing of iron oxide standards of varying and known surface area and bioavailability. Through the use of these material sources, a validation based on real-world samples in combination with well-defined standards was possible.

The objectives of the revised validation approach were to:

- Determine the relationship between BAFeIII and 6N HCl extractable Fe III (i.e., total Fe III) in samples from Thermochem site aquifer zones having widely different reducing conditions.

- Quantify the precision of the BAFeIII assay using Thermochem site samples and iron oxide standards.

- Using Thermochem site samples and iron oxide standards, characterize the relationship between the BAFeIII assay and confirmatory analyses including: 0.5N HCl extractable Fe III, 6N HCl extractable Fe III, microcosm-reducible Fe III in the absence and presence of *Shewanella alga* BrY (the bacteria utilized in the BAFeIII assay kit), and the relative iron oxide composition determined to the extent possible using electron microprobe analysis.

Technical reviewers for ESTCP suggested that two additional confirmatory analyses be conducted on samples including citrate dithionite bicarbonate extraction and ammonium oxalate extraction.

Field work at the Thermochem site was to be conducted in November, 2002. Unfortunately, the Thermochem site was not available, so the USCG Support Center in Elizabeth City, North

Carolina was utilized instead. This site has also been well characterized by Dr. Wilson and has a variety of TEAPs. Samples were collected from two different areas:

- **Fuel Farm Area –** Samples containing petroleum hydrocarbons and MTBE were collected from a background area (3 depths), a hot zone (2 depths), and two down gradient areas (3 depths each) for a total of 11 locations, each collected and analyzed in duplicate (22 total analyses).

- **North Beach Area –** Samples containing chlorinated volatile organic carbon (VOC) compounds, primarily trichloroethene (TCE), were collected from a background area (4 depths), and in the vicinity of two monitoring wells: GW3-30 (3 depths) and MW-1 (4 depths), for a total of 11 locations, each collected and analyzed in duplicate (22 total analyses).

The objectives of the revised demonstration plan utilizing the Elizabeth City site instead of the Thermochem site, described above, were maintained. All samples were homogenized in the field, sample splits were created, and the splits were sent to the following locations for the stated analyses:

- **U.S. EPA, Subsurface Protection and Remediation Division, National Risk Management Research Laboratory, Ada, Oklahoma (EPA/Ada), Dr. John Wilson -** All groundwater and soil. Groundwater analyses included PCE, TCE, cis-DCE, VC, methane, ethene, ethane, BTEX, MTBE, nitrate, and ferrous iron. Soil analyses included BAFeIII using the test kit, chemical extractions with citrate dithionite bicarbonate and ammonium oxalate, and total iron. One third of the sample splits were analyzed for extractable iron and total iron.

- **U.S. EPA, Ecosystems Research Division, National Exposure Research Laboratory, Athens, Georgia (EPA/Athens), Dr. Eric Weber –** Soil only. Analyses included 0.5N HCl extractable Fe II and Fe III, 6N HCl extractable Fe II and Fe III, volatile sulfide-bound Fe II, and pyrite Fe II. All sample splits were analyzed.

- **Georgia Institute of Technology (GIT), School of Biology, Dr. Thomas DiChristina –** Soil only. Analyses included microcosm reducible Fe III in the presence and absence of *Shewanella alga* BrY conducted on half of collected samples and their splits.

- **University of Colorado (UC), Geology Department, Dr. John Drexler –** Soil only. Analyses included electron microprobe analysis (EMPA) of collected soil samples and their splits. Analysis was used for qualitative evaluation of soil mineral types only.

- **CDM –** Soil only. BAFeIII (using the test kit) was conducted on all soil samples and their splits.

The iron oxide standards were synthesized by Dr. B.T. Thomas at EPA/Athens. The iron oxides were sent to the University of Iowa from EPA/Athens for Brunauer-Emmett-Teller (BET) surface area characterization of the iron oxides. The iron oxide standards and the corresponding BET results are summarized in **Table 1-1**.

Table 1-1: Iron Oxide Standards

Iron Oxide	Surface Area (m^2/g) [1]
2-Line Ferrihydrite [$Fe(OH)_3$]	233.2
6-Line Ferrihydrite [$Fe(OH)_3$]	177.6
Lepidocrocite (γ-FeOOH)	41.4
Goethite (α-FeOOH)	29.2
Hematite (α-Fe_2O_3)	34.8
Magnetite (Fe_3O_4)	18.2

[1] Brunauer-Emmett-Teller (BET) method.

Each of the oxides in **Table 1-1** was analyzed in duplicate by each laboratory. The analysis of the iron oxides by the BAFeIII assay and the confirmatory analyses were used to further validate the BAFeIII assay.

1.3 Regulatory Drivers

The analyte (BAFeIII) of interest in this demonstration is discussed in the EPA technical guidance on natural attenuation of chlorinated solvents (U.S. EPA, 1998). Analysis of BAFeIII is not required at this time and is considered optional by regulatory agencies. Additionally, no method for BAFeIII has been approved by any regulatory agency.

In practice, demonstration of MNA requires three basic lines of evidence. The first of these is direct evidence of contaminant removal. The second is geochemical indicators and electron balances used to indirectly demonstrate contaminant removal or potential for removal. And the third is microcosms used to demonstrate contaminant removal in a laboratory setting. BAFeIII primarily and directly relates to the second line of evidence, but also allows documentation of contaminant mass removal (first line). It is also potentially important with respect to control of DH and its effect on reductive dechlorination, EAB, and consumption of electron donors. Although no current regulatory requirement exists for BAFeIII, its measurement is an important consideration for the demonstration of MNA.

1.4 Stakeholder/End-User Issues

Generally several questions arise during demonstration of MNA. Key questions are:

- How much contaminant mass has been consumed historically?
- Is there enough BAFeIII left to sustain MNA?

- Is BAFeIII a sink for electrons produced by oxidation of natural or anthropogenic organic carbon and does it therefore limit elevation of dissolved hydrogen concentrations and in turn complete reductive dechlorination?

- Can iron-reducing bacteria limit migration of vinyl chloride or other incomplete degradation products of reductive dechlorination by promoting oxidative biodegradation of VC?

Additionally, EAB involves the supplementation of electron donors that can be consumed by iron-reducing bacteria. Key questions with respect to EAB are:

- What is the electron donor demand of BAFeIII and how much electron donor must be added to overcome this demand?

- What limitations are imposed on EAB by iron-reducing bacteria and their effect on establishment of steady-state dissolved hydrogen concentrations sufficient to promote complete reductive dechlorination?

- Can iron-reducing bacteria limit migration of vinyl chloride or other incomplete degradation products of reductive dechlorination commonly observed during EAB?

The BAFeIII assay is an important tool for answering these questions.

2.0 Technology Description

2.1 Technology Development and Application

The BAFeIII assay kit is pictured in **Figure 2-1**. The kit is manufactured by New Horizons Diagnostics Corporation (NHD) of Columbia, Maryland. The BAFeIII assay involves addition of a soil sample to a test tube that contains the lyophilized iron-reducing bacterium *Shewanella alga* BrY, lactate as an electron donor, and a mineral salts medium supplemented with reagents that accelerate the assay. The assay composition is presented in **Table 2-1**.

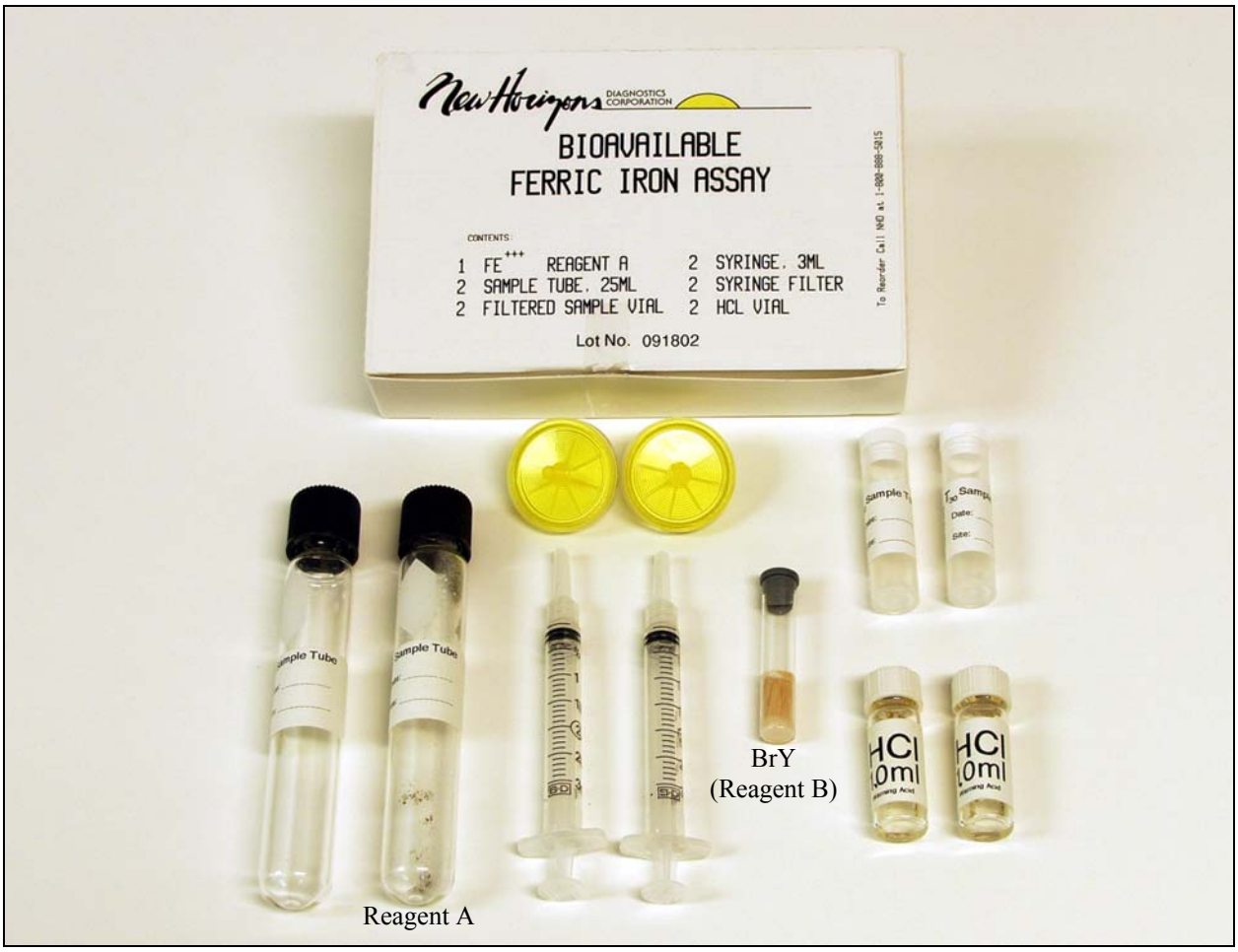


Figure 2-1. BAFeIII Assay Kit

Table 2-1: Bioavailable Ferric Iron Assay Composition

Component	Concentration (g/L)
Shewanella alga BrY	--
Sodium lactate	4.5
KH_2PO_4	2.2
K_2HPO_4	2.4
NH_4Cl	1.5
KCl	0.1
Humic acids, sodium salt [1]	1.0
AQDS [1]	0.041
$CaCl_2$-$2H_2O$	1.0
pH	6.5
NTA Nitrilotriacetic acid, trisodium salt	0.015
$MgSO_4 \cdot 7H_2O$	0.030
$MnSO_4 \cdot H_2O$	0.005
NaCl	0.010
$FeSO_4 \cdot 7H_2O$	0.001
$CoCl_2 \cdot 2H_2O$	0.001
ZnCl	0.0013
$CuSO_4$	0.0001
$AlK(SO_4)_2 \cdot 12H_2O$	0.0001
H_3BO_3	0.0001
Na_2MoO_4	0.00025
$NiCl_2 \cdot 6H_2O$	0.00024
$Na_2WO_4 \cdot 2H_2O$	0.00025

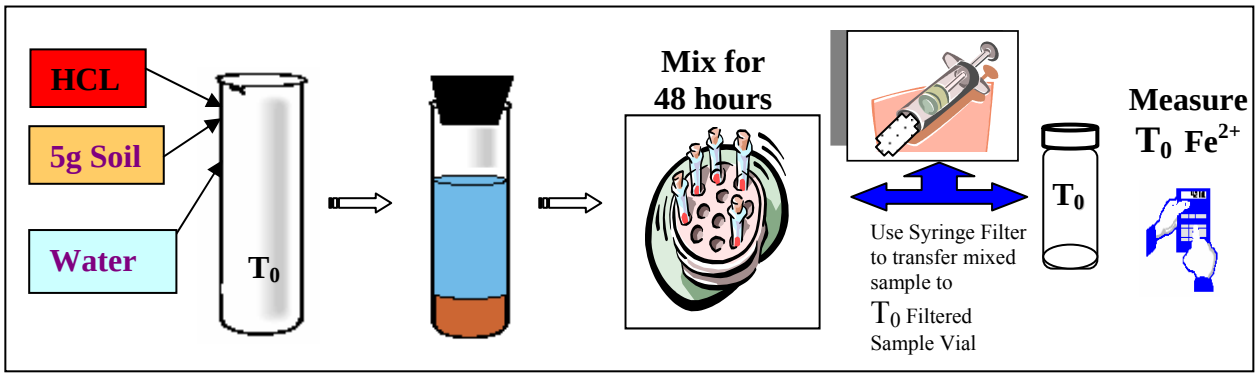
[1] Aldrich H1,675-2.
[2] Anthraquinone-1,5-disulfonic acid, disodium salt, 95% (Aldrich A9,040-3).

The BAFeIII assay composition was developed under a Small Business Innovative Research (SBIR) grant from the USAF. Further details of the BAFeIII assay development are provided in Section 2.2.

Soil samples are typically collected from the saturated zone. A four-ounce bottle of soil is more than sufficient for the BAFeIII assay. Bottles should be filled with water-saturated soil and kept refrigerated until analysis. Recommended holding times for soil samples have not been determined; preferably, analyses should be initiated within one week of sample collection.

If necessary to remove large gravel or debris from the soil sample, the soil sample should be wet-sieved through a 3/16-inch sieve. The procedure for the BAFeIII assay is graphically illustrated in **Figure 2-2**.

STEP 1:



STEP 2:

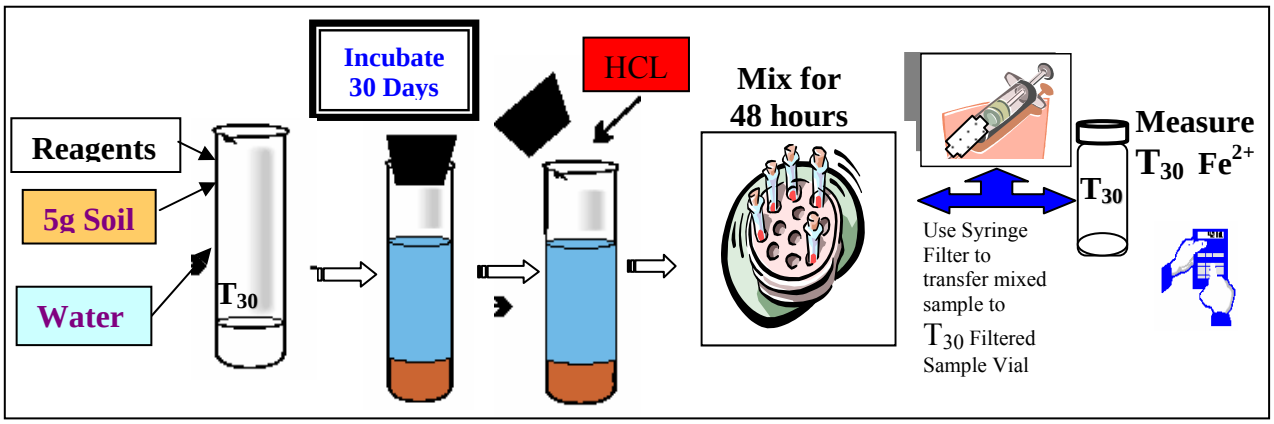


Figure 2-2. BAFeIII Assay Kit Procedure

Following homogenization, two 5-gram subsamples are placed into each of two 25-mL assay tubes labeled T_0 and T_{30}. The T_0 tube, which is used to determine the initial or ambient concentration of Fe II present in the soil immediately following sample collection, contains no reagents or BrY, is filled with distilled water and 1 mL concentrated HCl, capped, then placed on a tube rotator for 48 hours, during which time weakly associated Fe II is extracted from the soil. Following the extraction period, the T_0 extract liquid is filtered, if necessary, and analyzed for initial Fe II. The T_{30} tube is filled with distilled water plus the assay reagents (**Table 2-1**), capped, mixed by hand, and then incubated in the dark at room temperature for 30 days. During the incubation period the iron-reducing bacteria (i.e., BrY) consume lactate and reduce any bioavailable Fe III in the soil to Fe II. Following the incubation period, 1 mL of liquid is withdrawn from the T_{30} tube, discarded, replaced with 1 mL concentrated HCl to create a 0.5N HCl solution, then the tube is placed on a tube rotator for 48 hours, during which time both initial Fe II and Fe II produced by biological Fe III reduction is solubilized. Following the extraction period, the T_{30} liquid is filtered, if necessary, and analyzed for Fe II. The concentration of Fe II

in the T_{30} extract liquid is the total Fe II, and is the sum of the ambient Fe II (T_0 tube) and the BAFeIII. The following formula is used to convert the extract liquid Fe II concentration to initial Fe II (T_0 tube) or initial plus bacterial-reduced Fe II (T_{30} tube):

$$C_S = \frac{C_E\left(V_T - \frac{M_S}{D_S}\right)}{1000\, M_S F_S}$$

where:

- C_S = Equivalent concentration of Fe II in the soil (mg/kg dry soil).
- C_E = Measured concentration of Fe II in the extract liquid (mg/L).
- V_T = Volume of the assay tube that is filled with soil and liquid (25 mL).
- M_S = Mass of soil assayed (5 g).
- D_S = Soil particle density (g/mL). Measured separately or 2.6 g/mL can be assumed.
- F_S = Solids fraction (g dry soil/g wet soil). Measured separately or ignored if results expressed per kg wet soil are acceptable.

BAFeIII is determined as the difference between C_S in the T_{30} tube and C_S in the T_0 tube:

$$\text{BAFEIII (mg/kg)} = (C_S \text{ in } T_{30}) - (C_S \text{ in } T_0)$$

This equation can also be expressed in terms of the extract liquid Fe II concentrations (C_E):

$$\text{BAFEIII(mg/kg)} = \frac{(C_E \text{ in } T_{30}) - (C_E \text{ in } T_0)}{217\, F_S}$$

Extract liquid Fe II concentrations (C_E) can be measured colorimetrically using several different organic complexing reagents, including 1,10-phenanthroline and ferrozine in the absence of a reducing agent. The phenanthroline method is Standard Method 3500-Fe B (Greenberg et al., 1992) and is available as a Hach test kit (Hach Company, Method 8146). The Hach kit is recommended for use with the BAFeIII kit sold by NHD. Since the concentration of Fe II in the liquid extract may exceed the analytical range for the colorimetric phenanthroline method, dilution of the extract is typically required. Dilution is also required to eliminate effects of the 0.5N HCl which interferes with the Hach assay. **Appendix A** includes the NHD BAFeIII test kit protocol.

As described in Section 1, the BAFeIII assay can be used for site characterization and monitoring for MNA and EAB applications. Bioavailable ferric iron is a significant terminal electron acceptor supporting the oxidative biodegradation of contaminants in numerous MNA scenarios. Natural attenuation of benzene-toluene-ethylbenzene-xylenes (BTEX) is one common example. Initial site characterization for MNA involves the calculation of assimilative capacity of an aquifer for biodegradation of BTEX. Typically this involves measurement of dissolved

concentrations of oxygen, nitrate, Fe II, sulfate, and methane upgradient of the BTEX plume and in the plume. A quantitative determination of BTEX biodegradation potential by each TEAP can be calculated through this process. However, measurement of dissolved Fe II can dramatically underestimate the amount of iron reduction that may be occurring in an aquifer and thus the amount of BTEX that is being biodegraded. Furthermore, the dissolved Fe II concentration gives no indication of the amount of the Fe III reserve in the aquifer material that can support BTEX biodegradation in the future. Measurement of BAFeIII is the only way to measure this reserve and in doing so determine the total mass of BTEX that can be biodegraded in the aquifer. Such measurement can be conducted during initial site characterization by measuring BAFeIII upgradient of the plume and in the plume. Assay results can include both the ambient Fe II concentration in the soil and the BAFeIII in the soil. The ambient soil Fe II would be expected to be greater in the plume than upgradient of the plume because of more Fe III reduction. Similarly, the soil BAFeIII would be expected to be less in the plume than upgradient of the plume. These results can be used to determine the mass of BTEX that has been degraded previously and the potential for future BTEX biodegradation.

Monitoring of ongoing natural attenuation is another application of the BAFeIII assay. The concentration of BAFeIII can be measured some time after the initial characterization and then compared to the initially determined concentration. This comparison can be used to determine the amount of BAFeIII that has been consumed over time and the amount of BAFeIII that remains for continued BTEX biodegradation. Continued knowledge that the reserve of BAFeIII is sufficient to maintain a steady state or shrinking BTEX plume is of interest to regulators, site owners, and the public.

BAFeIII can also affect reductive dechlorination in MNA and EAB applications. Reductive dechlorination is based on chlorinated compounds such as trichloroethene (TCE) serving as a terminal electron acceptor. Complete dechlorination of TCE to ethene requires that each dechlorination product, i.e., cis-dichloroethene (cDCE) and vinyl chloride (VC), also serve as terminal electron acceptors. Terminal electron acceptors will be used preferentially according to thermodynamic and kinetic considerations. For example, VC may be dechlorinated to ethene under methanogenic conditions (and correct microbial populations) but not under aerobic or denitrifying conditions because the free energies for reduction of oxygen and nitrate are greater (i.e., more negative) than for reduction of VC. The free energy for reduction of several BAFeIII oxides is greater than that for reductive dechlorination of cDCE to VC (Evans and Koenigsburg, 2001). Thus, limited BAFeIII can result in TCE being reductively dechlorinated to cDCE only and further reductive dechlorination can be inhibited. Thus knowledge of the BAFeIII concentration can indicate the potential for complete reductive dechlorination of TCE. It can also be used for planning EAB remedies. If the BAFeIII concentration is sufficient to inhibit cDCE reductive dechlorination, reductive dechlorination of TCE to cDCE and VC followed by oxidative biodegradation of VC and possibly cDCE under iron-reducing conditions may be a better approach.

A challenge in applying BAFeIII results is that insufficient experience exists to use the results in quantitative models at this time. The results from the assay, however, can be used in either a

quantitative or qualitative manner. The BTEX example above represents a quantitative application of BAFeIII assay results. The enhanced anaerobic bioremediation application represents a qualitative application of the assay. Experience using the assay results in a qualitative fashion will, in time, lead to more quantitative applications as a database is developed. An example of a potential application is incorporation of BAFeIII as a variable in biodegradation computer modeling programs such as the EPA program BIOPLUME IV planned for release winter, 2004 (John Wilson, personal communication). BIOPLUME is a 2D, finite difference model for simulating the natural attenuation of organic contaminants in groundwater due to the processes of advection, dispersion, sorption, and biodegradation. The BIOPLUME program uses an USGS solute transport code and kinetic equations to determine the fate and transport of the organic contaminants and the electron acceptors (dissolve oxygen, nitrate, Fe III, sulfate, and carbon dioxide) and the reaction by-products (including dissolved Fe II).

2.2 Previous Testing of the Technology

Initial development and preliminary field-testing of the BAFeIII assay technology was conducted under a Phase I Small Business Innovative Research (SBIR) grant from the USAF. The focus of this work was on identifying and characterizing critical factors that control microbial Fe III reduction and developing a prototype bioassay. Identification of factors was based on a literature search and discussions with experts in the field of FeRB. Characterization involved completion of a factorial experimental design to evaluate several factors and their interactions simultaneously. The results of the factorial experiment were then used to formulate an optimal bioassay medium. The ability of this optimal medium to enhance microbial Fe III reduction was validated through the use of column studies, which confirmed that both iron oxide crystallinity and the presence of critical factors in the groundwater were important with respect to Fe III bioavailability. The use of this optimal medium was shown to greatly enhance the speed of the bioassay. The bioassay was statistically evaluated and then tested using actual soil and groundwater samples obtained from three fuel-contaminated sites: a jet fuel spill site in Hanahan, South Carolina; the USAF Natural Attenuation Test Site (NATS) in Columbus, Mississippi; and a crude oil spill site in Bemidji, Minnesota. The bioassay was determined to have an acceptable linear range, to have sufficient sensitivity for the soil samples, and have good reproducibility based on replicate measurements. Detailed results of the Phase I SBIR are provided by Evans (1997) and are summarized in Evans (2000).

Further development and field-testing of the technology was conducted under Phase II of the SBIR, which led to development of a field test kit. Field-testing was conducted at eight sites including Hill Air Force Base, the USAF NATS, and the Marine Corps Air Station at Laurel Bay. Other sites included industrial and commercial sites. Field testing focused on identification of operational problems with the prototype. These problems were corrected and a new version of the technology was delivered to the Air Force. Results of the Phase II work are provided by Evans and Jones (1999) and Evans et al. (1999). Additional evaluation of the importance of BAFeIII with respect to reductive dechlorination and EAB are presented in Evans and Koenigsberg (2001).

2.3 Factors Affecting Cost and Performance

The main factor affecting the cost of the BAFeIII assay is the cost of collecting soil samples, which usually requires the drilling of borings at key locations at a site. The number of sample locations necessary will depend on the complexity and size of the site. The cost of the analysis is primarily dependent on the test kit price and the labor used to conduct the test. These costs are further discussed in Section 5.

The BAFeIII assay contains lyophilized live bacteria that must be stored frozen. Storage of the assay at room temperature or use beyond the expiration date likely will result in poor performance because of viability loss. Toxic levels of heavy metals or other contaminants in site soil can potentially inhibit or kill the bacteria in the assay. However, if inhibitory concentrations of toxic compounds are present, they probably limit the applicability of MNA or bioremediation at the site anyway.

The BAFeIII assay is a practical method based on standardization of reagent composition and procedures. Assay results are dependent on incubation time and extraction procedures. The recommended incubation time is one month. Lesser or greater incubation times are not recommended based on laboratory evaluations conducted previously (Evans, 1997; Evans, 2000). Extraction must be conducted for the 48-hour time period as well using a tube rotator to promote mixing. Deviation from these procedures can result in poor precision and accuracy. As with most analyses of this type, performance is also affected by operator skill and experience.

The performance of the BAFeIII assay is also affected by soil heterogeneity: the more heterogeneity, the less likely a single BAFeIII assay measurement will be representative of overall site conditions. Heterogeneity is a common problem affecting analysis of soil and is not unique to the bioavailable ferric iron assay. For example, heterogeneity is often observed during sampling and analysis of soil for heavy metals. This performance limitation, however, can be minimized by collection and analysis of a larger number of samples, so as to provide a more representative distribution of conditions at complex sites, combined with replicates to enable evaluation of the degree of heterogeneity. In general, soil samples for BAFeIII assay should be homogenized prior to distribution and analysis unless discrete sample analysis is desired.

2.4 Advantages and Limitations of the Technology

Measurement of BAFeIII is important because it is generally the most abundant potential electron acceptor for organic matter oxidation in most soils and sediments, before anaerobic conditions develop (Lovley, 1991). Dissolved Fe II in groundwater is often measured to estimate total iron reduction. However, this method significantly underestimates this TEAP because most Fe II remains bound to the soil (Lovley, 1991). Dissolved Fe II also gives no indication of the amount of Fe III present in aquifer soil that is bioavailable for future reduction.

BAFeIII data allow site managers and regulators to evaluate MNA and EAB at sites more completely and accurately than with dissolved Fe II data alone. In the case of BTEX natural attenuation, dissolved Fe II data allow calculation of the mass of BTEX that has been

biodegraded historically and is being biodegraded currently. BAFeIII data allow calculation of the mass of BTEX that will be biodegraded in the future. It is impossible to calculate future potential for BTEX biodegradation using dissolved Fe II data alone. Furthermore, since most dissolved Fe II remains bound to the soil, the historical and current mass of biodegraded BTEX is underestimated using dissolved Fe II data for electron acceptor calculations. Completion of a mass balance and subsequent understanding of contaminant source fate is dependent on accurate electron acceptor calculations. In the case of EAB of TCE, BAFeIII data allow determination of the total electron donor demand. High electron donor demand can decrease the likelihood that TCE will be reductively dechlorinated beyond cDCE to VC and ethene upon addition of an electron donor such as molasses or HRC (Evans and Koenigsburg, 2001). High electron donor demand can also prevent complete reductive dechlorination under MNA conditions. Dissolved Fe II data alone give no indication of this electron donor demand.

While the BAFeIII assay provides these advantages over measurement of dissolved Fe II, it does depend on soil sampling in the saturated zone, which is costly and inconvenient for routine sampling. On the other hand, measurement of BAFeIII in soil likely does not require quarterly sampling of numerous locations. This decreased sampling frequency can minimize the additional cost associated with soil sample collection.

The BAFeIII assay evaluated in this report is in a sense a standardized microcosm study. Besides being the first of its kind, the assay has many advantages that make it an easy-to-use and reliable analytical tool. Unlike laboratory-based microcosm studies which are typically research projects, it is standardized, self-contained, portable, packaged for field or laboratory use, and includes lyophilized FeRB that are relatively stable. Care must be taken to store the lyophilized FeRB under freezing conditions for stability. The bioassay reagents other than FeRB (**Table 2-1**) are packaged separately from the FeRB and are stable at room temperature. These chemical components are present at optimal levels and are known to influence bioavailability. Their presence is intended to provide reproducible, standardized, and direct estimates of the maximum concentration of BAFeIII in a given soil sample. Recognition that the assay results are maximum values should be considered when using the data.

A potential limitation of the BAFeIII assay is that the indigenous FeRB may be different in their iron-reducing capabilities when compared to the strain used in the assay (i.e., *Shewanella alga* BrY). The assay may overestimate Fe III bioavailability if BrY is capable of greater iron reduction than indigenous FeRB. Inclusion of BrY in the assay was intended to make the assay standardized and reproducible. Additionally, since BrY is a facultative microorganism, storage under anaerobic conditions is not necessary, further increasing the test kit's ease-of-use. Thus the decision whether or not to use BrY in the bioassay represents a trade-off of obtaining site-specific results versus standardization, reproducibility, and ease-of-use. If results using only indigenous bacteria are desired, the BrY culture can be easily left out of the assay since it is packaged separately. Iron reduction would then be accomplished via FeRB that are indigenous to the soil sample used in the assay. A new limitation would be introduced by conducting the assay in this manner, however, since the required incubation time would be unknown. Monitoring of the assay over time would be required which would decrease the ease-of-use of

the assay. Direct comparison of BAFeIII results to results for other sites would also not be possible.

Another potential limitation of the BAFeIII assay involves the one-month incubation time. However, considering that standard turnaround time for most analytical laboratories is two weeks, this time requirement is probably acceptable in most cases.

Finally, no standardized technologies exist for directly measuring BAFeIII. Chemical extraction, sophisticated instrument-dependent methods, and microcosm studies have been evaluated, but each have significant disadvantages. Selective extraction using a variety of extractants, including various concentrations of HCl, hydroxylamine-HCl, ammonium oxalate, citrate, citrate dithionite bicarbonate and other compounds has been used to attempt to quantify BAFeIII. However, these extractants do not provide direct measurements and do not necessarily correlate to the concentration of BAFeIII (Lovley and Phillips, 1987). Also, extraction methods do not take into account the effect of groundwater chemistry on bioavailability. A laboratory method for BAFeIII quantitation involving redox titration of soil with the reduced form of AQDS also known as anthraquinol disulfonate (AHDS) has been evaluated (Hacherl et al., 2001). This method is also an indirect measurement of BAFeIII. Sophisticated instrumentation, including electron microscopy, electron microprobe analysis, near infrared spectrophotometry, and Mossbauer spectroscopy have been evaluated but are not especially useful. Furthermore, these techniques are expensive and not readily available. Microcosm studies have been conducted in various laboratories but with different methods and media. While microcosm studies are a direct approach to evaluation of BAFeIII, no standard method exists for conducting them; they are also time-consuming and expensive. Therefore, the major advantage of the BAFeIII assay over other methods is that it is a standardized, direct measurement of BAFeIII.

3.0 Demonstration Design

3.1 Performance Objectives

The BAFeIII assay is difficult to validate because no standard method exists to measure bioavailability of ferric iron. Nevertheless, performance criteria were developed *a priori* in order to be able to validate the BAFeIII assay. These criteria were based initially on the demonstrated relationship between Fe III bioavailability and Fe III oxide particle surface area (Roden and Zachara, 1996). Different Fe III oxides ranging from amorphous ferric oxyhydroxide to various crystalline forms have different specific surface areas. Oxides with greater specific surface area (amorphous oxides having the greatest) have been shown to be more bioavailable for iron reduction (Roden and Zachara, 1996). Thus the initial working hypothesis of the evaluation was that the BAFeIII concentration determined by the assay should correlate to the specific surface area of the oxide particles in a soil sample. In addition, other factors associated with groundwater may influence Fe III bioavailability (Evans, 2000; Roden and Urrutia, 2002) including:

- pH
- Specific conductivity
- Divalent cations
- Electron shuttles such as humic acids
- Chelators
- Adsorbed anions including ferrous iron

Based on the multiple factors that could affect BAFeIII, the following initial specific criteria were used in the evaluation:

- Relationship between results of the BAFeIII assay, the degree of iron oxide crystallinity/surface area, and results measured using microcosms.

- Range as demonstrated by a relative response that is consistent with other analytical techniques used to quantify different iron oxide species.

- Precision as demonstrated by a low relative percent deviation between duplicate analyses.

- Sample throughput as measured by labor time required for assay setup and final analysis.

- Versatility as demonstrated by consistent performance at all four sites.

These criteria were augmented based on results obtained at SUBASE Bangor, Ft. Lewis, and NAS Pensacola, and the revised demonstration plan. Additional criteria established based on the revised demonstration plan included:

- Relationship between the BAFeIII assay and 6 N HCl extractable Fe III (i.e., total Fe III) in samples from Elizabeth City aquifer zones having widely different reducing conditions.

- Precision of the BAFeIII assay using Elizabeth City site samples and iron oxide standards.

- Using Elizabeth City site samples and iron oxide standards, the relationship between the BAFeIII assay and confirmatory analyses including: 0.5N HCl extractable Fe III, 6N HCl extractable Fe III, citrate dithionite bicarbonate extractable Fe III, ammonium oxalate extractable Fe III, microcosm-reducible Fe III in the absence and presence of *Shewanella alga* BrY, and the relative iron oxide composition determined to the extent possible using electron microprobe analysis.

Table 3-1 summarizes the performance objectives established for this demonstration.

Table 3-1: Performance Objectives for BAFeIII Assay

Type of Performance Objective	Primary Performance Criteria	Expected Performance (Metric)	Actual Performance Objective Met?
Qualitative	Relationship between BAFeIII assay and degree of iron oxide crystallinity/surface area.	Positive association	Yes
	Relationship between BAFeIII assay and confirmatory analyses and other BAFeIII factors (total iron, groundwater chemistry, microbial composition).	Positive association	Yes
	Range of BAFeIII assay relative to other analytical techniques.	Similar or better range	Yes
	Sample throughput of BAFeIII assay.	Labor time ≤ similar methods	Yes
	Versatility of BAFeIII assay.	Consistent performance	Yes
Quantitative	Intra-laboratory precision of BAFeIII assay based on soil and laboratory replicates.	Absolute RPD ≤ 35	Yes
	Inter-laboratory precision of BAFeIII assay based on replicates analyzed by both CDM and EPA/Ada.	-35 ≥ RPD ≤ 35	Yes

3.2 Selection of Test Sites

Technical and administrative data associated with contaminated areas at each of the six DoD sites originally planned for inclusion in this demonstration were acquired and reviewed. Sites were selected based on this information. Specific information included:

- Availability of an existing groundwater monitoring well network.
- Geological and hydrogeological characteristics.
- TEAPs occurring in the aquifer.
- Concentrations of parent compounds and presence of daughter products.
- Groundwater chemistry.
- Ability to drill on site.
- Availability and quality of existing site characterization documentation.

The objective was to select sites that offered a range of DH and iron concentrations, geochemical characteristics, and TEAPs, to enable validation of the DH analyzer (presented in a separate report) and the BAFeIII assay. Originally, six DoD sites were selected (or proposed) for field testing, including:

- Bangor Naval Submarine Base in Kitsap County, Washington (SUBASE Bangor) – dissolved petroleum hydrocarbons and chlorinated VOCs.
- Fort Lewis Logistics Center near Tillicum, Washington (Fort Lewis) – chlorinated VOCs.
- Naval Air Station (NAS) in Pensacola, Florida (NAS Pensacola) – chlorobenzene and TCE plumes.
- Dover AFB in Dover, Delaware (Dover AFB) – chlorinated VOCs.
- Laurel Bay Exchange – Marine Corps Air Station in Beaufort, South Carolina – petroleum hydrocarbons and MTBE.
- Cape Canaveral, Florida, or Moody Air Force Base, Georgia (proposed only) – site information unavailable at time of selection.

Preliminary evaluations following field work at the first three sites (SUBASE Bangor, Fort Lewis, and NAS Pensacola) resulted in a revised demonstration plan that eliminated the last three sites and replaced them with the proposed Thermochem site in Muskegon, Michigan (proposed in revised demonstration plan) containing chlorinated VOCs. Unfortunately, the Thermochem site was not available for purposes of the demonstration, and so an alternative site was selected instead: the USCG Support Center in Elizabeth City, North Carolina. The Elizabeth City site had two areas: the Fuel Farm containing petroleum hydrocarbons and MTBE and the North Beach area containing chlorinated VOCs.

3.3 Test Site Description

Detailed descriptions of the six DoD sites originally selected for testing of the BAFeIII technology are provided in the Technology Demonstration Plan (CDM, 2001) and in associated reports as referenced in this section. Summaries of the three DoD sites actually used in the demonstration are provided in the following paragraphs along with a description of the USCG Elizabeth City site that was added per the revised scope of work.

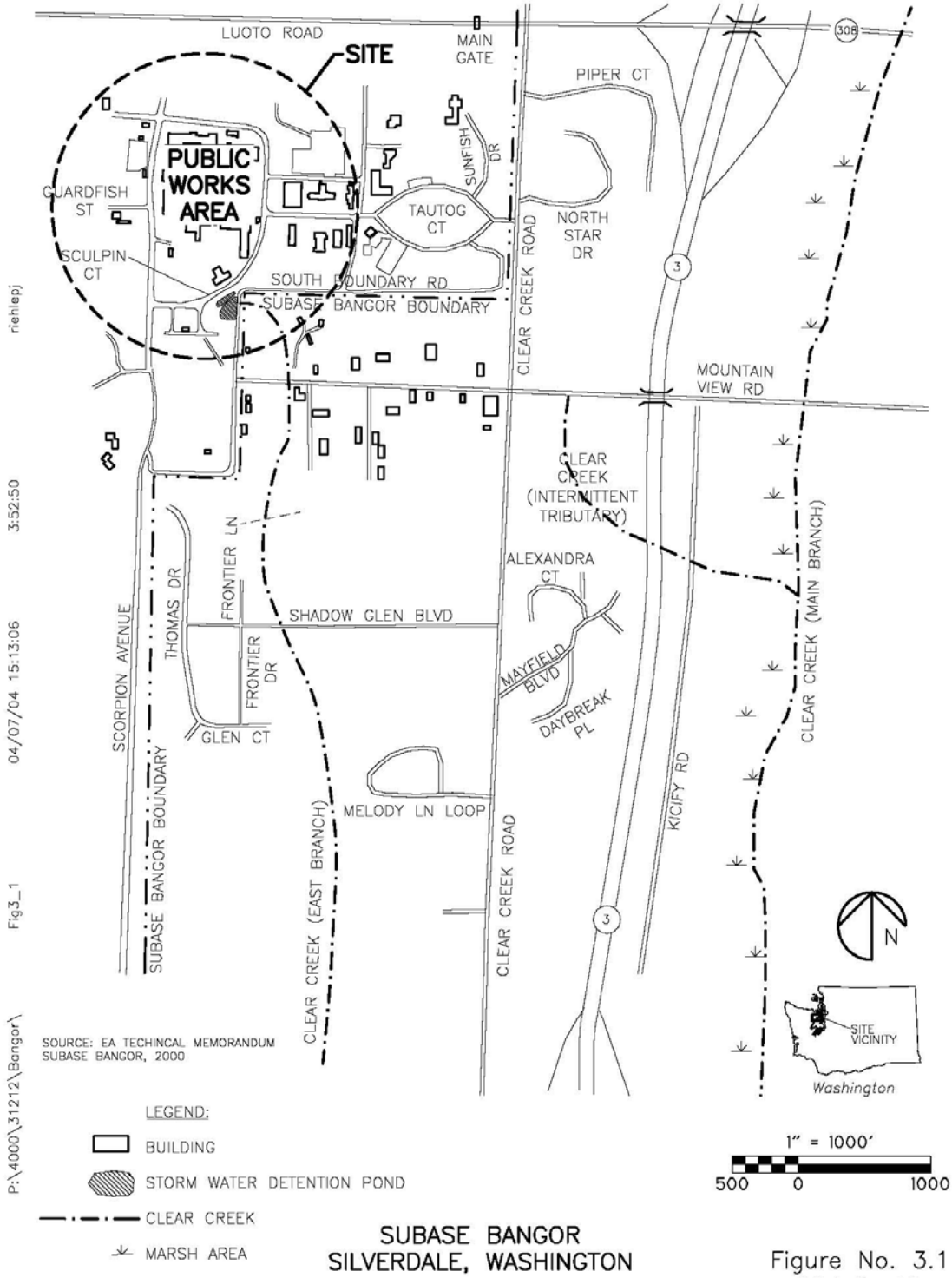
SUBASE Bangor

The study area for this demonstration is the vicinity of Operable Unit 8 (OU8), located in the Public Works Industrial Area (PWIA) of SUBASE Bangor (**Figure 3-1**). SUBASE Bangor is located near the town of Silverdale, Washington. An onsite UST is believed to be the source of a release of unleaded gasoline into the surrounding media between 1982 and 1986. In 1986, soil vapor extraction/air system and product recovery were implemented to clean up the site. To date, liquid petroleum hydrocarbons remain in several monitoring wells at the PWIA (EA, 2000). Chlorinated VOCs are also present in site groundwater (EA, 2000).

Geological conditions at OU8 at SUBASE Bangor have been highly characterized by drilling and monitoring well installation. The area consists of four stratigraphic units: construction fill, Vashon till (Qvt), Vashon Advance Outwash (Qva), and Lawton Clay. The construction fill can be found 2 to 3 feet bgs and consists of a sandy material. Underlying the construction fill and ranging to a depth of about 45 ft bgs is the Vashon till, which consists of silt, sand, gravel, and cobbles. This unit is 20 to 40 ft thick. The Vashon Advance Outwash (location of the shallow aquifer) is beneath the Vashon till and consists of sand, silt, and gravel. The thickness of the Vashon Advance Outwash is about 100 to 130 feet. Beneath the Vashon Advance Outwash is the Lawton Clay aquitard. A silty transition zone in the bottom of the Vashon Advance Outwash separates the shallow aquifer from the lower aquitard.

There are about 100 monitoring wells at OU8. The wells were installed at three different depth intervals: shallow, intermediate, and deep. The depth to groundwater is about 20 feet bgs and the general flow direction is southeast. The Vashon Advance Outwash lies beneath the Vashon till at OU8 and is the location of the shallow unconfined aquifer. The shallow aquifer contained in the Vashon Advance Outwash is about 125 feet thick. The shallow wells are screened within 30 feet of the water table, intermediate wells are screened within the middle 40 feet of the aquifer thickness, and the deeper wells are screened within 30 feet of the Lawton Clay aquitard. The DCA plume in the PWIA area contains dissolved petroleum contaminants (benzene) and 1,2-DCA. The majority of the contaminants are located in the shallow and intermediate zones of the Vashon Advance Outwash (EA, 2000).

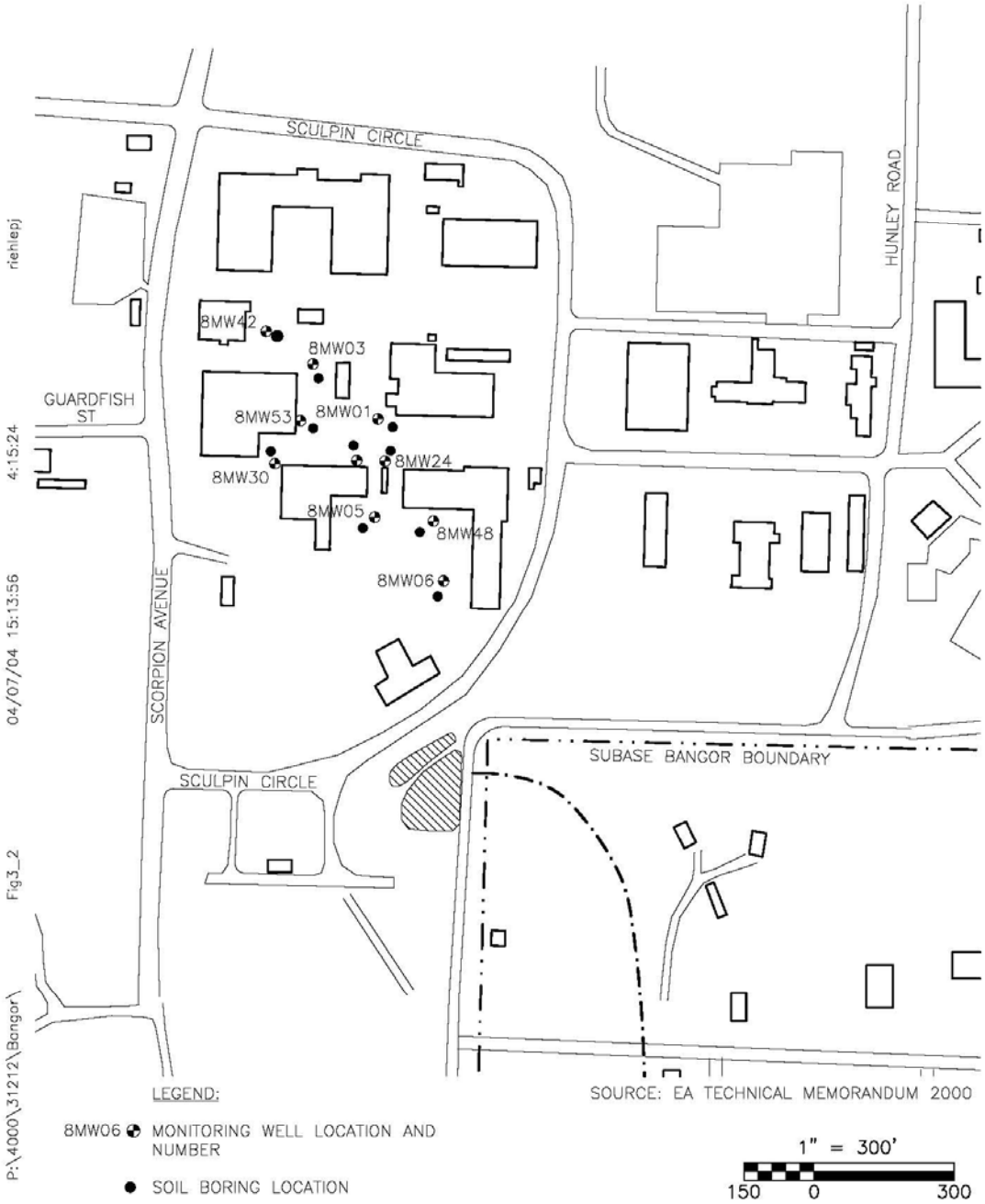
EA Engineering, Science, and Technology (EA) conducted an investigation to assess natural attenuation processes at OU8 of SUBASE Bangor, and further site characterization details are presented in their report (EA, 2000). **Figure 3-2** shows the sampling locations conducted at the SUBASE Bangor site in conjunction with this demonstration.



SUBASE BANGOR
SILVERDALE, WASHINGTON

Figure No. 3.1
Vicinity Map

CDM



SUBASE BANGOR
SILVERDALE, WASHINGTON

Figure No. 3.2
Sample Collection Points

CDM

Fort Lewis
The study area for this demonstration is the vicinity of the East Gate Disposal Yard (EGDY) of the Fort Lewis Logistics Center (Fort Lewis), located south of Tacoma, Washington (**Figure 3-3**). The EGDY, which is situated at the northwest corner of the base, originally was used for storage and disposal of various solid and liquid waste products. Since 1982, studies have been conducted at the EGDY to verify and delineate contamination at the site. Affected media were soil and groundwater, with the prominent contaminant being trichloroethene (TCE) (Battelle, 2000).

The upper portion of the EGDY at Fort Lewis consists of a brown to black alluvial sand and gravel matrix with local lenses of silts. The material gets coarse with depth. Underlying this formation at about 260 feet msl is the Vashon Till, which is a complex mixture of silt, sand, and clay. The Vashon Till has low permeability and serves as a barrier between the upper and deeper aquifers. At the source area the groundwater can be encountered between 8 and 15 feet bgs. Farther downgradient the groundwater is generally between 10 and 35 feet bgs. The upper aquifer is unconfined and mostly anaerobic. Groundwater flow is generally west to northwest. There are more than 80 monitoring wells and piezometers on site.

Battelle Memorial Institute (in cooperation with the Air Force Research Laboratory, USGS, EPA, and Cornell University) performed Reductive Anaerobic *In Situ* Treatment Technology (RABITT) at the EGDY of Fort Lewis, and further site characterization details can be found in their report (Battelle, 2000). **Figure 3-4** shows the sampling locations conducted at the Fort Lewis site in conjunction with this demonstration.

NAS Pensacola
The study area for this demonstration is the vicinity of the wastewater treatment plant at the NAS in Pensacola, Florida (NAS Pensacola), located near Pensacola Bay in the far northwest corner of the state (USGS, 1999). **Figure 3-5** shows the location of the site.

The area predominantly consists of marine and fluvial terrace deposits ranging from fine- to medium-grained sands, silts, clays, and gravel. The site has two aquifers, a shallow aquifer and a deeper confined aquifer (referred to as the underlying main producing zone). There is a 20-foot-thick confining barrier of low-permeable silts and clays that separate the upper and lower aquifers. The upper aquifer is composed of fine- to medium-grained sands. The main producing zone is used locally as a water supply and consists of permeable sands and gravel. Two plumes have been identified at the site, one comprised of chlorinated ethenes and the other chlorinated benzenes. Most of the contaminants on site are located in the upper aquifer region. The depth of contamination ranges from 20 to 40 feet bgs.

A monitoring plan for natural attenuation processes at the wastewater treatment plant at NAS Pensacola was conducted by the USGS in 1999, and further site characterization details can be found in their report (USGS, 1999). **Figure 3-6** shows the sampling locations conducted at the NAS Pensacola site in conjunction with this demonstration.

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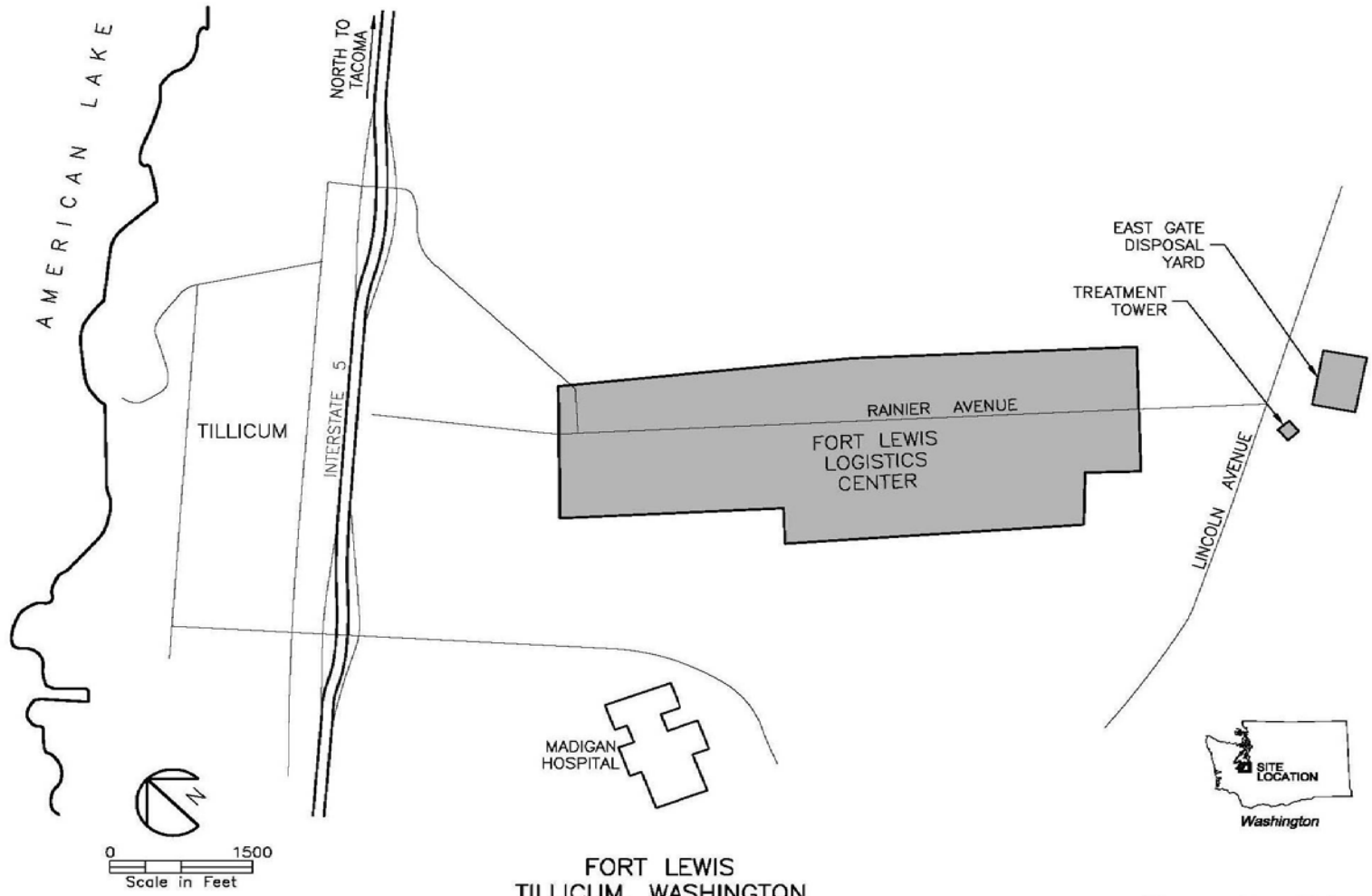

FORT LEWIS
TILLICUM, WASHINGTON

CDM

Figure No. 3.3
Vicinity Map

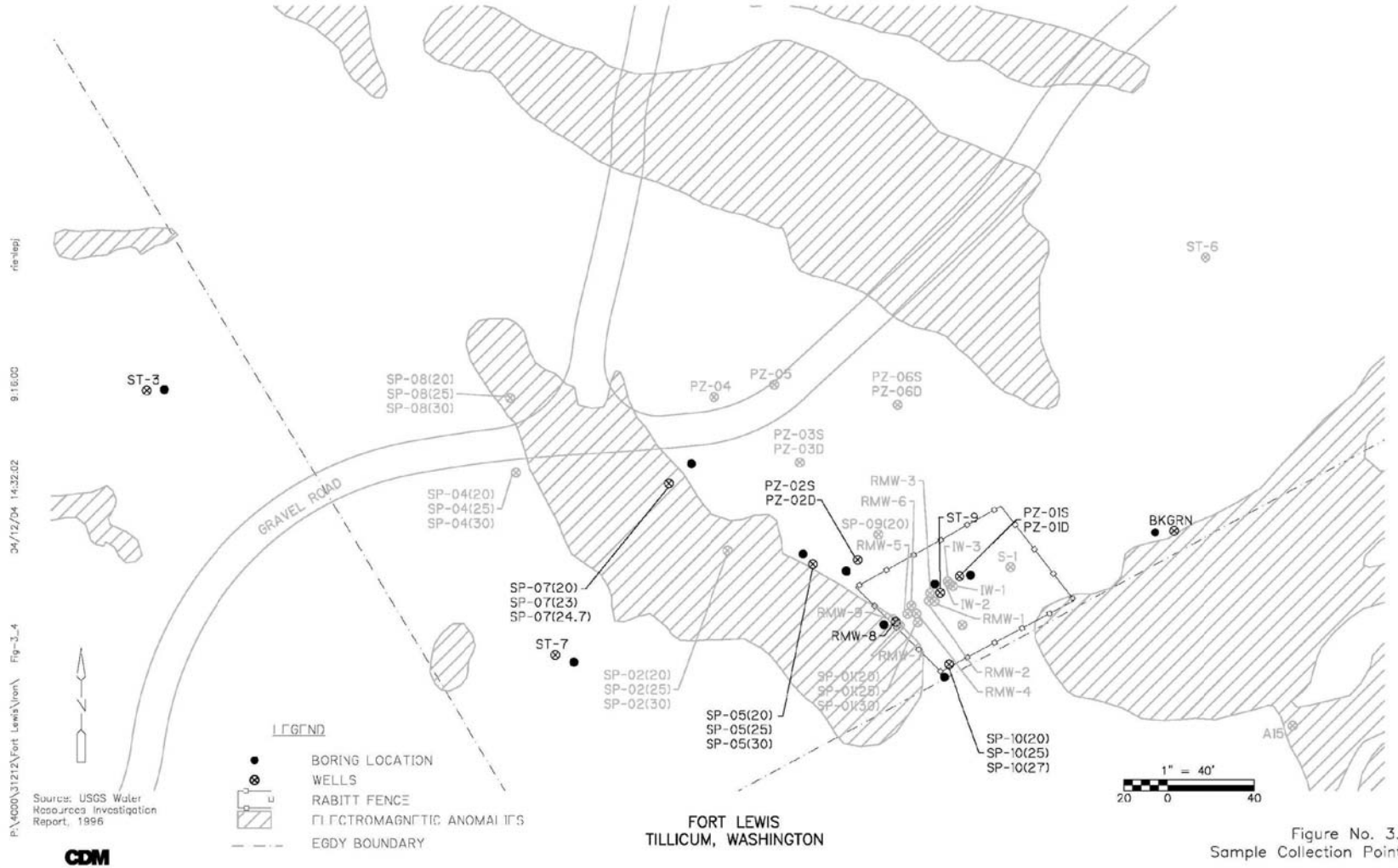

Figure No. 3.4
Sample Collection Points

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NAS PENSACOLA
PENSACOLA, FLORIDA

CDM

Figure No. 3.5
Vicinity Map

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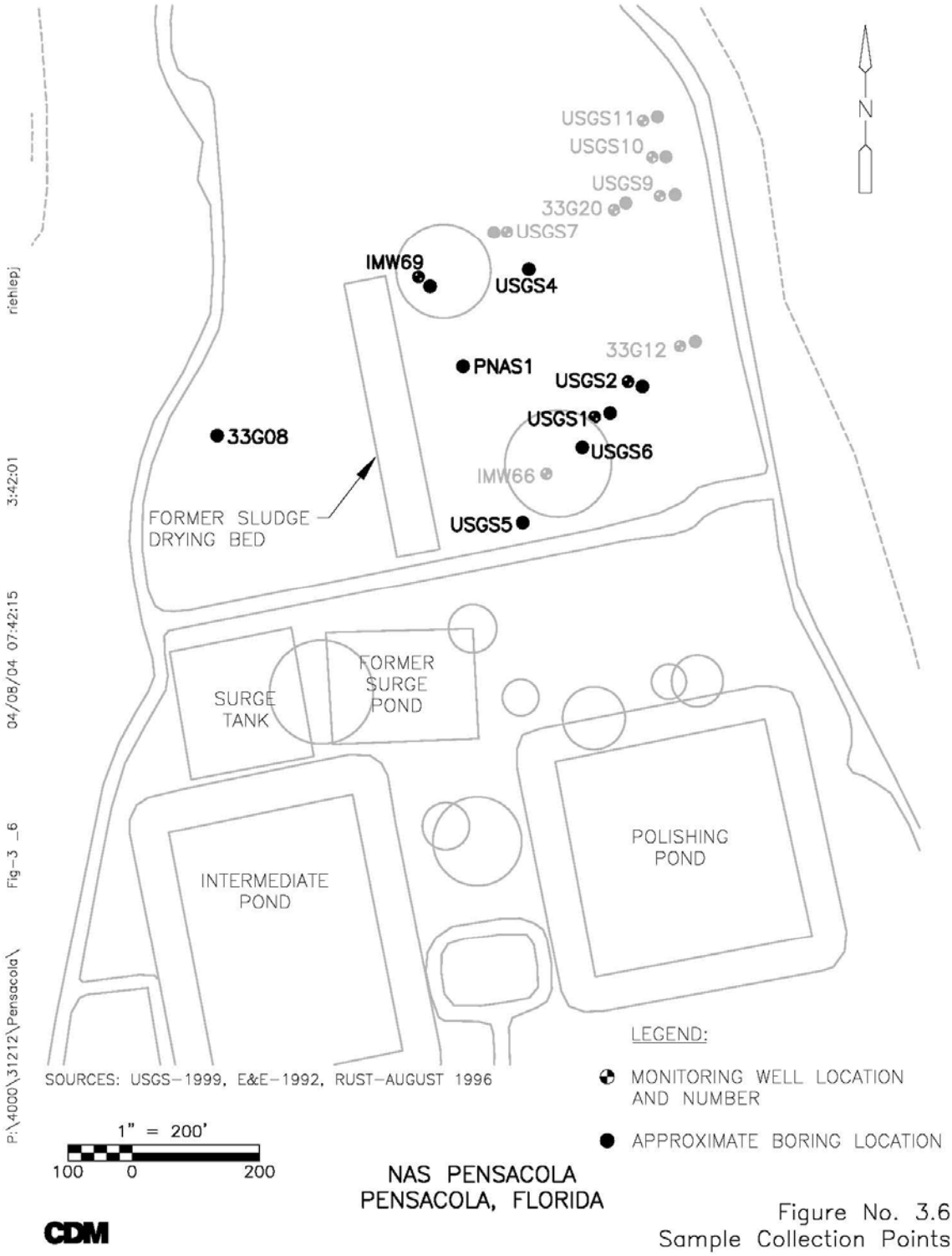


NAS PENSACOLA
PENSACOLA, FLORIDA

CDM

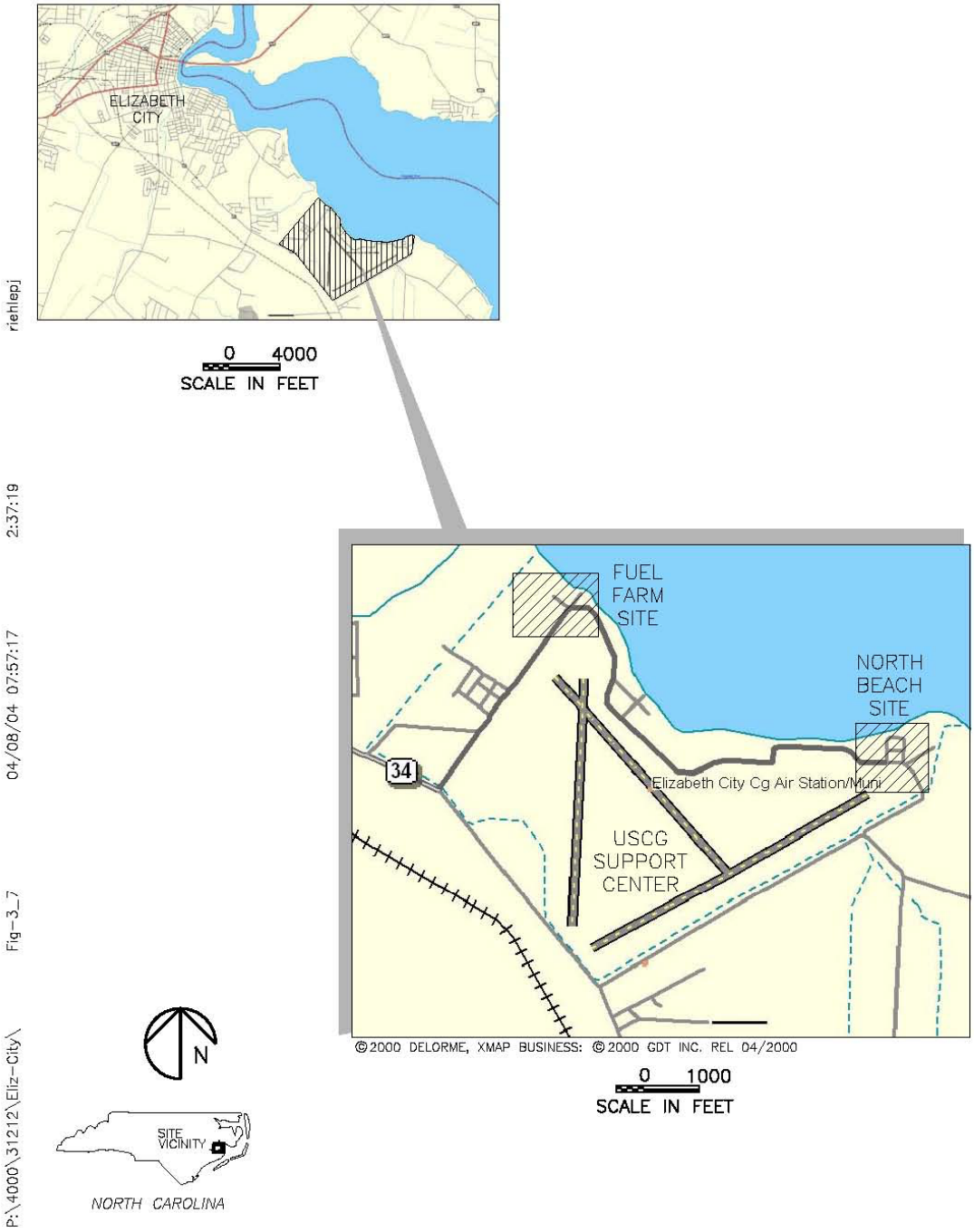
Figure No. 3.6
Sample Collection Points

Elizabeth City

The U.S. Coast Guard Support Center in Elizabeth City, North Carolina, is located on the southern bank of the Pasquotank River (**Figure 3-7**). As described previously, two separate areas at the site were used in this demonstration, the Fuel Farm (petroleum hydrocarbon) area and the North Beach (chlorinated VOC) area.

The following description of the Fuel Farm area was obtained from the report by Wilson, et al. (2000). The former fuel farm was located south of a concrete ramp used to recover seaplanes from the Pasquotank River. A plume of MTBE and fuel hydrocarbons in ground water emanates from a source area in the location of the former fuel farm, and flows under the concrete ramp toward the Pasquotank River to the north, and toward a drainage canal along the western side of the seaplane ramp. This source area corresponds to the former location of fuel storage tanks on the site. Fuel was stored at the site until December 31, 1991. The fuel farm had been in use since 1942, and originally consisted of a 50,000-gallon concrete underground storage tank and two steel underground storage tanks with a volume of 12,000-gallons and 15,000-gallons, respectively. The steel tanks were apparently removed in the mid-1980s. In addition to the underground storage tanks, two steel, aboveground storage tanks with a capacity of 50,000 gallons were installed in the mid-1980s. There was evidence of corrosion in the transfer lines from these tanks. They were taken out of service and removed from the site. No evidence of a release from the pipes was discovered. The U.S. Coast Guard began a free product recovery effort at the site in September 1990. Eight recovery wells were arranged around the source area in a circle. By March 1992, a total of 79,000 gallons of fuel was recovered. Parsons Engineering Science (1996) provides further description of the site in the Former Fuel Farm Work Plan, a part of the Remediation Feasibility Assessment Work Plan prepared for the U.S. Coast Guard Support Center, Elizabeth City (SCEC), North Carolina.

The following description of the North Beach Disposal Area was provided by ARCADIS (2004). The North Beach Disposal Area occupies 4.8 acres in the northeast corner of the SCEC. The site is bounded immediately north and west by the Pasquotank River and to the east by a drainage canal. The North Beach site is unpaved. Approximately half of the site is heavily wooded. The other half, where the majority of disposal activities may have occurred, consists of grass-covered open areas. Historical information and site investigation activities indicate that industrial wastes generated at the SCEC may have been buried at the North Beach Disposal Area. The exact quantity and nature of the wastes disposed of in the North Beach Disposal Area are unknown; however, it is suspected that the wastes may have included chlorinated solvents, batteries, petroleum wastes, scrap metals, paint sludges, and plating wastes. Disposal activities likely occurred from the 1940s to approximately 1975. Four separate areas of concern (i.e., Source Areas 1, 2, 3, and 4) were identified at the site and had elevated concentrations of metals, scrap-metal fragments, VOCs, and semivolatile organic compounds (SVOCs) in soil. Only PCE, TCE, cis-1,2-DCE, vinyl chloride and pentachlorophenol are present in groundwater at elevated concentrations. The groundwater constituents near Source Areas 1, 2, and 4 emanate from Area 2.



USCG SUPPORT CENTER
ELIZABETH CITY, NORTH CAROLINA

CDM

Figure No. 3.7
Vicinity Map

Hydrogen Release Compound™ (HRC™), a food-grade polylactate ester, was injected into the shallow aquifer zone at multiple points near Source Area 2 of the North Beach Disposal Area from January 21 to 25, 2003. The treatment area for HRC injection is a grid approximately 40 feet wide by 100 feet long, encompassing Monitor Wells GP20, GM315, GM330, and GM360. Within the grid area, Standard HRC was injected into 40 points while HRC Primer was injected into 9 points. A total of 5,545 pounds of HRC was injected across the grid, with between 110 and 135 pounds of Standard HRC or primer injected at each point. The depths for these injections were 5 feet below ground surface (ft bgs) to 45 ft bls within the primary interval impacted by chlorinated VOCs. Quarterly monitoring has been conducted for one year since the HRC injections. Results indicate that HRC does not appear to have significant influence on groundwater geochemistry beyond the immediate vicinity of the injection points within the grid.

Figures 3-8 (Fuel Farm) and **3-9** (North Beach) show the sampling locations conducted at the Elizabeth City site in conjunction with this demonstration.

3.4 Pre-Demonstration Testing and Analysis

Previous investigations have been conducted at each of the sites as described previously in Section 3.3 and detailed in various reports (EA, 2000; Battelle, 2000; USGS, 1999; EPA, 2000). This information was thoroughly reviewed as part of the selection process of these sites for this demonstration, and to select appropriate sampling locations.

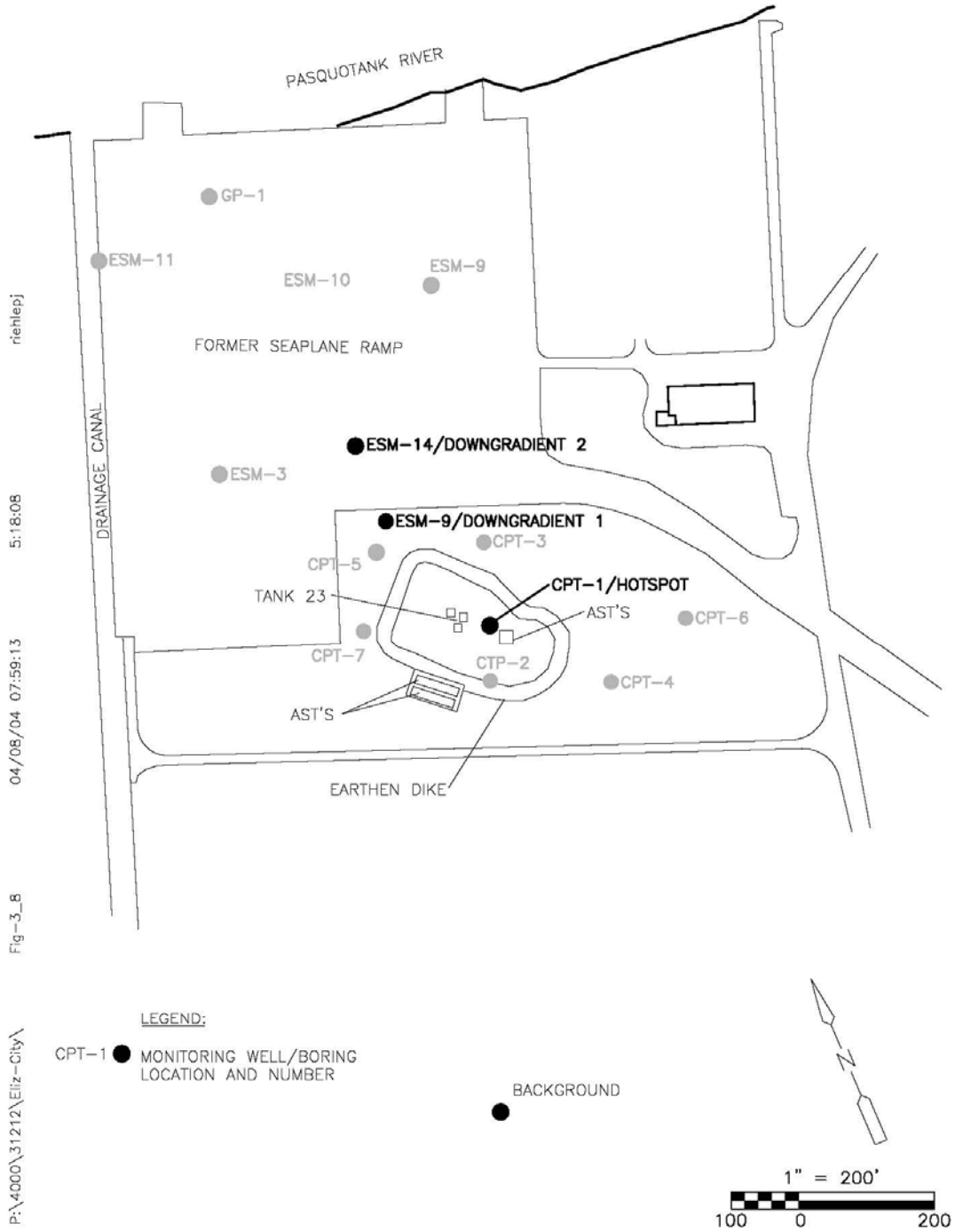
In addition, pre-demonstration development and field-testing of the BAFeIII assay was conducted as described in Section 2.2.

3.5 Testing and Evaluation Plans

The original testing and evaluation plans for this demonstration are provided in: *Technology Demonstration Plan, Development of a Dissolved Hydrogen Analyzer and a Bioavailable Ferric Iron Assay* (CDM, 2001). The Technology Demonstration Plan (TDP) provides: the objectives of the demonstration; a description of the technology; pre-demonstration activities; site/facility descriptions; the demonstration approach, including the sampling plan; data collection, storage, and archiving procedures; cost performance criteria; regulatory issues; the quality assurance plan; and the health and safety plan. As described in Section 1.2, the original objectives of the demonstration have been modified and revised over the course of the study as new information and results were obtained. However, the quality assurance plan and health and safety plan sections of the TDP have not changed and are provided in **Appendices B and C**, respectively, of this report. The sampling plan, including sample analysis and sample collection, with revisions, is provided in this section.

3.5.1 Demonstration Installation and Startup

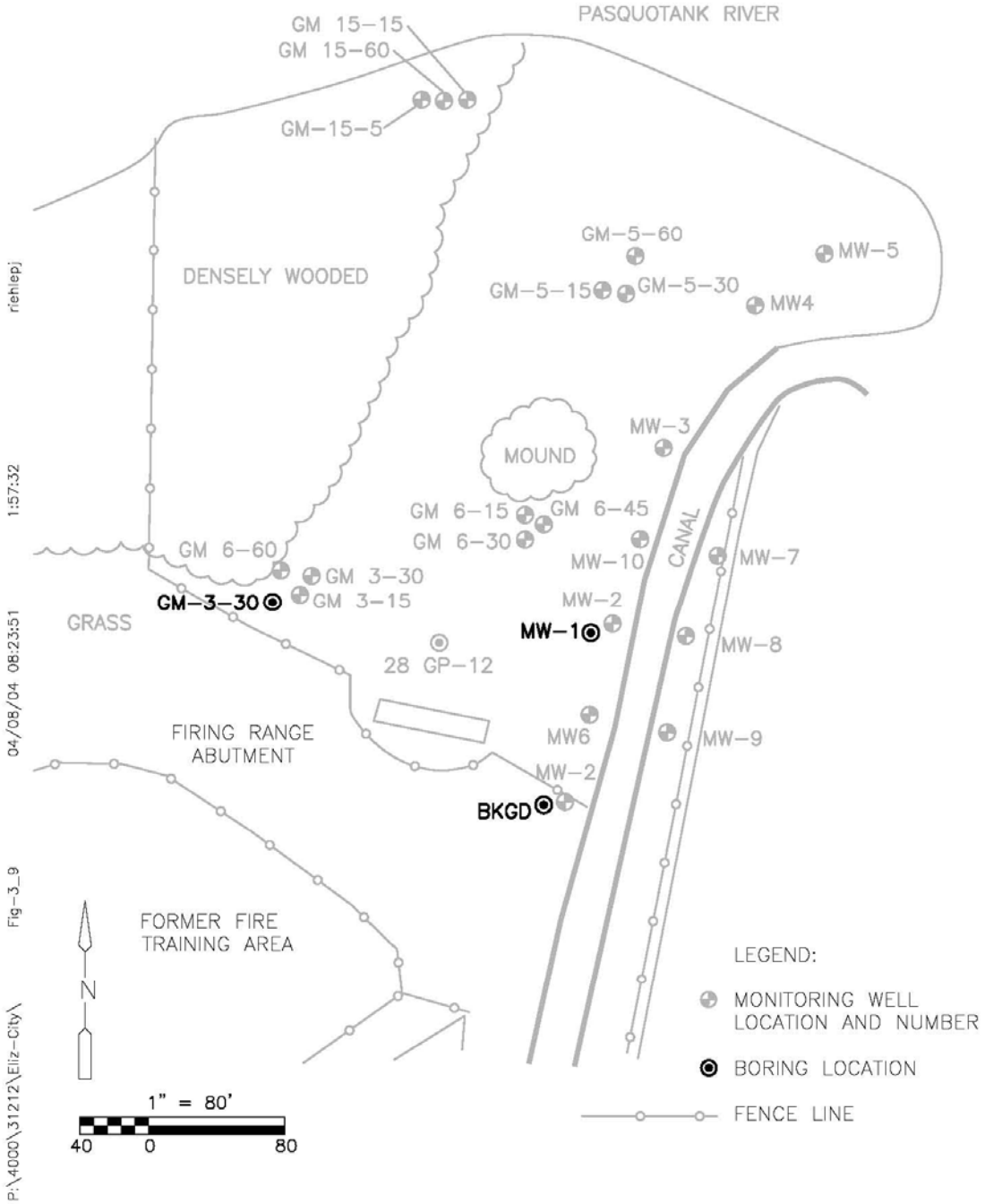
Site work involved collection of soil and groundwater samples. Refer to Section 3.3 for sample collection locations. All other work was conducted in individual laboratories as described in Sections 1.2 and 3.5.7.2.



FUEL FARM
USCG SUPPORT CENTER
ELIZABETH CITY, NORTH CAROLINA

CDM

Figure No. 3.8
Sample Collection Points



NORTH BEACH
USCG SUPPORT CENTER
ELIZABETH CITY, NORTH CAROLINA

CDM

Figure No. 3.9
Sample Collection Points

3.5.2 Period of Operation

The periods of sample collection for the BAFeIII assay were as follows:

Site 1 – SUBASE Bangor: January 22 to February 2, 2001
Site 2 – Fort Lewis: February 19 to March 2, 2001
Site 3 – Pensacola NAS: April 29 to May 3, 2002
Site 4 – Elizabeth City: October 23 to 25, 2002

Analyses were conducted immediately after sample collection with the following exceptions: 1) BAFeIII analysis of samples collected from SUBASE Bangor and Ft. Lewis were conducted on January 11, 2002 on archived samples, and 2) HCl extractions were repeated in March 2002 using the ferrozine analysis method.

3.5.3 Amount/Treatment Rate of Material to Be Treated

Since this demonstration involved a site characterization method, this subsection is not applicable.

3.5.4 Residuals Handling

Residuals included drill cuttings that were handled in accordance with Base requirements. These requirements varied from emplacement in drums and disposal to emplacement of cuttings back into the boring. Spent BAFeIII test kit contents were disposed by a licensed hazardous waste company.

3.5.5 Operating Parameters for the Technology

Operation of the BAFeIII assay on collected soil samples involved the following steps:

1. Vial T_0: Combine soil, HCl, and water. Vial T_{30}: Combine soil, water, and bioassay reagents.
2. Measure ferrous iron in Vial T_0.
3. After a 4-week incubation period, add HCl and measure ferrous iron in Vial T_{30}.

The first step takes approximately one half hour, depending on the number of samples to be run. Running the Hach kit ferrous iron analysis for Vial T_0 (step 2) typically takes 1.5 hours for up to five samples – this includes time to run standards and prepare dilutions as necessary. Following the 4-week incubation period, another hour and a half would be needed for step 3 to add the HCl to Vial T_{30} and analyze for ferrous iron. If an analytical lab is used for ferrous iron analysis, the required labor would include labeling, packing, shipping the sample containers and filling out the chain of custody forms.

3.5.6 Experimental Design

The experimental design involved collection of samples from multiple sites and reducing zones and completion of the BAFeIII assay and multiple confirmatory analyses on each sample. Confirmatory analyses included various chemical extractions, microcosms, and mineralogic characterization. Additionally, various iron oxides were tested using the BAFeIII assay and the

confirmatory analyses. Comparisons of the BAFeIII assay results to the confirmatory analyses involved statistical methods such as principal component analysis and correlations. These methods were used to determine the presence of significant positive associations between the BAFeIII assay and specific confirmatory analyses. Positive association between the BAFeIII assay and multiple confirmatory analyses was a primary means of technology validation. Intra-laboratory and inter-laboratory precision of the BAFeIII assay was also determined. Intra-laboratory precision was determined by comparison of BAFeIII assay results conducted on sample splits at the CDM laboratory. Inter-laboratory precision was determined by comparison of BAFeIII assay results conducted on sample splits by the CDM and EPA/Ada laboratories. Analysis of iron oxides by the BAFeIII assay and the confirmatory analyses were also compared to results reported in the literature for the oxides.

3.5.7 Sampling Plan

3.5.7.1 Sample Collection

Table 3-2 lists the samples collected for purposes of this demonstration. The sample identification number and the site and location where they were obtained are provided, along with the category of analyses conducted. Further descriptions of the sites and sample locations, including maps, were provided in Section 3.3.

Table 3-2: Sample Summary for BAFeIII Demonstration and Validation

Site	Sample Identification	Depth Intervals (ft bgs)	Analyses Category [1]
SUBASE Bangor	28MW01	30.0-31.5, 35.0-36.5	SD, BA, SE, MC, MS, GW
	8MW06	35.0-36.5, 42.0-43.5	“
	8MW24	33.0-34.5, 39.0-40.5	“
	8MW30	36.0-37.5, 40.0-41.5	“
	8MW42	31.0-32.5, 35.5-37.0	“
	8MW47	36.0-37.5, 40.0-41.5	“
	8MW48	25.0-30.0, 39.0-40.5	“
	8MW53	36.0-37.5, 39.0-40.5	“
	MW03	30.0-31.5, 35.0-36.5	“
	MW05	34.0-35.5, 38.0-39.5	“
Fort Lewis	BKGRND	20.0-27.5, 27.5-32.0	SD, BA, SE, MC, MS, GW
	MW9	20.0-26.5, 26.5-31.7	“
	PZ1	20.0-24.0, 24.0-31.0	“
	PZ2D	20.0-28.0	“
	SP5	20.0-28.0, 28.0-33.0	“
	SP07	20.0-28.0, 28.0-33.0	“
	SP10	20.0-26.5, 28.0-30.8	“
	ST3	15.0-24.0, 27.0-27.75, 28.0-29.5	“
	ST7	20.0-26.5, 26.5-32.0	“
	ST9	20.0-24.0, 24.0-28.0	“

Table 3-2: Sample Summary for BAFeIII Demonstration and Validation (cont.)

Site	Sample Identification	Depth Intervals (ft bgs)	Analyses Category 1
NAS Pensacola	33G08	0.0-1.0	SD, BA, SE, MC, MS
	IMW69	-- [2]	"
	PNAS1	-- [2]	"
	SMW8	-- [2]	"
	USGS1	4.0-6.0	"
	USGS2	0.0-1.0, 4.0-6.0	"
	USGS4	-- [2]	"
	USGS5	-- [2]	"
	USGS6	-- [2]	"
Elizabeth City – Fuel Farm	BKGND	15-18, 18-21, 21-24	SD, BA, SE, MS, GW, MC
	Downgradient 1	15-18, 18-21, 21-24	"
	Hot	18-21, 21-24	"
	Downgradient 2	15-18, 18-21, 21-24	"
– North Beach	BKGND	16-19, 21-24, 26-29	"
	MW1	16-19, 21-24, 26-29	"
	GM3-30	16-19, 21-24, 26-29	"
Iron Oxide Standards	Goethite	-- [2]	BA, SE, MC, MS
	2 line Ferrihydrite	-- [2]	"
	6 line Ferrihydrite	-- [2]	"
	Lepidocrocite	-- [2]	"
	Goethite	-- [2]	"
	Hematite	-- [2]	"
	Magnetite	-- [2]	"

[1] GW – Groundwater; BA – BAFeIII assay test kit; SE – Selective extractions; MC – Microcosms; MS – Mineral speciation; SD – Site data. See **Table 3-3** for further information. Not all analyses in the category were conducted for all samples.
[2] Not applicable or available.

Groundwater samples were collected from existing monitoring wells on each site using low flow techniques and a peristaltic and /or bladder pump system. Soil borings were completed for collection of soil samples using hollow-stem auger, direct push technology, or hand-auger. During drilling, a CDM engineer or scientist logged and sampled the borings. The soils were visually described and classified in accordance with the Unified Soil Classification System (USCS; ASTM D2488-84). Generally two sections of each boring were collected (i.e., the top and bottom portions). Attempts were made to obtain different types of soil samples as defined by USCS. The soil from each section was homogenized by hand (mixing with stainless steel spoon in bowl) and then placed in 4- or 8-ounce glass jars, capped with Teflon-lined lids, and labeled prior to shipment to the labs. Samples were shipped in coolers with ice to maintain temperature between 2 and 6 ^{o}C.

Soil samples were sent to the CDM laboratory in Bellevue, Washington for BAFeIII analysis. Soil samples were also sent to other organizations for analysis as detailed in **Tables 3-2** and **3-3**.

3.5.7.2 Sample Analysis

Table 3-3 lists the analyses that were conducted to demonstrate and validate the BAFeIII assay. The significance of each analysis relative to dominant TEAPs and BAFeIII is provided, along with the method description and the organization that conducted the analysis. **Appendix A** includes a detailed description of the methods used for each analysis.

Table 3-3: Analyses for BAFeIII Demonstration and Validation

Analysis	Significance	Method	Organization [1]
Groundwater – GW			
Specific Conductivity	BAFeIII factor	Field/probe	CDM
pH	BAFeIII factor	Field/probe	CDM
ORP	Redox Conditions	Field/probe	CDM
Dissolved Oxygen	Redox Conditions	Field/probe	CDM
Dissolved Hydrogen	Dominant TEAP	Field/bubble strip	CDM
Dissolved Sulfide	Redox Conditions	Field/colorimetric	CDM
Dissolved Fe II	Redox Conditions	Field/colorimetric	CDM, EPA/Ada
Dissolved Methane	Redox Conditions	Field/bubble strip	EPA/Ada
BAFeIII Assay Kit (Soil) – BA			
Fe II – ambient (T_0)	Ambient Fe II	BAFeIII assay kit	CDM, EPA/Ada
Fe II – anaerobic incubation (T_{30})	Bioavailable Fe III	BAFeIII assay kit	CDM, EPA/Ada
Lactate	Lactate consumption	Sigma test kit	CDM
Selective Extraction (Soil) [2] – SE			
Fe II – 0.5 *N* HCl	Extractable Fe II	Extraction/colorimetric	EPA/Athens
Fe III – 0.5 *N* HCl	Extractable Fe III	Extraction/colorimetric	EPA/Athens
Fe II – 6 *N* HCl	Extractable Fe II	Extraction/colorimetric	EPA/Athens
Fe III – 6 *N* HCl	Extractable Fe III	Extraction/colorimetric	EPA/Athens
AVS – Acid volatile sulfides [3]	AVS-bound Fe II	Extraction/colorimetric	EPA/Athens
CRS – Chromium reducible sulfides [4]	Pyrite-bound Fe II	Extraction/colorimetric	EPA/Athens
Fe – Citrate dithionite bicarbonate	Extractable Fe	Extraction/colorimetric	EPA/Ada
Fe – Ammonium oxalate	Extractable Fe	Extraction/colorimetric	EPA/Ada
Fe – Total	Total Fe	Digestion	EPA/Ada
Microcosms (Soil) – MC			
Fe III	Bioavailable Fe III	Microcosm	GIT
Fe III with BrY	Bioavailable Fe III	Microcosm	GIT
Fe III with FeOOH	Bioavailable Fe III	Microcosm	GIT
Fe III with BrY and FeOOH	Bioavailable Fe III	Microcosm	GIT
Microbial Composition [5]	BAFeIII factor	Most Probable Number	GIT
Mineral Speciation (Soil) – MS			
Fe mineralogy [6]	Iron oxide speciation	Electron Microprobe	UC
Surface area [7]	BAFeIII factor	BET	University of Iowa
Site Data (Soil) – SD			
Unified Soil Classification	BAFeIII factor	Field	CDM
Soil Type and Description	BAFeIII factor	Field	CDM

[1] Indicates the organizations conducting the analyses. The analyses were not always conducted at all sites and the organizations conducting the analyses may have changed from site to site.
[2] Extraction (Kennedy, et al. 1999 and 2001); Fe analyses (Lovley and Phillips 1987); sulfide analyses (Cline 1969).
[3] Sulfide – 6 *N* HCl with Zn trap.
[4] Sulfide – 6 *N* HCl/Cr II with Zn trap.
[5] Aerobes, nitrate reducers, FeIII reducers, MnIV reducers, sulfate reducers.
[6] Relative mass percentages of Fe in hematite, goethite, limonite, $FeSiO_4$, magnetite, ilmenite, pyrite, jarosite, Fe-metal, Ti-Mn spinel, stainless steel, Mn-FeOOH, Cr-spinel, brookite, organic matter.
[7] Brunauer-Emmett-Teller (BET) method. Conducted on iron oxides only.

3.5.7.3 Experimental Control

Abiotic iron reduction was evaluated by measurement of lactate consumption in the BAFeIII assay T_{30} tubes for SUBASE Bangor and Ft. Lewis samples. Lactic acid consumption was measured using a Sigma lactate dehydrogenase colorimetric test kit. An electron balance was

then conducted to determine the relationship between lactic acid consumption and Fe II production. The average observed lactate consumption for the samples was 3.0 g/L (SD = 0.7 g/L, N = 44). The average, calculated lactate consumption based on an electron balance for complete lactate oxidation coupled to observed BAFeIII reduction was 0.030 g/L (SD = 0.016 g/L, N = 44). Iron reduction by the BAFeIII assay was concluded to not be abiotic because the observed lactate consumption was much greater than the calculated consumption. The large difference between the observed and calculated lactate consumptions was probably due to incomplete oxidation of lactate to various volatile fatty acids such as acetate and propionate.

3.5.7.4 Quality Assurance & Quality Control

A discussion of general QA/QC elements followed by laboratory-specific elements is presented here. Further QA/QC discussion is presented in the QAQC Plan in **Appendix C**.

General

Data Quality Parameters

Samples were collected from each boring and homogenized in the field. The samples were then split into individual jars and shipped to each laboratory to ensure representative and comparable results. An attempt was made to distribute all samples to all laboratories but in certain cases insufficient sample mass was available. Accuracy of iron analyses was ensured by making fresh standards especially in the case of ferrous iron which can oxidize. The stable ferrous iron standard ferrous ethylene ammonium sulfate was used to minimize oxidation. Precision was monitored by completing analyses in duplicate or triplicate. Additionally, each laboratory was responsible for its own QA/QC.

The analyses were considered to be representative of a core or subcore of sediment form the field. The core samples were blended prior to analysis. The core samples were considered to be representative of the location on the map where the aquifer was sampled.

Data Quality Indicators

Precision of the BAFeIII assay was quantified by calculation of relative percent difference (RPD) as described in Sections 4.3.1 and 4.3.2. RPD was also used to quantify the precision of the ferrous iron analyses.

The method reporting limit was determined by analysis intra-laboratory precision data as a function of concentration. A discussion is presented in Section 4.3.1.

Calibration Procedures, Quality Control Checks, and Corrective Action

Refer to the specific laboratory sections below for discussion of these items.

CDM
CDM conducted ferrous iron analyses on T_0 and T_{30} samples from the BAFeIII assay. The ferrozine method was conducted (Lovley and Phillips, 1987) in combination with a Hach DR2000 specotrophotometer. Calibration procedures included construction of 5-point standard curves for set of samples. Linear correlation coefficients were $r > 0.99$. All ferrous iron analyses were conducted in duplicate.

A corrective action was undertaken when a difference was observed between the EPA/Ada and the CDM BAFeIII results for Elizabeth City wherein the CDM results were biased high. The reason was though to be attributable to use of different methods for ferrous iron analysis. Creation and analysis of blind ferrous iron standards for each lab was conducted to ascertain the nature of this difference. Further description of this testing is presented in Section 4.3.2.

EPA/Athens
Soil samples were stored in the cold room (- 20°C) prior to extraction. Samples were allowed to equilibrate to room temperature for 1 hr and then put into the anaerobic chamber (96% N_2/4% H_2). Each sample was weighed into a tared 120-ml serum bottle. A range of about 300 - 800 mg of soil was used. Either 10 ml of 0.5 N or 6.0 N HCl was added to the serum bottle. The 6.0N samples containing 6.0 N HCl contained 2.5 ml 2% zinc acetate in 3.0-ml test-tubes to trap volatile sulfides. Samples were crimped and removed from the chamber. All analyses were conducted in duplicate.

The samples treated with 0.5 N HCl were shaken for 2 days and the 6.0 N HCl treated samples were shaken for 3 days room temperature (24 to 25 ^{0}C). About 1.5 ml of sample was removed and centrifuged at 8000 rpm for 10 min. A 0.1-ml aliquot was taken from the supernatant and 5.0 ml ferrozine solution added. Color was allowed to develop for at least 2hr, then read at 562 nm. For total Fe, 0.1 ml of 1.4-M hydroxylamine was added to the 0.1-ml sample and reacted for 10-min prior to adding the ferrozine. After 2 hrs, the color complex was found to be stable for well over 24 hr. A 100 ppm standard of Fe(II) and Fe(III) was measured after every 10 samples to account for changes in lamp intensity. Fe(II) was determined in ppm based on a linear curve using standards of 10 to 200 ppm. For total Fe, a standard curve was developed using Fe(III) solutions from 10 to 200 ppm. The 100 ppm standard of Fe(III) yielded no color complex without hydroxylamine. Both standard curves yielded the same linear equation with $r^2 = 0.999$.

Georgia Institute of Technology
All experiments and assays conducted during the project included appropriate biotic and abiotic controls. All Most Probable Number (MPN) counts of bacterial populations and geochemical analyses were conducted in triplicate to ensure precision and accuracy. Standard errors were calculated to test data quality. All pipettors used in the laboratory work were calibrated on a bi-monthly basis to ensure accuracy. Permanent records of all experiments and data were maintained in dedicated notebooks in the DiChristina lab. All personnel conducting research on the project were trained in the proper use and disposal of biologicals and chemicals. Regular lab group meetings were held on a biweekly basis to discuss on-going experiments and to provide

opportunities for discussions. In addition, all students (graduate and undergraduate) presented their results at least once every semester during lab meetings. Results of the research will be submitted as research manuscripts to peer-reviewed journals. Databases have maintained for culture collections and all geochemical results.

University of Colorado

The University of Colorado was not responsible for data review or interpretation, therefore, quality control and quality assurance was limited to sample analyses.

EMPA

Calibration of our JOEL Electron Microprobe was performed on a daily basis prior to analyses of CDM samples. The instruments magnification marker was checked for accuracy using a certified standard (#17490 Ernest Fullam, Inc.). If found out of calibration a Joel service engineer would be notified and be required to re-calibrate the instrument. This corrective action was never required.

The wavelength spectrometers (WDS) system was calibrated daily for oxygen and iron analyses. Peak locations and concentrations were calibrated using certified mineral standards (quartz for oxygen and ilmenite for iron). Counting times were adjusted to provide 1% reproducibility on standards.

XRD

The Scintag X-ray diffractometer was calibrated prior to analysis of CDM samples by reduction of a certified quartz standard. All major lines were compared to certified values (∀ 0.01 degrees 2-theta) for quartz. If locations were outside these limits the goniometer was re-initialized according to manufacturers procedures and re-calibrated.

Spectroscopy

The FieldSpec Pro FR spectroradiometer was calibrated twice each day prior to analyses using a manufacture provided template. Reflectance was measured between 350 to 2500 nm. Acquired spectra were then compared to stored standard spectra from the USGS (US Geological Survey) spectral library using ENVI v. 3.4 software.

EPA/Ada

The results of the assay were calculated from the weight of the sediment extracted, and from the concentration of Fe II in the extract. The sediment was weighed on a laboratory precision balance to an accuracy of 0.001 gram. The calibration of the balance is checked and confirmed by the R.S. Kerr Center's QA officer Mr. Steve Vandegrift every 6 months.

Concentrations of Fe II were determined using program 255 on a Hach DR/2010 Spectrophotometer. The concentrations of Fe II was determined in serial dilutions of the original extract, with the intention to report more than 0.3 and less than 3.0 mg/L iron in the dilution. There was an indication that acid in the extract could interfere with the analysis. The analysis was accepted if two separate dilutions returned a value of the original concentration of Fe II that

agreed within 20% of each other. If the agreement was not with 20% the analyses were discarded and the extraction repeated if sediment was available.

The goal for completeness was to successfully analyze at least 90% of the samples acquired. More than 98% of the samples acquired were successfully analyzed. To ensure comparability of results, the data were all reported in units of mg/kg sediment.

3.5.8 Demobilization

Demobilization activities at each site were minimal and consisted of finishing borings either with grout or bentonite pellets and management of waste. Drill rigs were decontaminated as part of demobilization. Samples were packaged and shipped to individual laboratories.

3.6 Selection of Analytical/Testing Methods

No standard method exists for BAFeIII analysis. This analysis is operationally defined. Therefore, validation of the BAFeIII test kit involved use of multiple confirmatory analytical methods and an interdisciplinary team of investigators. These confirmatory analytical methods included selective chemical extractions, microcosm tests, and mineralogic determinations, as detailed in Section 3.5.2. Selection of these methods was based on known or expected associations or relationships between them and actual or expected BAFeIII. In addition, the BAFeIII assay itself was conducted in replicate to assess its precision.

3.7 Selection of Analytical/Testing Laboratory

A number of analytical/testing laboratories were selected for this demonstration based on the interdisciplinary team of investigators assembled, that is, the laboratories with which the investigators are affiliated. As detailed in previous sections of this report, the laboratories selected included: EPA/Ada, EPA/Athens, Georgia Institute of Technology (GIT), University of Colorado (UC), and CDM. **Figure 3-10** is a flow chart illustrating sample disposition and analysis.

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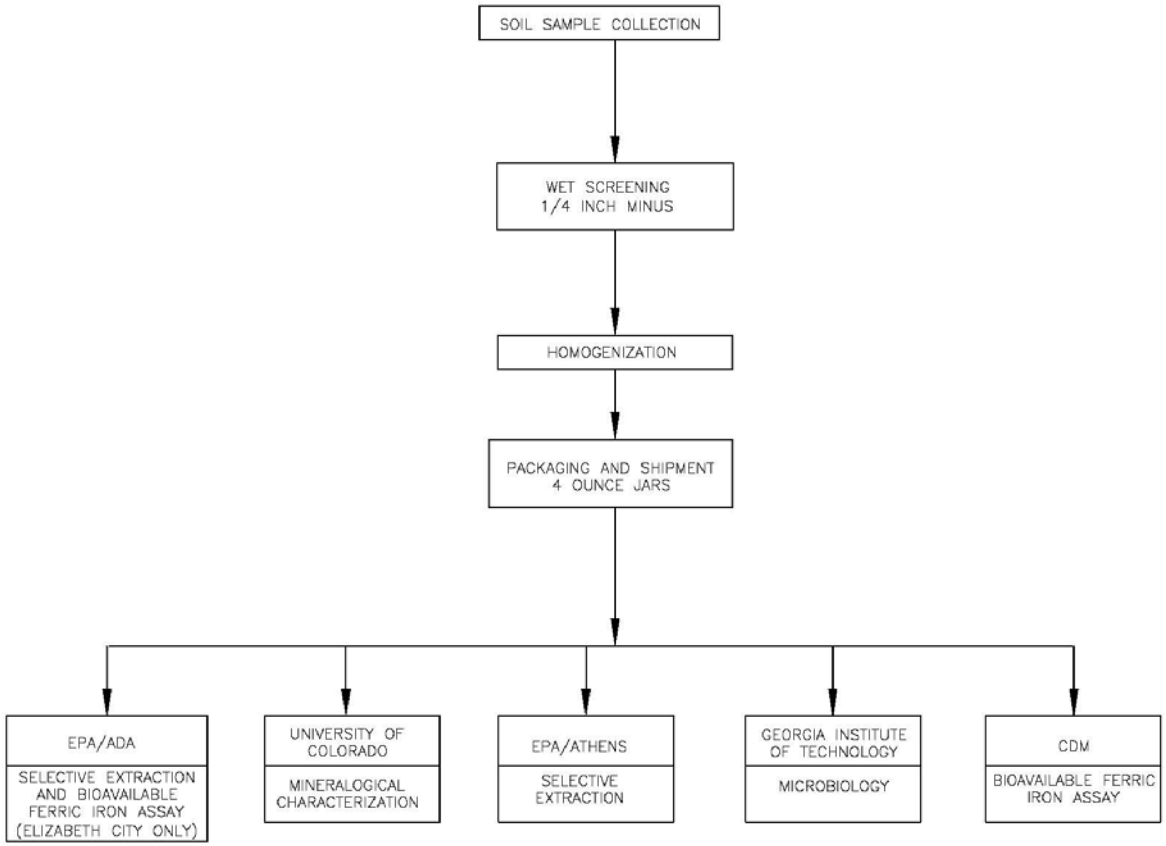

CDM

Figure No. 3.10
Soil Sample Allocation

4.0 Performance Assessment

4.1 Performance Criteria

The performance objectives established for this demonstration are provided in **Table 3-1** of Section 3. The general performance criteria that were used to evaluate the performance of the BAFeIII assay (including the performance objectives) are described in **Table 4-1**.

Table 4-1: Performance Criteria for BAFeIII Assay

Performance Criteria	Description	Primary or Secondary
Contaminants	The BAFeIII assay technology measures bioavailable ferric iron and is applicable to sites that contain various contaminants, including chlorinated solvents, petroleum hydrocarbons, MTBE, pesticides, and other inorganic and organic chemicals.	Secondary
Process Waste	The completed BAFeIII assay contains 0.5 N HCl at a pH of about 2 and requires disposal in a safe manner. Additionally the completed BAFeIII assay may contain contaminated site soil requiring disposal. Excess soil samples used for iron assay should be contained in appropriate waste containers and disposed of by a licensed waste facility. Soil cuttings from drilling should be contained in 55-gallon drums and disposed properly.	Secondary
Factors Affecting Technology Performance	The BAFeIII assay contains lyophilized live bacteria. Storage of the assay unfrozen or use beyond the expiration date may result in poor performance. Toxic levels of heavy metals or other contaminants can potentially inhibit or kill the bacteria in the assay, although if such toxic compounds are present, they probably limit the applicability of natural attenuation or bioremediation at the site. Heterogeneity is a common problem affecting analysis of soil and although not unique to this technology may introduce additional interpretive uncertainty; sample homogenization and collection of additional samples is typically necessary to offset this uncertainty.	Secondary
Reliability	The BAFeIII assay is manufactured by New Horizons Diagnostics Corporation, which has QA/QC procedures in place to ensure a high level of reliability.	Secondary
Ease-of-Use	The BAFeIII assay was specifically designed to be a standardized and easy-to-use technology. Individuals performing the BAFeIII assay should have a minimum of an Associated Arts or Bachelors degree in science or engineering and have experience in soil and groundwater sampling techniques and use of field test kits and instrumentation. OSHA health and safety training is required if the technology is to be used within an exclusion zone.	Secondary

Table 4-1: Performance Criteria for BAFeIII Assay (cont.)

Performance Criteria	Description	Primary or Secondary
Versatility	The BAFeIII assay is applicable to various sites and remediation of various contaminants such as fuel hydrocarbons, MTBE, chlorinated hydrocarbons, and various volatile and semivolatile organic compounds. It can be applied to various treatment technologies such as natural attenuation and enhanced anaerobic bioremediation (e.g., RABITT).	Primary
Sample Throughput	The BAFeIII assay is setup quickly similar to other chemical extraction methods and faster than laboratory microcosms. The BAFeIII assay requires two 48-hour extraction steps and one 30-day incubation step. These steps do not affect sample throughput rates and do not require user involvement.	Primary
Procurement and Scale-Up	The BAFeIII assay is available for purchase from New Horizons Diagnostics Corporation. Scale-up constraints are not applicable since any number of analyses can be accomplished with the technology.	Secondary
Maintenance	The BAFeIII assay produces an extract liquid for which dissolved Fe II measurement is required using a colorimeter or spectrophotometer that may require periodic maintenance according to the manufacturers instructions. Otherwise, no maintenance is required.	Secondary
Range	The BAFeIII assay produces results within an analytical range that is consistent with other analytical methods used historically to assess MNA and EAB.	Primary
Intra-Laboratory Precision	The BAFeIII assay is capable of analytical precision comparable to other methods used to analyze soil samples, generally within the uncertainty due to the inherent heterogeneity of soils (absolute RPD $\leq$ 35).	Primary
Inter-Laboratory Precision	The BAFeIII assay yields similar results when conducted by two different laboratories on split samples (-35 $\leq$ RPD $\leq$ 35).	Primary
Relationship between BAFeIII Assay and Degree of Iron Oxide Crystallinity/Surface Area	The BAFeIII assay results show positive associations with results expected to be important factors in the bioavailability of Fe III including iron oxide crystallinity and surface area.	Primary
Relationship between BAFeIII Assay and Confirmatory Analyses and other BAFeIII Factors	No standard method for measuring BAFeIII exists for comparison with, or direct demonstration of, the BAFeIII assay. However, the BAFeIII assay results show a positive association with other methods used to assess BAFeIII, including selective extraction and microcosm tests.	Primary

4.2 Performance Confirmation Methods

The results of the BAFeIII assay conducted during this demonstration and the effectiveness of this technology were evaluated primarily by comparison with confirmatory analytical methods designed to measure the factors associated with the bioavailability of Fe III in soils at contaminated sites. These comparisons required statistical analyses of the analytical data

obtained during the demonstration in order to evaluate both qualitative and quantitative performance objectives.

In addition, as with any analytical method conducted to measure a soil parameter, the assessment of quality assurance indicators (precision, reliability, etc.) was an important component of the demonstration. These assessments also required statistical analyses of the analytical data for certain indicators, such as replicate precision, in order to evaluate performance objectives. A quality assurance plan was provided in the Technology Demonstration Plan and is reproduced in Appendix B of this report.

The confirmatory methods used to evaluate performance of the BAFeIII assay are summarized in **Table 4-2**.

Table 4-2: Expected Performance and Performance Confirmation Methods for BAFeIII Assay

Performance Criteria	Expected Performance Metric (pre demo)	Performance Confirmation Method	Actual (post demo)
Primary Criteria (Performance Objectives) (Qualitative)			
Relationship between BAFeIII assay and degree of iron oxide crystallinity/surface area.	Positive association	Measurement of both BAFeIII and BET surface area for iron oxide standards with varying degrees of crystallinity and surface areas.	Generally a positive association with the exception of lepidocrocite which is expected.
Relationship between BAFeIII assay and confirmatory analyses and other BAFeIII factors (total iron, groundwater chemistry, microbial composition).	Positive association	Multivariate statistical analysis (principal components analysis). Loadings ≥0.45 for original variables within a principal component demonstrate positive association.	Most of the variance in the original variables (about 41%) accounted for by two principal components. Component 1 showed positive associations (loadings ≥0.45) for 6N HCl extractable FeII, FeIII and total Fe; 0.5N HCl extractable FeII, FeIII and total Fe; microcosm reducible FeIII with BrY; and relative mass percent magnetite.

Table 4-2: Expected Performance and Performance Confirmation Methods for BAFeIII (cont.)

Performance Criteria	Expected Performance Metric (pre demo)	Performance Confirmation Method	Actual (post demo)
Relationship between BAFeIII assay and confirmatory analyses and other BAFeIII factors (total iron, groundwater chemistry, microbial composition). (cont.)			Component 2 showed positive associations (loadings ≥0.45) for 6N HCl extractable FeIII, 0.5N extractable FeIII and total Fe; microcosm reducible FeIII with BrY and with BrY and FeOOH; citrate dithionite bicarbonate extractable Fe; ammonium oxalate extractable Fe; total Fe; and CDM and EPA BAFeIII assay results. Positive associations with BAFeIII assay results were not demonstrated for groundwater chemistry and microbial composition factors. These factors appeared to be independent of BAFeIII in the dataset.
Range of BAFeIII assay relative to other analytical techniques.	Similar Range	Comparison of analytical range to citrate dithionite bicarbonate extractable Fe, ammonium oxalate extractable Fe, total Fe, 0.5N and 6.0N HCl extractable Fe III, and to microcosm reducible FeIII with BrY.	Range was similar to or greater than all comparable methods examined.
Sample throughput of BAFeIII assay.	Labor time ≤ similar methods	Comparison with other methods used to characterize BAFeIII.	Labor time was less than or approximately the same as other methods.
Versatility of BAFeIII assay.	Consistent performance	BAFeIII assay conducted on a wide variety of soils and standards, at a wide variety of sites, and under a wide variety of environmental conditions.	Performance was consistent with other methods used to characterize BAFeIII.

Table 4-2: Expected Performance and Performance Confirmation Methods for BAFeIII (cont.)

Performance Criteria	Expected Performance Metric (pre demo)	Performance Confirmation Method	Actual (post demo)
Primary Criteria (Performance Objectives) (Quantitative)			
Intra-laboratory precision of BAFeIII assay based on soil and laboratory replicates.	Absolute RPD ≤ 35	Field and laboratory replicate sample collection and analyses.	Average absolute RPD = 29.7. RPD pattern consistent with expected pattern from other analytical methods. Deviations assumed due to inherently nonhomogeneous soils.
Inter-laboratory precision of BAFeIII assay based on replicates analyzed by both CDM and EPA/Ada.	-35 ≥ RPD ≤ 35	Field and laboratory replicate sample collection and analyses. Blind standard analysis.	Average RPD = 11.6. CDM BAFeIII results generally higher than EPA BAFeIII results due to use of two different colorimetric methods for the T_{30} extract. Preferred colorimetric method is 1,10-phenanthroline method.

4.3 Data Analysis, Interpretation and Evaluation

The analytical data obtained during this demonstration have been tabulated and are provided in **Appendix E**. **Appendix E** also includes reports provided by each of the laboratories conducting confirmatory analyses. Analysis, interpretation and evaluation of these data are provided in this section.

4.3.1 Intra-Laboratory Precision

Replicate analyses were conducted to demonstrate the intra-laboratory precision of the BAFeIII assay. Two types of replicate analyses were conducted: (1) splits created during sample collection (field replicates) and (2) splits created at the laboratory (lab replicates). BAFeIII replicate analyses were conducted by CDM for selected samples from SUBASE Bangor (2 lab, 1 field), Fort Lewis (2 lab, 1 field), NAS Pensacola (10 field), Elizabeth City (22 field), and the iron oxide standards (8 lab, 6 of which were analyzed in triplicate). BAFeIII replicate analyses were conducted by EPA/Ada for the iron oxide standards (6 lab, all of which were analyzed in triplicate). Absolute relative percent differences (RPDs) for the replicates were calculated according to the following formula:

$$\text{absolute RPD} = \frac{|A - B|}{(A + B)/2}(100)$$

where A represents replicate A for a particular laboratory and B represents replicate B for the same laboratory. In total, the CDM replicate analyses resulted in 58 absolute RPD measurements and the EPA/Ada replicate analyses resulted in 18 absolute RPD measurements, for a total of 76 absolute RPD measurements.

An absolute RPD of 35 was used to evaluate the replicate data. This value was selected as an approximate criterion for analyses of replicates of inherently non-homogeneous soils. That is, even the most precise analytical method would be limited in its reproducibility due to the inherently non-homogeneous nature of soils. And although such reproducibility will vary depending on how well the sample is homogenized prior to sample splitting, as well as the size and representativeness of the sample subjected to the analysis, an absolute RPD of 35 is considered appropriate for evaluation of intra-laboratory precision. Further discussion of the valid use of an RPD of 35 can be found in EPA guidance on analysis of solid matrices (U.S. EPA, 2002).

The overall average absolute RPD for the 76 CDM intra-laboratory replicates was 29.7, which met the RPD ≤ 35 criterion, with absolute RPDs for most of the individual replicates (77.6%) also meeting the criterion. As with other types of soil analyses, intra-laboratory precision as measured by absolute RPD generally improved toward the higher end of the BAFeIII concentration range. As shown in **Figure 4-1**, improvement in precision was noted for the BAFeIII assay when concentrations exceeded about 1 g/kg. At concentrations below about 1 g/kg the frequency of very high RPD measurements (RPDs as high as 200) increased. These higher RPDs occurred at the lower concentrations because, although the differences between the replicate results were small, their relative differences tended to be large.

The minimum reporting limit (MRL) for the BAFeIII assay depends on the method used to measure Fe II in the T_0 and T_{30} liquid samples. Hach method 8146 for Fe II analysis is one such method and can be conducted using the inexpensive Hach color wheel system or the more expensive, accurate, and precise Hach spectrophotometer system. The MRL for these methods varies from 0.02 to greater than 1 mg/L depending on dilution. The T_0 and T_{30} samples require dilution of at least 20 prior to Fe II analysis to eliminate interferences by the BAFeIII assay reagents. Thus the effective MRL for Fe II ranges from 0.4 to greater than 20 mg/L. These values are divided by 217 to obtain BAFeIII concentration in units of g/kg as described in Section 2.1. Therefore the MRL for the BAFeIII assay ranges from 0.001 to greater than 0.09 g/kg. For general use, an MRL of 0.1 g/kg is recommended for the BAFeIII assay. This recommended MRL is substantiated by the data presented in **Figure 4-1**.

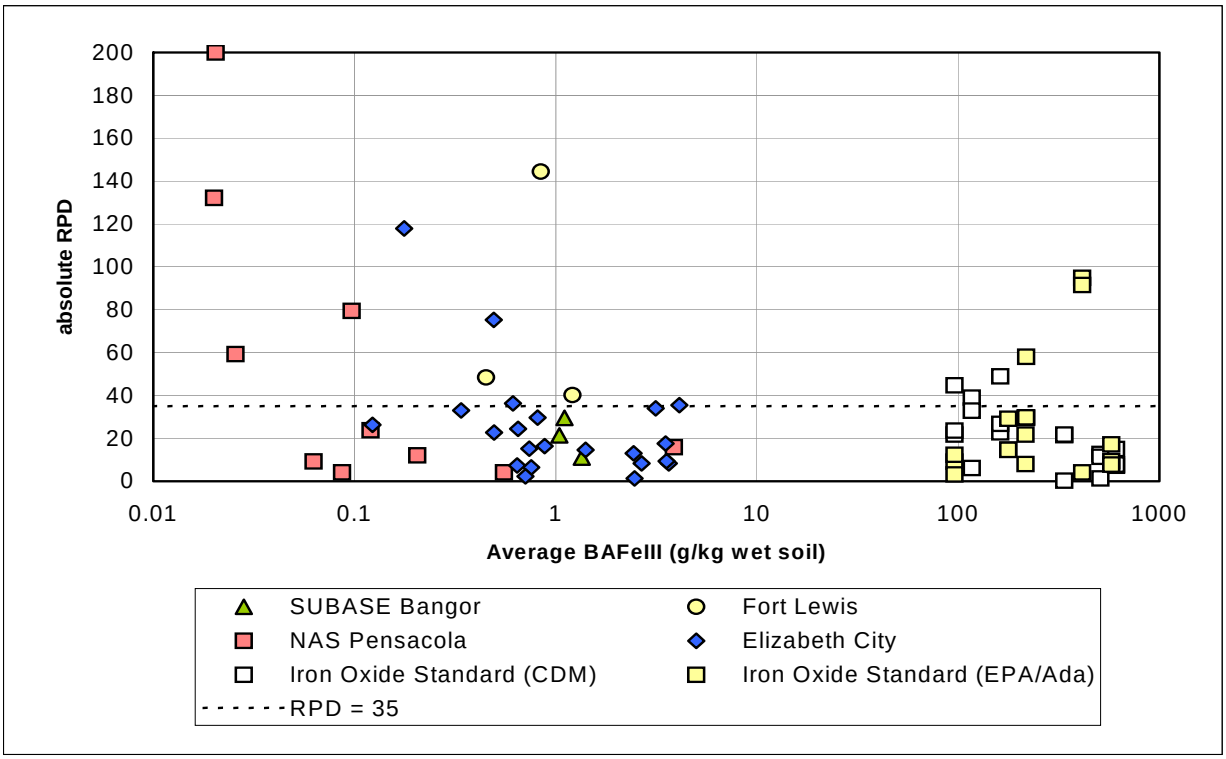

Figure 4-1. Intra-Laboratory Replicate Precision for BAFeIII Assay

Additionally, EPA/Ada conducted independent analyses to evaluate intra-laboratory precision. The precision of the assay was determined by analyzing triplicate samples of sediment from four different aquifers and calculating the mean and the coefficient of variation (CV) of the analyses. CV was calculated as the standard deviation divided by the mean. Results are shown in **Table 4-3**. Samples were selected from an aquifer that had high, average, and very low concentrations of biologically available iron. The precision was excellent (CV < 20%) for all samples except the Port Hueneme sample which contained BAFeIII at a concentration less than the recommended reporting limit of 100 mg/kg.

Table 4-3: Intra-laboratory Precision for the BAFeIII Assay Conducted on Multiple Samples By EPA/Ada

Location	Description	BAFeIII (mg/kg)	CV (%)
North Beach at Elizabeth City, NC	"higher iron content" Sample	2339	18.0
Fuel Farm at Elizabeth City, NC	"lower iron content" Sample	590	2.6
Sand Aquifer in Minnesota (TCAAP)	Glacial outwash sand	616	15.8
Sand Aquifer in Minnesota (TCAAP)	Glacial outwash sand	606	13.7
Port Hueneme, CA	Anaerobic aquifer	14.3	50

4.3.2 Inter-Laboratory Precision

Replicate analyses were also conducted to demonstrate the inter-laboratory precision of the BAFeIII assay. Again, two types of replicate analyses were conducted: (1) splits created during

sample collection (field replicates) and (2) splits created at the laboratory (lab replicates). BAFeIII inter-laboratory replicate analyses were conducted by the CDM and EPA/Ada laboratories for 22 field replicates for Elizabeth City samples and 18 laboratory replicates for the iron oxide standards, for a total of 40 replicate analyses. Relative percent differences (RPDs) were calculated using the following formula:

$$\mathrm{RPD} = \frac{\mathrm{A} - \mathrm{B}}{(\mathrm{A} + \mathrm{B})/2}(100)$$

where A represents the replicate analyzed by the CDM laboratory and B represents the replicate analyzed by the EPA/Ada laboratory.

An RPD of 0 was used to evaluate the replicate data. This value was selected to represent no difference between the analyses as conducted by the two laboratories, i.e., a perfect 1:1 correlation and no inter-laboratory bias. In addition, a paired data t-test was conducted to test the hypothesis of no statistically discernable difference between the means of the two sets of data.

The overall average RPD for the 40 inter-laboratory replicates was 12, which indicated that the CDM results were slightly higher, on average, than the EPA results. A plot of CDM BAFeIII versus EPA BAFeIII is provided in **Figure 4-2**. The data in **Figure 4-2** are shown on a log-log scale to allow a more representative comparison by spreading the data more evenly across the measurement scales; although the true associations between the two measurements may be linear, the data obtained by the two sets of replicate measurements more closely resembled lognormal distributions. As shown in **Figure 4-2**, the replicate results closely approximated a straight line with a correlation coefficient (r) of 0.98 for the natural log transformed data. The paired data t-test for an hypothesized mean difference of 0 resulted in a two-tailed p-value of 0.410, indicating no statistically discernable difference relative to a critical false-positive probability of 0.05.

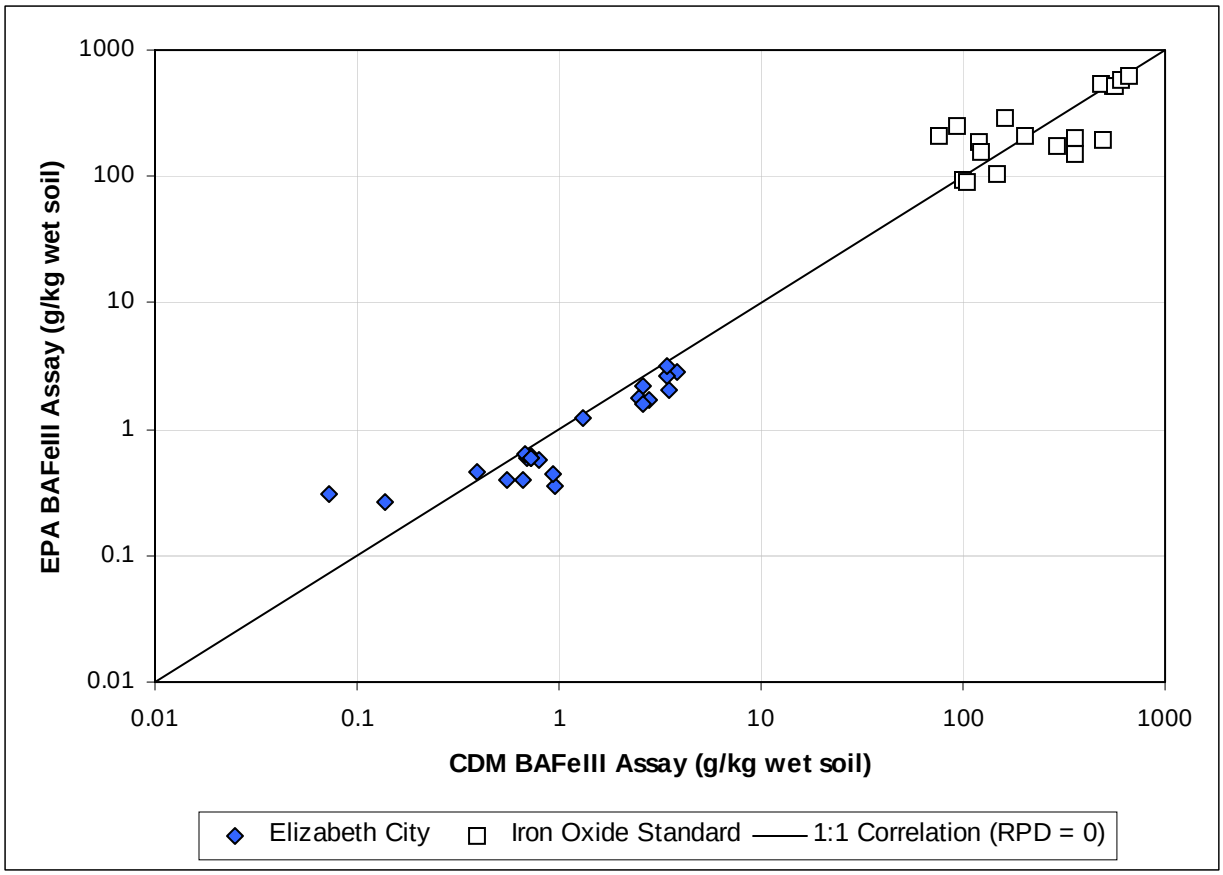

Figure 4-2. Inter-Laboratory Replicate Precision for BAFeIII Assay

A hypothesis for the slight inter-laboratory replicate bias (CDM higher than EPA/Ada) was the use of two different methods for the colorimetric analysis of dissolved Fe II. CDM used the ferrozine method (Lovley and Phillips, 1987) and EPA/Ada used the Hach 1,10-phenanthroline method. To test this hypothesis, standard Fe II solutions were prepared using the stable Fe II standard ferrous ethylene ammonium sulfate (FEAS) and shipped to both laboratories in triplicate. The Fe II range of the standards was selected to approximate the range of dissolved Fe II observed in the BAFeIII T_{30} assay extracts, and the standards were prepared in 0.5N HCl solutions to approximate the acidity of the T_{30} extracts. Results are shown in **Table 4-4**.

Table 4-4: Double-Blind Ferrous Iron Analysis Results

Ferrous Iron Concentration (mg/L)			
Standard	**Ferrozine (CDM)**	**1,10-Phenanthroline (CDM)**	**1,10-Phenanthroline (EPA/Ada)**
0 (Blank)	2.3	0.1	0.2
0 (Blank)	4.2	0.0	0.0
0 (Blank)	3.1	0.0	0.0
13	15	1.7	12.6
13	17	5.6	13.1
13	16	9.6	12.9
130	124	132	124
130	142	128	128
130	132	159	129
1300	1266	1305	1263
1300	1199	1195	1243
1300	1257	1260	1235

These results demonstrate that the ferrozine and 1,10-phenanthroline methods generally provided consistent results except when dissolved Fe II concentrations were low (<13 mg/L), in which case the ferrozine method provided higher values relative to both 1,10-phenanthroline and the standard concentrations. The bias was especially evident in the 0 (blank) mg/L standards where the ferrozine method reported concentrations ranging between 2.3 – 4.2 mg/L. This bias may partially explain the slight CDM versus EPA/Ada inter-laboratory bias discussed above. Because of this bias, the 1,10-phenanthroline method is considered the preferred method for analyzing the BAFeIII assay extracts.

4.3.3 Range Comparison

The analytical range of the BAFeIII assay was demonstrated by comparison with the ranges determined by seven other analytical methods conducted on the soil samples and iron oxide standards. The seven methods selected for comparison with the BAFeIII assay were: citrate dithionite bicarbonate extractable Fe, ammonium oxalate extractable Fe, 0.5N extractable Fe III, 6N extractable Fe III, microcosm reducible Fe III, microcosm reducible Fe III with BrY, and microcosm reducible Fe III with BrY and FeOOH added. Each of these methods, though not standard methods for measuring bioavailable Fe III, were expected to provide minimum acceptable ranges. As discussed earlier, these methods are generally considered alternatives to the BAFeIII assay.

As shown in **Figure 4-3** and **Table 4-5**, the analytical range for the BAFeIII assay was generally similar to the chemical extraction methods and greater than the microcosm methods.

Table 4-5: Range of the BAFeIII Assay

Method	N	Analytical Range (g/kg wet soil)
CDM BAFeIII Assay	125	0.01 – 658
Citrate Dithionite Bicarbonate Extractable Fe	39	0.096 – 909
Ammonium Oxalate Extractable Fe	46	0.098 – 726
0.5N HCl Extractable FeIII	98	0.002 – 535
6.0N HCl Extractable FeIII	110	0.004 – 663
Microcosm Reducible FeIII with BrY/FeOOH	92	0.920 – 115
Microcosm Reducible FeIII	77	0.005 – 2.09
Microcosm Reducible FeIII with BrY	80	0.202 – 7.33

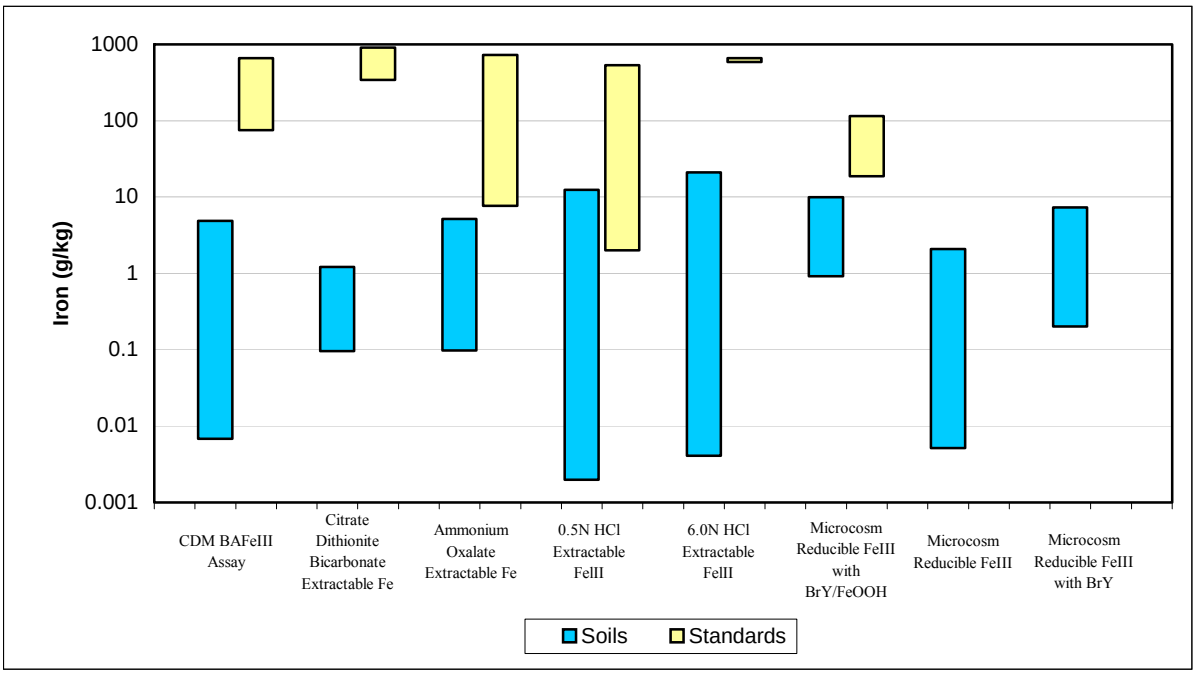

Figure 4-3. Analytical Range Comparison

4.3.4 Association with Bioavailable Fe III Factors

Principal components analysis (PCA) was conducted to evaluate the relationships and associations among the various potential bioavailable Fe III factors. PCA is a multivariate statistical method of identifying and characterizing the correlations of a large number of variables by grouping the variables into "components" such that variables within each component are related or highly correlated, thus allowing interpretation of each component according to its variables and the summarizing of many variables by a few components.

The PCA was conducted only on the soil data, i.e., the iron oxide standard data were not included. Since the soil data were most complete for the Elizabeth City data, the PCA results provided in this section primarily reflect the variances and relationships for variables within this data subset. The set of 30 variables included in the PCA are presented in **Table 4-6**.

Table 4-6: Principle Component Analysis Variable Set

Variable Name	Units	Variable Code
Acid Volatile Sulfide Bound FeII	(mg/kg)	AVSFEII
Ammonium Oxalate Extractable Fe	(g/kg wet soil)	AOFE
Citrate Dithionite Bicarbonate Extractable Fe	(g/kg wet soil)	CDBFE
CDM BAFeIII Assay	(g/kg wet soil)	CDMBAFEIII
Chromium Reducible Sulfide Bound Fe II	(mg/kg wet soil)	CRSFEII
Dissolved FeII	(mg/L)	DFEII
Dissolved Methane	(mg/L)	DMETH
Microcosm Reducible FeIII	(g/kg dry soil)	MFE
Microcosm Reducible FeIII with BrY	(g/kg dry soil)	MFEBRY
Microcosm Reducible FeIII with BrY/FeOOH	(g/kg dry soil)	MFEBRYFEOOH
Microcosm Reducible FeIII with FeOOH	(g/kg dry soil)	MFEFEOOH
EMPA RM Fe-metal	RM % Fe	RMFEM
EMPA RM FeSiO4	RM % Fe	RMFES
EMPA RM Geothite	RM % Fe	RMGEO
EMPA RM Hematite	RM % Fe	RMHEM
EMPA RM Ilmenite	RM % Fe	RMILM
EMPA RM Limonite	RM % Fe	RMLIM
EMPA RM Magnetite	RM % Fe	RMMAG
EMPA RM Organic	RM % Fe	RMORG
EMPA RM Pyrite	RM % Fe	RMPYR
EMPA RM Stainless Steel	RM % Fe	RMSTA
EMPA RM Ti-Mn Spinel	RM % Fe	RMTIS
EPA BAFeIII Assay	(g/kg wet soil)	EPABAFEIII
Total Fe	(g/kg wet soil)	TFE
0.5N HCl Extractable FeII	(g/kg wet soil)	V05FEII
0.5N HCl Extractable FelII	(g/kg wet soil)	V05FEIII
0.5N HCl Extractable Fe total	(g/kg wet soil)	V05FETOT
6.0N HCl Extractable FelI	(g/kg wet soil)	V6FEII
6.0N HCl Extractable FelII	(g/kg wet soil)	V6FEIII
6.0N HCl Extractable Fe total	(g/kg wet soil)	V6FETOT

The PCA was conducted on a correlation matrix using pairwise deletion to account for any missing data values, and variomax rotation, an orthogonal rotation method that minimizes the number of variables that have high loadings on each component and therefore simplifies the interpretation of the components.

Results of the PCA are summarized as follows. **Figure 4-4** shows the percentages of the total variance in the data set explained by, or contributed to, the first 10 components (i.e., grouping of the original 30 variables into the 10 components with the highest variances).

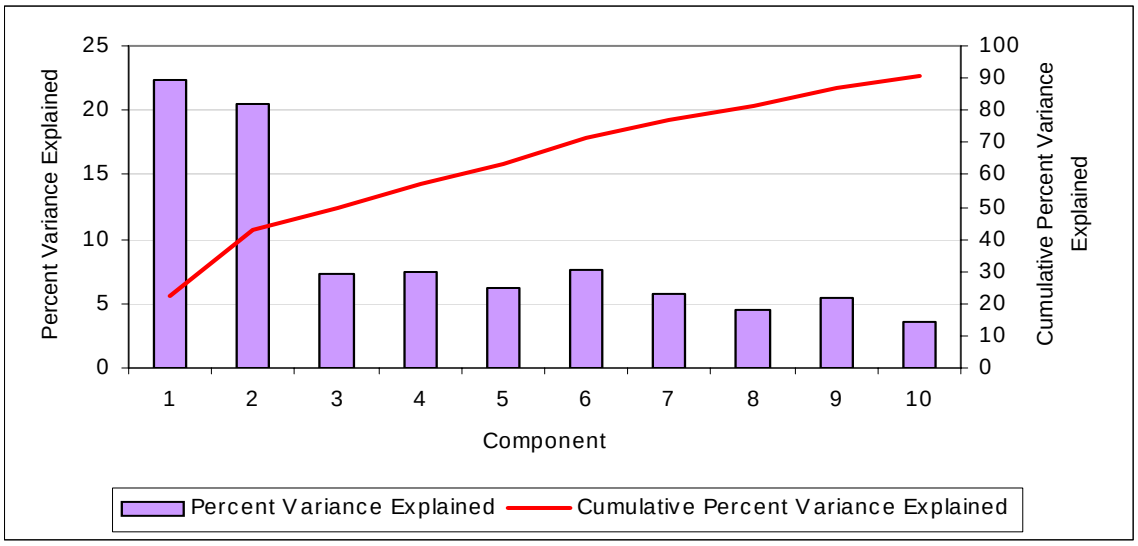

Figure 4-4. Percent Variance Explained by PCA Components

These results indicate that approximately 43% of the total variance is explained by the first two components, and that approximately 77% of the total variance is explained by the first seven components. Components 4-10 are relatively similar with regard to percentage of variance explained.

The correlations between the original variables and the components are referred to as "loadings". Thus, variables with high loadings in a particular component are associated with each other (i.e., they are intra-correlated). A listing of the variables with loadings greater than 0.45 in each of the 10 components is provided in **Table 4-7**. A negative sign indicates a negative loading.

Table 4-7: Component Loadings

Component	Variables with Loadings > 0.45
1	V6FETOT, V6FEII, RMMAG, V6FEIII, V05FETOT, V05FEIII, MFEBRY, V05FEII
2	V6FEIII, V05FETOT, V05FEIII, MFEBRY, AOFE, EPABAFEIII, TFE, CDBFE, MFEBRYFEOOH, CDMBAFEIII
3	RMHEM, -RMILM, -RMFES
4	MFEFEOOH, MFE
5	RMORG, RMSTA
6	DMETH, DFEII
7	RMPYR, RMGEO
8	RMFEM
9	RMLIM
10	RMTIS

Examination of the components resulted in the following interpretation. Component 1, which accounted for approximately 22% of the total variance in the data set, contained a number of possible BAFeIII-related factors that are associated with each other but that are not particularly well associated with the BAFeIII assay results (loadings ≤ 0.45). These include 0.5N and 6N extractable Fe II and relative mass Fe in magnetite. Component 1 was concluded to be associated with iron.

Component 2, which accounted for approximately 20% of the total variance in the data set, also contained several of the same variables that loaded highly into Component 1, including 0.5N extractable Fe III and 0.5N HCl extractable total Fe, 6N HCl extractable Fe III, and microcosm reducible Fe III with BrY. Component 2 also contained citrate dithionite bicarbonate and ammonium oxalate extractable Fe, total Fe, and microcosm reducible Fe III with BrY and FeOOH, in addition to both the CDM and EPA BAFeIII assay variable. Component 2 therefore indicates the relatively high association of BAFeIII with these variables. Component 2 is concluded to be associated with BAFeIII as opposed to Component 1 that is associated with iron.

The remaining components (3 – 10) are difficult to evaluate but nevertheless correspond to variables that are essentially independent of BAFeIII.

Therefore, the PCA results indicated that Components 1 and 2 were the key components relative to the association of BAFeIII with bioavailable Fe III factors. **Figures 4-5 and 4-6** illustrate the actual loadings for these two components.

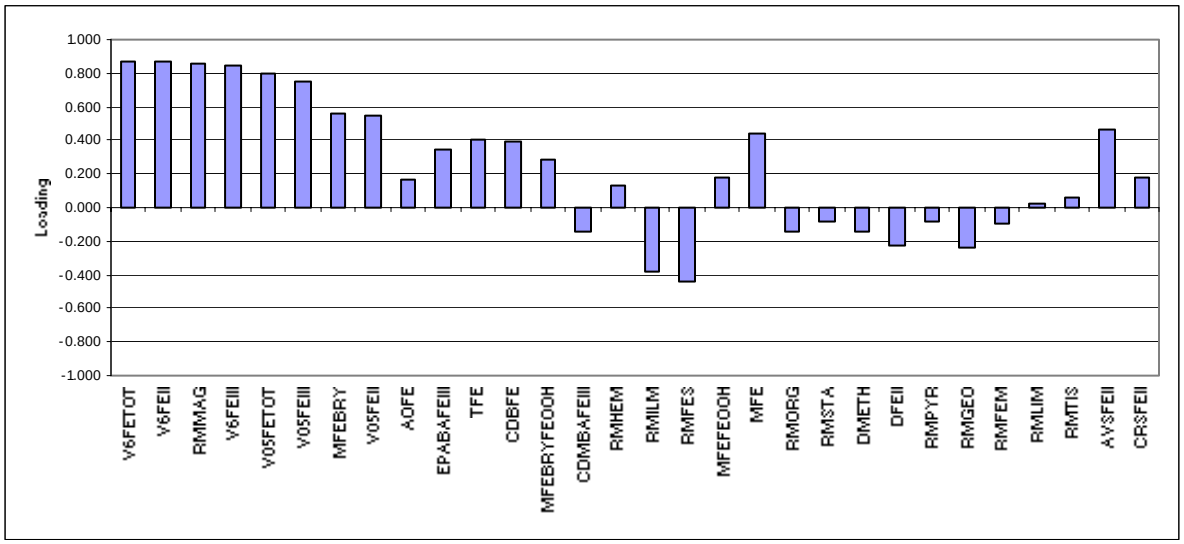

Figure 4-5. Loadings for PCA Component 1

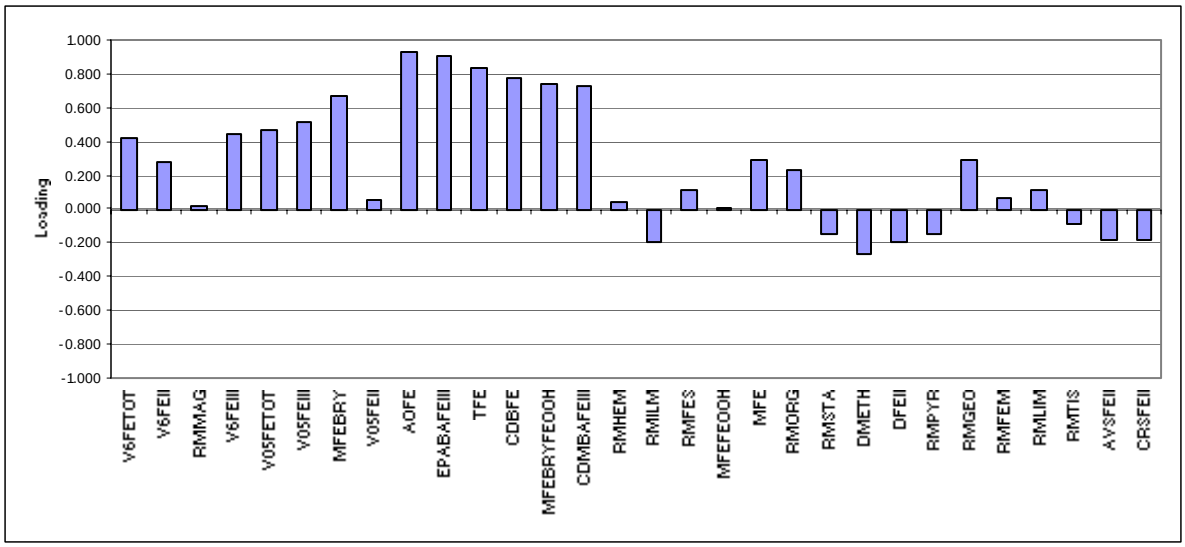

Figure 4-6. Loadings for PCA Component 2

4.3.5 Association with Microcosm Reducible Fe III

A primary performance objective was to demonstrate association between the BAFeIII assay and microcosm reducible Fe III both in the presence and absence of BrY. Data were plotted after natural log transformation as shown in **Figures 4-7** and **4-8**. Natural log transformation was conducted to allow a more representative comparison by spreading the data more evenly across the measurement scales; although the true associations between the methods may be linear, the data obtained by the methods at the demonstration sites more closely resembled lognormal distributions.

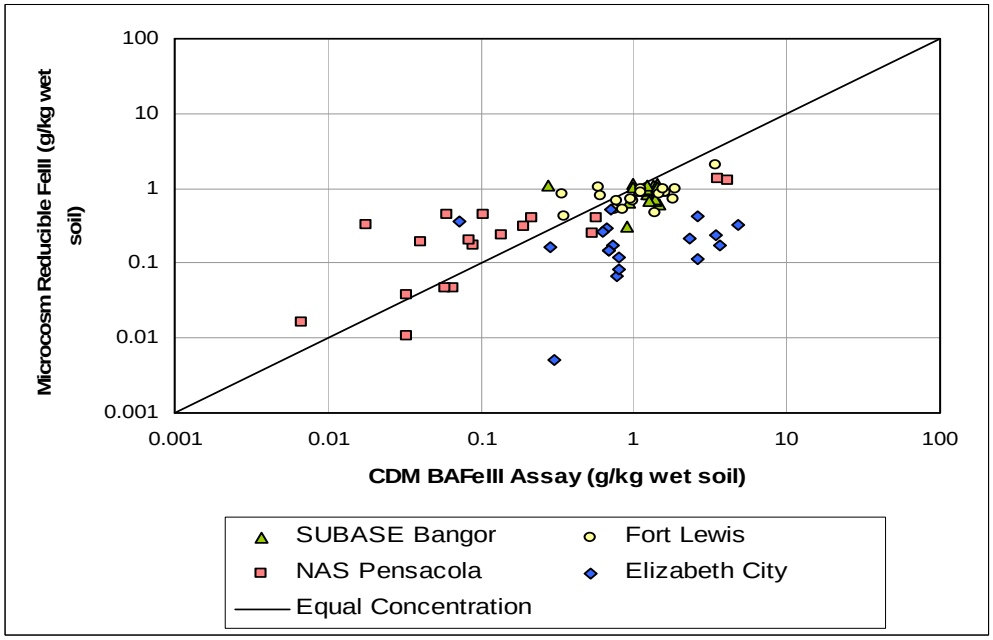

Figure 4-7. Association between BAFeIII and Microcosm Reducible FeIII

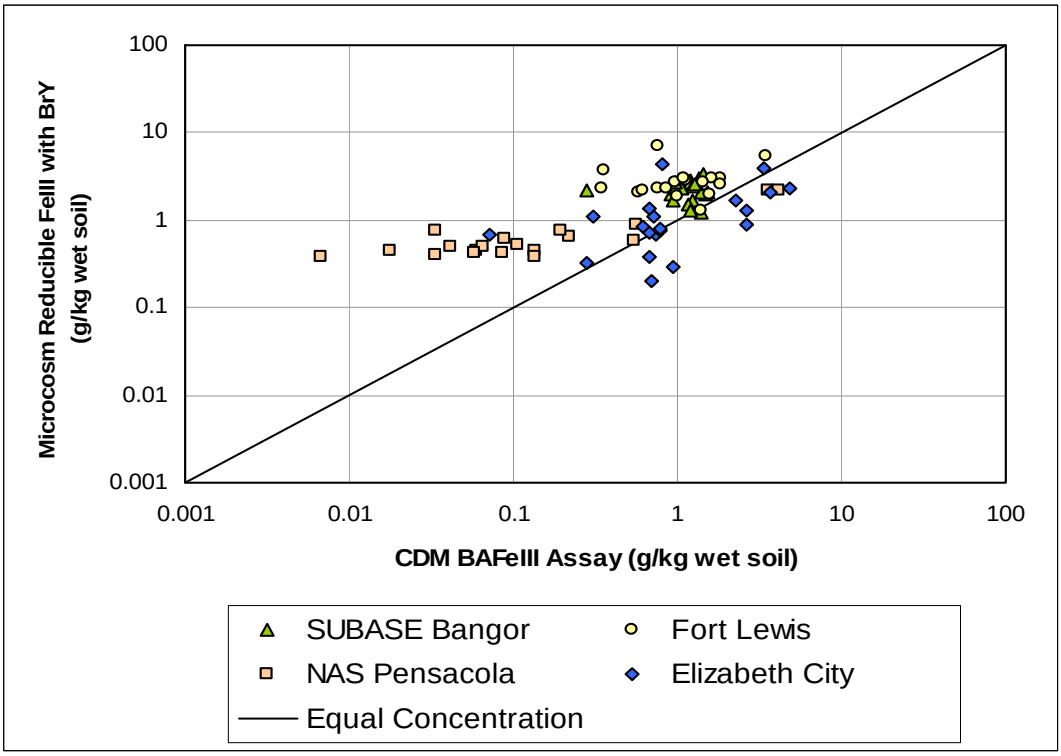

Figure 4-8. Association between BAFeIII and Microcosm Reducible FeIII with BrY

Both microcosm reducible Fe III in the absence of BrY and microcosm reducible Fe III in the presence of BrY were positively associated with the BAFeIII assay as discussed in Section 4.3.4. The presence of BrY in the microcosm was not necessary to obtain a positive association with the test kit. This result is significant because it demonstrates that the BAFeIII test kit containing BrY generates results that are representative of iron reduction in the absence of BrY.

4.3.6 Association with 0.5N and 6.0N HCl Extractable Fe III

A primary performance objective was to demonstrate association between the BAFeIII assay and 0.5N and 6N HCl extractable Fe III. Data were plotted after natural log transformation as shown in **Figures 4-9** and **4-10**. Natural log transformation was conducted to allow a more representative comparison by spreading the data more evenly across the measurement scales; although the true associations between the methods may be linear, the data obtained by the methods at the demonstration sites more closely resembled lognormal distributions.

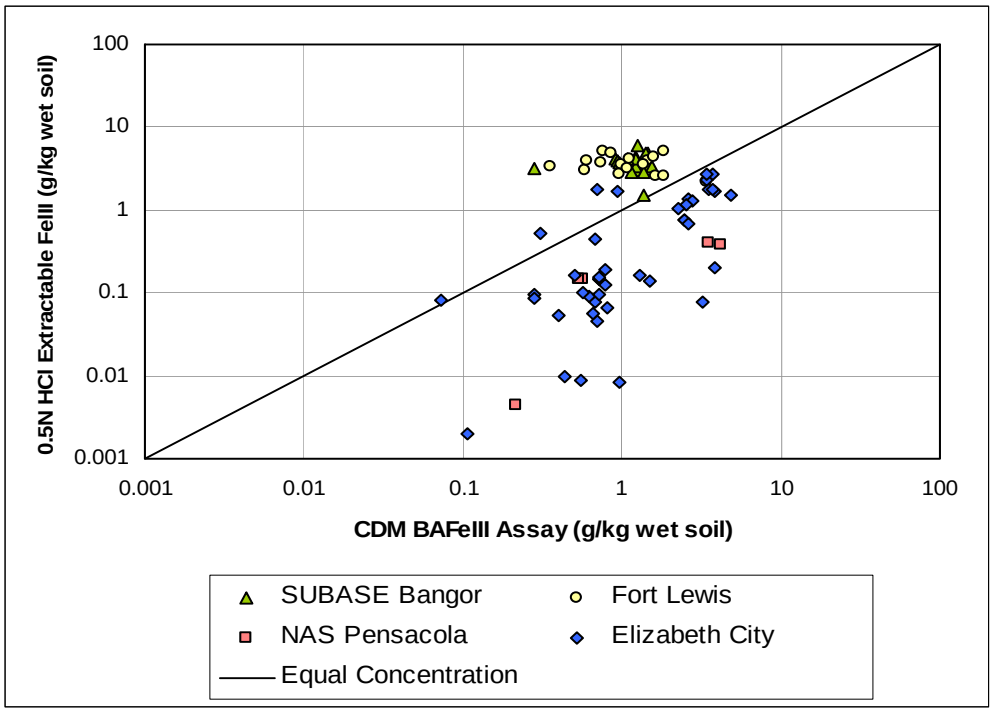

Figure 4-9. Association between BAFeIII and 0.5N HCl Extractable FeIII

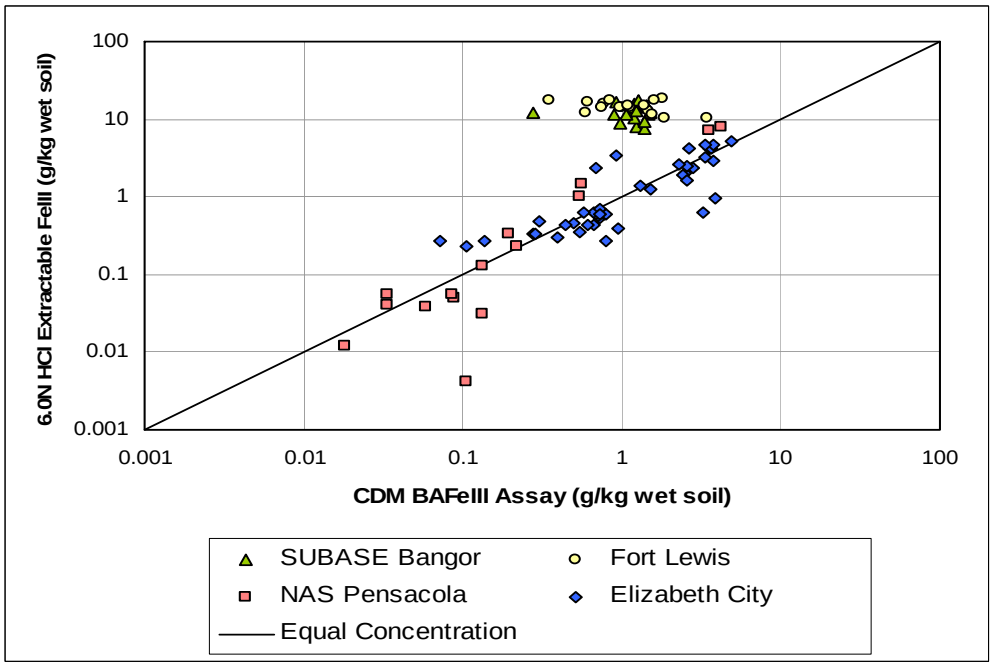

Figure 4-10. Association between BAFeIII and 6.0N HCl Extractable FeIII

Both 0.5N extractable Fe III and 6N extractable Fe III were positively associated with the BAFeIII assay as discussed in Section 4.3.4. BAFeIII results tended to exceed 0.5N HCl extractable Fe III results at the lower concentrations, probably because the extraction removes only the more easily extractable Fe III while a relatively larger percentage of non-extractable Fe III is bioavailable. These results are consistent with those previously observed (Evans and Jones 1999). The greater results obtained using the BAFeIII test kit give an indication that it is more robust with respect to quantification of BAFeIII. BAFeIII results tended to be similar to 6N HCl extraction results for the NAS Pensacola and Elizabeth City data sets. BAFeIII results tended to be less than the 6N HCl extraction results for the SUBASE Bangor and Ft. Lewis data sets. The differences noted with the latter data sets may be attributable to the samples being stored and later analyzed rather than immediately analyzed (see Section 3.5.2). NAS Pensacola and Elizabeth City samples were analyzed immediately after collection.

4.3.7 Association with Citrate Dithionite Bicarbonate Extractable Fe, Ammonium Oxalate Extractable Fe, and Total Fe

A primary performance objective was to demonstrate association between the BAFeIII assay and citrate dithionite bicarbonate extractable Fe and ammonium oxalate extractable Fe. The association between the BAFeIII assay and total Fe was also determined. Data were plotted after natural log transformation as shown in **Figures 4-11** through **4-13**. Natural log transformation was conducted to allow a more representative comparison by spreading the data more evenly across the measurement scales; although the true associations between the methods may be linear, the data obtained by the methods at the demonstration sites more closely resembled lognormal distributions.

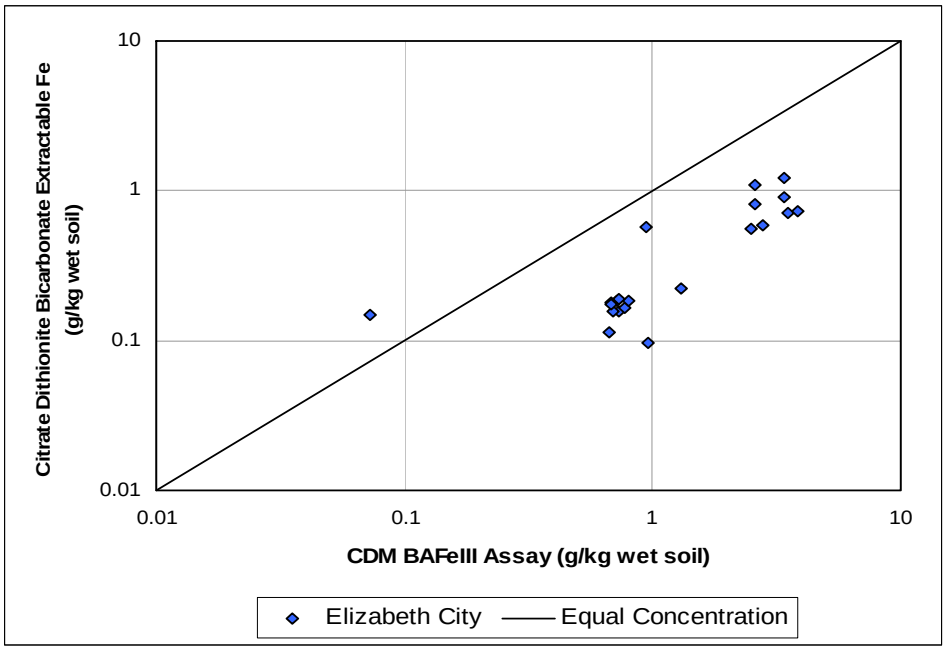

Figure 4-11. Association between BAFeIII and Citrate Dithionite Bicarbonate Extractable Fe

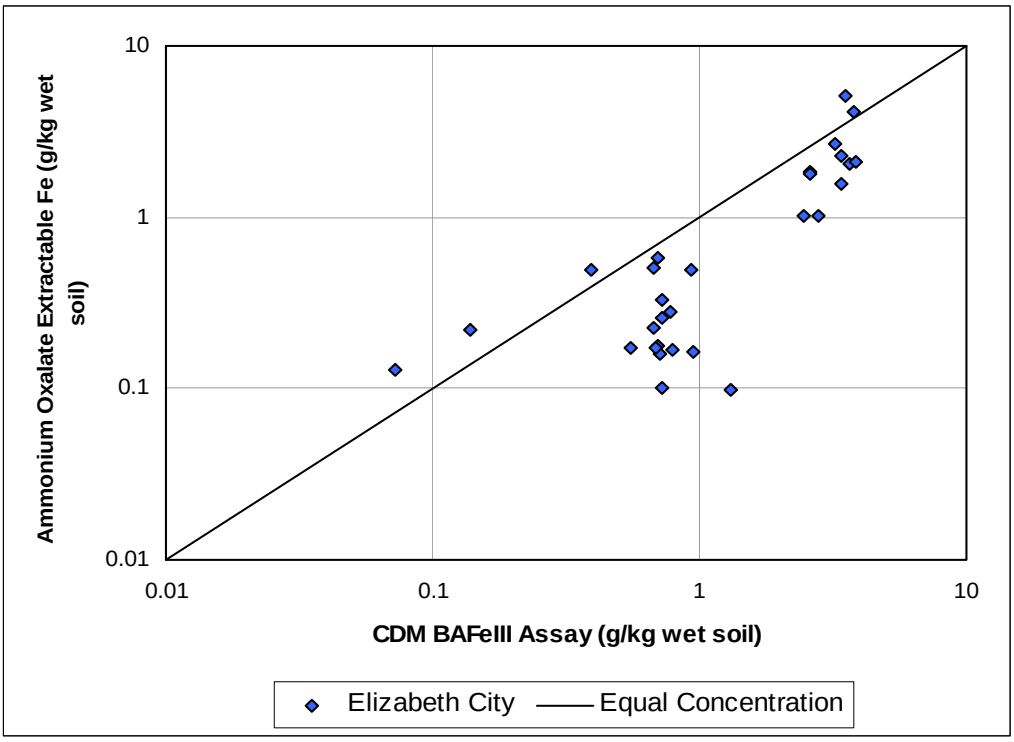

Figure 4-12. Association between BAFeIII and Ammonium Oxalate Extractable Fe

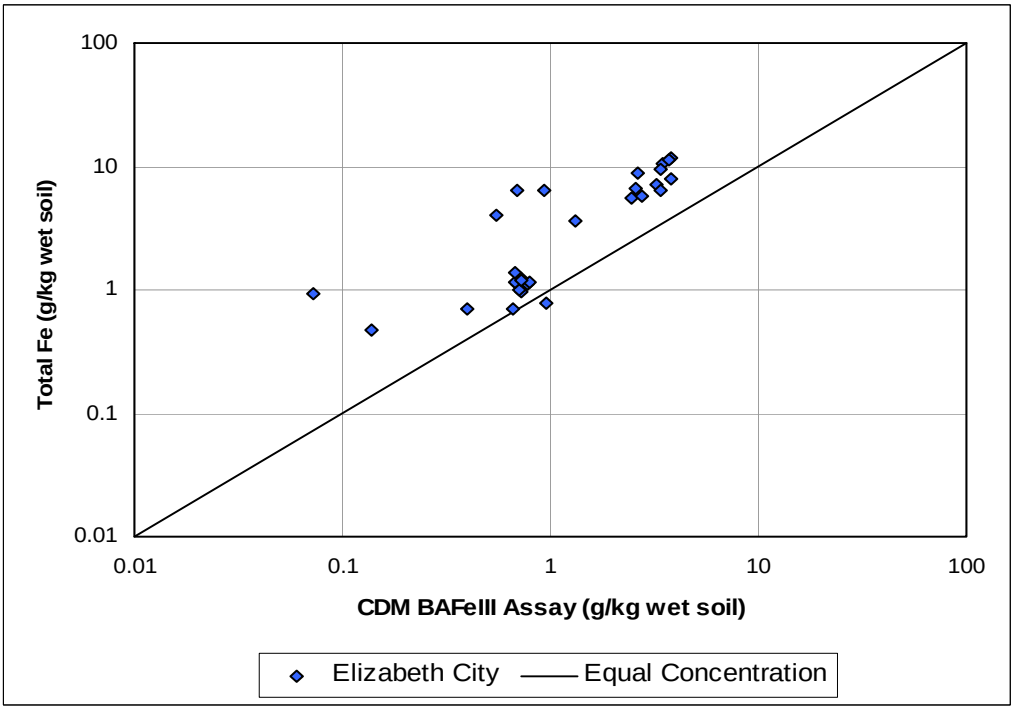

Figure 4-13. Assoication between BAFeIII and Total Fe

All three methods (BAFeIII versus citrate dithionite bicarbonate extractable Fe, ammonium oxalate extractable Fe, and total Fe) showed a positive association with the BAFeIII assay as discussed in Section 4.3.4. BAFeIII assay results were consistently greater than those for the citrate dithionite bicarbonate extraction. The BAFeIII assay results were consistently less than those for the total iron analysis as expected; not all iron is bioavailable. The BAFeIII assay results were greater than the ammonium oxalate extraction results for most but not all samples. Thus the BAFeIII assay appears to be more robust than chemical extraction methods with respect to its ability to quantify the maximum amount of BAFeIII in soil.

4.3.8 Relationship with Fe III Mineral Crystallinity and Surface Area

A significant amount of research has been conducted by others evaluating the bioavailability of various synthetic iron oxides. The crystallinity and surface area of Fe III minerals have been shown to be an important factor affecting in the bioavailability of Fe III. To investigate this factor, several different iron oxide standards of varying degrees of crystallinity and surface area were analyzed by BAFeIII assay, microcosm reducible Fe III with BrY, 0.5N HCl extractable Fe III, 6N HCl extractable Fe III, citrate dithionite bicarbonate extractable Fe, ammonium oxalate extractable Fe, and total Fe. Surface areas of the standards were measured using the BET method. The results are shown graphically in **Figures 4-14 through 4-20** and discussed below.

As shown in **Figure 4-14**, poorly crystalline, high surface area iron oxides such as 2-line and 6-line ferrihydrite demonstrated greater BAFeIII than highly crystalline, low surface area iron oxides such as magnetite, hematite, and goethite. Such a relationship between bioavailability and surface area has been demonstrated by Roden and Zachara (1996). Reduced bioavailability of 2-line ferrihydrite may have been due to oxide particle aggregation as has been observed by Roden (2003). On the other hand, lepidocrocite had a relatively low surface area and yet the highest BAFeIII. Additionally, 6-line ferrihydrite (the more crystalline and lower surface area of the two forms) had a relatively higher BAFeIII when compared to 2-line ferrihydrite. This pattern was also observed for the more crystalline/lower surface area minerals, where BAFeIII generally increased in the order: magnetite > goethite > hematite, or the opposite of what might have been expected based on surface area measurements alone.

Although crystallinity and surface area are generally thought to be the dominant factors affecting bioavailability of iron oxides, other investigators have observed deviations in the presumed relationship between surface area and bioavailability. For example, anaerobic reduction of ferrihydrite, lepidocrocite, goethite, and hematite by the anaerobic bacterium *Corynebacterium* demonstrated a positive relationship between the rate of reduction and surface area for all oxides except lepidocrocite (Schwertmann et al., 1986). Lepidocrocite was reduced at the same rate as ferrihydrite and significantly faster than goethite and hematite. The best correlation was between reduction rate and oxalate extraction (Schwertmann et al., 1986). Roden (2003) has also observed greater biological reduction rates of lepidocrocite when compared to goethite with a similar surface area. This difference in reduction rates appears to be attributable to structural differences between lepidocrocite and goethite (Cooper et al., 2000; Hersman et al., 2001; Roden, 2003). These data further indicate that factors other than surface area affect iron oxide

bioavailability. Indirect measurement of BAFeIII is unlikely to account for the myriad factors that affect biological iron oxide reduction.

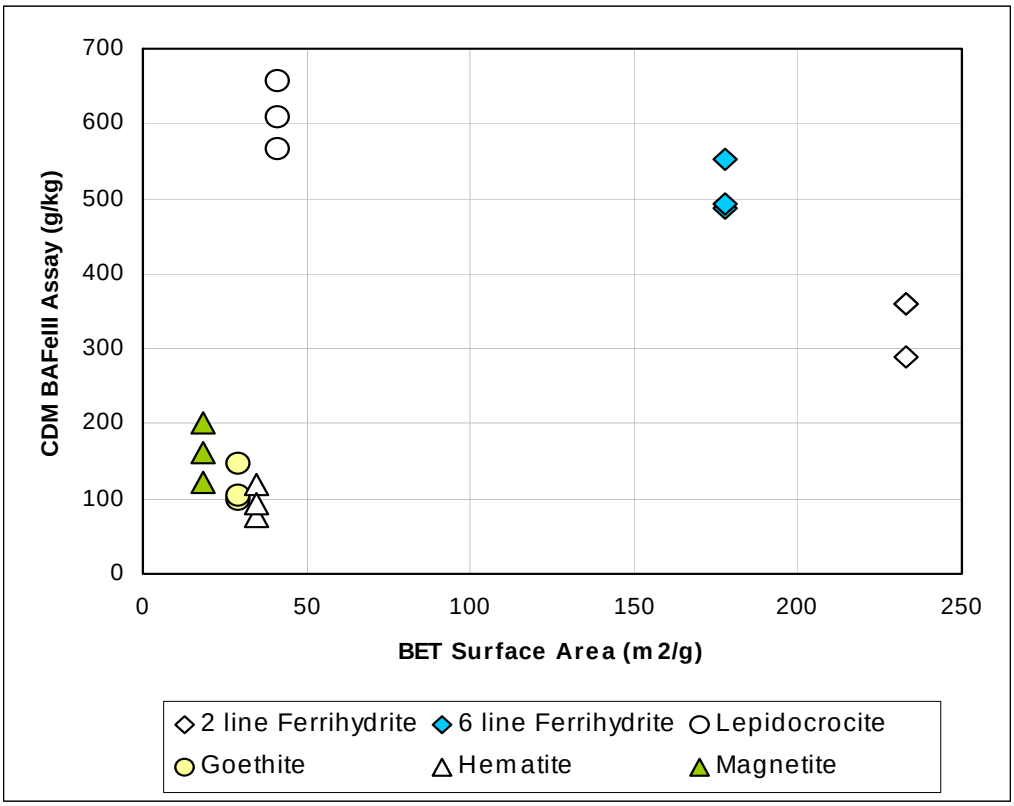

Figure 4-14. BAFeIII Assay versus Surface Area for Iron Oxide Standards

As shown in **Figure 4-15**, a similar relationship existed between microcosm reducible Fe III with BrY and crystallinity/surface area (cf., **Figures 4-14** and **4-15**). Although microcosm reducible Fe III with BrY results were lower overall than BAFeIII results, the same general pattern was observed, indicating that at least the two methods are consistent with regard to crystallinity and surface area factors. The microcosm data and the bioassay showed that goethite and hematite were partially bioavailable which is in contrast to 0.5 N HCl and ammonium oxalate chemical extraction data presented below. Again, the bioavailability of Fe III is not solely a function of crystallinity/surface area.

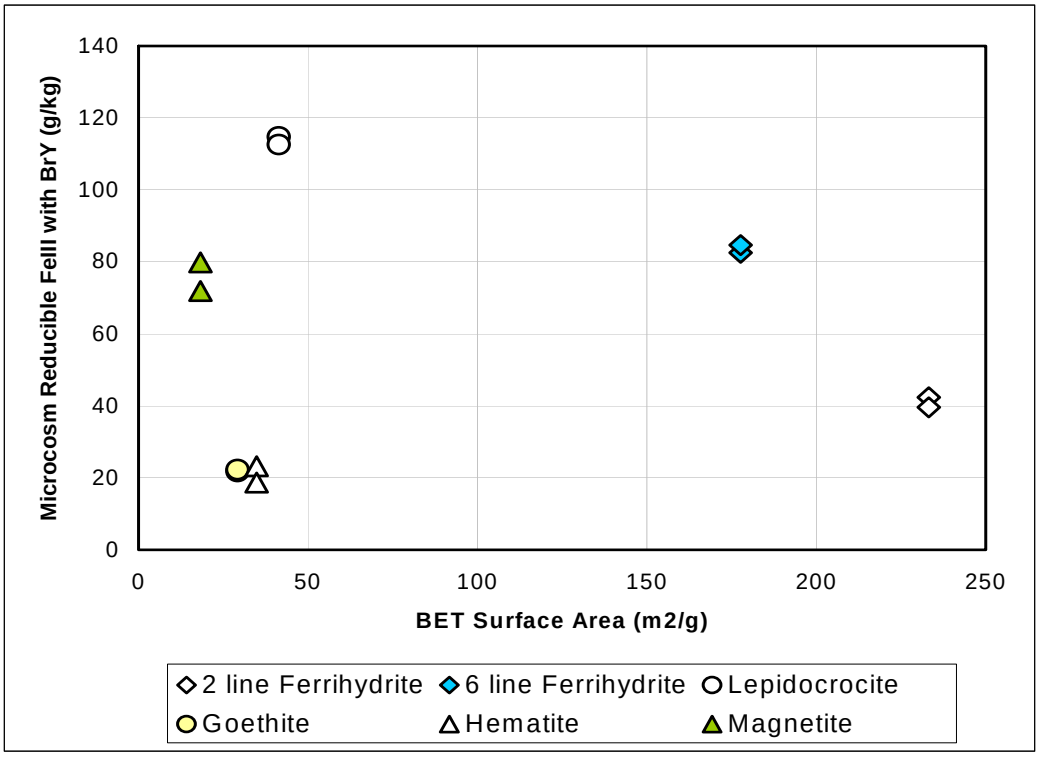

Figure 4-15. Microcosm Reducible Fe III with BrY versus Surface Area for Iron Oxide Standards

As shown in **Figure 4-16**, 0.5N HCl extractable Fe III and crystallinity/surface area were more closely but not completely related. The highest 0.5N HCl extractable Fe III was obtained for the least crystalline and highest surface area iron oxide standard, 2 line ferrihydrite, gradually decreasing in the order: 2 line ferrihydrite > 6 line ferrihydrite > lepidocrocite > magnetite. Of note here, however, was the relative insolubility of goethite and hematite relative to the standards with similar surface areas (lepidocrocite and magnetite). These data and the data presented in **Figures 4-14** and **4-15** demonstrate that the 0.5 N HCl extraction underestimates the bioavailability of certain crystalline iron oxides. Lovley and Phillips (1987) have also presented data indicating that chemical extractions with HCl-hydroxylamine underestimate bioavailability of crystalline iron oxides. Additionally, magnetite and 2-line ferrihydrite, for example, had about a 40 percent difference in acid extractability but a 1200 percent difference in surface area. On the other hand, with the more concentrated 6.0N HCl extractable Fe III, all of the standards exhibited similar degrees of dissolution, as shown in **Figure 4-17**.

As shown in **Figure 4-18**, citrate dithionite bicarbonate extractable Fe III was scattered but in general constant and independent of crystallinity/surface area. The results in **Figure 4-19**, on the other hand, were somewhat similar to results for 0.5 N HCl extractable iron shown in **Figure 4-16**. Goethite and hematite demonstrated very low extractability and the other iron oxides were similar in their extractability. As shown in **Figure 4-20**, total iron was constant around 600 g/kg. Iron oxide with the formula FeOOH is calculated to have an iron content of 630 g/kg.

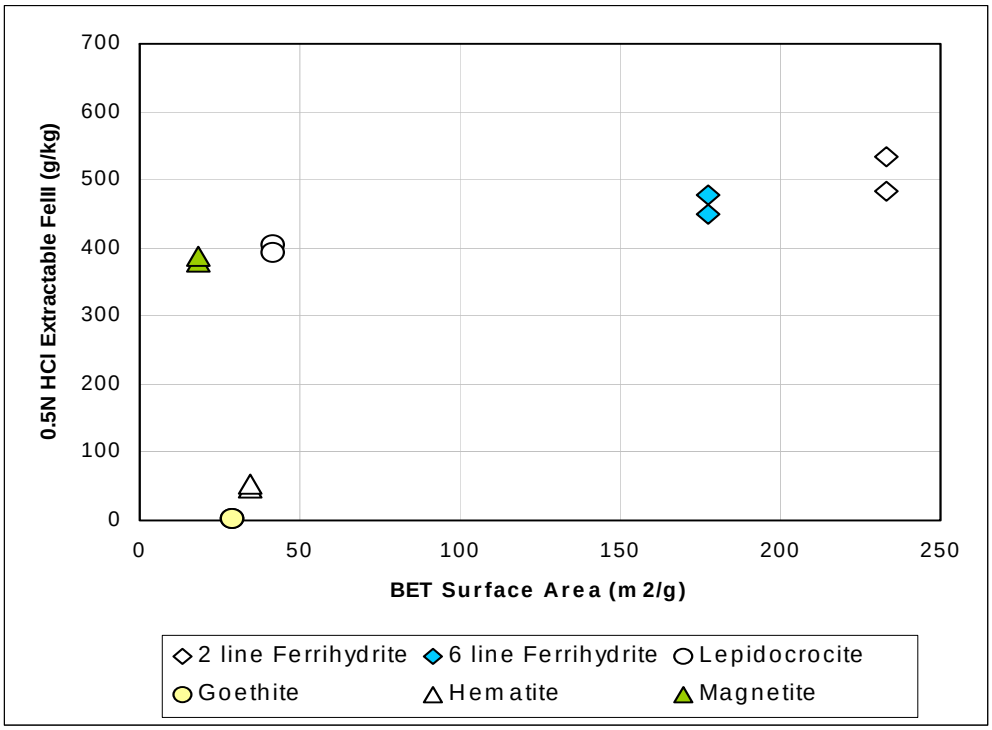

Figure 4-16. 0.5N HCl Extractable Fe III versus Surface Area for Iron Oxide Standards

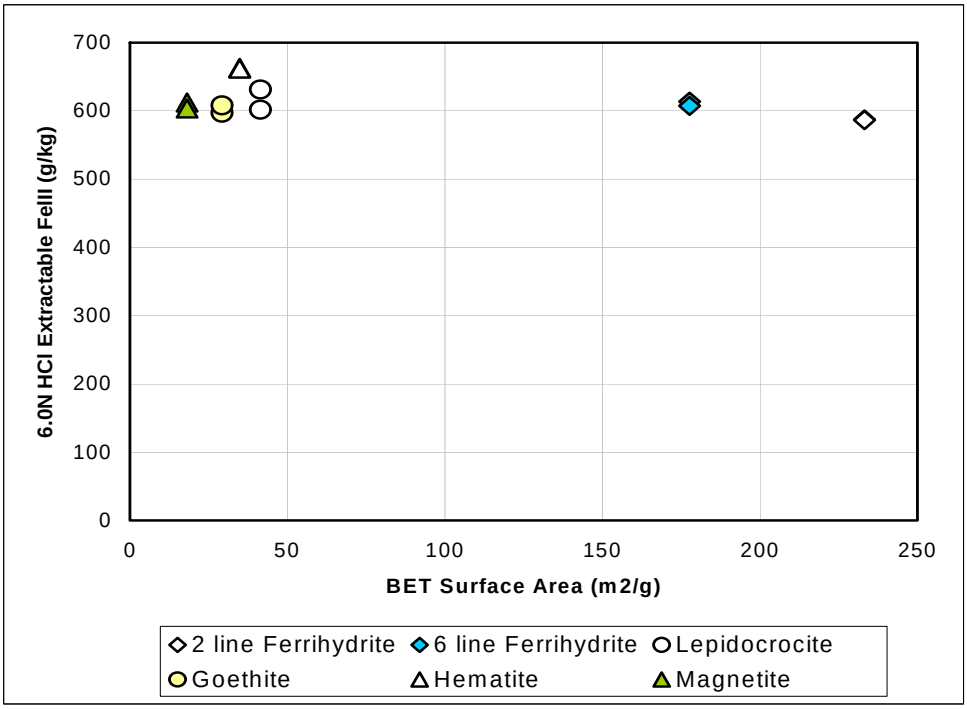

Figure 4-17. 6.0N HCL Extractable Fe III versus Surface Area for Iron Oxide Standards

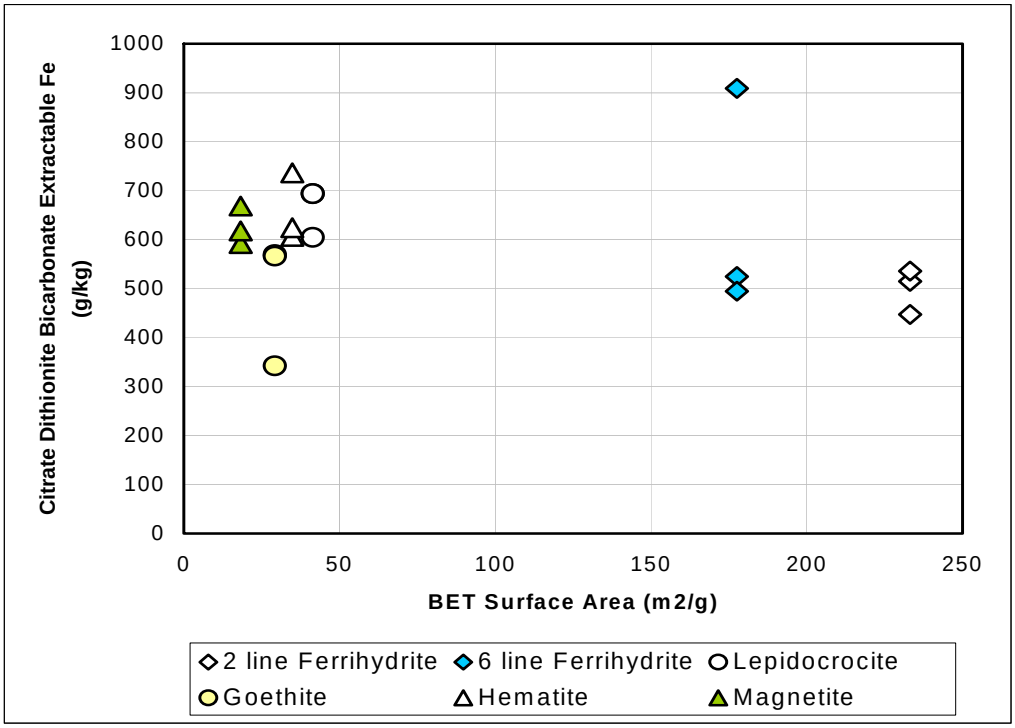

Figure 4-18. Citrate Dithionite Bicarbonate Extractable Fe versus Surface Area for Iron Oxide Standards

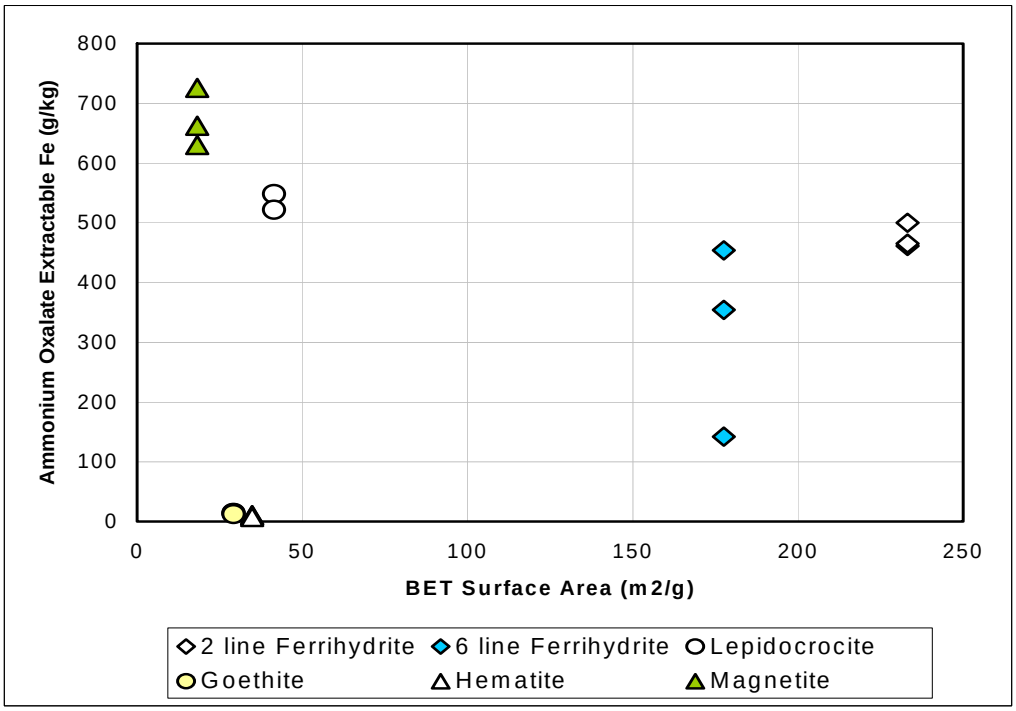

Figure 4-19. Ammonium Oxalate Extractable Fe versus Surface Area for Iron Oxide Standards

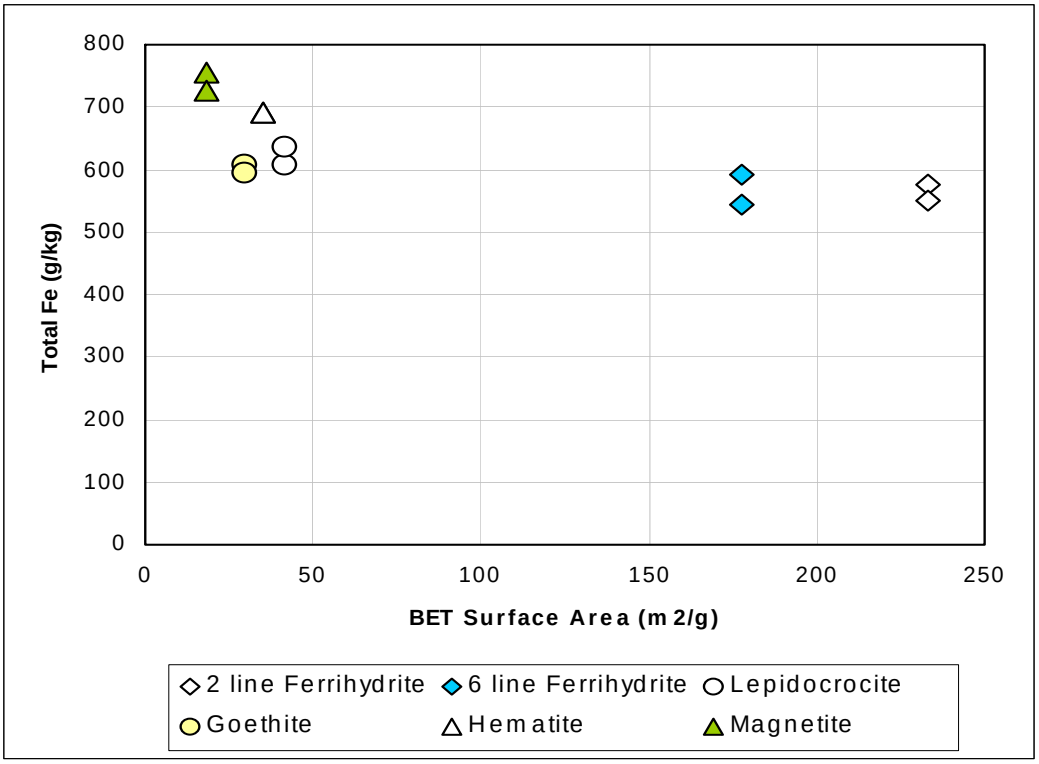

Figure 4-20. Total Fe versus Surface Area for Iron Oxide Standards

Figure 4-21 shows iron concentrations measured using the BAFeIII assay, microcosms, and chemical extractions expressed as fractions of total iron concentrations. The BAFeIII assay conducted by the CDM and EPA laboratories gave qualitatively and quantitatively similar results. The BAFeIII assay results for 2-line ferrihydrite were greater at the CDM laboratory. The BAFeIII assay results for hematite were greater at the EPA laboratory. Nevertheless, 6-line ferrihydrite and lepidocrocite were more bioavailable using the BAFeIII assay compared to goethite, hematite, and magnetite. The bioavailabilities of goethite, hematite, and magnetite using the BAFeIII assay were nonzero. The values of the goethite iron fraction divided by the lepidocrocite iron fraction (defined here as the goethite-lepidocrocite ratio) were 0.20 and 0.17 for the CDM and EPA BAFeIII assay results, respectively. The results for the microcosms conducted with *Shewanella alga* BrY were qualitatively similar to the BAFeIII results but quantitatively less. The microcosm results were 12 to 47 percent of the CDM BAFeIII assay results. The goethite-lepidocrocite ratio for the microcosm was 0.20 which is identical to the CDM BAFeIII assay ratio. Extractions with 0.5N HCl and ammonium oxalate had similar patterns to each other. Goethite was essentially unextractable with either reagent and the goethite-lepidocrocite ratios were 0.01 for 0.5 N HCl and 0.03 for ammonium oxalate and significantly less than for the BAFeIII assay and microcosm. Ammonium oxalate and 0.5N HCl iron fractions were greater than BAFeIII assay iron fractions for 2-line ferrihydrite and magnetite. BAFeIII assay iron fractions were greater than 0.5 N HCl extraction iron fractions for lepidocrocite. Citrate-dithionite-bicarbonate 6N HCl were capable of nearly complete extraction

of each iron oxide. Major extractability differences amongst the different iron oxides were not observed for either of these two extractants. The goethite-lepidocrocite ratios were 0.77 and 1.01 for citrate-dithionite-bicarbonate and 6N HCl, respectively and significantly greater than that for the BAFeIII assay and microcosm. Similar comparisons between the different analytical methods were also observed using a goethite to 6-line ferrihydrite ratio.

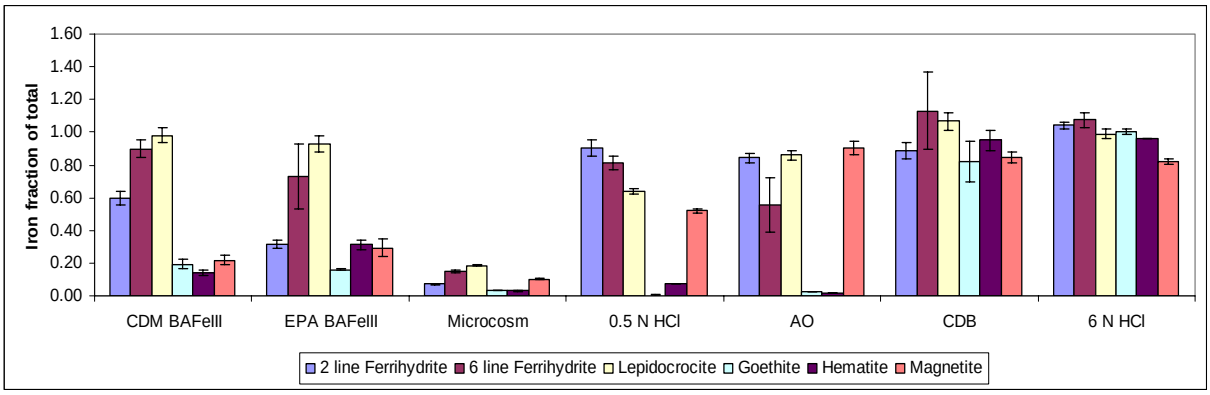

Figure 4-21. Summary of Iron Oxide Analytical Results

4.3.9 Mass Balance

A mass balance calculation on iron at the Elizabeth City Fuel Farm site was conducted to further validate the BAFeIII assay and illustrate the use of BAFeIII data. This calculation was based on data obtained during this demonstration (Appendix D) plus data and pertinent information obtained from the EPA (Wilson et al., 2000). The mass balance was conducted by calculating the mass of BAFeIII originally present in the area impacted by hydrocarbons, calculating the mass of ferrous iron removed in soluble form via downgradient groundwater transport, and then comparing the two values.

First the mass of BAFeIII originally present in the area impacted by hydrocarbons was calculated. The area of impacted soil at the Fuel Farm site was assumed to be 11,000 m^2 (Wilson et al., 2000, p.13). Given an average thickness of 2 m (Dr. John Wilson, personal communication) the estimated volume of impacted soil at the site is: (11,000 m^2)(2 m) = 22,000 m^3. Assuming an average porosity of 0.3 (Wilson et al., 2000, p.23) and soil particle density of 2.6 kg/L, the average bulk soil density is 1.82 kg/L (Wilson et al., 2000, p.11); and therefore the estimated total mass of impacted soil at the site is: (22,000 m^3)(1.82 kg/L)(1,000 L/m^3) = 40,040,000 kg. The original BAFeIII in the impacted soil prior to the release of fuel contaminants can be estimated using measurements taken on the background soil samples. The average background soil BAFeIII measured by CDM and EPA/Ada at three depth intervals (Appendix D) was 0.733 g/kg and 0.590 g/kg, respectively. This results in an original mass of BAFeIII in the impacted soil of: (0.733 g/kg)(40,040,000 kg)/(454 g/lb) = 64,600 lb based on CDM BAFeIII and (0.590 g/kg)(40,040,000 kg)/(454 g/lb) = 52,000 lb based on EPA/Ada BAFeIII. Average background soil concentrations determined using chemical extraction methods were 0.5 N HCl - 0.12 g/kg, 6 N HCl – 0.60 g/kg, ammonium oxalate - 0.18 g/kg, citrate dithionite bicarbonate – 0.17 g/kg, total iron 1.09 g/kg. Based on these concentrations, the

masses of extractable iron present in impacted soil were 0.5 N HCl - 10,500 lb, 6 N HCl - 52,900 lb, ammonium oxalate – 15,900 lb, citrate dithionite bicarbonate – 15,000 lb, and total iron – 96,000 lb.

Next the mass of soluble ferrous iron transported downgradient was calculated. The estimated average contaminant plume width at the site is 230 ft (Wilson et al., 2000, p.16) with an average vertical thickness of 10 ft (Wilson et al., 2000, estimated from **Figures 3.14** and **3.15**). Thus the average plume cross sectional area relative to groundwater flow is: (230 ft)(10 ft) = 2,300 ft^2. Leakage of fuel contaminants from underground storage tanks (USTs) at the site began in 1942 and stopped in 1991 (Wilson et al., 2000, p.11 and Dr. John Wilson, personal communication), and BAFeIII measurements were taken on samples collected in 2002; thus the maximum number of years of advective Fe II release from the site is 60 yrs. A minimum time for advective Fe II release was assumed to be 20 yrs. Given an average groundwater flow velocity of 82 m/yr (Wilson et al., 2000, Table 4.1) and porosity of 0.3, the estimated total volume of groundwater transported through the plume is: (2,300 ft^2)(82 m/yr)(3.281 ft/m)(60 yr)(7.48 gal/ft^3) = 83,314,774 gal based on the maximum timeframe and (2,300 ft^2)(82 m/yr)(3.281 ft/m)(20 yr)(7.48 gal/ft^3) = 27,771,591 gal based on the assumed minimum timeframe. Therefore, given an average dissolved Fe II concentration of 57 mg/L (Wilson et al., 2000, Table 4.2), the total mass of Fe advectively removed as Fe II is: (83,314,774 gal)(3.785 L/gal)(57 mg/L)/(1,000 mg/g)/(454 g/lb) = 39,592 lbs Fe over the maximum timeframe and (27,771,591 gal)(3.785 L/gal)(57 mg/L)/(1,000 mg/g)/(454 g/lb) = 13,197 lbs Fe over the minimum timeframe.

Comparisons of the calculated iron masses are presented in **Table 4-8.** In summary, the total mass of BAFeIII in the impacted soil determined using the BAFeIII assay is estimated to range between about 52,000 – 65,000 lbs, of which between about 13,000 – 40,000 lbs have been removed historically via biodegradation of fuel contaminants, reduction of BAFeIII to Fe II, and advective groundwater transport of dissolved Fe II from the site. This result indicates that the BAFeIII assay did not underestimate the amount of BAFeIII iron present in the soil. Estimates of BAFeIII obtained using 0.5 N HCl, ammonium oxalate, and citrate dithionite bicarbonate all underestimated the mass of BAFeIII. The total iron mass gives an upper bound for the calculation and is greater than all other values as expected. The 6N HCl extractable iron is similar to the BAFeIII estimate.

Table 4-8: Iron Mass Balance for Elizabeth City Fuel Farm

Parameter	Estimated Mass (lb)
Minimum advectively removed Fe II	13,000
Maximum advectively removed Fe II	40,000
Minimum BAFeIII assay estimate	52,000
Maximum BAFeIII assay estimate	65,000
0.5 N HCl estimate	11,000
6 N HCL estimate	53,000
Ammonium oxalate estimate	16,000
Citrate dithionite bicarbonate estimate	15,000
Total iron estimate	96,000

Further evaluation of Elizabeth City Fuel Farm data involved calculation of BAFeIII consumption. BAFeIII consumption was calculated as the BAFeIII originally present (as estimated from measurements of the background soil) minus the current BAFeIII (as determined from measurements of the impacted soils), that is:

BAFeIII consumed = BAFeIII background soil – BAFeIII impacted soil

Using the average background soil BAFeIII measured by CDM and EPA/Ada at three depth intervals (0.733 g/kg and 0.590 g/kg, respectively), this calculation was conducted for samples collected from the three impacted soil locations (Downgradient 1, Downgradient 2 and Hot Zone) and at the three depth intervals (15-18, 18-21 and 21-24 ft for Downgradient 1 and Downgradient 2, and 18-21 and 21-24 ft for the Hot Zone). The results are summarized in **Table 4-9** along with measurements of dissolved methane, total petroleum hydrocarbon (TPH) + volatile organic carbon (VOC), and dissolved Fe II conducted during soil sample collection.

Table 4-9: Consumed BAFeIII and Dissolved CH4, TPH + VOC, and Fe II at Elizabeth City Fuel Farm

Sample ID	Depth (ft)	Average BAFeIII Consumed[1]	CH_4 (mg/L)	TPH + VOC (μg/L)	Fe II (mg/L)
Downgradient 1	15-18	0.422	5.99	1,774.2	66.4
	18-21	0.046	5.31	3,303.4	11.2
	21-24	0.101	3.79	3,376.1	7.12
Hot Zone	18-21	0.141	0.218	5,461.7	21.9
	21-24	0.072	0.556	388.1	10.0
Downgradient 2	15-18	0.468	11	858.9	109
	18-21	0.259	9.47	1,465	83
	21-24	0.058	2.99	1,442.4	46

[1] Average of CDM and EPA/Ada measurements.

As shown in **Figure 4-22**, the amount of consumed BAFeIII generally decreases with depth. This is reasonable because the fuel hydrocarbons, which are light non-aqueous phase liquids, would be present primarily in the shallower zones of the aquifer; and, therefore, biodegradation would tend to proceed more rapidly in the shallower zones where the hydrocarbons provide a larger carbon source reservoir. This is illustrated in **Figure 4-23**, which as expected shows that the concentration of dissolved TPH + VOC, representing remaining fuel hydrocarbons, is generally lower when the amount of consumed BAFeIII is higher. Conversely, the concentrations of dissolved methane and Fe II generally increase with higher consumed BAFeIII, as shown in **Figures 4-24 and 4-25**. This is expected because the generation of CH_4 and Fe II should be higher in the zones where more biodegradation and consumption of BAFeIII has occurred.

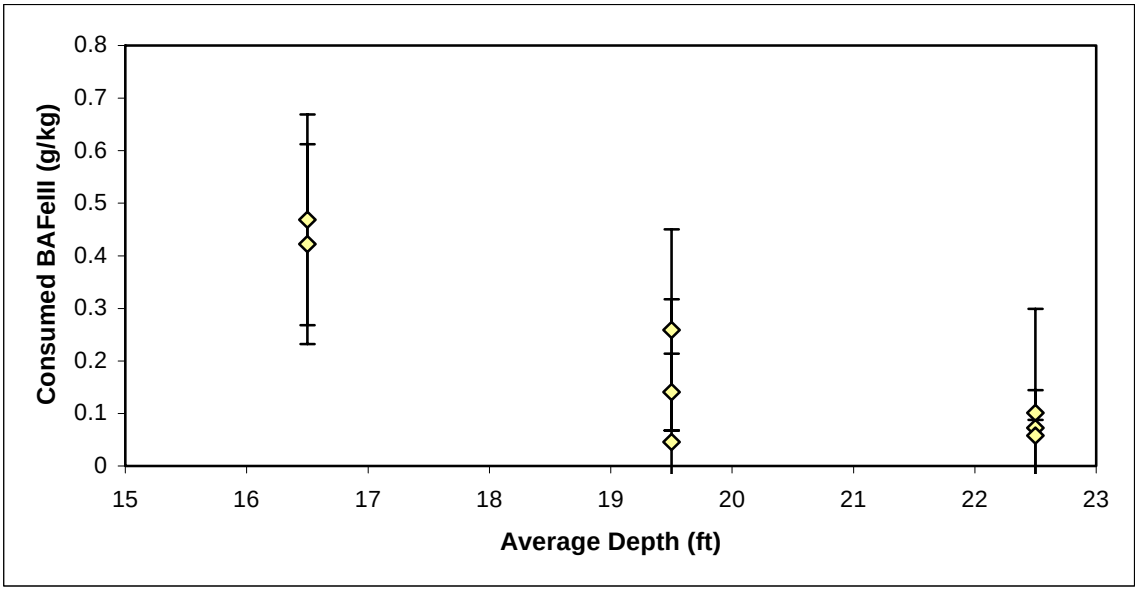

Figure 4-22. Consumed BAFeIII versus Depth at Elizabeth City – Fuel Farm. Diamond Symbols are Average BAFeIII Calculated from CDM and EPA/Ada Measurements and Error Bars Represent ± 1 Standard Deviation

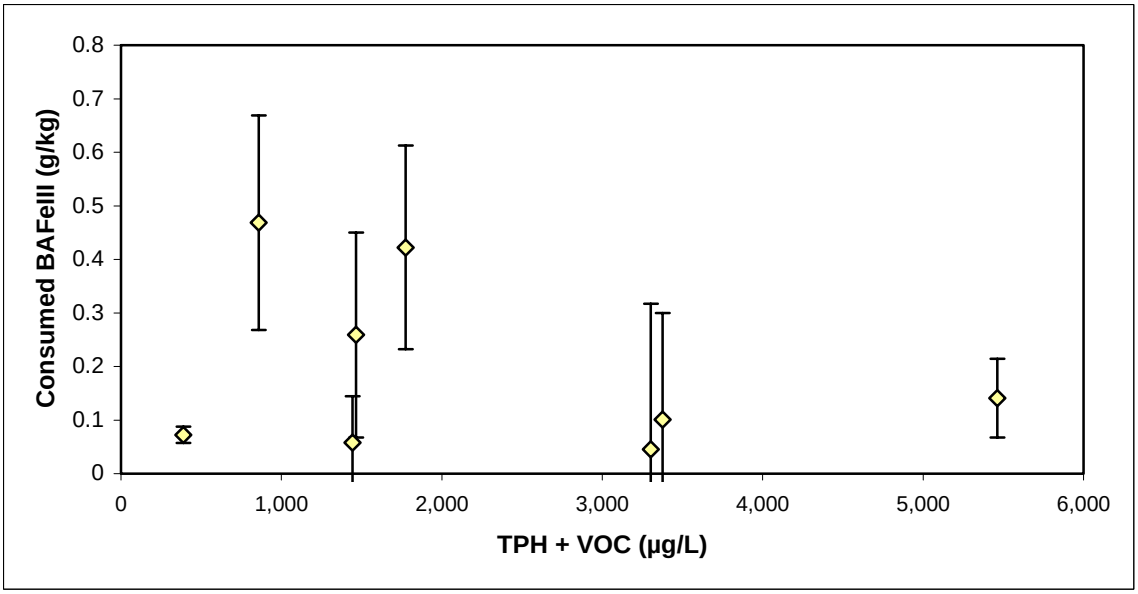

Figure 4-23. Consumed BAFeIII versus TPH + VOC at Elizabeth City – Fuel Farm. Diamond Symbols are Average BAFeIII Calculated from CDM and EPA/Ada Measurements and Error Bars represent ± 1 Standard Deviation

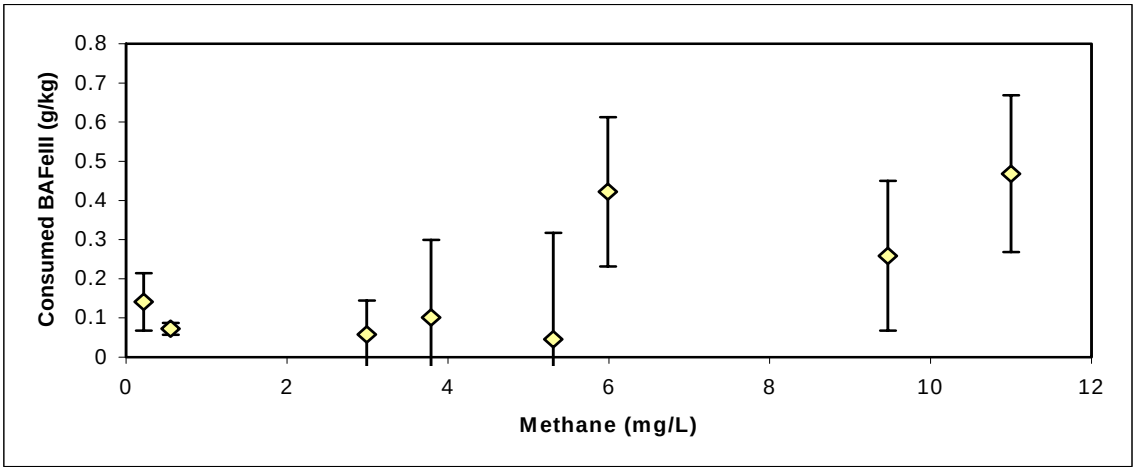

Figure 4-24. Consumed BAFeIII versus Methane at Elizabeth City – Fuel Farm. Diamond Symbols are Average BAFeIII Calculated from CDM and EPA/Ada Measurements and Error Bars represent ± 1 Standard Deviation

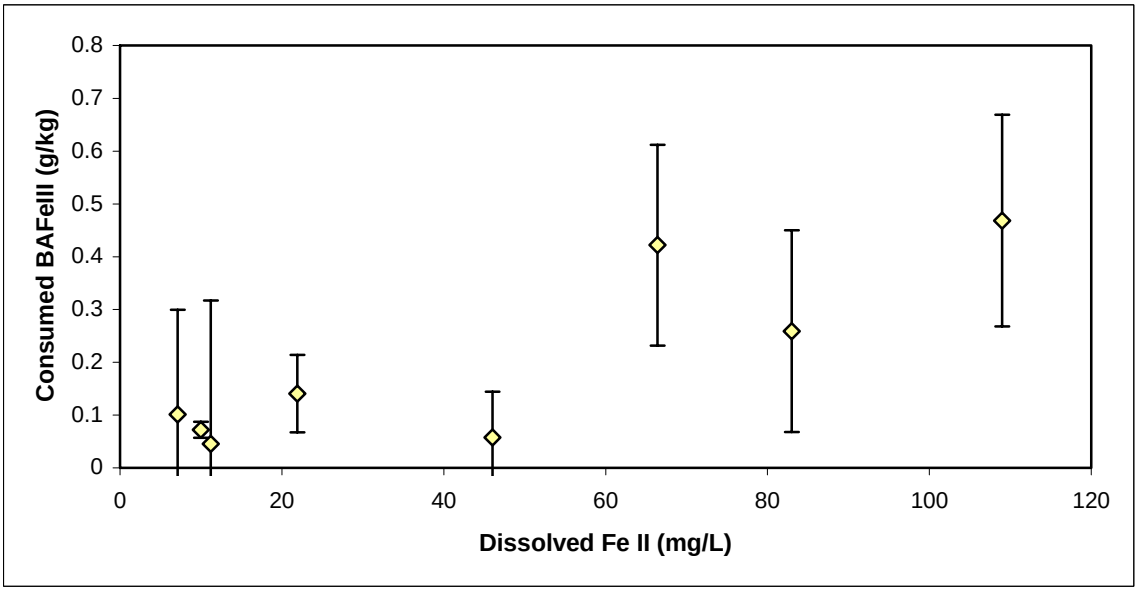

Figure 4-25. Consumed BAFeIII versus Fe II at Elizabeth City – Fuel Farm. Diamond Symbols are Average BAFeIII Calculated from CDM and EPA/Ada Measurements and Error Bars represent ± 1 Standard Deviation

A mass distribution of BAFeIII at the three impacted locations and at the different depth intervals can also be estimated using the average CDM and EPA/Ada BAFeIII data plus measured dissolved Fe II. Such a mass distribution for the Elizabeth City Fuel Farm site is provided in **Figure 4-26**, which shows the fractions of BAFeIII consumed historically along with the BAFeIII available for future MNA. Perhaps noteworthy in this illustration is the relatively

higher percentages of consumed BAFeIII in the shallower depth intervals at the three locations. Again, as discussed above, this is reasonable because the fuel hydrocarbons, which are light non-aqueous phase liquids, would be present primarily in the shallower zones of the aquifer.

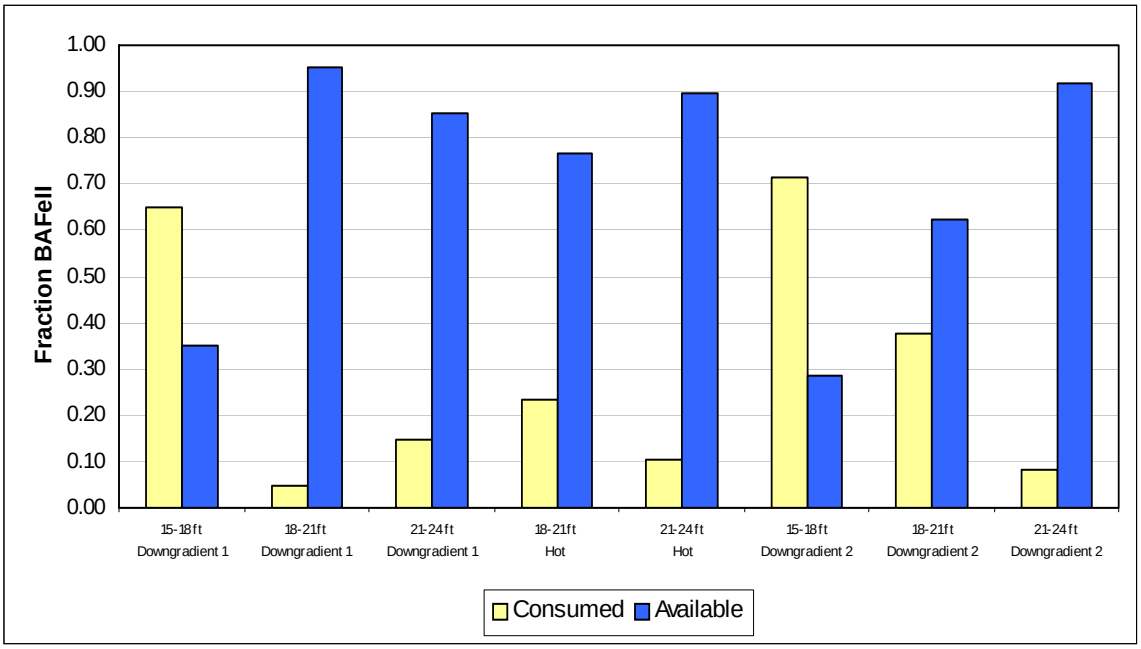

Figure 4-26. BAFeIII Mass Distribution for Elizabeth City – Fuel Farm

5.0 Cost Assessment

ESTCP guidance states that costs should be reported in this section in the recommended Federal Remediation Technologies Roundtable (FRTR) format. However, this format is primarily suited for presenting costs associated with remedial process technologies where costs need to be broken down into categories such as capital, operational and maintenance, and life cycle costs. Costs associated with purchasing and using the bioavailable iron assays do not fall into these categories, and so the FRTR format has not been used. The subsections below have been prepared to describe all costs associated with obtaining the assays and using them to analyze soil samples that have already been collected from a given site.

5.1 Cost Reporting

Purchasing the Assay Kits

The test kit is currently commercially available as the "Bioavailable Ferric Iron Assay" produced by New Horizons Diagnostics Inc. of Columbia, Maryland. Information about the kit and how to order can be found online at www.nhdiag.com . Orders can be placed at 800-888-5015 or 410-992-9357. As of the writing of this report, the costs of the kits were:

- 1 to 11 kits: $75 each
- 12 kits: $60 each
- 20 kits: $50 each

Since the kits include a reagent that contains bacteria which are temperature-sensitive, overnight shipping (not included) is required. The kits contain syringes, syringe filters, hydrochloric acid, incubation and sample vials, and the lyophilized BrY inoculum.

Additional Supplies/Equipment

To analyze ferrous iron before and after incubation with BrY, a Hach kit is typically used. The reagent needed to run the 1,10-phenanthroline ferrous iron method (Hach Method 8146) costs $15 for 100 reagent "pillows" (Item #103769), or $18.35 for 25 "Accuvac ampules". The Hach method also requires colorimetric analysis to quantify the ferrous iron. This can be done by one of three methods:

1. Using a high quality bench model spectrophotometer (Hach models run $2,000 to $2,200)
2. Using a Hach DR/800 series portable colorimeter ($580 to $930)
3. Using a Hach color disc ($29.20)

The choice of which of these methods to use will depend primarily on the number of samples that are to be analyzed in the long term, whether analyses are to be performed in the field, and on the availability of the required equipment. The color disc method is semi-quantitative, and is not recommended due to its low level of accuracy relative to the other two methods.

If only a few samples are to be analyzed, it will likely be most economical to have the samples (before and after incubation) analyzed for ferrous iron by an analytical laboratory. Typically, this analysis can be performed for approximately $30 per sample (i.e., $60/bioavailable iron sample since both the T_0 and T_{30} measurements must be conducted).

A small tumbler or orbital shaker is needed for the HCl extraction steps of the assay to rotate the vials and provide mixing of the soil with the acid. This item can be purchased from most lab supply companies for approximately $250. Miscellaneous other supplies for performing the assay and ferrous iron analyses include pipettes, beakers, a small field balance (accuracy to 0.1 gram), and safety ware (gloves and glasses). An approximate cost for these supplies is $300.

Labor

The labor time required to perform the assay can be divided into three steps:

1. Vial T_0: Combine soil, HCl, and water. Vial T_{30}: Combine soil, water, and bioassay reagent.
2. Measure ferrous iron in Vial T_0
3. After a 4-week incubation period, add HCl and measure ferrous iron in Vial T_{30}

The first step takes approximately one half hour, depending on the number of samples to be run. Running the Hach kit ferrous iron analysis for Vial T_0 (step 2) typically takes 1.5 hours for up to five samples – this includes time to run standards and prepare dilutions as necessary. Following the 4-week incubation period, another hour and a half would be needed for step 3 to add the HCl to Vial T_{30} and analyze for ferrous iron. If an analytical lab is used for ferrous iron analysis, the required labor would include labeling, packing, shipping the sample containers and filling out the chain of custody forms.

Cost Example

As a costing example, consider the following scenario:

- Six soil samples are to be analyzed for bioavailable iron at a given site
- The samples have been collected
- A field technician and bench space are available to perform the extraction steps
- Neither a spectrophotometer nor a Hach color-measuring equipment are available for the ferrous iron analysis

Costs under this scenario are shown in **Table 5-1**.

Table 5-1: BAFeIII Assay Costs

Item	Units	No. of Units	Unit Cost	Cost
Assay Kits	Each	6	$75	$450
Ferrous Fe Analysis (commercial lab)	Sample	12	$30	$360
Supplies	Lump Sum	1	$100	$100
Labor	Hour	6	$60	$360
Total				**$1,270**

The unit cost per sample is thus $212.

5.2 Cost Analysis

Cost Comparison

For comparison purposes, the contract analytical lab costs for conducting standard precipitation leaching procedure (SPLP) or toxicity characteristic leaching procedure (TCLP) analyses with zero headspace extraction (ZHE) conducted on soil samples is on the order of $90. ZHE is required to prevent oxidation of Fe II to Fe III. The extractions would be modified to use a particular chemical extractant such as 6N HCl. However, it is important to note that extraction with 6N HCl overestimates the bioavailability of many iron oxides as discussed in Section 4.3.8 and shown in **Figures 4-17 and 4-21**. Analysis of extracts for total Fe and Fe II is on the order of $50. Thus the total cost is on the order of $140. There would be some labor required for labeling, packing, shipping the sample containers and filling out the chain of custody forms. This cost 30 percent less than the BAFe III assay cost. As discussed in Section 4, the results obtained using chemical extraction are indirect measurements of BAFeIII.

The cost of laboratory microcosms varies widely but typically is at least $10,000 and can be as high as $50,000. These costs are clearly greater than the BAFeIII assay.

Cost Basis

The analytical costs listed above are based on discussions with laboratories for performing an extraction procedure similar to the Toxicity Characteristic Leaching Procedure (TCLP) as described in 40 CFR 261/SW846 Method 1311 or Synthetic Precipitation Leaching Procedure (SPLP) as described in 40 CFR 261/SW846 Method 1312. Only the extraction acid would be modified from the TCLP or SPLP method. The extractant would be analyzed for ferrous and ferric iron using the phenanthroline method number 3500-Fe D (Greenberg et al., 1992)with appropriate controls for acidity of the extracts.

Cost Drivers

While not directly related to the cost of performing the bioavailable iron kit method, the 4-week incubation period may in some circumstances result in higher indirect costs compared to a method that gives results over a 2-week period typically associated with analytical lab turnaround times. Such indirect costs need to be considered on a case-by-case basis.

If, based on the results of analyzing initial soil samples, it is determined that additional analysis is warranted, then additional costs associated with obtaining additional soil samples would be necessary. These costs would be highly site-specific and would depend on the depth of sample needed, number of samples to be collected, and site access issues.

6.0 Implementation Issues

6.1 Environmental Checklist

No special permits are required to apply the BAFeIII assay technology during investigations at a facility. No special regulations apply beyond those necessary for any field investigation or study of MNA or EAB.

6.2 Other Regulatory Issues

Currently no other regulatory issues have been identified. BAFeIII is not required by any regulatory agency at this time. However, it is one line of evidence that can be used in support of MNA. This line of evidence, though not specifically required, can be used to strengthen the scientific justification for MNA at a site.

6.3 End-User Issues

The BAFeIII assay technology is expected to provide useful information for demonstration of MNA and EAB, including the mass of contaminant consumed historically, the quantity of BAFeIII remaining to sustain MNA, and potential impact on EAB. The BAFeIII assay kit used in this demonstration is available commercially from New Horizons Diagnostics Corporation. A test kit for the preferred 1,10-phenanthroline colorimetric determination of the T_0 and T_{30} extracts is available commercially from the Hach Company, Method 8146.

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_____ _____
Signature of Project Lead Date

Appendix A
Bioavailable Ferric Iron Assay Protocol

The Bioavailable Ferric Iron Assay measures the amount of ferric iron in soil or sediment that can be reduced to ferrous iron (Fe^{2+}) by iron-reducing bacteria. Bioavailable ferric iron is one indicator of natural attenuation and can be important for other in situ bioremediation technologies such as enhanced anaerobic bioremediation.

Reagents Material Provided:

1. Foil Pouch:
   A: 1 ea: T_0 Sample Tube (25mL)
   B: 1 ea: T_{30} Sample Tube (25mL) containing Reagent "A".
   C: 2ea: Filtered Sample Vial (4mL)
   D. 2ea: Syringes, (3mL)
   E. 2ea: Syringe Filters (0.45µm)
   F. 2ea: Vials Hydrochloric acid
   G. 1ea: Weigh boat
2. 1 ea: Bioassay Reagent "B" (Keep frozen until ready for use to ensure stability)
3. Funnel with adapter

Materials Not Provided:

1. Sample Jar (2 or 4 oz)
2. Distilled Water
3. Gloves
4. Safety Glasses
5. Fe^{2+} analysis
6. Tube mixer
7. Work Station Rack
8. Portable Standard Balance

Accessories Available:

1. Work Station Rack
2. Portable Standard Balance
3. Fe^{2+} analysis
4. Tube mixer

Sample Collection

1. Collect saturated soil sample into Sample Jar. Try to get as homogenous and representative a sample as possible. Wet sieve sample to 3/16" minus if necessary.
2. Use the Scoop to weigh out 5 grams (± 0.5 grams) of soil into the weigh boat. Transfer the soil to the T_0 Sample Tube. Use of the provided funnel may be helpful. Mark date and site location onto label of tube. Weigh out another 5 grams of soil and put into the T_{30} Sample Tube. Mark date and site location on vial.

Step 1: T_0 Assay Procedure

1. Fill T_0 Sample Tube with distilled water, (~20 mL), leaving enough room for 1 mL of concentrated hydrochloric acid (HCl).
2. Add contents of 1 mL vial of HCl to sample. Top off with additional distilled water if necessary. Cap and invert to mix.
3. Place on tube mixer and mix for 48 hours.
4. Remove tube from tube mixer and allow soil to settle.
5. Remove cap and, with 3mL syringe, extract 3 mL of liquid, being careful not to disrupt the soil.

6. Attach Syringe Filter syringe and filter sample into T_0 Filtered Sample Vial (4mL).
7. Perform ferrous iron (Fe^{2+}) analysis on filtered sample.
8. Record results in mg/L.

Step 2: T_{30} Assay Procedure (<u>with</u> reagents)

1. Add contents of Bioassay Reagent vial "B" to Sample Tube T_{30} using the follow procedure. Gently tap vial "B" until the freeze-dried reagent moves freely. Remove the cap, invert the vial over Sample Tube T_{30}, and tap again to transfer the reagent. If the reagent does not move use a small spatula or other instrument to dislodge and transfer the reagent. If necessary, rinse vial "B" with distilled water (to ensure all reagents are collected) and pour rinsate into Sample Tube T_{30}.
2. Fill to the top of the neck of the T_{30} Sample Tube with distilled water. Tap tube as necessary to cause air bubble to rise to the surface and top off with additional distilled water. Invert the tube several times to mix soil, water, and reagents.
3. Store at room temperature for 30 days in the dark in an upright position.
4. After 30 days, remove cap and remove 1 mL of liquid. Dispose of this 1 mL.
5. Add contents of 1 mL vial of concentrated hydrochloric acid (HCl).
6. Place tube on tube mixer for 48 hours.
7. Remove tube from tube mixer and allow soil to settle.
8. Remove cap and, with 3-mL syringe, extract 3 mL of liquid, being careful not to disrupt soil.
9. Attach Syringe Filter syringe and filter sample into T_{30} Filtered Sample Vial (4mL).
10. Perform ferrous iron (Fe^{2+}) analysis on filtered sample.
11. Record results in mg/L.

Ferrous Iron (Fe^{2+}) Analysis Method (recommended)

1. Determine Fe^{2+} concentration range using Quantofix® Iron 1000 test strips (VWR Part No. 60787-724). Use the test strip directly and do NOT use the Iron 1 reagent which reduces Fe^{3+} to Fe^{2+}.
2. Dilute sample with distilled water so that Fe^{2+} is 3 mg/L or less. Dilute sample a minimum of 1:20 even if the test strip result is less than 30 mg/L in order to decrease the acidity which interferes with the Hach method.
3. Measure Fe^{2+} using the 1,10-phenanthroline method (Hach Method 8146). Make sure the Hach test kit directions are followed carefully including the incubation time.

Calculate Bioavailable Ferric (Fe^{3+}) Iron

Results from T_0 samples (without reagents) indicate initial ferrous iron (Fe^{2+}) in the soil sample. Results from T_{30} samples (with reagents) indicate initial ferrous iron (Fe^{2+}) in the soil sample plus bioavailable ferric iron (Fe^{3+}) that has been reduced to ferrous iron (Fe^{2+}). Calculation gives grams of bioavailable ferric iron per kilogram of wet weight of soil. To convert to a dry weight basis, multiply answer by the percent solids in the sample used to conduct the assay.

Bioavailable Ferric Iron (g/kg Fe^{3+}) =
[T_{30} (mg/L Fe^{2+}) – T_0 (mg/L Fe^{2+})] ÷ 217

Background

Ferric iron (Fe III) is a widespread terminal electron acceptor used by iron-reducing bacteria under anaerobic conditions. These bacteria can oxidize various organic compounds and in turn reduce ferric iron (Fe III) to ferrous iron (Fe II). Some of the organic compounds that can be oxidized by certain iron-reducing bacteria include benzene, toluene, vinyl chloride (VC), *cis*-dichloroethene (cDCE), and methyl tertiary butyl ether (MTBE). Additionally, iron oxides play an important role in the immobilization of metals in aquifers and bacterial iron reduction is one factor affecting the transport of metals in aquifers.

Not all ferric iron can be biologically reduced. A definition of bioavailable ferric iron is:

> *Ferric iron (Fe III) that is capable of being reduced by microorganisms that oxidize another chemical species and derive energy from the electron transfer.*

Prediction of the amount of bioavailable ferric iron is difficult because it is affected by many factors. Factors that can determine whether ferric iron is bioavailable include iron oxide crystallinity and surface area, groundwater pH and specific conductivity, concentrations of divalent cations, concentrations of electron shuttles such as humic acids, and adsorbed ferrous iron.

Assay Description

The assay is a bioassay that uses an iron-reducing bacterium to give an estimate of the maximum concentration of bioavailable ferric iron in soil or other solid materials. A five-gram soil sample is incubated in the assay medium along with the bacteria for a period of one month. During this time bioavailable ferric iron is reduced to ferrous iron. The newly formed ferrous iron plus the originally present ambient ferrous iron is extracted with weak acid (0.5 N HCl) at the end of the incubation period and measured using a Hach kit following dilution. The ambient ferrous iron concentration is measured by similarly extracting a soil sample that has not been incubated or exposed to the assay reagents. The ambient ferrous iron concentration is subtracted from the concentration in the incubated sample to obtain the bioavailable ferric iron concentration.

Assay Method

Soil samples are typically collected from the saturated zone. A four-ounce jar of soil is sufficient for the bioavailable ferric iron assay. Jars should be filled with water-saturated soil and kept refrigerated until analysis. Recommended holding times for soil samples have not been determined. Preferably, analyses should be initiated within one week of sample collection.

The sample is wet-sieved through a 3/16-inch sieve if necessary and two five-gram sub-samples of the sieved material are placed in each of two assay tubes labeled T_0 and T_{30}. The T_0 tube is filled with distilled water and one milliliter of concentrated HCl. The tube is capped and then placed on a tube rotator for 48 hours during which time the acid extracts weakly associated ferrous iron (Fe II) from the soil. Following the incubation period, the extract liquid is filtered if necessary and diluted prior to measurement of the ferrous iron concentration using the Hach

phenanthroline method. The T_{30} tube, which also contains the assay reagent and lyophilized bacteria, is filled with distilled water, capped, mixed by hand, and then incubated in the dark at room temperature for 30 days. Following the incubation one milliliter of liquid is withdrawn, discarded, and replaced with one milliliter of concentrated hydrochloric acid. The tube is then rotated for 48 hours and analyzed for ferrous iron. This concentration is the final ferrous iron concentration and is the sum of the ambient ferrous iron and the bioavailable ferric iron. The ambient ferrous iron and the bioavailable ferric iron concentrations on the soil are calculated as follows:

Ambient Fe II = (T_0 Fe II)/{(217)(solids fraction)}

Bioavailable Fe III = (T_{30} Fe II - T_0 Fe II)/{(217)(solids fraction)}

The terms in these equations are defined as follows:

Ambient Fe II – The concentration of Fe II in the soil sample (units of grams Fe per kilogram dry soil) prior to conducting the assay.

Bioavailable Fe III – The concentration of biologically reducible Fe III in the soil sample (units of grams Fe per kilogram dry soil) determined using the assay.

T_0 Fe II – The Fe II concentration measured in the T_0 tube (units of milligrams Fe II per liter) following acid extraction. Measured using a Hach phenanthroline kit.

T_{30} Fe II – The Fe II concentration measured in the T_{30} tube (units of milligrams Fe II per liter) following acid extraction. Measured using a Hach phenanthroline kit.

217 – A conversion factor to convert the liquid Fe II concentration to the soil concentration. It incorporates tube volume (25 milliliters), soil mass (5 grams), soil particle density (2.6 grams per milliliter), and unit conversions.

Solids fraction – Solids fraction in the soil sample (units of grams dry soil per gram wet soil). Measured separately and used to convert the ambient and bioavailable iron results from a wet-soil basis to a dry-soil basis. This term is optional if results expressed per kilogram of wet soil are acceptable.

Applications

The concentration of bioavailable ferric iron in soil is one parameter that may be used to determine the potential for oxidative degradation of organic chemicals and the transport of metals. It also may be used to determine the potential for inhibition of reductive dechlorination of chlorinated ethenes by maintenance of low dissolved hydrogen concentrations.

The assimilative capacity for oxidation of organic chemicals can be calculated by calculation of the electron equivalents that can potentially be accepted by the bioavailable ferric iron. This is calculated as follows:

Electron accepting equivalents = (Bioavailable Fe III)(eq./56 g) equiv./kg

As an example, vinyl chloride oxidation to carbon dioxide requires 10 electron equivalents/mole or 6 g/equiv. If the measured bioavailable ferric iron concentration is 1 g/kg, the assimilative capacity for vinyl chloride oxidation is:

0.11 g VC/kg soil = (1 g bioavailable Fe III/kg soil)(1 equiv. /56 g Fe III)(6 g VC/equiv.)

Another application is calculation of how much historical contaminant oxidation is attributable to iron reduction. This calculation requires comparison of background samples to samples in the contaminant plume. The difference in bioavailable ferric iron between these two samples can be used to calculate the amount contaminant that has been oxidized as shown in the following example. If the background sample contains 1 g/kg and the sample in the plume contains 0.5 g/kg bioavailable ferric iron, then the amount of vinyl chloride oxidation theoretically attributable to iron reduction is:

0.054 g VC/kg soil = {(1-0.5) g bioavailable Fe III/kg soil}(1 equiv. /56 g Fe III)(6 g VC/equiv.)

Note that oxidation of organic chemicals other than VC is also possible. The above calculations do not take into account oxidation of other chemicals and are provided for example only. They should be modified to meet the requirements of specific sites.

Disclaimer

The bioavailable ferric iron assay is an analytical method that was developed for the U.S. Air Force under the Small Business Innovative Research (SBIR) program. It is currently being evaluated by the Department of Defense under the Environmental Security Technology Certification Program (ESTCP) and by the EPA. It is not an EPA-approved test method. The results of the assay are only one of several types of analytical data that should be considered in assessing soil conditions and are intended to be used in combination with these other data. Users of the bioavailable ferric iron assay should not rely solely on the assay results and should exercise best professional judgment in determining the extent to which reliance on the assay results is appropriate in a particular instance. The user shall be solely responsible for inconsistent or erroneous assay results or injuries that occur due to improper or negligent use or handling of the test kit or of any results of assays performed with the test kit.

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Extraction of Solid Phase Fe and S (EPA/Athens)

This amended plan for extraction of solid phase Fe and S reflects the request of the Cleanup ESTCP Committee in the Spring 2002 IPR to quantify the hydrous ferric oxide concentrations in soil by performing the citrate-dithionite-bicarbonate (CDB) and acid ammonium oxalate tests. A general discussion and experimental procedure for each of these extraction procedures, as well as the weak and strong acid extraction procedures, is provided below. The extraction procedures and targeted minerals are summarized in Table A-1.

Citrate-Dithionite-Bicarbonate Extraction

Extraction of sediments with citrate-dithionite-bicarbonate (CDB) is performed to determine the amount of "free" or total iron oxides, which is defined as the iron in the form of oxide minerals such as goethite and hematite (*1*). Dithionite is a strong reductant that reductively dissolves the Fe(III) to Fe(II) in iron oxides. During the reductive dissolution process, hydrogen sulfite is formed resulting in a significant drop in pH (pH 2.6-3.5), which can result in the precipitation of FeS and elemental S. To prevent the precipitation process, citrate is used to chelate Fe(II) and sodium bicarbonate to buffer the solution near pH 7. Because particle size is known to affect dissolution efficiency, grinding of large particles of iron oxides such as magnetite, goethite and hematite is necessary to insure complete dissolution. Extraction with CDB will also recover small amounts of exchangeable, organically bound Fe, and Fe(III) found in layer-silicate minerals such as nontronite, montmorillonite, and vermiculite.

Experimental Protocol for Citrate-Dithionite-Bicarbonate Extraction of Aquifer Samples

The general procedure for the citrate-dithionite-bicarbonate extraction is summarized as follows. Five g of soil (ground to pass a 250-mesh sieve) are transferred to a 100-mL polypropylene centrifuge tube. The sample size is adjusted according to the expected amount of extractable Fe. To the centrifuge tube is added 40 mL of 0.3 M sodium citrate and 5 mL of 1 M $NaHCO_3$. The tube is shook to mix the contents, and then heated in a water bath at 75 to 80°C for several minutes while stirring the suspension with a glass rod. At the elevated temperature, 1.0 g of sodium dithionite is added. The suspension is stirred for 1 min, and then intermittently for 5 min. A second 1-g portion of sodium dithionite is added, and intermittent stirring is continued for 10 min. The sample is allowed to cool to room temperature, and then 10 mL of saturated NaCl is added to promote flocculation. The sample is then centrifuged for 5 min at 1600 to 2200 rpm. The supernatant is then transferred to a 500-mL volumetric flask. If necessary, flocculation will be promoted by the addition of acetone or Superfloc solution. The extraction procedure is repeated for those samples in which a brown or red color persists in the extract solution.

Ammonium Oxalate Extraction

Extraction of sediments and soils treatment with acidic ammonium oxalate has been the most common method used for the quantification of the noncrystalline, or amorphous iron oxides (*1*). These amorphous iron oxides (e.g., ferrihydrite) are considered the most "biologically reactive" due to their high surface area. Because the ammonium oxalate extraction is kinetically controlled, the quantity of Fe recovered is strongly dependent on extraction times and

temperature. Consequently, these variables must be carefully controlled. Furthermore, the extraction procedure is conducted in the dark to prevent the photoreduction of Fe and to minimize the dissolution rate of crystalline iron oxides.

A limitation of this extraction procedure is that extraction of crystalline iron oxides can also occur. Heron et al. (*2*) and Boggard et al. (*3*) reported the dissolution of crystalline oxides such as magnetite (Fe_3O_4) and akageneite (β-FeOOH). Because Fe(II) has been shown to catalyze the dissolution of crystalline oxides, extraction with oxalate may also dissolve crystalline goethite and hematite. As a result, Heron et al. (*2*) have concluded that ammonium oxalate extraction can potentially over estimate the amount of amorphous Fe(III) in sediments containing mixed Fe(II)-Fe(III).

Experimental Protocol for Acid Ammonium Oxalate Extraction of Aquifer Samples
The acidified ammonium oxalate (pH 3.0) solution is prepared by the addition of 0.175 M ammonium oxalate to 0.1 M oxalic acid. This solution is adjusted to pH 3.0 by the addition of NH_4OH or HCl, and diluted to a final volume of 1 L. A 1.0 M solution of ammonium acetate (pH 5.0) is prepared by the addition of 60 g of glacial acetic acid to 600 ml of deionized water. The pH is adjusted to pH 5.5 with NH_4OAc, and diluted to a final volume of 1 L.

The ammonium acetate solution (30 mL) is added to 500 mg of sediment in a 50-mL polypropylene centrifuge tube. The slurry is allowed to react for 1 hr with intermittent stirring of the vented container, and readjustment of the pH to 5.5 as needed with acetic acid. Adjustment of the pH is performed hourly until a constant pH of 5.5 is obtained. The slurry is then centrifuged, decanted, and washed with deionized water to remove dissolved Ca and acetate. Ammonium oxalate solution (pH 3.0, 30 mL) is then added to the tube, which is then stoppered and placed on a reciprocating shaker in the dark for 2 hr. The samples are then centrifuged, decanted and analyzed for Fe.

Weak and Strong Acid Extraction

The weak acid (0.5 N HCl) and strong acid (6.0 N HCl) extraction methods were developed in support of the concept of AMIBA (Aqueous and Mineralogical Intrinsic Bioremediation Assessment) (*4,5*). AMIBA is a tool for natural attenuation assessment that incorporates a mineralogical evaluation. The need to incorporate a mineralogical evaluation results from the realization that (1) Fe(III) and sulfate often dominate the electron acceptor pool in many aquifer systems and (2) the microbially-mediated reduction of Fe(III) and sulfate involve mineral phases. Consequently, natural attenuation studies that focus primarily on quantifying aqueous-electron acceptors can potentially lead to incorrect conclusions concerning the extent of historical natural attenuation and the potential for future natural attenuation in contaminated aquifers. The mineral phase analyses developed in support of AMIBA focused on quantifying bioavailable Fe(III), biologically produced Fe(II), bulk Fe(II) and Fe(III), acid volatile sulfides, and chromium reducible sulfides.

Experimental Protocol for Weak and Strong Acid Extraction of Aquifer Samples

Extraction with 0.5 N HCl. Approximately 100 to 300 mg of sediment is transferred by spatula to a tarred 120-mL serum bottle in an anaerobic chamber (97% N_2/3% H_2). Dry sediment

weights are calculated based on sediment moisture content determined in triplicate from oven-dried sediment (100°C for 48 h). Ten mL of 0.5 N trace-metal HCl is added to the 120-mL serum bottles. The serum bottle are crimp-sealed and removed from the anaerobic chamber and placed on a tabletop shaker. The sediment slurries are gently agitated for 48 h. Approximately 1.5 ml of the acid extract is transferred to a 1.5-mL polypropylene centrifuge tube and centrifuged for 10 min at 14,000 rpm (Eppendorf 5415 C microcentrifuge). The supernatant is analyzed for Fe(II) and Fe(III) according to the method of Lovely and Phillips (*6*). If necessary the sample is diluted with 0.1 N HCl prior to analysis.

Extraction with 6.0 N HCl. Approximately 100 to 300 mg of sediment is transferred by spatula to a tarred 120 mL serum bottle in an anaerobic chamber (97% N_2/3% H_2). Dry sediment weights are calculated based on sediment moisture content as previously described. A 3-mL test-tube containing 2.5 mL of 2% $Zn(C_2H_3O_2)_2$ solution to serve as a sulfide trap is placed in the bottom of the serum bottle. Three ml of 6.0 N HCl (trace-metal) is then added by pipette to the serum bottle. The bottle and contents are removed from the anaerobic chamber and placed on a tabletop shaker for 72 h. The serum bottle is then returned to the anaerobic chamber and 1.5 ml of the acidic solution and the entire zinc acetate solution is removed for analysis. Approximately 1.5 ml of the acid extract is transferred to a 1.5-mL polypropylene centrifuge tube and centrifuged for 10 min at 14,000 rpm (Eppendorf 5415 C microcentrifuge). The supernatant is analyzed for iron content according to the method of Lovely and Phillips (*6*). If necessary the acid extract is diluted with 0.1 N HCl prior to analysis for iron. The same sediment is then used for determination of pyrite. The sulfide trap is replenished with fresh zinc acetate and the bottle is again sealed. 2.5 mL of 1 N Cr^{2+}(aq) and 1.0 ml of 12 N HCl solution is added to the sediment slurry and the bottle placed onto a tabletop shaker for 3 days. At the end of this period the Cr^{2+} solution is discarded and the zinc acetate analyzed for sulfide. Sulfide was determined according to the Cline method (*7*).

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Table A-1 Targeted mineral phases and extraction procedures

Target Phases	Target Minerals	Extractant	Method for Analysis
Total Fe(III) oxides	Crystalline and Amorphous Fe(III) oxides	Citrate-bicarbonate-dithionite	Ferrozine Method
Bioavailable Fe(III)	Amorphous Fe(III) oxides	Acidic Ammonium Oxalate	Ferrozine Method
Bioavailable Fe(III)	Amorphous Fe(III) oxides	0.5 N HCl	Ferrozine Method
Biologically produced Fe(II)	$FeCO_3$, FeS, $Fe_3(PO_4)_2$	0.5 N HCl	Ferrozine Method
Bulk Fe(II) and Fe(III)	Crystalline Fe(III) oxides (e.g., hematite, goethite, and magnetite)	6.0 N HCl	Ferrozine Method
Acid volatile sulfides (AVS)	Amorphous FeS, mackinawite, greigite and pyrrhotite	6.0 N HCl with Zn trap	Cline Method
Chromium reducible sulfides (CRS)	FeS_2 and S^0	6.0 N HCl/Cr(II) with Zn trap	Cline Method

Redox Characterization of Sediment and Aquifer Samples (GIT)

Microbiological Methods

The soil samples will also be analyzed for the presence of anaerobic microbial populations that are capable of mediating nitrate reduction, iron reduction, manganese reduction, sulfate reduction, and methanogenesis. Enumeration of redox-specific microbial populations will be determined by Most Probable Number (MPN) analysis of viable cells in defined liquid medium.

Most Probable Number analysis in liquid medium. Viable cells will be quantified via MPN analysis as described by Colwell, et al. (1979) and Ludvigsen, et al. (1995). Since prior knowledge on cell numbers is not available, the initial MPN strategy will use 10 replicate cultures at each of three successive 10-fold dilutions for examination of cell growth. In subsequent MPN analyses, a narrower range of dilution factors may be used, thereby leading to a greater accuracy in calculating cell numbers. The liquid growth medium will consist of a basal oligotrophic mineral medium (OAM; Albrechtsen and Christensen 1994) supplemented with a cocktail of potential electron donors (yeast extract, tryptone, lactate, and acetate) and a specific anaerobic electron acceptor (at concentrations identical to those described by Ludvigsen, et al. 1995): sulfate (mixture of $CaSO_4$, $MgSO_4$, $FeSO_4$), Fe III-oxides (amorphous ferrihydrite), Mn IV-oxides (MnO_2), and nitrate (KNO_3). The MPN tubes will be incubated anaerobically at ambient temperature in the dark and scored periodically for a positive growth response. The indicators used to score positive growth will be as follows: sulfate reducers (formation of a black FeS precipitant), Fe III- and Mn IV-reducers (reductive dissolution of solid phase electron acceptor), and nitrate reducers (NO_3 depletion).

Microcosms

Bioassays experiments will be conducted for the purpose of validating the bioavailable ferric iron assay. The bioassays are designed to determine the activity and capacity of the natural redox processes using iron as terminal electron acceptor. The results of these experiments will be compared to those obtained from the bioavailable ferric iron assay in which lyophilized iron-reducing bacterium *Shewanella alga* BrY has been added, and to the extraction experiments in which bioavailable iron is operationally defined as the fraction of Fe(III) that can be extracted by treatment with ascorbate.

In order to mimic the bioavailable ferric iron assay, the same mineral supplement used for the bioavailable ferric iron assay will be used for incubation of the aquifer material (see the Technology Demonstration Plan for details). Samples will be processed in quadruplicate. The four incubations are designed to differentiate in situ and potential rates of bioavailable iron reduction. The first serum bottle will be left unamended and the second serum bottle will be amended with *Shewanella alga* BrY, the third second serum bottle will be amended with 5 mM hydrous ferric oxide, and the fourth serum bottle will be amended with *Shewanella alga* BrY and 5 mM hydrous ferric oxide. For each sample, 10 g of sediment will be added to each of four 60-mL serum bottles. Under a nitrogen atmosphere, 50-ml of nitrogen-purged mineral supplement will be added to each serum bottle. The serum bottles will be analyzed for soluble Fe(II) on a

weekly basis over a period of 3 to 4 months, or until the concentration of Fe(II) reaches a constant level (± 5% over a 2-week period). At the end of the incubation period, concentrated HCl will be added to recover biogenically formed Fe(II) associated with the sediment. Soil moisture content of the sediment will be determined by drying ~10 g wet sediment from each sample interval for 48 h at 95°C.

Soluble ferrous and ferric iron concentrations will be determined in a modified Ferrozine method, which allows us to establish the Fe(II)/Fe(III) speciation on a single aliquot volume (Viollier, et al., 2000). In this modified protocol, the absorbance of a colored Fe(II)-Ferrozine complex is measured spectrophotometrically before and after a Fe(III) reduction step by hydroxylamine.

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Mineralogical Characterization of Precipitants (UC)

The purpose of this study is to validate a series of ferric iron bioassays. To accomplish this, the Laboratory for Environmental and Geological Studies (LEGS) at the University of Colorado-Boulder will examine well precipitants from sites across the country. Dr. John Drexler will coordinate precipitant characterization at the university. The university will utilize three primary analytical techniques: x-ray diffraction (XRD), electron microprobe analyses (EMPA), and infrared reflectance spectroscopy (IRS).

The forms of iron oxide that will precipitate from solutions and the stability of these forms are highly dependent on the iron concentration in solution, pH, redox potential, pCO_2 and pO_2, and the concentration of complexing ligands (SO_4^{2-}, S_2^{2-}, CO_3^{2-}, etc.). The iron oxyhydroxides commonly found in groundwater wells are ferrihydrite ($Fe_5HO_8\ 4H_2O$), goethite (α FeOOH), and, less commonly, lepidocrocite (γ FeOOH).

Ferrihydrite is generally associated with either goethite or lepidocrocite, but not with hematite (α Fe_2O_3). Its crystals are a rusty-red color, poorly ordered and small (2-5 nM) in size; therefore, the large surface area makes this iron phase highly reactive. Ferriyhydrite is the least stable of the Fe^{+3} oxides, with solubility products of approximately 10^{-38}. The slow transformation of ferrihydrite to goethite involves dissolution and recrystallization, which accounts for their common association.

Goethite is a yellow-brown Fe^{+3} oxide. It is the most stable form in the surface environment with a solubility product of 10^{-44}. Goethite precipitation is favored when organic compounds, capable of complexing Fe, are present. Due to its slow formation from ferrihydrite, Al will often substitute for Fe in its structure.

Lepidocrocite occurs much less frequently than its polymorph, goethite. It is bright orange in color. Its presence is a good indicator of oxygen deficiency. Lepidocrocite is metastable with respect to goethite as high pCO_2 and Si waters favor goethite precipitation.

EMPA

The morphology and chemistry of the iron precipitants will be determined on a JEOL 8600 electron microprobe. For morphological examination a portion of the dried precipitant will be mounted onto carbon stubs with conductive tape and gold coated. Once an iron oxide is identified using energy dispersive spectrometry (EDS), the morphology can be studied either using secondary (SEM) or backscatter (BEI) electron detectors. Most iron oxides have a typical morphology, as shown in Table A-2. These morphologies can provide important insight into the formation and reactivity of the precipitant structure.

Table A-2 Typical morphology of iron oxyhydroxides

Oxide	Principal Morphology	Other Morphologies
Goethite	Acicular (spiked)	Stars (twins), hexagons, rods, cubes
Lepidocrocite	Laths	Tablets, plates, diamonds, cubes
Akaganeite	Somatoids, rods	Stars, crosses, hexagons, prisms
Schwertmannite	Aggregates	
FeOOH	Plates	Thin rolled films
Feroxyhyte	Plates	Needles
Ferrihydrite	Spheres	
Hematite	Hexagonal plates, rombs	Spindles, rods, ellipsoids, cubes, discs, spheres, stars
Magnetite	Octahedra	Intergrown octahedra, rhombs
Maghemite	Laths or cubes	Plates, spindles
FeO	Cubes	Irregular pieces
Fe(OH)2	Hexagonal plates	

For chemical analysis, a portion of the dried precipitant will be embedded in an epoxy plug and its surface will be optically polished. Once coated with carbon, the surface-exposed iron oxides can be analyzed using wavelength dispersive spectrometers (WDS) on the EMPA. All major elements including oxygen will be determined. Certified mineral standards will be used along with recognized ZAF correction programs.

The purpose of this study is to validate a series of ferric iron bioassays. To accomplish this, the Laboratory for Environmental and Geological Studies (LEGS) at the University of Colorado-Boulder will examine well precipitants from six assayed sites across the country. Dr. John Drexler will coordinate precipitant characterization at the university. The university will utilize three primary analytical techniques: x-ray diffraction (XRD), electron microprobe analyses (EMPA), and infrared reflectance spectroscopy (IRS).

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large surface area makes this iron phase highly reactive. Ferriyhydrite is the least stable of the Fe^{+3} oxides, with solubility products of approximately 10^{-38}. The slow transformation of ferrihydrite to goethite involves dissolution and recrystallization, which accounts for their common association.

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Lepidocrocite occurs much less frequently than its polymorph, goethite. It is bright orange in color. Its presence is a good indicator of oxygen deficiency. Lepidocrocite is metastable with respect to goethite as high pCO_2 and Si waters favor goethite precipitation.

XRD

The mineralogy of precipitants will be determined using a SCINTAG x-ray diffractometer. A portion of the sample will be ground using an agate mortar and pestle and pressed into leucite frames. The Scintag diffractometer is fitted with a Cu x-ray tube (Cu $K\alpha$ = 1.542 A) and a graphite-focusing monochromator for analysis. The instrument will scan a 2θ range from 5° to 75° continuously in 0.05 degree steps. The diffractometer is operated at an accelerating voltage of 40kV and a beam current of 25mA.

X-ray powder scans from Cornell and Schwertmann (1996) for Fe^{+3} oxides will be used as references along with files from JCPDS.

IRS

Spectrometers are in use in the laboratory to acquire reflectance, and emittance spectroscopy of natural precipitants is sensitive to specific chemical bonds in materials. Spectroscopy has the advantage of being sensitive to both crystalline and amorphous materials, unlike some diagnostic methods like X-ray diffraction. The variations in material composition often cause shifts in the position and shape of absorption bands in the spectrum. Thus, with the vast variety of chemistry typically encountered in the real world, spectral signatures can be quite complex and sometimes unintelligible. However, that is now changing with increased knowledge of the natural variation in spectral features and the causes of the shifts.

Iron oxides and hydroxides are cases where spectroscopy detects at very low levels because of the strong absorption bands in the visible and ultraviolet. In nature, there appear to be many amorphous iron oxides and hydroxides with equally intense absorptions. Thus, spectroscopy can not only detect them at levels below other methods (e.g., X-ray diffraction), but in the case of amorphous materials, detect them when other methods are not sensitive to their presence when they are major fractions of the sample.

There is a whole suite of iron oxides, iron hydroxides, and amorphous phases, all with similar electronic absorption bands in the visible and near infrared. Hematite has a narrower absorption

at a slightly shorter wavelength than goethite. However, a coarse-grained hematite has a broader absorption, approaching the position and width of a fine-grained goethite (or a thin-film goethite). Jarosite has a narrow absorption near 0.43 μm, but it sometimes appears weak because of the saturated UV absorption. Jarosite, an iron sulfate, has a diagnostic absorption at 2.27 μm due to a combination OH stretch and Fe-OH bend. Ferrihydrite is an amorphous iron oxide, and its spectrum appears very similar to the orange precipitant, an amorphous iron hydroxide. If we remove the continuum and compare the positions and shapes of the bands, we can see they are indeed different.

Appendix B
Quality Assurance Plan

Appendix B
Quality Assurance Project Plan (QAPP)

1.1 Purpose and Scope of the Plan

The data from the laboratory analyses should be of such known quality that informed decisions can be made and results are considered reliable. The quality-assurance (QA) program of the laboratory is designed under the PARCC scenario (i.e., precision, accuracy, representativeness, completeness, and comparability) of quality assurance/quality control.

1.2 Quality Assurance Responsibilities

The process of data reduction, review, and reporting is applicable to all aspects of the project and is required for technical and managerial data. Documented verification of this data is crucial. Consistent, documented data reduction techniques for hand calculations and computer analyses and standardized technical data validation are equally important in the verification of the technical data.

To ensure that all aspects of the demonstration remain in conformance with ESTCP-approved data quality objectives, we have defined specific project roles and responsibilities as shown in the organization chart (**Figure 1**) and summarized below.

Project Management — Carmen Lebron of the Naval Facilities Engineering Service Center will serve in a project management capacity. As such, she will be responsible for establishing site contacts, coordinating site access, and assisting in review and implementation of the Technology Demonstration Plan.

Laboratory Director —Dr. Patrick J. Evans, who serves as the Laboratory Director at CDM in Bellevue, Washington, will oversee all aspects of data management. He will be responsible for establishing QA/QC policies and ensuring those policies are followed. He will also be responsible for reviewing QA/QC results to verify if data are acceptable for use or if an analytical batch or sequence needs to be reanalyzed.

Specific responsibilities of the Laboratory Director include:

Ensuring that the necessary staff and resources to produce quality results in a timely manner are committed to the project.
Ensuring that the staff are adequately trained in the procedures so that they are capable of producing high quality results and detecting situations that are not within the method or project QA limits.
Ensuring that the analytical methods and laboratory procedures are followed and well documented.

Maintaining the laboratory's Quality Assurance Manual and documenting that its procedures are followed.
Ensuring that laboratory reports are complete and reported in the required deliverable format.
Communicating, managing, and documenting all corrections initiated at the laboratory.

Validation and Characterization - Dr. Eric J. Weber (EPA/Athens, GA), in cooperation with Dr. Thomas DiChristina (Georgia Institute of Technology), will perform redox characterization of sediment and aquifer samples at EPA-NERL and Georgia Institute of Technology laboratories. These analyses will support validation of the bioavailable ferric iron assay.

Dr. Frank Chapelle will evaluate results of the DH analyzer by comparing them with the bubble-strip method. Dr. Chapelle is with the U.S. Geological Survey in South Carolina.

Dr. John Drexler will conduct mineral characterization for precipitants. He is Associate Professor and Director of Analytical Facilities in the Department of Geological Sciences at the University of Colorado in Boulder, Colorado.

Analysts – The Laboratory Director will be supported by one analyst who will be responsible for maintaining a laboratory notebook for each project and updating the laboratory's sample receipt, standard preparation, and instrument calibration/maintenance notebooks.

Specific responsibilities of the analysts include:

Ensuring that appropriate testing, measurement, and record keeping procedures are followed.
Ensuring the proper use of standard operating procedures associated with data collection and equipment operation.
Ensuring that the proper number and type of QC samples are analyzed.
Informing the Laboratory Director when problems occur, and communicating and documenting any corrective actions that are taken.

1.3 Data Quality Parameters

1.3.1 REPRESENTATIVENESS

Representativeness is a measure of how closely measured results reflect the actual concentration or distribution of chemical compounds in a sampled media. The number, location, and frequency of samples, sampling techniques, and sample custody and shipment are developed at the start of the project to ensure that data are representative of site conditions.

1.3.2 COMPLETENESS

Completeness is a measure of the amount of valid data obtained from the requested analytical method. Completeness may be defined as the number of samples with acceptable chemical analyses compared to the total number of samples collected in the field. The target completeness objective will be 95 percent.

1.3.3 COMPARABILITY

Data comparability expresses the confidence with which each sampling event can be compared to another. Comparability will be maintained by use of consistent sampling procedures, EPA-approved analytical methods, consistent detection limits, and consistent units.

1.3.4 ACCURACY

Accuracy is assessed by determining how close a measured value lies to its actual value. One approach is to spike a sample with an analyte of known concentration and calculate the average percent recovery (%R). A second procedure is to analyze a standard and calculate the percent difference (%D) between the measured value and the statistically determined value of the standard.

Two types of percent recoveries generally are measured for organic analyses: matrix spike and surrogate spike. For a matrix spike, analytes with a known concentration are added to the sample. A matrix spike will be conducted for the bioavailable ferric iron assay. This will involve addition of a known concentration of a specific iron oxide to the bioavailable ferric iron assay tubes either with or without soil. The assay will be run and bioavailable ferric iron assay results will be compared to expected results in order to calculate percent recovery. Initially, an iron oxide standard with a predetermined concentration will be tested using the bioavailable ferric iron assay without soil. Subsequently, this standard will be spiked into one soil sample per site and the bioavailable ferric iron assay will be run. The surrogate spike is not applicable for DH and bioavailable ferric iron assays.

1.3.5 PRECISION

Precision is a measure of the reproducibility of an analytical result (i.e., to obtain the same or similar results on replicate measurements of the same sample or of duplicate samples). Reproducibility is affected by matrix variations, the extraction procedure, and the analytical method used. For duplicate and replicate samples, precision is expressed as the relative percent difference (RPD). For each site, a duplicate soil sample will be analyzed by the bioavailable ferric iron assay and the analyses listed in Table 2.

1.4 Calibration Procedures, Quality Control Checks, and Corrective Action

1.4.1 STANDARD PREPARATION

New standards are prepared at the beginning of each project. New standards are based on the chemical nature of the constituents. Standards that are used for analysis will be traceable to a nationally recognized source (e.g., NIST). The following frequency of stock standard preparation generally is performed by the laboratory:

All stock standard solutions are entered into the standard preparation logbook immediately after preparation. The standard preparation logbook identifies the following for each stock standard: preparation date, preparer's initials, chemical constituents, solvent, volume, and disposal date (completed when standard is destroyed).

1.4.2 INSTRUMENT CALIBRATION

Analytical instruments are calibrated at the beginning of each project or when calibration verification results indicate that instrument recalibration is required. Instrument calibration QC parameters are specified in the analytical methods, but generally three-point calibration curves are generated with a correlation coefficient [r] requirement of >0.95 and/or with a percent relative standard deviation (%RSD) of the calibration standard response factors of 30 percent.

1.4.3 CALIBRATION VERIFICATION

The instrument calibration average response factor is verified at the beginning and end of each analytical sequence (of no more than 20 samples) or within a 12-hour window. Calibration verification QC parameters are specified in the analytical methods, but generally a %D criterion of 25 percent is used. The %D value is derived by assessing the difference between the average response factor from the initial calibration and the response factor from a mid-range calibration verification standard.

1.4.4 METHOD BLANKS

Method blanks are analyzed per sample preparation batch (of no more than 20 samples). Method blanks are used to determine the cleanliness of the analytical system. If method blanks are shown to contain reportable concentrations of an analyte, the associated sample batch containing concentrations up to five time the level in the associated blank will be reanalyzed.

1.4.5 SURROGATE SPIKES

Surrogate spike compounds are added to each sample before the preparation steps of the analysis begin. Surrogate spike compound percent recovery values are evaluated against laboratory and method requirements. Surrogate spikes are not applicable as described above.

1.4.6 DUPLICATE SAMPLE ANALYSES

One sample is selected for duplicate analysis per sample batch. The sample and the duplicate are taken from the same sample container and carried through the sample preparation and analysis steps as discrete samples. Performance of the duplicate sample analysis is evaluated against laboratory and method RPD criteria.

1.4.7 LABORATORY CONTROL SAMPLES

A laboratory control sample/laboratory control sample duplicate (LCS/LCSD) is analyzed per analytical batch. The LCS/LCSD samples are prepared by adding the target analyte spiking solutions to clean silica sand (soil analyses) or distilled water (groundwater analyses) and carrying the spiked samples through the preparation and analysis steps. Performance of the LCS/LCSD analyses is evaluated against laboratory and method %R and RPD criteria. For the bioavailable ferric iron assay evaluation, samples of clean silica sand spikes with known quantities of various iron oxide standards will be submitted for analysis. These samples will be blind standards.

1.4.8 MATRIX SPIKE SAMPLES

A matrix spike/matrix spike duplicate (MS/MSD) is analyzed per analytical batch. The MS/MSD samples are prepared by adding the target analyte spiking solutions to an aliquot of a field sample and carrying the spiked samples through the preparation and analysis steps. Performance of the MS/MSD analyses is evaluated against laboratory and method %R and RPD criteria.

1.4.9 CORRECTIVE ACTIONS

In the event that data quality falls outside of established acceptance criteria, correction involves the following steps:

1. Discovery of a nonconformance.
2. Identification of the responsible party.
3. Plan and schedule of corrections.
4. Confirmation that the desired results were produced.

The intent of the quality assurance process is to minimize corrections through the development and implementation of effective internal controls. To accomplish this, procedures will be implemented, as described in this section, to activate a correction for each measurement system when acceptance criteria have been exceeded. In addition, reviews and audits will be conducted on a periodic basis to check this implementation as described in Section 9.7. Results of quality assurance reviews and audits typically identify the requirements for corrections. When this occurs, a correction plan will be prepared to include: identification of the correction, organizational level of responsibility for the action taken, steps to be taken for correction, and approval for the correction.

Procedures for ensuring the correctness of the data reduction process are discussed in this section. Data are reduced either manually on calculation sheets or by computer on formatted printouts. Responsibilities for the data reduction process are delegated as follows:

- Technical personnel will document and review their own work and are responsible for the correctness of the work.
- Major calculations will receive a method and calculation check by a secondary reviewer prior to reporting (peer review).
- The Laboratory Director will be responsible for ensuring that data reduction is performed according to protocols discussed in this QA Plan.

The need for correction(s) in the analytical laboratory may come from several sources: equipment malfunction, failure of internal QA/QC checks, method blank contamination, failure of performance or system audits, and/or noncompliance with QA requirements. When measurement equipment or analytical methods fail QA/QC checks, the problem will immediately be brought to the attention of Laboratory Director and the analysts in accordance with laboratory and method protocols. If failure is due to equipment malfunction, the equipment will be repaired, precision and accuracy will be reassessed, and the analysis will be rerun. Attempts will be made to reanalyze all affected parts of the analysis so that, in the end, the product is not

affected by failure of QA requirements. All incidents of QA failure and the correction tasks will be documented in the instrument logbook and in the associated project notebook. Corrections also will be taken promptly for deficiencies noted during spot checks of raw data. As soon as sufficient time has elapsed for corrections to be implemented, evidence of correction of deficiencies will be presented to the Laboratory Director.

1.5 Demonstration Procedures

1.5.1 DH ANALYZER

The DH analyzer will be inspected in CDM's laboratory in Bellevue, Washington prior to shipment to each site. Inspection of mechanical and electrical systems will be conducted to ensure reliable operation. Test runs will be conducted in the laboratory to assure acceptable operation. Upon arrival at each site, the DH analyzer will once again be inspected to determine if damage occurred during shipment. A calibration run will be conducted to further ensure proper operation. During operation at each site, the DH analyzer will be calibrated at least once daily and variations in the calibration factor will be noted. If unacceptable variation is observed, associated problems will be identified and corrected. Mechanical or electrical failure while on site will be addressed upon discovery through repair and/or replacement of malfunctioning parts.

1.5.2 BIOAVAILABLE FERRIC IRON ASSAY

The bioavailable ferric iron assay is manufactured by New Horizons Diagnostics Corporation (NHD). Standard manufacturing procedures at NHD will include QA/QC checks that cover microorganism viability, chemical composition, pH, and parts inventory. Details of NHD's QA/QC procedure is in **Appendix J**. Upon receipt at CDM's laboratory, the assay will be reviewed for completeness and at least one assay out of every batch will be subjected to in-house testing. This testing will involve measurement of solution pH and lactic acid concentration followed by testing of the assay with an iron oxide standard. Measurement of pH and lactic acid concentration will be done as an QA/QC check that is supplemental to NHD's procedures. Testing with the iron oxide standard is not part of NHD's QA/QC procedures and will be conducted by CDM.

1.6 Calculation of Data Quality Indicators

Technical validation requires comparison of QC and instrument performance standard results to the required control limits. The following QC elements will be reviewed (as appropriate):

Analytical holding times.
Blank contamination.
Instrument calibration.
Continuing calibration standards.
Internal standards.
Analytical accuracy (matrix spike compound recoveries, laboratory control sample spike compound recoveries, surrogate compound recoveries).
Analytical precision (comparison of duplicate sample results and duplicate spike results, expressed as RPD).

Compound identification.
Compound quantitation and reported detection limits.

Any outliers from laboratory or method QC criteria for the above-mentioned parameters will be qualified through an in-house data validation process. Results will be flagged with the appropriate data qualifier and the effect on data usability. The following data flags are used by the laboratory (Source: National Functional Guidelines for Organic Data Review, USEPA 2/94):

U – The analyte was analyzed for, but was not detected at concentrations greater than the sample reporting limit.
J – The analyte was positively identified; the associated numerical value is the approximate concentration of the analyte in the sample.
UJ – The analytes were not detected at concentrations greater than the sample reporting limit. However, the sample reporting limit is approximate and may or may not represent the actual limit of quantitation necessary to accurately and precisely measure the analyte in the sample.

The data qualifier "R" (data are rejected) is not used. Any situation requiring rejection of data will require corrective action and reanalysis with acceptable results.

1.7 Performance and System Audit

Data quality audits will be performed on each data set for comparison to instrumental and laboratory QC parameters. If the data quality audit indicates a significant laboratory problem, then performance audits of the laboratory will be conducted to identify and correct specific problems.

1.8 Quality Assurance Reports

All data will be reviewed for quality assurance. This will include checking for appropriate holding times, preservation, chains-of-custody, and field and laboratory quality control samples.

Appendix C
Health and Safety Plan (HASP)

Prepared For:
Environmental Security Technology Certification Program

Project Health and Safety Plan

DEVELOPMENT OF A DISSOLVED HYDROGEN ANALYZER AND A BIOAVAILABLE FERRIC IRON ASSAY

July 26, 2001

CDM
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CDM Project No. 4000-31212

TABLE OF CONTENTS

1.0 INTRODUCTION 116
1.1 GENERAL 116
1.2 CONTACT PERSONNEL 116
1.3 PROJECT RESPONSIBILITIES 116
1.4 SITE LOCATION AND DESCRIPTION 118
1.4.1 FORT LEWIS, WASHINGTON 118
1.4.2 NAVAL SUBMARINE BASE BANGOR, WASHINGTON 118
1.4.3 LAUREL BAY EXCHANGE, BEAUFORT, SOUTH CAROLINA 118
1.4.4 DOVER AIR FORCE BASE, DELEWARE 118
1.4.5 NAVAL AIR STATION PENSACOLA, FLORIDA 119
2.0 SCOPE OF WORK 119
3.0 CHEMICAL HAZARD ASSESSMENT 120
3.1 CHLORINATED SOLVENTS 120
3.2 PETROLEUM PRODUCTS 121
4.0 PHYSICAL HAZARD ASSESSMENT 122
4.1 TEMPERATURE-RELATED HAZARDS 122
4.2 FIRE AND EXPLOSION HAZARDS 122
4.3 NOISE HAZARDS 122
4.4 OXYGEN DEFICIENCY HAZARDS 123
4.5 UTILITY HAZARDS 123
4.6 CONSTRUCTION HAZARDS 123
5.0 SITE WORK ZONES 123
5.1 EXCLUSION ZONE 123
5.2 CONTAMINATION REDUCTION ZONE 123
5.3 SUPPORT ZONE 124
6.0 AIR MONITORING AND SAMPLING 124
6.1 AIR MONITORING 124
6.2 AIR SAMPLING 125
7.0 PERSONNEL PROTECTION 125
7.1 EXCLUSION ZONES AND CONTAMINATION REDUCTION ZONES 125
7.2 SUPPORT ZONES 126
7.3 SUMMARY 126

TABLE OF CONTENTS

8.0 DECONTAMINATION PROCEDURES 126
8.1 PERSONNEL 127
8.2 SAMPLING EQUIPMENT 127
8.3 HEAVY EQUIPMENT 127
9.0 GENERAL SAFE WORK PRACTICES 127
10.0 EMERGENCY PROCEDURES 128
10.1 PHYSICAL INJURIES 128
10.2 CHEMICAL EXPOSURES 128
10.2.1 Eye Exposures 128
10.2.2 Skin Exposures 128
10.2.3 Inhalation 129
10.2.4 Ingestion 129
10.3 FIRES 129
10.4 UNCONTROLLED RELEASE OF HAZARDOUS MATERIALS 129
10.5 EMERGENCY NOTIFICATION SYSTEM 129
10.6 EMERGENCY SERVICES 130
10.6.1 Hospital Route 130
10.6.2 Emergency Telephone Numbers 130
11.0 TRAINING 131
11.1 MEDICAL SURVEILANCE 131
12.0 HASP MODIFICATIONS 132

Attachments

Attachment A: Subcontractor Safety Agreement Form
Attachment B: Field Team Review Form
Attachment C: Supplementary Record of Occupational Injuries and Illnesses Form

1.0 INTRODUCTION

The purpose of this project is to demonstrate and validate two innovative analytical tools that can measure dissolved hydrogen (DH) and bioavailable ferrous iron. The efficiency of these two tools will be assessed at six Department of Defense sites (Fort Lewis and SUBASE Bangor in Washington; Laurel Bay in Beaufort, South Carolina; Dover Air Force Base in Delaware; and Naval Air Station Pensacola in Florida). The sixth site (Cape Canaveral in Florida or Moody Air Force Base in Georgia) will be selected at a later date.

1.1 General

This project Health and Safety Plan (HASP) provides guidance and procedures to Camp Dresser & McKee Incorporated (CDM) personnel involved in field activities at the five sites listed in Section 1.4. This HASP applies to CDM personnel working within the scope of work outlined in Section 2.0.

If, during the course of work, information is obtained indicating additional hazards or a change in scope, field work will be temporarily halted, information regarding potential hazards reevaluated, and this HASP updated or modified as necessary. Project work will resume after field personnel are notified of modifications to the HASP.

1.2 Contact Personnel

CDM Project/Field Manager	Patrick Evans	(425) 453-8383
CDM Health and Safety Manager	Monica Beckman	(425) 453-8383
CDM Occupational Physician	Dr. Calvin Jones	(425) 822-3651

1.3 Project Responsibilities

The CDM Health and Safety Manager (HSM) and CDM Project Manager (PM) are responsible for ensuring this HASP is implemented during project operations. The CDM Field Manager will act as the designated Site Safety Officer (SSO) and is responsible for day-to-day safety requirements during fieldwork. CDM personnel are responsible for following the procedures set forth in this HASP. When no policies or regulations apply, CDM employees should act in a manner to reduce potential risk of injury or health effects. Project-related safety responsibilities include the following:

Project Manager:

Ensure subcontractors have submitted a completed Subcontractor Safety Agreement Form, included as **Appendix A**.

Ensure site personnel and visitors comply with the requirements of the project HASP.

Ensure site personnel meet the required qualifications.

Health and Safety Manager:

Write and amend the project HASP.

Investigate accidents, injuries, and illnesses.

Conduct specialized and site-specific training as required.

Address questions raised by the PM, SSO, or site personnel.

Site Safety Officer:

Ensure site personnel comply with the requirements of the HASP and have submitted a completed Field Team Review Form (included as Appendix B) to the HSM.

Monitor the site and work areas for health and safety hazards and address any unusual situations; consult the HSM if necessary.

Investigate accidents, injuries, and illnesses; contact the HSM.

Oversee the proper use, maintenance, and care of safety equipment and ensure proper decontamination procedures are followed.

Conduct regular site safety meetings.

Stop work if necessary (i.e., an imminent danger or health hazard exists) and contact the HSM.

Site Personnel:

Read and follow the HASP.

Report accidents, illnesses, or unsafe conditions to the SSO or HSM.

Properly clean and maintain safety equipment.

Prior to working at the site, each employee will receive a copy of this HASP from the PM or HSM. Employees are required to read the HASP and forward a completed copy of the Field Team Review Form to the HSM. Employees are expected to conduct site work in a safe manner and comply with this HASP and federal, state, and local regulations.

CDM may hire subcontractors to assist with field operations at the site. Subcontractors should follow the CDM project HASP or provide and implement their own project HASP. Subcontractor personnel should follow their company's HASP and conduct site work in a safe manner. If a subcontractor is hired, an individual authorized to commit the company will read

the CDM HASP and forward a completed copy of the Subcontractor Safety Agreement Form to the PM. Work performed on the site by subcontractors may include utilizing a Geoprobe to collect soil samples and decontaminating equipment.

1.4 Site Location and Description

Five Department of Defense sites have been designated for field work. An additional site will be selected at a later date.

1.4.1 Fort Lewis, Washington. Fort Lewis Logistics Center is located in Washington State between the cities of Tacoma and Olympia. The source area for this study is the East Gate Disposal Yard (EGDY), which is in the northwest corner of the base. Originally the s EGDY was used for storage and disposal of various solid and liquid wastes, from the Fort Lewis Logistic Center. Studies have been conducted at the EGDY since 1982 to verify and delineate contamination. Affected media include soil and groundwater, with the prominent contaminant being trichloroethene. Additional information is available in Battelle Technology Demonstration Plan (2000).

1.4.2 Naval Submarine Base Bangor, Washington (SUBASE Bangor). The source area for this study is Operable Unit (OU) 8, which is located in the Public Works Industrial Area (PWIA) of the base. SUBASE Bangor is located near the town of Silverdale, Washington. A UST located onsite is believed to be the source of a release of unleaded gasoline into the surrounding media for years spanning 1982 to 1986. In1986, remediation efforts were undertaken to clean up the site of the release (soil vapor extraction/air sparging and product recovery). To date, liquid petroleum hydrocarbons remain in several monitoring wells at the PWIA. Chlorinated VOCs are also present in groundwater. Additional information is available in EA's Final Technical Memorandum (2000).

1.4.3 Laurel Bay Exchange, Beaufort, South Carolina. Laurel Bay is situated on Port Royal Island in the Sea Islands of the Atlantic Coastal Plain, near Beaufort , South Carolina. Contamination onsite was first discovered in March/April of 1993. Soil and groundwater had concentrations of contaminants above maximum contaminant levels (MCLs). The source of this contamination was a gasoline leakage from a UST system associated with an onsite service station. The USTs and surrounding sediments were excavated and removed as part of the site remediation. Additional information is available in the USGS Water-Resources Investigations Report (1996).

1.4.4 Dover Air Force Base, Delaware. The source area for this study is Target Area 1. It is situated in the West Management Unit (WMU) of Dover Air Force Base. The likely contaminant source is the WP21 impoundment, which received hazardous waste from 1963 to 1984. Contamination was first discovered in the water supply of a trailer park during the 1980s. Contaminated soil has been excavated and the area backfilled with clean soil. Additional information is available in an investigation report by the U.S. Army Corps of Engineers (1999).

1.4.5 Naval Air Station Pensacola, Florida. The source area for this study is the wastewater treatment plant located in the corner of the base (USGS, 1999). Naval Air Station Pensacola, Florida is situated in Pensacola Bay in the far northwest corner of the state. At the time this HASP was prepared, no further historical or facility information was available.

2.0 SCOPE OF WORK

CDM will be collecting environmental samples from each site. This HASP describes procedures to be followed and personal protective equipment (PPE) to be used by CDM personnel performing the following field tasks:

Collect groundwater samples from existing site monitoring wells(10 total).

Measure DH with Dissolved Hydrogen analyzer

Measure DH by Bubble-Strip method

Measure chemical parameters of groundwater

Obtain pore water samples from wells

Contain purge water

Collect soil samples.

Drill borings using a hollow-stem auger, Geoprobe, or hand auger.

Collect 20 subsurface soil samples from each site.

Contain excess soil cuttings and decontamination water.

Conduct air monitoring using an organic vapor meter equipped with a photoionization detector (OVM-PID).

Perform laboratory analysis.

Bioavailable Ferric Iron Assay (performed by CDM in Bellevue, Washington)

Redox characterization of sediment and Aquifer samples (work will be conducted at Georgia Institute of Technology, Georgia)

Mineralogical Characterization of Precipitants (work will be conducted at the University of Colorado, Colorado)

3.0 CHEMICAL HAZARD ASSESSMENT

Personnel may be exposed to hazardous chemicals during field operations at the site. Exposure could result from physical contact with, inhalation of compounds volatilizing from, or inadvertent ingestion of contaminated soil or water. The following potential contaminants are present at each site:

Fort Lewis: trichloroethene, cis1,2-dichloroethene, trans-1,2-dichloroethene, ethene, vinyl chloride

- SUBASE Bangor: dichloroethane, benzene, trichloroethane
- Laurel Bay: benzene, toluene, ethylbenzene, xylenes, methyl-tert-butyl-ether
- Dover AFB: trichloroethane, tetrachloroethene, trichloroethene, dichloroethene

NAS Pensacola: 1,3-dichlorobenzene, 1,2-dichlorbenzene, 1,4-dichlorobenzene, chlorobenzene

In general, acute short-term exposure to potential site contaminants may result in eye, nose, skin, and upper respiratory tract irritation. Mild narcosis, chest pain, difficulty breathing, nausea, vomiting, and diarrhea are indications of severe exposure. Some potential site contaminants are considered carcinogenic; therefore, exposure should be minimized. Observable symptoms in site personnel may indicate a chemical's permissible exposure level (PEL) is being exceeded. If such symptoms are observed, CDM personnel should leave the site and inform the CDM HSM, who will reevaluate conditions at the site and implement engineering controls before allowing CDM personnel to reenter.

3.1 Chlorinated Solvents

The most common chlorinated solvents include trichloroethene (TCE), trichloroethane (TCA, methyl chloroform), tetrachloroethene (PERC, perchloroethylene), dichloroethane (DCA, ethylene dichloride), and vinyl chloride. Dermal contact, inhalation, and ingestion are considered the most common exposure routes for chlorinated solvents. Many chlorinated solvents act as central nervous system depressants and are considered carcinogens of the liver, lung, skin, and blood-forming tissues.

Eye and skin irritation may result from prolonged or repeated dermal contact. Contact with some of these compounds may result in skin tissue freezing due to rapid evaporation. Toxic effects may result from repeated exposures to concentrations too low to cause narcotic effects that would normally produce an adequate warning of exposure; individual susceptibility varies widely. Symptoms of exposure include hallucinations and distorted perceptions, dizziness, drowsiness, lack of coordination, confusion, nausea, vomiting, and other gastrointestinal effects.

Symptoms indicating acute exposure to TCE include narcosis and anesthesia; death may occur and are attributed to ventricular fibrillation resulting in cardiac failure. Symptoms of chronic

exposure include eye effects, somnolence, hallucinations or distorted perceptions, gastrointestinal changes, and jaundice. Prolonged inhalation of moderate concentrations of TCE may result in headaches and drowsiness. TCE is also considered an eye and severe skin irritant. Chronic exposure to TCE may result in damage to the liver and other organs. In addition, TCE is considered a potential carcinogen by some organizations. ACGIH recommends an 8-hour TWA-TLV of 50 ppm and STEL of 100 ppm for occupational exposure to TCE. The OSHA PEL for TCE is 100 ppm and the OSHA STEL is 200 ppm.

Symptoms indicating acute exposure to TCA include headache, lassitude, central nervous system depression, poor equilibrium, irritated eyes, dermatitis, and cardiac arrhythmia. Chronic exposure to TCA may result in damage to the skin, central nervous system, cardiovascular system, and eyes. TCA is also considered an eye and severe skin irritant. ACGIH recommends an 8-hour TWA-TLV of 350 ppm and STEL of 450 ppm for occupational exposure to TCA. The OSHA PEL for TCA is 350 ppm.

Symptoms indicating acute exposure to PERC include irritated eyes and skin, respiratory system anesthetic, and depression of the central nervous system. Chronic exposure to PERC may result in dermatitis and irritation of the gastrointestinal system in addition to those systems affected by acute exposure. ACGIH recommends an 8-hour TWA-TLV of 25 ppm and a STEL of 100 ppm for occupational exposure to PERC. The OSHA PEL established for PERC is 100 ppm and the OSHA STEL is 200 ppm.

Symptoms indicating exposure to DCA include irritated eyes, skin, and respiratory system; and depression of the central nervous system. Chronic exposure to DCA may result in damage to the respiratory system, eyes, and central nervous system. ACGIH recommends an 8-hour TWA-TLV of 10 ppm for occupational exposure to DCA. The OSHA PEL established for DCA is 50 ppm and the OSHA STEL is 100 ppm. DCA is considered a potential carcinogen.

Symptoms indicating acute exposure to vinyl chloride include severe irritation of the skin, eyes, and mucous membranes. Skin exposure may result in burns due to rapid evaporation and consequent freezing. At high concentrations, vinyl chloride acts as an anesthetic. Chronic exposure to vinyl chloride may result in damage to the reproductive system and liver. ACGIH recommends an 8-hour TWA-TLV of 5 ppm for occupational exposure to vinyl chloride. The OSHA PEL established for vinyl chloride is 1 ppm. Vinyl chloride is considered a potential carcinogen.

3.2 Petroleum Products

Petroleum products usually include benzene, ethylbenzene, toluene, and xylenes (BETX). Petroleum products also may contain cyclohexane, methyl tert butyl ether, and tetraethyl lead (leaded gasoline only.) The most common exposure routes for these compounds include inhalation and skin contact or absorption. Acute short-term inhalation of petroleum hydrocarbon concentrations up to 1,000 parts per million (ppm) may result in headache, dizziness, loss of appetite, weakness, loss of coordination, and upper respiratory tract irritation. Inhalation of vapor concentrations in excess of 5,000 ppm may result in loss of conscious-ness, coma, and

death. Dermal contact may result in eye and skin irritation. Benzene is considered carcinogenic; therefore, exposure should be minimized.

Symptoms indicating acute exposure to benzene compounds include irritated eyes, nose, and respiratory system; giddiness; headache; nausea; staggered gait; fatigue; and dermatitis. Chronic exposure to benzene may result in damage to the blood, central nervous system, skin, bone marrow, eyes, and respiratory system. The American Council of Governmental Industrial Hygienists (ACGIH) recommends an 8-hour time weighted average-threshold limit value (TWA-TLV) of 0.5 ppm for occupational exposure to benzene. The Occupational Safety and Health Act (OSHA) permissible exposure level (PEL) for benzene is 1.0 ppm and the short-term exposure limit (STEL) is 5 ppm.

4.0 PHYSICAL HAZARD ASSESSMENT

4.1 Temperature-Related Hazards

Ambient work site temperatures and the amount of physical activity performed may contribute to temperature-related illnesses in employees ranging from heat stress to hypothermia. Personnel performing physical labor while wearing protective clothing at temperatures greater than 70°F are subject to developing heat-related disorders. Employee temperatures and radial pulse rates should be monitored to ensure an adequate work/rest regimen is followed and heat-related illnesses are prevented. If temperatures exceed 80°F, personnel should take a 15-minute rest from strenuous activity every hour and drink plenty of water or an electrolytic beverage (e.g., Gatorade). Appropriate clothing should be worn if outside temperatures decrease to less than 40°F for more than 2 hours.

4.2 Fire and Explosion Hazards

The risk of fire or explosion may be present during field activities. A combustible gas meter (CGM) should be utilized if OVM-PID readings indicate elevated volatile organic vapors in the work zone. If the CGM indicates combustible gas levels in the general work area at 20 percent of the lower explosive limit (LEL), work shall cease and the tasks will be reevaluated. Work involving welding or cutting shall not be performed if the CGM indicates concentrations have reached 10 percent of the LEL in the general work area. Engineering controls, such as ventilation, will be implemented to control combustible gas levels. As a precautionary measure, smoking will not be permitted on site at any time.

4.3 Noiss Hazards

Heavy equipment and drill rigs(Geoprobe) may be a source of high noise levels. Because noise levels vary for each piece of equipment, hearing protection will be provided as necessary. Personnel should utilize hearing protection while working within 15 feet of operating heavy equipment and drill rigs.

4.4 Oxygen Deficiency Hazards

Site personnel are not expected to encounter an oxygen-depleted atmosphere during site activities. Entry into a confined space is considered a last resort and requires an addendum to

this HASP. Confined spaces are defined as any space having a limited means of egress and subject to the accumulation of toxic or flammable contaminants or an oxygen-deficient atmosphere. This definition includes, but is not limited to, tanks, silos, utility vaults, trenches over 4 feet deep, and open-topped vessels with walls greater than 4 feet high.

4.5 Utility Hazards

CDM personnel should be aware of any overhead power lines located within 20 feet of the work area. If such lines are present, they should be guarded, insulated, or turned off. In addition, the Geoprobe contractor should utilize a locating service to determine whether underground utilities are in the area prior to beginning soil-sampling activities. Most State laws require a minimum 48-hour notice to utilities prior to the start of underground work. CDM personnel should be satisfied utilities have been located and that this notice has been given. Since subsurface soil samples will be collected, any required dig permits will be acquired prior to fieldwork activities.

4.6 Construction Hazards

The principal construction hazards are expected to be those associated with Geoprobe sampling and traffic movement. Operation of the Geoprobe will be conducted by a qualified subcontractor and will be performed in accordance with applicable regulations.

When equipment is being loaded and unloaded, CDM personnel should stand clear to prevent injuries in case the load falls. CDM personnel should be aware of moving equipment and traffic at the site and stay out of the way; particular attention should be paid when backup alarms are sounding because operator visibility in the direction of travel may be decreased.

5.0 SITE WORK ZONES

Three work zones, described in the following paragraphs, will be established during site activities as a contamination control measure.

5.1 Exclusion Zone

The exclusion (or work) zone is the area that contains or is suspected of containing contaminated soil or open monitoring wells. An area having an approximately 15-foot radius should be established around each sampling location to serve as the exclusion zone during work activities. These areas will cease being exclusion zones when contamination is no longer present or has been contained. Personnel should not be allowed to enter an exclusion zone unless they have been given permission by the SSO and otherwise follow all applicable portions of this HASP.

5.2 Contamination Reduction Zone

A contamination reduction zone will be established adjacent to each exclusion zone to act as a transition area for personnel and equipment decontamination. The contamination reduction zone is also considered a restricted area; therefore, personnel must meet training and medical surveillance qualifications.

5.3 Support Zone

The support zone is the area considered uncontaminated. This area is used to stage clean equipment and other support facilities. Visitors must stay in the support zone unless proof of training and medical clearance is shown to the SSO.

6.0 AIR MONITORING AND SAMPLING

Air monitoring will be conducted during site operations having a high potential to release contaminants. Monitoring will be used to document exposure levels and confirm that necessary precautions are taken to protect on-site personnel and the general public. In addition, air sampling may be performed if personnel exposures to organic vapors are suspected of exceeding established exposure limits.

Monitoring and sampling equipment will be calibrated daily in accordance with manufacturers' requirements. Calibration data, background readings, predominant wind direction, air monitoring readings, and air sampling information will be recorded as part of the daily field logs. If instrument readings are questionable or abnormal, the HSM should be notified.

6.1 Air Monitoring

The organic vapor action level is based on readings obtained with an OVM-PID. Measurements are taken in the breathing zone, which is considered to encompass a sphere of 1-foot radius around a worker's nose during normal work operations.

Because the OVM-PID measures total organic vapors and cannot readily distinguish between compounds, a conservative organic vapor action level has been established. The organic vapor action level will be a sustained (5-minute) reading of 2.5 ppm (one-half the STEL for benzene) above background, measured in the breathing zone. If organic vapor levels exceed 2.5 ppm above background and cannot be controlled utilizing engineering controls, half-face respirators should be worn. If levels exceed 5 ppm above background, full-face respirators should be worn. If organic vapor concentrations exceed 50 ppm above background, work should cease and personnel should leave the site.

The action levels discussed above were determined to be sufficient based on a comparison of air sampling analytical results to air monitoring readings obtained using an OVM-PID or OVM equipped with a flame ionization detector (OVM-FID) during sampling. Action levels may be adjusted as additional information is obtained. CDM employees are instructed to stay outside the exclusion zone or upwind as much as possible. Such work practices will minimize the potential for exposures above established PELs.

6.2 Air Sampling

Air samples have been collected for CDM employees observing, directing, and documenting operations at hazardous waste sites to document exposure of CDM personnel to benzene and total petroleum hydrocarbons (TPH). These air samples have been collected at various project locations during different phases of site operations. Analytical results received from these samples indicate no exposures to benzene greater than the PEL of 1 ppm measured as an 8-hour TWA at any site.

Additional air sampling may be conducted at the discretion of the CDM HSM, PM, or SSO. Personnel air sampling of organic vapors may be conducted using organic vapor diffusion (OVD) badges or a charcoal tube and pump assembly. For personnel sampling, the OVD badge or charcoal tube should be placed within the breathing zone of the individual with the greatest potential exposure for 8 to 10 hours. OVD badges and charcoal tubes may be exposed for shorter durations if personnel leave the exclusion zone. Upon sampling completion, the OVD badges or charcoal tubes are collected and sealed, exposure times recorded, and the badges are sent to an independent laboratory accredited by the American Board of Industrial Hygiene (ABIH) to perform industrial hygiene analysis.

7.0 PERSONNEL PROTECTION

7.1 Exclusion Zones and Contamination Reduction Zones

This section describes the PPE to be worn by personnel performing field operations within site exclusion and contamination reduction zones. Appropriate PPE was determined using information in Sections 3.0 and 4.0.

Head protection - American National Standards Institute (ANSI) approved hard hats shall be worn near heavy equipment and drill rigs, and whenever there is an overhead hazard.

Eye and face protection - Safety glasses shall be worn during sampling activities. When there is a high splash potential, face shields also shall be worn.

Foot protection - Steel-toe and -shank work boots shall be worn.

Skin protection - Coveralls should be worn. If direct contact with contaminated material is expected, Tyvek coveralls also should be worn. If the probability of being splashed or coming in contact with wet contaminants is high, personnel should wear polyvinyl chloride (PVC) rainsuits or Saranax-coated Tyvek coveralls.

Hand protection - Personnel should wear two pairs of chemically protective gloves during sampling activities. An inner, surgical-type glove should be worn to lessen the chance of cross contamination during decontamination activities. Outer gloves should be made of Nitrile. If necessary, heavy-duty work gloves also may be worn. If work gloves are worn over chemically protective gloves, they should be considered disposable. An alternative is to wear the work gloves under the chemically protective gloves.

Respiratory protection - If organic vapor concentrations (measured in the breathing zone) exceed sustained (i.e., 5 minutes) readings of 2.5 ppm, personnel should wear National Institute of Occupational Safety and Health (NIOSH) approved, properly fitted half-face respirators. Respirators should be equipped with organic vapor (OV) cartridges. Cartridges should be changed a minimum of once per day or more often if break-through is suspected. At organic vapor levels between 5 and 50 ppm measured in the breathing zone, personnel should wear full-face respirators equipped with the same type cartridge. At sustained concentrations greater than 50 ppm, work shall cease. Additional information concerning air monitoring is included in Section 6.0.

7.2 Support Zones

Personnel working in a support zone, or in an exclusion or contamination reduction zone before or after contaminated material is present, are not required to wear protective clothing or respirators. Regular work clothing should provide adequate protection during operations in these areas. Hard hats, safety glasses, and steel-toe and -shank boots must be worn while heavy equipment is being mobilized.

7.3 Summary

Levels of protective clothing have been assigned to each field task. Level D is considered general work clothing; Level C is considered general work clothing with the addition of chemically protective clothing and respirators. In some cases, personnel may wear respirators and no chemically protective clothing; this is referred to as Modified Level C protection. The levels of protection listed below may be altered based on additional information and field conditions. Final determinations concerning levels of protection will be made by the SSO and are subject to approval of the HSM. The following is a list of field tasks and the levels of protective clothing assigned to them:

- Collect groundwater samples from existing site monitoring wells - Level C or D (as determined on site).

Collect soil samples - Level C or D (as determined on site).

Perform laboratory analysis - Level D.

8.0 DECONTAMINATION PROCEDURES

Decontamination procedures should be used for equipment and personnel to ensure contamination is controlled and not spread from the site. In addition, contact with contaminated material should be limited. Methods to minimize the spread of contamination include using plastic covers over field equipment and limiting personnel contact rates and areas. Used disposable protective equipment and decontamination water will be contained for off-site disposal.

8.1 Personnel

Personnel should don protective equipment before entering exclusion zone and follow decontamination procedures before reentering the support zone. The level of protective equipment and therefore decontamination procedures may be altered based on additional information and field conditions. Decontamination should include the following steps:

Wash and rinse outer clothing, boots, and gloves. A soap and water solution should be used for the wash.

Remove outer gloves and protective clothing (if worn).

Remove respirator and cartridge assembly (if worn); clean respirator.

Remove inner gloves.

Wash hands and face.

Shower as soon as possible after leaving the site.

8.2 Sampling Equipment

Sampling equipment should be brought through the decontamination line with personnel and cleaned before returning it to CDM. Samples and sample coolers should be wiped down to prevent contaminating laboratory personnel.

8.3 Heavy Equipment

Heavy equipment should be decontaminated before leaving the site. Heavy equipment is difficult to decontaminate; methods generally include washing with high pressure water or steam cleaning while scrubbing accessible parts. Particular care should be given tires, tracks, augers, buckets, and other components in direct contact with potentially contaminated material.

9.0 GENERAL SAFE WORK PRACTICES

If respiratory protection is required, a buddy system will be used to readily detect when emergency aid is required. No person will be allowed to work out of sight of other personnel.

A first aid kit and fire extinguisher will be available during site activities. Fire extinguishers should be within 50 feet of the work operation. A first aid kit , cell phone, and fire extinguisher will be present in CDM onsite vehicle.

Personnel shall not eat, drink, chew gum or tobacco, smoke, or perform any other practice that increases the probability of hand-to-mouth contact in site exclusion zones or contamination reduction zones.

The use of controlled substances or alcohol is forbidden at the site. In addition, personnel shall not work at the site while under the influence of such substances.

10.0 EMERGENCY PROCEDURES

Emergency response procedures have been developed for extraordinary events that could occur during field operations. These events include injuries, chemical exposures, fires, and spills. In general, the following actions should be implemented in the event of an emergency:

First aid or other appropriate initial action should be administered by those closest to the accident or emergency situation. This assistance should be conducted such that those giving assistance are not placed in a situation of unacceptable risk.

The CDM PM and HSM should be contacted immediately.

A Supplementary Record of Occupational Injuries and Illnesses Form (included as **Appendix C**) should be completed by the injured individual or witness and forwarded to the PM. The PM will review the form prior to forwarding it to the HSM. Changes to the operation should be made to prevent the same event from occurring in the future.

10.1 Physical Injuries

If a person is physically injured or suffers a medical emergency, first aid procedures should be followed. Depending on the severity of the injury or medical condition, emergency medical response may be sought. Contaminated clothing may need to be decontaminated and removed prior to transport to an emergency medical facility.

10.2 Chemical Exposures

If the injury to the worker is chemical in nature, the following first aid procedures should be followed.

10.2.1 Eye Exposures

If contaminated solid or liquid enters the eyes, they should be flushed immediately with large amounts of clean water while occasionally lifting the upper and lower eyelids. Medical attention should be obtained immediately.

10.2.2 Skin Exposures

If contaminated material contacts the skin, the affected area should be washed promptly with soap and water. If contaminated materials penetrate clothing or protective equipment, the items should be removed and affected skin areas washed. Medical attention should be obtained if symptoms warrant.

10.2.3 Inhalation
Anyone inhaling a large volume of potentially toxic vapors should be moved to fresh air at once. If breathing has stopped, artificial respiration should be performed. Medical attention should be obtained immediately.

10.2.4 Ingestion
If contaminated material is swallowed, medical attention should be obtained immediately and the poison control center contacted for further directions.

10.3 Fires
Fire extinguishers should be available on site and in vehicle cabs. In case of fire at the site, the following actions should be taken:

Evacuate personnel from the site to an upwind location.

Notify the fire department and emergency response agencies.

Attempt to extinguish the fire using portable fire extinguishers or by smothering (only if the fire is small).

10.4 Uncontrolled Release of Hazardous Materials
The primary considerations during a hazardous materials spill are to prevent additional personnel from entering the area, contain existing spillage, and prevent further spillage. In the event of a hazardous materials spill at the site, the following actions should be taken:

Evacuate personnel from the area.

Summon emergency medical or fire services if the spill involves extremely toxic or flammable materials.

Contain the spill with absorbent booms and block off the area. Drains, sewers, etc. should be blocked to prevent material from migrating.

Attempt to stop the flow of material from its point of origin.

10.5 Emergency Notification System
Generally, emergency notification is given by an air horn or car horn. The following signals are considered standard:

One Long Blast - Warning; personnel should give necessary aid, prepare to evacuate, and await further instructions.

Two Long Blasts - Evacuate; all personnel should evacuate the area.

Three Long Blasts - All Clear; personnel may reenter the site.

10.6 Emergency Services

The telephone closest to each site should be located by the SSO prior to starting site work. If outside services (e.g., ambulance, fire, police) are required, field personnel should immediately telephone the local emergency number (911). The SSO should notify CDM at (425) 453-8383 after the emergency situation has been stabilized. If medical attention is needed but the situation is not an emergency, the injured employee may be transported to the hospital by other field personnel.

10.6.1 Hospital Route

Hospital route maps are shown on the following figures for each of the five sites:

Figure 1 Fort Lewis

Figure 2 SUBASE Bangor

Figure 3 Laurel Bay

Figure 4 Dover AFB

Figure 5 NAS Pensacola

In cases involving severe emergencies, personnel should await emergency medical transport.

10.6.2 Emergency Telephone Numbers

The following emergency telephone numbers should be available at the site:

Fire 911

Ambulance 911

Paramedics 911

Police.....911

Poison Control Center.....911

Occupational Medical Consultant
(Dr. Calvin Jones) .....(425) 822-3651

CDM Health and Safety Manager (Monica Beckman).....(425) 453-8383
(Home) .....(206) 760-1013

11.0 TRAINING

Personnel working at the sites will have received the required 40-hour training for work at hazardous waste sites in accordance with Occupational Safety and Health Administration (OSHA) regulations. Site personnel also will be up to date with respect to 8-hour annual refresher training requirements. At least one individual working at the site will be currently certified in First Aid and Cardiopulmonary Resuscitation (CPR) procedures. The PM will have completed 8 hours of specialized training for supervising workers at hazardous waste sites in accordance with OSHA requirements. Training records are maintained at CDM by the HSM.

11.1 Medical Surveilance

CDM employees working at the sites will participate in a Medical Surveillance Program. The CDM Medical Surveillance Program is administered by Dr. Calvin Jones of Virginia Mason Occupational Medicine Clinic in Bellevue, Washington. Medical surveillance documentation is maintained at CDM by the HSM; actual medical examination results are maintained at the Virginia Mason Occupational Medicine Clinic.

Direct hire and new employees are given a baseline physical and annual examinations thereafter. The examining physician verifies in writing whether each individual is fit to work at hazardous waste sites and utilize protective equipment, including respirators. Additional medical examinations may be required during the course of the project if overexposure to site contaminants or an injury occurs.

The content of the medical examinations has been determined by the CDM Occupational Physician. The following are the minimum requirements of the medical surveillance examinations:

Baseline head-to-toe examination

Medical history, including work history, past exposures, hobbies, and family history

Complete blood count and blood chemistries (including liver function, kidney function, heart function, and thyroid function screening)

Urinalysis

Spirometry

EKG (every 2 years)

Chest X-ray (every 2 years)

Audiogram

Vision acuity test

Additional tests may be conducted at the discretion of the examining physician.

12.0 HASP MODIFICATIONS

This project HASP should be reviewed and amended when:

Applicable regulations are revised.

Additional information concerning site contaminants, operations, personnel, and emergency services is obtained.

Site operations are revised.

When the HASP is revised or addenda prepared, personnel shall review the changes or addenda and file a new Field Team Review Form with the HSM.

ATTACHMMENTS

Attachment A

SUBCONTRACTOR SAFETY AGREEMENT FORM

SUBCONTRACTOR SAFETY AGREEMENT FORM

_____ (hereafter called Subcontractor) has been retained by CDM Technologies (CDM) to assist CDM with field work at (_____). Subcontractor has read and understands the project Health and Safety Plan (HASP) dated July 26, 2000 for this project. Subcontractor is aware that their employees may be exposed to potentially hazardous materials and physical hazards during the performance of work at the above-referenced site.

Subcontractor shall ensure their employees, agents, subcontractors, and other invitees to the project site comply with all applicable health and safety laws and regulations, and the most recent version of their project HASP. Subcontractor is responsible for examining regulatory requirements and determining whether additional or more stringent health and safety provisions are required for their portion of work.

_____
Authorized Signature

_____
Printed Name

_____
Title

_____
Date

Completed copies of this form should be forwarded to the CDM Project Manager.

Attachment B

FIELD TEAM REVIEW FORM

FIELD TEAM REVIEW FORM

I have read and reviewed the most recent revision dated July 26, 2000 of the project Health and Safety Plan (HASP) for fieldwork at (_____). I have been given a chance to ask questions regarding the project HASP and understand the information contained therein. I agree to comply with all aspects of the project HASP.

Name: _____

Signature: _____

Company: _____

Date: _____

Completed copies of this form should be forwarded to the CDM Health and Safety Manager.

Attachment C

SUPPLEMENTARY RECORD OF OCCUPATIONAL INJURIES AND ILLNESSES FORM

SUPPLEMENTARY RECORD OF OCCUPATIONAL

INJURIES AND ILLNESSES FORM
CASE NO:

THIS IS AN OFFICIAL DOCUMENT, BE THOROUGH AND ACCURATE.

This section to be completed by injured employee or witness:

Employer Name: CDM Technologies

Employer Address: 11811 N.E. 1st Street, Suite 201, Bellevue, Washington 98005

Project Name/Location: _____

Date of Accident/Incident: _____ Time: _____

Was place of accident/incident on employer's premises? Yes( ), No( )

Employee Name: _____

Employee Home Address: _____

Social Security Number: _____ Age: _____ Sex: M( ), F( )

Occupation/Department: _____

What was being done at time of accident/incident?_____

_____

_____

_____

How did the accident/incident occur? _____

_____

_____

Employee Signature: _____ Date: _____

SUPPLEMENTARY RECORD OF OCCUPATIONAL INJURIES AND ILLNESSES FORM (CONTINUED)

CASE NO:

THIS IS AN OFFICIAL DOCUMENT, BE THOROUGH AND ACCURATE.

This section to be completed by the Project Manager/Supervisor:

Time reported: _____ Did employee leave work? _____ When: _____

Date and time returned: _____

Nature of injury: _____ Exact body part affected: _____

Check one: Near Miss ( ), First Aid ( ), Doctor ( ), Hospitalized ( )

Doctor/Hospital Name: _____ Address: _____

Why did accident/incident occur? _____

_____

_____

What corrective action has been initiated to prevent recurrence? _____

_____

_____

Project Manager/Supervisor Signature: _____ Date: _____

This section to be completed by Health and Safety Manager:

Concur with action taken? Yes ( ), No ( ); Remarks: _____

_____

_____

Health and Safety Manager Signature: _____ Date:_____

Appendix D
Field Notes

Appendix E
Analytical Data