FINAL REPORT

Evaluating the Efficacy of Bioaugmentation for *In-Situ* Treatment of PCB Impacted Sediments

ESTCP Project ER-201215

SEPTEMBER 2018

Dr. Kevin R. Sowers
University of Maryland Baltimore County

Dr. Upal Ghosh
Sediment Solutions

Dr. Harold D. May
Medical University of South Carolina

ESTCP

Page Intentionally Left Blank

Page Intentionally Left Blank

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*
05-Sep-2018

2. REPORT TYPE
Final Technical Report

3. DATES COVERED *(From - To)*
12-APR-2012 – 12-APR-2017

4. TITLE AND SUBTITLE
Evaluating the efficacy of bioaugmentation for *in-situ* treatment of PCB impacted sediments

5a. CONTRACT NUMBER
W912HQ-12-C-0006

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Kevin R. Sowers, Upal Ghosh, Harold D. May

5d. PROJECT NUMBER
ER-201215

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) AND ADDRESS(ES)
Univ. of Maryland Baltimore County, 1000 Hilltop Cir Baltimore MD 21250-0001

Sediment Solutions, P.O. Box 7011, Ellicott City, MD 21042

Medical Univ of So. Carolina, 173 Ashley Ave Charleston, SC 29425

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
ENVR SECURITY TECHNO CERT PROG
901 NORTH STUART STREET
ARLINGTON, VA 22203

10. SPONSOR/MONITOR'S ACRONYM(S)
ESTCP

11. SPONSOR/MONITOR'S REPORT NUMBER(S) ER-201215

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The objective of the project was to demonstrate and validate a recently developed *in-situ* treatment for degrading polychlorinated biphenyls (PCBs) in contaminated sediments under field conditions. The innovative aspect of the technology is the application of activated carbon pellets as a solid substrate for: 1) delivery of microorganisms into sediments and 2) sequestration and concentration of hydrophobic PCBs in close proximity to the PCB transforming bacteria. Both anaerobic halorespiring and aerobic PCB degrading bioamendments were mass cultured, transported to a site and delivered through a water column to sediments without loss of viability. Treatment with the bioamendment mixture reduced the mean total PCB concentration by an average of 52% and the aqueous PCB fraction by 95% after 13.5 months. Costs of the treatment were less than thick caps and dredging and the approach is uniquely suited to treat ecologically sensitive and difficult to reach areas.

15. SUBJECT TERMS
PCBs, environmental restoration, bioremediation

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
					Kevin R. Sowers
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
U	U	U	UU	154	410.234.8878

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Page Intentionally Left Blank

FINAL REPORT
Project: ER-201215

TABLE OF CONTENTS

Page

TABLE OF CONTENTS (Continued)

Page

TABLE OF CONTENTS (Continued)

Page

LIST OF FIGURES

Page

LIST OF FIGURES

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

LIST OF TABLES

Page

ACRONYMS AND ABBREVIATIONS

AC	Activated carbon
ARARs	Applicable or Relevant and Appropriate Requirements
Aroclor	Commercial polychlorinated biphenyl mixtures produced by Monsanto
BAC	Bioamended Activated Carbon
BC	Black carbon
BphC	2,3-dihydroxybiphenyl-1,2-dioxygenase
Bioamended	Addition of PCB dechlorinating and degrading microorganisms
BSL-1	Biosafety Level 1
CE	Chloroethenes
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
COC	Chemicals of concern
DDx	Pesticide dichlorodiphenyltrichloroethane and associated congeners
DoD	U.S. Department of Defense
dHPLC	Denaturing high pressure liquid chromatography for community analysis
ECD	Electron capture detector
ESTCP	Environmental Security Technology Certification Program
FID	Flame ionization detector
GAC	Granulated activated carbon
GC	Gas chromatography
Kd	Partitioning coefficient
MCBQ	Marine Corps Base Quantico
MCCDC	Marine Corps Combat Development Command
NAVFAC	Naval Facilities Engineering Command
NDIR	Non-dispersive infrared gas analyzer
NIEHS-SRP	National Institute of Environmental Health Sciences Superfund Research Program
OCS	Marine Corps Officer Candidates School
ONR	Office of Naval Research
OOD	Officer of the Deck
PCB	Polychlorinated biphenyl
PCE	Perchloroethene
PCR	polymerase chain reaction
PE	Polyethylene

POM	Polyoxymethylene
POP	Persistent organic pollutant
PVC	Polyvinyl chloride
QA/QC	Quality assurance and quality control
QPCR	Quantitative polymerase chain reaction for enumerating bacterial gene copies
RDH	Reductive dehalogenase
RPM	Remedial Project Manager
SBIR	Small Business Innovation Research Program
SediMite™	pelleted AC, a trademark of Sediment Solutions
SERDP	Strategic Environmental Research and Development Program
SOP	Standard operating procedure
TEF	Toxic equivalency factor
TEQ	Toxic equivalency
TOC	Total organic carbon
USEPA	United States Environmental Protection Agency
VHI	Ventrui horn induction system
Vortex	Vortex TR-Aquatic system developed by Vortex Granular Systems, LLC

ACKNOWLEDGEMENTS

This project was a joint effort between the University of Maryland Baltimore County, Sediment Solutions and Medical University of South Carolina with support of The Department of Defense (DoD) Environmental Security Technology Certification Program (ESTCP) Project No. 201215.

Fredrick Evans, RPM NAVFAC, and Victoria Waranoski, RPM NAVFAC, provided us access to the Marine Corps Base Quantico (MCBQ) field site. Brian Ventura, RPM MCBQ, coordinated all visits to the site for treatment and sampling. We are also grateful to Captain Gulliksen, S4 OCS, for providing us access to the field site between training sessions. Dr. Rayford Payne conducted the mesocosm studies and analyses of field samples. Ms. Ethel Apolinario conducted quantitative PCR analyses for monitoring the bioamendments. Dr. Chris Marshall, University of Pittsburgh, conducted the Illumina MiSeq community sequencing and bioinformatics analyses of both the mesocosms and field sediments. Jeffrey Vance supported by Victoria Brisbing, Daniel Harnett and Emma Reinemann, Brightfields Inc., provided logistical support for field tests and sampling, and was responsible for design and testing of the venture horn induction deployment device. Dr. Seokjoon Kwon of Sediment Solutions assisted with the testing of SediMite application using a modified Vortex spreader and the field deployment.

ABSTRACT

Introduction and objective. Polychlorinated biphenyls (PCBs) are one of the most frequently reported contaminants associated with sediments in the country and ranks second after mercury for the basis for fish consumption advisories. Recent *in-situ* studies have demonstrated the feasibility of PCB bioavailability reduction using activated carbon (AC) as an amendment. A desirable goal is to ultimately reduce the inventory of legacy PCBs in sediments while also reducing bioavailability to the food chain. The objective of this project was to test the efficacy of *in-situ* application of AC amended with PCB degrading microorganisms to reduce the total mass and bioavailability of PCBs in sediments.

Technology description. Based on the optimal loading cell titer and carbon loading rates determined from a mesocosm study, we conducted a pilot-scale field application of bioamended AC in Abraham's Creek (Marine Corp Base Quantico) with the following objectives: 1) demonstrate the scalability of growing PCB respiring microorganisms for field application, 2) develop and test the application of PCB halorespiring and degrading bacteria using pelleted AC as a delivery system, 3) assess the benefits of bioamended AC treatment on concentrations of PCBs in sediments and porewater, 4) assess the fate of the bioamendment over time, and 5) evaluate the impact of treatment on the indigenous microbial populations.

Performance and Cost Assessment. Treatments with an AC agglomerate bioamended with PCB halorespiring and degrading bacteria reduced the PCB concentration in the top 7.5 cm by up to 52% and aqueous concentrations of tri- to nona-chlorobiphenyl PCB congeners by as much as 95% in 409 days. Co-planer congeners were reduced by up to 80% in sediment and were undetectable in the porewater. There was no significant decrease in PCB concentrations in non-bioamended plots. The titer of the bioamendments decreased but were still detectable after 409 days, whereas indigenous microbial diversity was not significantly affected by treatments. *In situ* treatment of PCBs using an AC agglomerate as a delivery system for bioamendments is particularly well-suited for environmentally sensitive sites where there is a need to reduce exposure of the aquatic food web to sediment-bound PCBs with minimal disruption to the environment. The net cost for the full remediation of the 7.8-acre site using bioamended SediMite application was estimated at $1.8M compared to $4M for an isolation cap and $25M for full excavation and disposal off-site. The annual average maintenance costs for bioamended AC is estimated to be in the range of costs for Monitored Natural Attenuation or capping which are estimated at about $100k/year for the first 5 years.

Implementation issues. The effectiveness of bioamended AC for reducing concentrations of total and soluble PCBs was affected by the homogeneity of the application. The VHI device used in this study is appropriate for application in water margin areas and difficult to access areas such as below piers. For large areas, a boat mounted belt spreader or land based telebelt are required to evenly distribute the bioamendments and obtain consistent maximum effectiveness.

Publications. 1) Payne, R.P.,Ghosh, U., May, H.D., Marshall, C.W. and Sowers, K.R. 2017. Mesocosm studies on the efficacy of bioamended activated carbon for treating PCB-impacted sediment. Environ. Sci. Technol. 51 (18): 10691-10699; 2) Payne, R.P., Ghosh, U., May, H.D., Marshall, C.W. and Sowers, K.R. A Pilot-Scale Field Study: *In Situ* Bioremediation of PCB-Impacted Sediments with Bioamended Activated Carbon. Submitted.

EXECUTIVE SUMMARY

Objectives of the Demonstration. The objective of the project was to demonstrate and validate a recently developed *in-situ* treatment for degrading polychlorinated biphenyls (PCBs) in contaminated sediments under field conditions. Department of Defense (DoD) facilities across the country are impacted with persistent pollutants such as PCBs and the DoD is challenged with the remediation of these sites. This work addresses the DoD need for cost effective, *in-situ* remediation technologies for PCBs and can be applied in principle to other persistent organic pollutants (POPs) such as pesticides. Most importantly, this work will enable extensive *in situ* treatment at DoD sites that include both shallow and deep sediments with minimum impact to environmentally sensitive areas. This integrated approach utilizes activated carbon to serve concurrently as an agent to sequester PCBs from the food chain and as a delivery system and solid substrate to enhance both anaerobic and aerobic microbial processes for complete *in situ* degradation. This technology demonstration project was conducted in a ponded waterbody in Abraham's Creek, Marine Corps Base Quantico, located in the southeastern portion of Chopawamsic Creek near the confluence with the Potomac River. The treatment utilizes an activated carbon agglomerate, SediMite™, as a delivery system for deploying PCB degrading microorganisms into PCB impacted sediments to accelerate the reduction of PCB levels within a period of months. In addition to evaluating the performance of the treatment, the demonstration project provides estimates on the cost of deploying the technology in the field and provides data for assessment of its regulatory acceptance.

Description of the Technology. This project is the culmination of more than a decade of laboratory research on PCB dechlorination (Sowers and May) and *in-situ* remediation of PCBs (Ghosh) that was ready for synergistic implementation in the field. The innovative aspect of the technology is the application of bioamended activated carbon agglomerate, SediMite™, as a solid substrate for: 1) delivery of microorganisms into sediments, 2) formation of microbial biofilms, and 3) sequestration and concentration of hydrophobic PCBs in close proximity to the biofilm of PCB transforming bacteria. Recent laboratory mesocosm scale experiments demonstrated the long-term activity of the microorganisms delivered with activated carbon and the overall feasibility of the approach.

Performance Assessment. Key performance objectives of the project included demonstration of reduction of total PCB concentration and PCB bioavailability in sediment after bioamendment. The project was conducted in two phases. In the first phase a laboratory treatability study was conducted to evaluate performance in laboratory mesocosms using sediments from the field site. This study determined the optimal loading of bioamendment, optimal type of dechlorinating inoculum, effect of organic carbon supplement, and most effective scale up and application methods. The treatability study demonstrated that:

- Levels of both higher and lower chlorinated congeners were reduced indicating that both anaerobic reductive dechlorination and aerobic degradation occurred concurrently.
- Total levels of PCBs were reduced by a mean of 78% after 375 days compared with no significant change in untreated sediments.
- The overall toxicity was reduced by up to 90% after treatment based on toxic equivalency of dioxin-like congeners in the sediments.

- Porewater concentrations of all PCB homologs were reduced after bioaugmentation by up to 88% after 120 days and up to 97% after 375 days.
- PCB levels were reduced throughout the 10 cm sediment column including both the aerobic and anaerobic zones.
- Overall results indicate the SediMite™/cellulose with 10^5 DF1 and LB400 g-1 sediment was most effective for PCB mass reduction for the field demonstration project.
- Sufficient amount of bioamendments were produced to complete the proposed pilot study.
- The Venturi Horn Induction device (VHI) was successfully calibrated to deliver 10^7 cell g-1 SediMite™ with no significant loss of inoculum during pellet inoculation.
- There was no significant loss in viability during storage, transport, and passage of bioamended SediMite™ through a 1m water column.

Based on the treatability study results, a decision was made to move forward with the pilot-scale demonstration in the field and a demonstration work plan was developed. Field testing was performed in close coordination with the RPMs and after approval of the demonstration plan by ESTCP and NAVFAC. The testing involved three phases: 1) initial baseline sampling, 2) application of treatment amendments in the field, and 3) subsequent monitoring visits to collect post-treatment samples. The application of treatment amendments was completed in 3 days and each sampling visit was performed in a day. The pilot demonstration was implemented safely and within the target timeline. Key outcomes of the field demonstration are provided below:

- Both anaerobic halorespiring and aerobic biphenyl degrading bioamendments were mass cultured, transported to the site and delivered through a water column to sediments without significant loss of viability.
- Treatment with the bioamendment mixture on 3% bioamended SediMite™ reduced the mean total PCB concentration by 30% in Plot 3 and 52% in Plot 4 based on 5 sediment cores. Even after excluding two outliers in plot 4 that had exceptionally high concentrations of black carbon (15.3 and 16.6%) due to variability in application, the decrease in total PCB concentration was 43% instead of 52% compared with Day 0 and was still statistically significant ($p>0.05$). The tri+ PCB congeners in sediment porewater were reduced by 84% and 95% in the two bioamended field plots after 409 days. Co-planer congener levels were reduced by up to 80% in the sediment and were undetectable in the porewater.
- All homolog groups were reduced in sediment and porewater indicating that both anaerobic halorespiration and aerobic degradation occurred within the benthic zone of the field sediments.
- The effectiveness of bioamended SediMite™ for reducing concentrations of total and soluble PCBs was affected by the homogeneity of the application. Although the mean values in bioamended plot 4 met the performance objectives, this was not the case for identically treated Plot 3. However, there was also wide variation within each bioamended plot and maximum values exceeded the performance objectives for total and porewater concentrations of PCBs in both bioamended plots. There was a direct relationship between the extent of degradation and the amount of black carbon detected in an individual sediment sample, which indicated that for full-scale treatment more consistent application of bioamended AC would be required to achieve consistent degradation throughout the site.

The VHI device is appropriate for application in water margin areas, wetlands and difficult to access areas such as below piers and under overhanging trees. For larger areas, methods that ensure even distribution such as a boat mounted belt spreader or land based telebelt are required to evenly distribute the bioamendments and obtain consistent maximum effectiveness.

- The titer of the bioamendments decreased over two orders of magnitude, but were still detectable after 409 days in the field. Indigenous microbial diversity was not significantly different between any other sites, time points, or depths. Therefore, bioaugmentation and the addition of activated carbon did not significantly alter total microbial diversity on a macroscale.
- The bioamended SediMite™ was stable and did not migrate downstream of the treatment area.

The study was limited to two post-assessments 140 and 409 days after treatment. Multi-year post-treatment assessments would be necessary to fully validate the long-term effectiveness of the bioamended AC to reduce total and porewater concentrations of PCBs in sediments. In addition, we observed a gradual reduction in the abundance of the bioamendments over time in the field. Future work should explore the feasibility of a second application to further reduce the concentrations of PCBs achieved with a single application. Our experience with the field application suggests that two applications may be helpful not just in further reducing the total PCB concentrations, but also potentially reduce the spatial heterogeneity in application observed after a single application.

Cost Assessment. The costs determined for the bioamended SediMite™ technology were compared with costs estimated for other technologies evaluated in the 2008 Feasibility Study conducted for this site. The net present cost for the 8 technologies evaluated in the Feasibility Study ranged from $0 for no further action scenario to $25M for full excavation and disposal off-site. Implementation of an isolation cap with or without reactive media is estimated at $4M. In comparison, the estimated cost of bioamended SediMite™ application is $1.8M. The capping design involves an 18” sand cap with a 6” topsoil habitat layer creating a total of 24” of cap thickness. This can have a major effect of altering the nature of the wetland/pond system. In comparison the amount of material to be added as bioamended SediMite™ will barely alter the bathymetry of the pond and wetland system with minimal impact on the existing ecosystem. We anticipate the annual monitoring and maintenance costs for bioamended SediMite™ to be in the range of costs for Monitored Natural Attenuation or capping which are estimated at about $100k/year for the first 5 years.

Overall, the project demonstrated the successful treatment of PCB impacted sediments using a combination of *in-situ* treatment with PCB degrading microbes and activated carbon. The activated carbon was effective in reducing the bioavailability of the PCBs in sediments (as demonstrated in previous studies) and also facilitates efficient delivery of the bioamendments into sediments, which accelerates mass reduction of PCBs in the treated zone. This combined remedy can be appropriate and effective in sites that are ecologically sensitive and provide a hydrodynamically stable environment where the amendments will remain embedded in the sediments.

Page Intentionally Left Blank

1.0 INTRODUCTION

This report describes the laboratory treatability studies and field demonstration performed for ESTCP Project Number ER-201215, "Evaluating the efficacy of bioaugmentation for *in-situ* treatment of PCB impacted sediments". The purpose of the project was to demonstrate and validate a recently developed *in-situ* treatment for degrading polychlorinated biphenyls (PCBs) in contaminated sediments under field conditions at Abraham's Creek located on the grounds of Marine Corps Base Quantico, Virginia (MCBQ). The treatment utilizes an activated carbon agglomerate, SediMite™, as a delivery system for deploying PCB degrading microorganisms into PCB impacted sediments to accelerate the reduction of PCB levels in sediment and in the sediment porewater within a period of months. In addition to evaluating the performance of the treatment, the demonstration project provides estimates on the cost of deploying the technology in the field and provides data for assessment of its regulatory acceptance.

1.1 BACKGROUND

Department of Defense (DoD) facilities across the country are impacted with persistent pollutants such as PCBs and the DoD is challenged with the remediation of these sites. This work addresses the DoD need for cost effective, *in-situ* remediation technologies for PCBs and can be applied in principle to other persistent organic pollutants (POPs) such as pesticides. Most importantly, this work will enable extensive *in situ* treatment at DoD sites that include both shallow and deep sediments with minimum impact to environmentally sensitive areas. This integrated approach utilizes activated carbon to serve concurrently as an agent to sequester PCBs from the food chain and as a delivery system and solid substrate to enhance both anaerobic and aerobic microbial processes for complete *in situ* degradation. Development of a tractable microbial *in situ* treatment system would provide a practical, cost-effective, and environmentally sustainable means of treating persistent pollutants. Several advantages of the technology compared with current technologies are shown in **Table 1**.

Table 1. Comparison of Bioamended SediMite™ with Dredging, Capping and Activated Carbon.

	Dredging	Capping	Activated carbon	Bioamended activated carbon
Reduces PCB level at site	Yes	No	No	Yes
Health risks	High	Low	Low	Low
Environmental disruption	High	High	Low	Low
Cost of energy use	High	Low	Low	Low
Long-term waste management	Long-term storage	20-100 year life [a]	None	None
Habitat restoration cost	High	Low	None	None
Wetland environments	Not suitable	Not suitable	Well suited	Well suited

[a] expected lifespan depends on environment

In terms of the life cycle assessment, use of bioamended SediMite™ has a significantly lower impact compared with dredging by reducing the health risks associated with sediment disruption, reducing overall energy use, and effectively negating the requirement for extensive waste management and habitat restoration.

1.2 OBJECTIVE OF THE DEMONSTRATION

The field demonstration involved deployment of bioamended SediMite™ in two test plots of sediments contaminated with PCBs using the optimal inoculum loading on SediMite™ and the appropriate loading rate of treatment material into the sediment determined in the prior Treatability Study associated with this project. The test plots were located in Site 102 – Abraham's Creek, located in the southeastern portion of Chopawamsic Creek near the confluence with the Potomac River (**Figure 1**). The site selection was based on sampling and assessment of sediments throughout the site by Battelle in June 2012 and a detailed assessment of the proposed plot sites by for this project in October 2012. Criteria considered include presence of PCBs at adequate concentrations, characteristics of the sediment and water column, and field logistics as described below in Section 4.1. Bioamendments were deployed in the site and the test plots were monitored over a period of one year. Two parallel plots served as controls: one with no treatment, and a second control with SediMite™ only treatment with no bioamendments. The efficacy of the *in situ* treatment was evaluated by measuring relative changes between treated and control plots. The project assessed two major effects of the treatment: 1) reduction of *in situ* total PCB mass in impacted sediments by microbial dechlorination and subsequent aerobic biodegradation of the PCBs, and 2) immediate and long-term reduction of aqueous PCB concentrations through sequestration on the carbon surface and microbial degradation. Measurements included PCB congener analysis of sediments, PCB congener analysis of pore water using passive samplers and an assessment of the sustainability of the bioamendment over time. A detailed Work Plan was developed that describes the procedures used for the field demonstration.

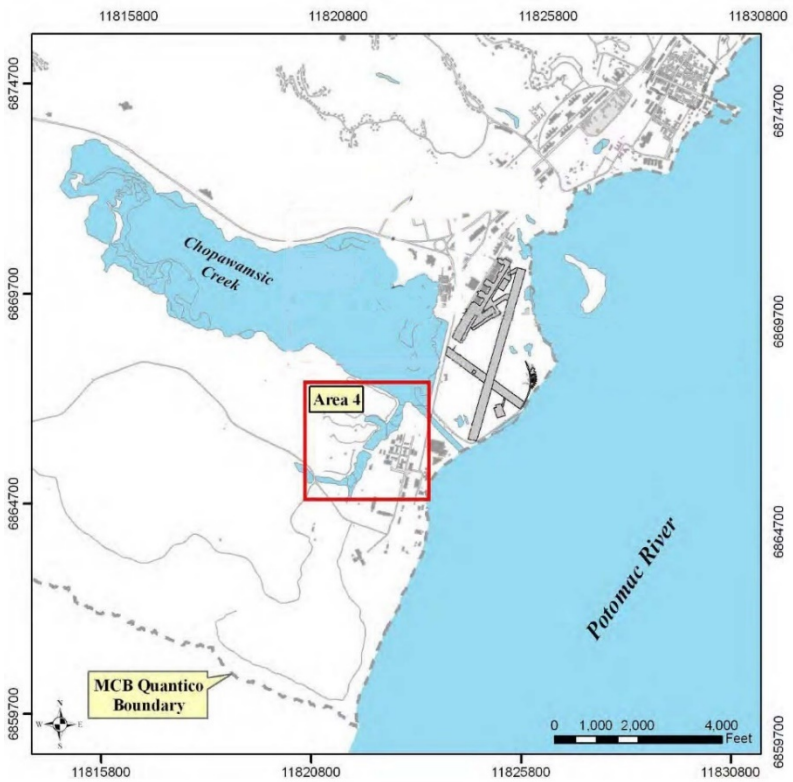


Figure 1. Location of Proposed Test Site in Abrahams's Creek, MCBQ (outlined in red)

1.3 REGULATORY DRIVERS

PCBs are still present in the environment despite a U.S. production ban in 1979 as a result of the Toxic Substances Control Act and a worldwide ban in 2001 by the Stockholm Convention on POPs. DoD facilities across the country are impacted with persistent pollutants such as PCBs and the DoD is challenged with the remediation of these sites. The Marine Corps Combat Development Command (MCCDC) is a 56,000-acre military training facility located in Quantico, Virginia, 35 miles south of Washington, D.C. Specifically, Site 102 Abraham's Creek is located in the middle of the Officer Candidate School training area. Because of this, the Marine Corps is interested in identifying remedies that would minimize impacts to training. EPA Region 3 has recommended evaluating remedies that include sediment amendments. Chemicals of Concern (COCs) in sediment at this site include dichlorodiphenyltrichloroethane and related congeners (DDx) and polychlorinated biphenyls (PCBs). This site is currently in the Remedial Investigation/Feasibility Study phase under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) and bench-scale treatability studies and treatment options are currently being evaluated. CERCLA requires that remedial actions selected for hazardous substances at sites 1) are protective of both human health and the environment, 2) are cost effective, and 3) comply with identified, promulgated Applicable or Relevant and Appropriate Requirements (ARARs), criteria, standards, or limitations.

Page Intentionally Left Blank

2.0 TECHNOLOGY

2.1 TECHNOLOGY DESCRIPTION

This project is the culmination of more than a decade of laboratory research on PCB dechlorination (Sowers and May) and *in-situ* remediation of PCBs (Ghosh) that is ready for synergistic implementation in the field. Although Sowers and May demonstrated the feasibility of bioaugmentation for PCB dechlorination in the laboratory, an effective delivery system to introduce microorganisms into sediments was not available. Recently, Ghosh and Menzie through SERDP and USEPA Small Business Innovation Research Program (SBIR) projects developed a pelletized delivery system, SediMite™, for introducing sorbent amendments into sediments. The innovative aspect of the proposed technology is the application of "bioamended" SediMite™ as a solid substrate for: 1) delivery of microorganisms into sediments, 2) formation of microbial biofilms, and 3) sequestration and concentration of hydrophobic PCBs in close proximity to the biofilm of PCB transforming bacteria. Recent laboratory mesocosm scale experiments demonstrated the long-term activity of the microorganisms delivered with SediMite™ and the overall feasibility of the approach.

The protocol for treatment of bioamended SediMite™ to PCB impacted sediments is illustrated in **Figure 2**. Laboratory mesocosm-scale treatability studies are conducted for a selected site to determine if the site is amenable to bioaugmentation and to identify the optimal ratio of microorganisms to activated carbon (AC). Methodology has been developed for scale-up of both the PCB halorespiring microorganisms and the aerobic PCB degrading microorganisms in the scale-up facility at IMET, which has a 250 L pilot-scale bioreactor and high volume continuous centrifugation capabilities. Aerobic and anaerobic cells are grown and harvested separately and transferred to 20 liter Cornelius flasks under a headspace of air or nitrogen, respectively, and transported to the site. SediMite™ is designed to slowly disaggregate once it is exposed to water and must be inoculated with microorganisms immediately before dispersion. A system for distributing pellets such as a (Vortex Granular System, LLC, http://www.vortexspreader. com/) or modified VHI is used to disperse the bioamended SediMite™. The bioamended AC is designed to achieve the following: 1) targeted dispersion of bioaugmented material to the contaminated sediment without loss to the water column during delivery, 2) initiate and accelerate complete degradation of weathered PCBs by providing a solid substrate and a source of slow release electron donor to sustain the biocatalysts in biofilms, and 3) concurrently concentrate and sequester PCBs in close proximity to the biofilm. The development of a system that can deliver microbial bioamendments through water columns and a solid substrate for sustaining both anaerobic and aerobic activity are significant innovations for treating PCB impacted sediments over large areas.

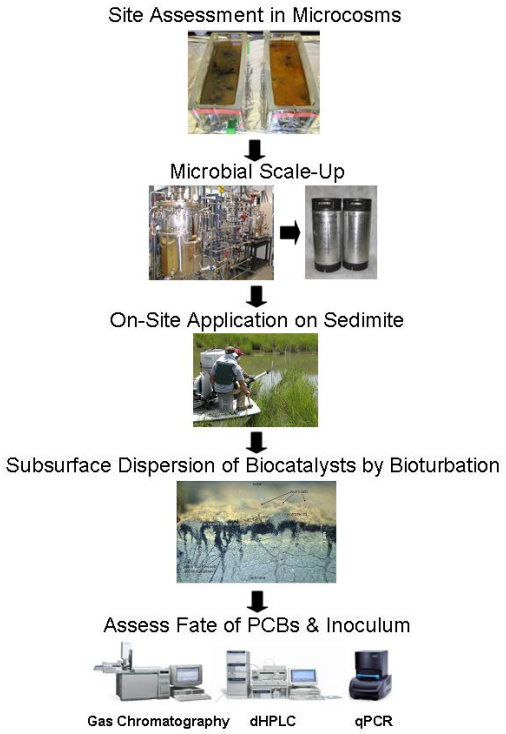


Figure 2. Graphic Scheme for in Situ Treatment of PCB Impacted Sediments Using Bioamended SediMite.

The approach is the culmination of DOD-funded research (Office of Naval Research, ONR, and SERDP) and funding from the National Institute of Environmental Health Sciences Superfund Research Program (NIEHS-SRP) that successfully identified and addressed technical issues that precluded successful treatment by bioaugmentation (**Figure 3**), including:

1) *Identification and isolation of biocatalysts*- PIs Sowers and May identified and isolated several PCB halorespiring species and demonstrated in mesocosms that these strains effectively dechlorinate Aroclors in sediment, are sustainable throughout the dechlorination process and can co-catabolize with aerobic PCB degrading bacteria for complete degradation of PCBs (U.S. Patent No. 6,946,248).

2) *High throughput monitoring methods*- PI Sowers' lab developed a molecular-based method for selectively detecting and monitoring PCB transforming bacteria in sediment communities without isolation and culturing [1-3].

3) *Scale-up of biocatalysts*- PIs Sowers and May developed a scale-up protocol for PCB halorespiring microorganisms that effectively eliminates residual PCBs or other regulated POPs from the bioaugmentation inoculum (U.S. Patent No. 7,462,480).

4) *Effective delivery system*- PI Sowers and co-PI Ghosh demonstrated that activated carbon can be used as a carrier for PCB transforming bacteria and concurrently enhances the transformation process by concentrating PCBs and acting as a substrate for biofilm formation (U.S. Patent Application No. 13/177,436). SediMite™, an activated carbon material that was developed under an SBIR by co-PI Ghosh, is used to deliver treatment material to sediments contaminated by PCBs (US Patent No. 7,824,129).

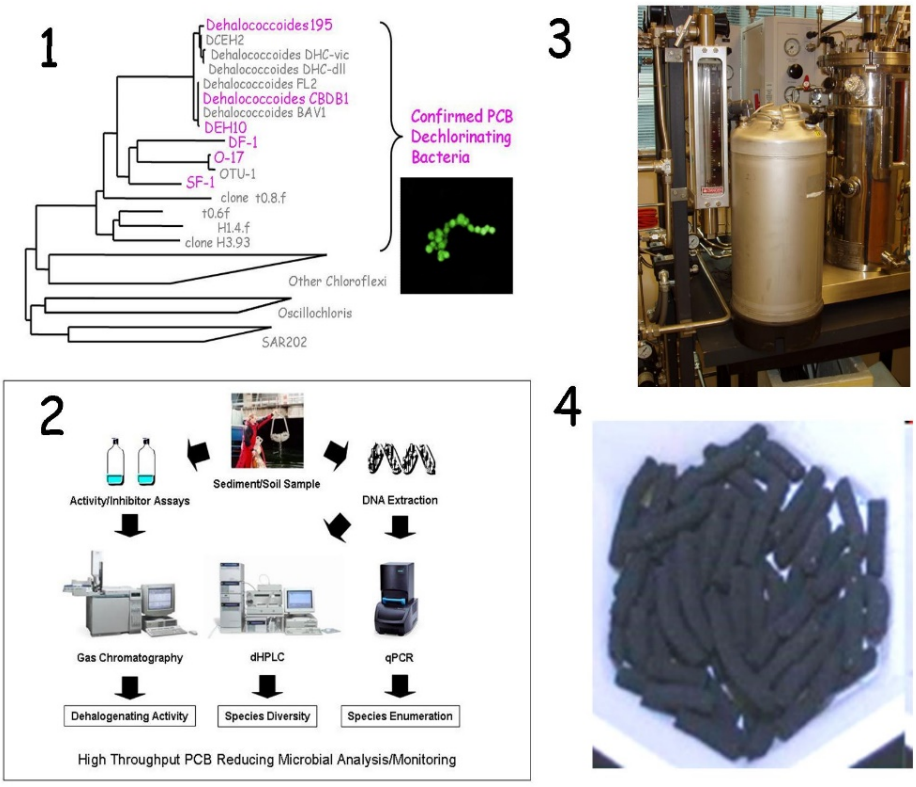


Figure 3. Illustration of Technical Achievements. 1) availability of biocatalysts, 2) development of post-treatment monitoring methods, 3) development of scale-up methodology, 4) in situ dispersion of inoculum on AC using the SediMite™ delivery system.

In the Treatability Report we demonstrated the following: 1) production level scale-up of the microorganisms sufficient for the proposed field study; 2) production of modified SediMite™ containing cellulose as an electron donor; 3) design and testing of dispersion systems to introduce active PCB transforming microorganisms into SediMite™ pellets during dispersal of the pellets at the site; and 4) the effectiveness of the treatment for reducing PCB levels in sediments from Abraham's Creek in mesocosms.

This technology is applicable for the treatment of sediments contaminated with PCBs and can also compliment other technologies such as dredging and capping. Examples include coastal sediments, lakes and rivers where the bioamendment must be delivered to sediment through a column of water. However, the unique properties of the technology allows treatment of sediments where conventional approaches are not practical. For example, the technology is uniquely suited for treating environmentally sensitive wetland area where technologies such as dredging or capping would be too disruptive. Also, the ability to deploy bioamended SediMite at distances up to 30 feet with pneumatic dispersion systems makes this technology uniquely suited for treating sediment in inaccessible area such as between pilings, below structures, and under vegetation. Finally, unlike all other available technologies, this is the first technology to combine reduction of PCB bioavailability by active microbial degradation and adsorption of residual PCBs to activated carbon as an *in-situ* process.

2.2 TECHNOLOGY DEVELOPMENT

NIEHS-SRP and SERDP supported laboratory testing of bioaugmentation with anaerobic halorespiring Dehalobium chlorocoercia DF1 and aerobic Paraburkholderia xenovorans LB400 added concurrently with granulated activated carbon (GAC) as a delivery system was published in 2013 [3]. Testing was conducted in 2 L laboratory mesocosms containing weathered Aroclor-contaminated sediment from Baltimore Harbor, MD, USA. Bioaugmentation resulted in an 80% decrease by mass of PCBs, from 8 to <2 mg/kg after 120 days (Figure 4). There was no significant increase in lesser chlorinated congeners, indicating that both anaerobic dechlorination and aerobic degradation occurred. In contrast, there was no significant decrease in the total concentration of PCBs observed in non-bioaugmented controls containing filtered culture supernatant within the same period of time. Direct colony counts and molecular analysis targeting a putative reductive dehalogenase gene of D. chlorocoercia or the bphA encoding gene of LB400 showed the presence of viable DF1 and LB400 in bioaugmented mesocosms after 365 days, indicating that both nonindigenous strains were sustainable within the indigenous microbial community. These results showed that an in situ treatment employing the simultaneous application of anaerobic and aerobic microorganisms had potential as an effective and environmentally sustainable strategy to reduce PCBs levels in contaminated sediment. A second study (unpublished) was conducted in situ in a wastewater treatment pond in Altavista, VA contaminated with Aroclor 1248 with partial support from the Town of Altavista. Treatments were conducted in caissons that consisted of 208 L steel drums with the ends removed inserted into the sediment. Treatments included SediMite™ with and without bioamendment. Once again 80% reduction in total PCB levels was observed in the benthic region of the bioamended treatment after 1 year, with levels decreasing from over 300 mg/kg to 60 mg/kg (**Figure 4**).

Although the rate of aerobic degradation appeared to slow significantly after a year, anaerobic dechlorination was observed after nearly 500 days. The PCB levels at this site were exceptionally high, particularly in the lower sediment where levels were 1300 mg/kg, and would require a subsequent treatment to achieve target levels of 50 mg/kg. We are continuing to monitor the site to determine the long-term effects and assess subsequent treatment options. The results of both these studies demonstrated that treatment with bioamended AC or SediMite™ significantly accelerated the rate of PCB dechlorination and degradation in sediments.

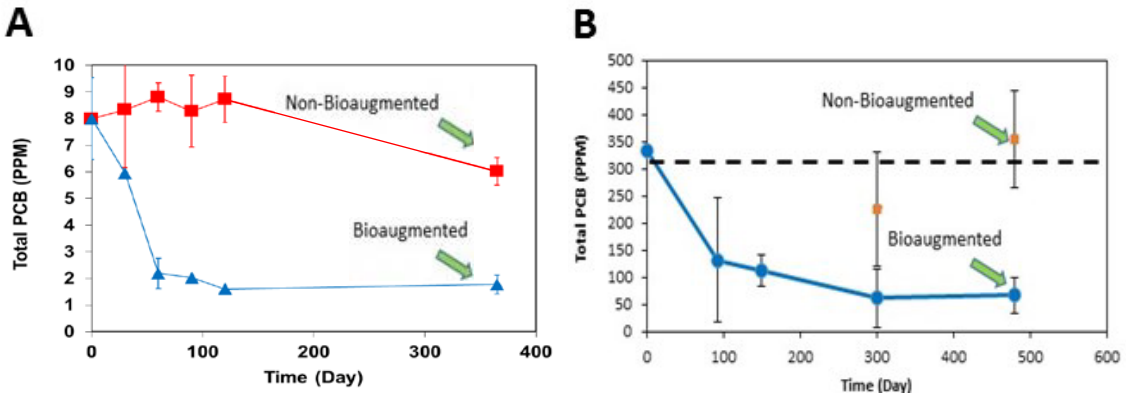


Figure 4. Effects of Bioamended SediMite on Reduction of PCB Levels in Mesocosms Containing Sediment from Baltimore Harbor (A) and in an In-situ Caisson Located in a Wastewater Treatment Pond in Altavista VA (B). *Activity was only detected in the bioaugmented treatment of sediments at both sites and in both studies the extent of degradation was approximately 80% with a single treatment.*

2.3 ADVANTAGES AND LIMITATIONS OF THE TECHNOLOGY

Advantages. This work addresses the DoD need for cost effective, *in-situ* remediation technologies to treat PCB impacted sediments and can be applied in principle to the treatment of other POPs. Most importantly, this work will enable treatment at DoD sites that include both shallow and deep water sediments with minimum impact to environmentally sensitive areas. This integrated approach utilizes activated carbon in the form of SediMite™ to serve concurrently as an agent to sequester PCBs from the food chain and as a delivery system and solid substrate to enhance both anaerobic and aerobic microbial processes for *in situ* degradation. In terms of the life cycle assessment, use of bioamended SediMite™ would have a significantly reduced impact compared with dredging by lowering the health risks associated with sediment disruption, reducing overall energy use and effectively negating the requirement for extensive waste management and habitat restoration.

Limitations. Our laboratory results suggest that activity slows down or stops after approximately 80% of the PCBs are degraded, which usually results in up to two orders of magnitude reduction in porewater concentrations. However, porewater analysis suggest that the remaining 20% of PCBs are no longer bioavailable thereby mitigating the risk. In addition, this technology requires natural processes such as bioturbation to mix the bioamendment into the sediment and the treatment is limited to the bioactive zone of sediments. This results in PCB degradation in the bioturbation zone of sediment overlying more contaminated sediment. In instances where it is desirable to remove PCBs from sub-benthic sediments, dredging and disposal might be appropriate with subsequent treatment with bioamended SediMite™ to clean up residual PCBs. The decision to use bioamended SediMite™ *in situ* technology would be mediated by the final cleanup goals for a particular site.

Environmental Risks. Deployment of bioamended SediMite™ is not expected to have adverse environmental impacts. SediMite™ has been used commercially in several pilot- and full-scale applications and has been featured in a recent guidance document by the USEPA on emerging technologies for the *in-situ* treatment of contaminated sediments [4]. The microorganisms used as bioamendments are from environmental sources, they are non-recombinant and have no known pathogenicity to humans or animals (Biosafety Level 1, BSL 1). Species of *Paraburkholderia* are globally ubiquitous soil microorganisms commonly associated with soil rhizospheres. *P. xenovorans*, which was isolated from a landfill in New York State, has a large number of oxygenases and comes from a phylogenetic group that is commonly isolated from grass rhizospheres and soils with a variety of complex naturally-occurring aromatic compounds [5]. Degradation of PCBs is cometabolic and depends on the presence of both oxygen and naturally occurring aromatic compounds to sustain the population. *Dehalobium chlorocoercia* is closely related to *Dehalococcoides* spp. that have been used extensively for bioremediation of chlorinated ethenes in groundwater and are available commercially as mixed consortia of microorganisms under trade names such as SDC-9, KB-1 and Bio-Dechlor INOCULUM® Plus. In contrast to these commercial mixtures, *D. chlorocoercia* is a defined coculture isolated on PCB from sediment in Charleston Harbor, SC [6]. This strain is capable of growth by anaerobic respiration with several PCB congeners in addition to selected chlorobenzenes and chloroethenes. In the absence of these chlorinated POPs growth is not sustainable. Any residual chlorinated POPs used to scale up the microorganisms is removed from the concentrated microorganisms prior to deployment.

Mesocosm studies show that addition of bioamended SediMite™ to sediments from Abraham's Creek had no observable impact on the indigenous microbial population based on comparative 16S rRNA gene analysis of DNA extracted from sediments before and after treatment.

3.0 PERFORMANCE OBJECTIVES

Performance of treatment with bioactive SediMite™ was assessed by evaluating: 1) physical delivery and spatial uniformity of application; 2) persistence of AC delivered as SediMite™ and survival of introduced microorganisms 140 and 409 days after delivery; 3) effect of introduced microorganisms on reduction of PCB concentrations in sediment and porewater. The approaches used to evaluate these metrics are summarized in **Table 2** and described below.

Table 2. Performance Objectives for Pilot Test

Performance Objective	Data Requirements	Success Criteria
Delivery & spatial uniformity of application	Analysis of sediment cores to evaluate the uniformity of SediMite™ & biocatalyst delivery in the top 15 cm of sediment	Uniform spatial distribution: More than 70% of the samples should have ± 50% of target dose of carbon; 60% of the sampling sites should indicate presence of biocatalysts at an abundance of at least 1 x 10^5 cells/g
Persistence of AC and viable biocatalysts in sediments	Stability of AC and sustainability of viable inoculated anaerobic and aerobic biocatalysts in the sediments	Minimal mobilization of AC from site of application and sustained viability of bioamendment for duration of test
Effect of treatment on PCB degradation	PCB depletion rate, change in risk factors including bioavailability & formation/depletion of toxic congeners, effect on indigenous community	>50% reduction of total PCB concentration and >80% combined risk reduction resulting from reduction of total PCBs, no accumulation of coplanar congeners & reduction in bioavailability; insignificant effect on indigenous bacteria
Effect of treatment on reduction of porewater PCB concentration in surficial sediment	Measurement of porewater PCB concentration in the top 3" of surficial sediment using passive samplers	> 80% reduction in porewater PCB concentration resulting from a combination of PCB degradation and sequestration in carbon
Performance of biocatalyst deployment system	Delivery system monitored for ease of operation, any issues of clogging of the nozzle during delivery, rates of application,	Consistent delivery of SediMite™ & microorganisms, no clogging of delivery nozzle, no clogging of TeeJet nozzle
Health and safety	Potential for hazards during application will be evaluated and documented. Potential formation of airborne dust or mist during application will be monitored.	Minimal dust during delivery of SediMite™, minimal aerosols during delivery of SediMite™
Scalability of technology	Any scale up issues related to microorganism culture or field application will be documented	Successful scale-up, concentration, transport of viable biocatalysts to field and field distribution
Technology transfer/regulatory acceptance	The demonstrated technology should receive favorable feedback from the regulatory and industry community.	Feedback will be sought and documented from representatives in the regulatory community and industry representatives

3.1 PERFORMANCE OBJECTIVE: DELIVERY & SPATIAL UNIFORMITY OF APPLICATION

An effective delivery system is critical for uniform delivery and even distribution of the biocatalyst into the sediments. Successful bioremediation of PCB-impacted sediments depends on even distribution of biocatalyst on the SediMite™ and even distribution of the inoculated SediMite™ into the sediments.

3.1.1 Data Requirements

Spatial uniformity of application was tested by placing ten collection trays on the sediment bed within the treatment area, and retrieving the trays immediately after application to measure the dose of SediMite™ achieved at each test plot. Total organic carbon (TOC) & black carbon analysis was conducted in the top 15 cm layer of sediment to confirm uniform distribution of SediMite™. Molecular analysis with microbial strain specific PCR primers was conducted to confirm uniform distribution of biocatalysts. In addition, the overall microbial communities was examined by comparative 16S rRNA sequence analyses.

3.1.2 Success Criteria

The goal was to achieve uniform spatial distribution and vertical mixing of AC and biocatalysts in sediments. While, some spatial variability is expected from a field application, more than 70% of the samples should have ± 50% of target dose of carbon. Distribution of biocatalysts was set at 10^5 cell per g dry sediment in more than 60% of discrete samples collected throughout plot.

3.2 PERFORMANCE OBJECTIVE: PERSISTENCE OF AC AND VIABLE BIOCATALYSTS IN SEDIMENTS

Sustained presence of AC and viability of biocatalysts is critical for maintaining reduced bioavailability and increasing degradation of PCBs.

3.2.1 Data Requirements

Black carbon analysis were carried out in sediment cores to evaluate the uniformity of SediMite™ delivery and its mixing into the top 7.5-cm layer of sediment. Five sediment cores were collected from each sampling plot to evaluate the pre and post-treatment distribution of native and introduced microorganisms in the sediment profile. The sediment cores were segmented into six intervals: 0 to 7.5 cm; 7.5 to 15 cm; 15-30 cm. These core samples were collected at 140 and 409 days after treatment. Molecular analysis with strain specific PCR primers were conducted to enumerate biocatalysts and viability assays were conducted to confirm that the biocatalysts are active throughout the trial.

3.2.2 Success Criteria

Immobilization of AC in test plots and sustained viability of biocatalysts at 10^5 cell per g dry sediment throughout plot.

3.3 PERFORMANCE OBJECTIVE: EFFECT OF TREATMENT ON PCB DEGRADATION

Effectiveness of treatments were determined by comparisons of treated and untreated plots and also by comparing before and after application at each treatment plot. The effect of the bioaugmentation was determined by comparing treated with control plots that are either not treated or treated with only abiotic SediMite™.

3.3.1 Data Requirements

The performance of bioaugmentation in these field applications were evaluated based on the total reduction in PCB levels and the sustainability of the inoculated anaerobic and aerobic biocatalysts in the sediments. Monitoring occurred at the time of treatment and at intervals of approximately two and twelve months after application. Monitoring included quantitative PCB congener analysis to determine efficacy and rate of total degradation and identification of any recalcitrant congeners. Qualitative and quantitative molecular analyses of the microbial community was also conducted to assess the sustainability of the inoculum and the response of the community at large. Rates of anaerobic reductive dechlorination and aerobic degradation was used to assess the process endpoint. Assessment of the endpoint for reductive dechlorination was based on reduction in the highly chlorinated PCBs. The endpoint for aerobic degradation was assessed based on total PCB removal. We have successfully used these monitoring methods in laboratory mesocosm studies for site analyses. Molecular assays specific for anaerobic halorespiring bacteria using the strain-specific RDH gene and aerobic degrading bacteria based on the *bph*C gene was used to assess the sustainability of the inocula at two and twelve months. Effects on the indigenous microbial community was monitored by 16S amplicon Illumina Hi-Seq analysis. Polyethylene (PE) passive samplers were deployed within the sediment and also just above the sediment surface to probe in-situ porewater and overlying water PCB concentrations. PCBs were extracted from the passive sampler by dialysis into hexane followed by chemical analysis and interpretation of freely dissolved concentrations in water. The in situ passive samplers allowed us to measure porewater depth profile of aqueous PCB concentrations and compare those values across treatment plots and also with overlying water concentrations.

3.3.2 Success Criteria

Greater than 50% reduction of total PCBs and greater than 80% combined risk reduction resulting from reduction of coplanar congeners, no accumulation of coplanar congeners, reduction in bioavailability, and an insignificant effect on indigenous bacteria.

3.4 PERFORMANCE OBJECTIVE: PERFORMANCE OF BIOCATALYST DEPLOYMENT SYSTEM

A critical component of this technology is the use of SediMite™ as a delivery medium for the biocatalysts. Since some biocatalysts are sensitive to oxygen, SediMite™ must be inoculated immediately before deployment using an inoculation manifold designed specifically for this purpose.

3.4.1 Data Requirements

The delivery of SediMite™ through a spray of biological inocula was monitored for ease of operation. The modified Vortex broadcaster was tested for issues of clogging of the nozzle during delivery, production rates of application, and potential formation of airborne dust or mist during application.

3.4.2 Success Criteria

Issues were identified and resolved prior to deployment at the site including uniform distribution of viable microorganisms into sediment, minimizing dust/mist to an acceptable level and minimal interruptions due to equipment performance such as clogging of inoculation nozzles.

3.5 DEVIATIONS FROM ORIGINAL SUCCESS CRITERIA

The following modifications were applied to the defined success criteria:

1) Sampling intervals were 140 and 409 days instead of 60 and 365 days because access had to be scheduled around training periods
2) Effects on the indigenous microbial community was monitored by 16S amplicon Illumina Hi-Seq analysis instead of denaturing HPLC. This was the result of an offer by Dr. Chris Marshall to conduct direct sequencing, a more precise and informative approach than dHPLC, at no extra cost to the project.
3) Polyethylene was used instead of polyoxymethylene for passive sampling, because it equilibrates more rapidly and performance characteristics with PCBs were available after the original proposal was submitted.
4) Temperature was not monitored due to loss of data logger prior to day 140 sampling event

All other criteria in the performance objective were as originally proposed.

4.0 SITE SELECTION: ABRAHAMS CREEK, QUANTICO MCB

4.1 SITE LOCATION AND HISTORY

Abraham's Creek is located near the MCBQ Officer Candidates School (OCS) and is an active training area. PCBs in Abraham's Creek are historical and the precise source is not known (**Figure 5**). Since the base has been an active training site since 1917 a possible source of contamination was the common practice of including 5 to 25 parts per hundred by weight of a PCB Aroclor with volatile insecticides to increase the effective kill life of the treatment. This practice reduced costs by suppressing rapid evaporation of the volatile insecticide and extending effectiveness by 2-3 months. It is also possible that contaminants associated with various base activities migrated to Chopawamsic and/or Abraham's Creek via deposition, surface water runoff, and groundwater discharge from nearby operations including fire training activities, oil/water separators, washracks and wastewater discharge directly to Chopawamsic Creek at the mouth of Abraham's Creek.

Figure 5. Aerial View of Abraham's Creek Showing Sampling Proposed Test Site (not to scale).

4.2 SITE GEOLOGY/HYDROGEOLOGY

Abraham's Creek is subject to tidal fluctuations in the northern section of the creek (AC-Area 1, 3.7 acres), but upstream regions of the creek are largely unaffected by tidal effects (AC-Area 2, 5.15 acres and AC-Area 3, 7.8 acres) because of a beaver dam and land bridge. Any water flow resulting from a flooding event in upstream area 3 flows through culverts beneath the land bridge and discharges downstream through areas 1 an 2 into Chopawamsic Creek. Area 3 consists of small streams feeding into the main water body, which extends from the land bridge to the upstream end of Abraham's Creek (**Figure 6**). The water depths in this area are variable throughout the year and can range from approximately 122 cm in the northernmost area located

at the road, to a wetland area, and finally to then to upland streams that drain into the water body. The northern section consists of open water surrounded by steep walls and forest along much of the western shoreline. The eastern side is adjacent to a cleared training area and parade grounds that is separated by trees and vegetation. Much of the stream bank is surrounded steep banks with trees and vegetation up to the shoreline.

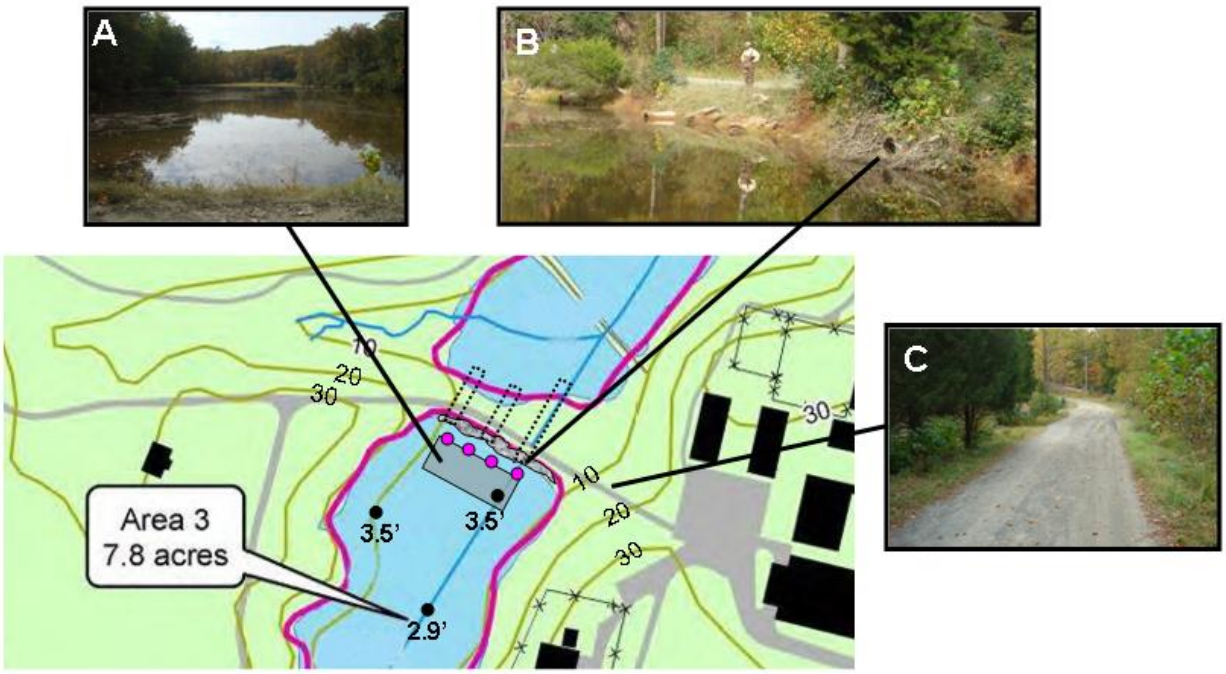


Figure 6. Proposed Test Site Showing View Form Access Road (A), Shore Along Access Road Showing Three Corrugated Steel Culverts Connecting Areas 2 and 3 (B) and Access Road (C).

Sediment sampling sites are shown in red and the proposed test area is shown in grey. Water depths and land contours are shown in feet.

4.3 CONTAMINANT DISTRIBUTION

Samples taken on 30 June 2012 by Battelle as part of a remedial investigation project for the Department of the Navy Environmental Program (Project No. G922014) were provided to our laboratory for PCB congener analysis using the protocol described in the Field Demonstration Plan (ER-201215). Congeners of DDx reported previously at the site were detected in our samples. Since DDx congeners can co-elute with some PCB congeners and they are detectable by GC-ECD, the retention times of the two parent and four transformation products were determined using the same GC parameters used for PCBs. **Figure 7** shows that only two DDx congeners co-elute with PCB congeners. Congener *p,p*-DDE co-elutes with PCB 145; however, this congener is tetra-*ortho*-chlorinated and does not occur in Aroclors or dechlorination products of Aroclors. Congener *p,p*-DDT co-elutes with co-eluting PCB congeners 130/137/176. All three congeners make up no more than 0.6 % of some Aroclors and we detect a peak that is less than 1% of the total PCB concentration from multiple samples retrieved form the site. Based on our analysis DDx congeners do not interfere with the analysis of PCBs using the extraction/analysis protocol.

Prior measurements of PCB levels in sediment at locations close to site 15 were estimated in the range of 0.5-1.5 ppm using an immunoassay kit for Aroclor 1260 and the NOAA NS&T methods based on extrapolation from 18 congeners in Aroclors. However, our analysis indicated that ten out of fifteen samples tested by GG-ECD analysis of individual congeners met the criterion of >1ppm originally proposed (**Figure 8**).

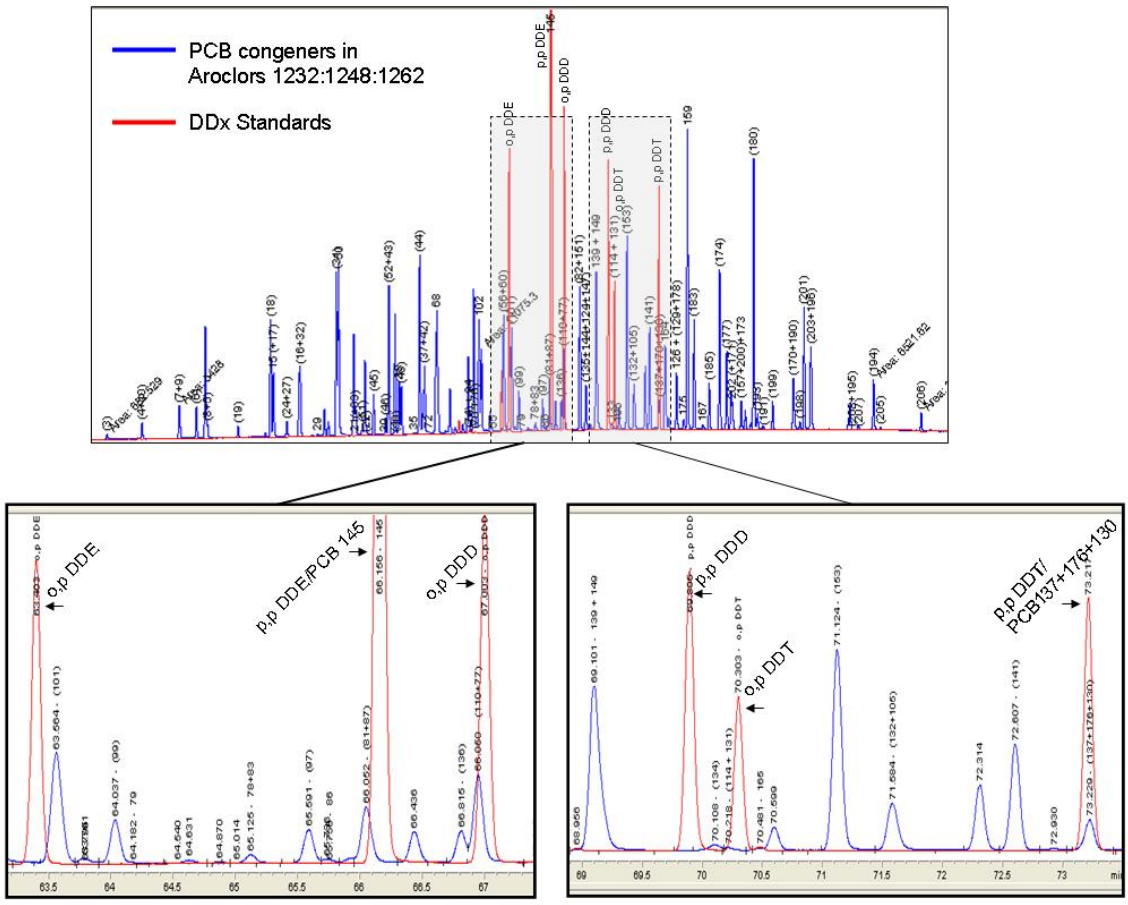
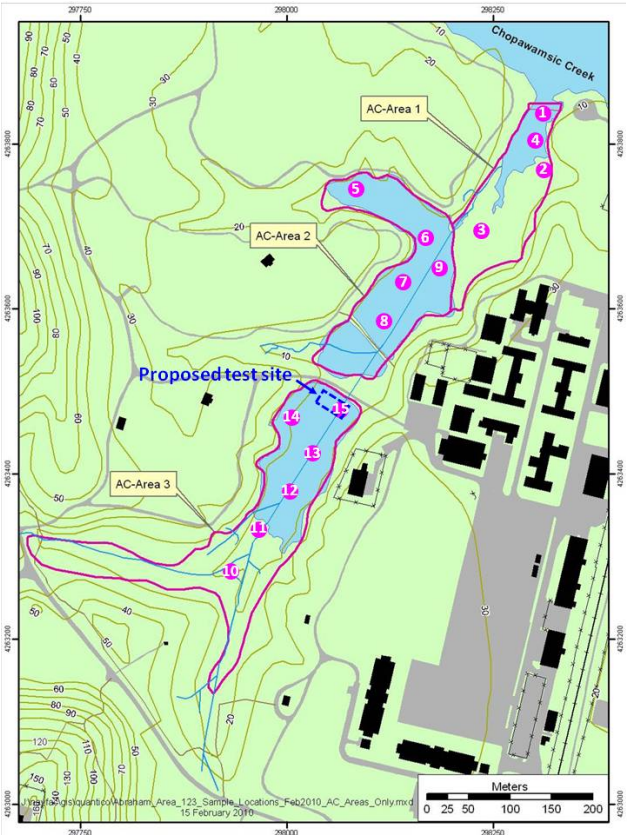


Figure 7. GC-EDC Profile of PCB Congeners from Aroclors 1232, 1248 and 1262 and Six DDx Congeners. Top Panel Shows Chromatogram of all PCB and DDx Congeners and Lower Panels are Magnified to Show Detailed Resolution between PCB and DDx Congeners.

Based on the total PCB concentrations and relative consistency of the concentrations site 15 was proposed as the target site to test for this project because it met all of the criteria as shown in **Table 3**.



Site number	Total PCBs in ppm
1	0.6
2	2.9
3	0.6
4	2.2
5	0.8
6	1.5
7	1.5
8	1.4
9	0.6
10	0.4
11	1.0
12	2.2
13	1.3
14	1.1
15	6.9

Figure 8. Total PCBs Determined by Congener Analysis from Samples Collected by Battelle from Sites Shown Map.

Total PCB concentrations were calculated from quantification of individual congeners as described in Appendix D of the Treatability Study Work Plan.

In order to assess the concentration of PCBs in the proposed test site for the Site Selection Memo four cores were extracted from across the length of the site between 38°29'49.37"N, 77°18'57.16"W and 38°29'48.74"N, 77°18'55.79"Won 18 October 2012 to determine the homogeneity of PCB distribution (**Figure 9**). Sediment samples were taken in 3 to 3.5 feet of water using a petite Ponar sampler and were transferred to pre-cleaned glass sample jars sealed with Teflon lined lids for transport back to the Sowers' lab. All samples were stored in the dark at 4 °C prior to use. Assessment of all samples indicates that PCB concentrations were sufficiently high (5.1) and homogeneous (±1 ppm) across the length of the plot site. These values were confirmed by independent GC-ECD analysis of samples by the Ghosh lab (4.8 ± 0.6 ppm) with total PCB levels within 6% of the values detected by the Sowers' lab. The homolog distribution showed higher amounts of hepta-, tetra- and tri-chlorbiphenyls (**Figure 10)**. The lack of a bell-curve distribution commonly observed for individual commercial mixtures suggests that either there was more than one Aroclor contamination source or the large amount of tetra- and tri-chlorobiphenyls could have resulted from reductive dechlorination of a higher chlorinated Aroclor and accumulation of the less chlorinated congeners. Based on the total PCB concentrations and relative consistency of the concentrations site 15 was proposed as the target site to test for this project.

Figure 9. Views of Proposed Test Site and Sampling Conducted on 18 October 2012.

Aerial view of proposed study area shows approximate locations of core samples (yellow).

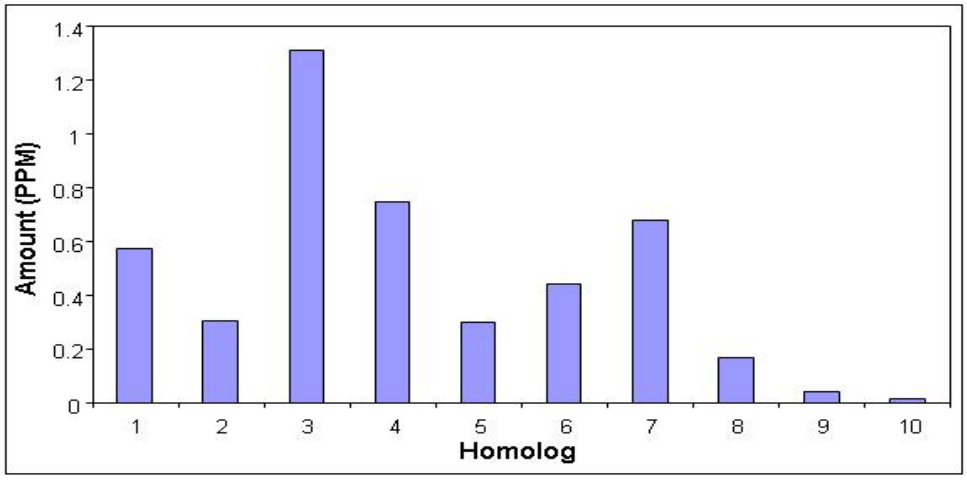


Figure 10. Congener Homolog Distribution Based on the Mean of Five Sediment Samples at Site 15. Presence of Relatively Large Amounts of Hexa- and Hepta-Chlorobiphenyls Suggests Historical Contamination by a Higher Chlorinated Aroclor Such as A1260 and Presence of Mono-, Di- and Tri-chlorinated Congeners Suggest Some Weathering as a Result of Microbial Reductive Dechlorination.

Page Intentionally Left Blank

5.0 TEST DESIGN FOR ABRAHAM'S CREEK

5.1 EXPERIMENTAL DESIGN

The treatment design in the field involved four plots, each approximately 0.1 acres (400 m^2) in area. The final plot size and configuration was confirmed in consultation with site managers and contractors after pre-treatment assessment. The four treatments as illustrated in **Figure 11** included: 1) control plot with no amendments, 2) SediMite™ with cellulose as an electron donor, 3) SediMite™ with cellulose and microorganisms, and 4) replicate SediMite™ with cellulose and microorganisms. We originally proposed a plot treated with SediMite™ without cellulose. However, our treatability studies indicated that addition of cellulose with the bioamended with SediMite™ supported the most extensive degradation of PCBs. Instead, we substituted a replicate plot of bioamended SediMite™ with cellulose to: 1) provide a replicate treatment, and 2) provide the opportunity to test effects of a second treatment at a later date. The pilot testing site is a shallow marsh/wetland type area. Thus, there is limited water movement and limited potential of amendments in one treatment plot influencing adjacent plots. The treatment plots were marked in the field using buoys and GPS coordinates. The delivery device was deployed on a boat similar to work done in two previous pilot studies with SediMite™ at Canal Creek, Aberdeen Proving Grounds, MD, and Bailey Creek, Ft. Eustis, VA.

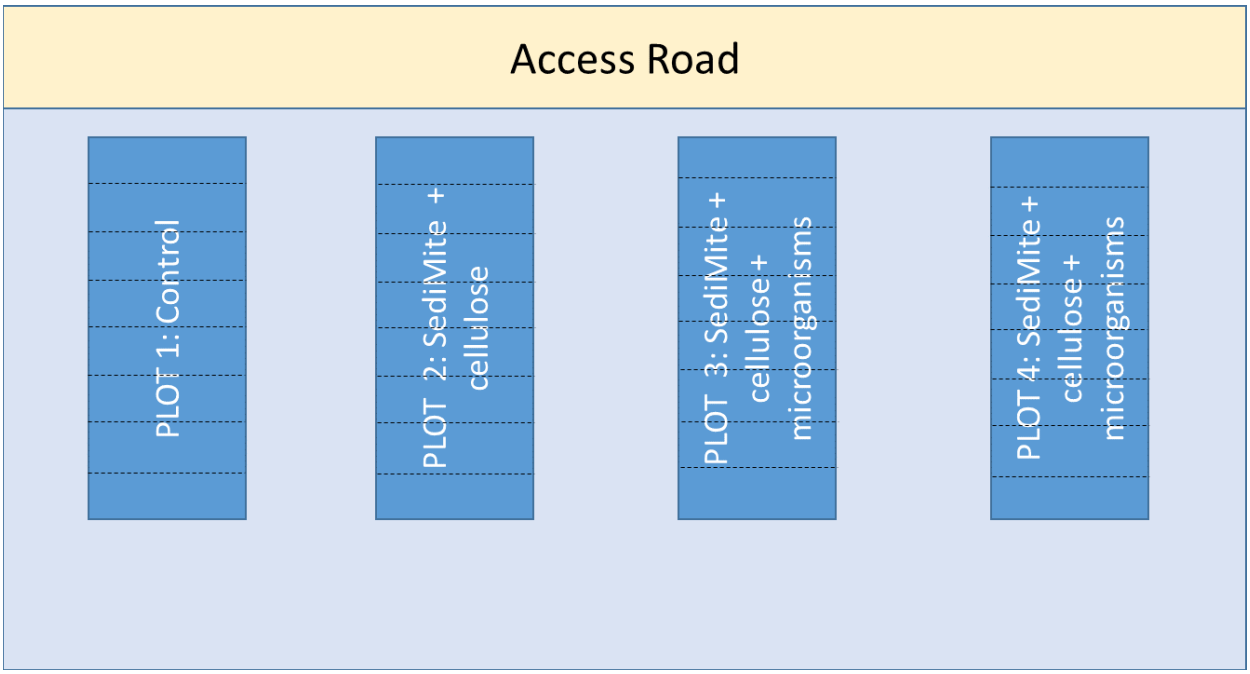


Figure 11. Schematic Image of the Four 400 m^2 Treatment Plots in Abraham's Creek.

Each treatment plot of 400 m^2 was split into eight equal subsections marked with floats as described below (Section 5.4.2), and the calculated amount of amendments was delivered within each subsection to minimize variability of dose across the site. The primary performance criteria were reduction of PCB congener concentration in sediment in the treatment plots over time and comparison with the no-treatment control plot. We anticipated changes in PCB concentration in sediment over time through natural attenuation processes; hence we also tracked that in the control plot.

To address spatial variability of PCB concentration in sediment, five replicate samples were collected from each treatment plot at each sampling time. In addition to sampling of PCBs in sediment, PCB concentration in sediment porewater was monitored to assess changes in PCB bioavailability over time. Performance metrics also included measurement of spatial distribution of activated carbon and bioamendments in sediment core samples collected from each of the treatment plots.

5.2 BASELINE CHARACTERIZATION

We originally proposed MCBQ as the candidate site for the pilot study, and two alternative sites that are contaminated with PCBs: Ft. Eustis Army Base in Virginia, and NAVFAC Midwest, Crane, Indiana. Quantico was selected for the pilot study because in addition to meeting all of the site criteria shown in **Table 3**, remediation of this site is being actively pursued and the relative proximity to Maryland reduces the logistical complexity.

Table 3. Site Selection Criteria from Site Selection Memorandum

Parameter	Target Value	Actual Value	Importance[a]
PCB concentration [b]	> 1ppm	5.1± 1.4 ppm	1
Inhibitory co-contaminants (such as toxic metals)[c]	No	No	1
Water depth[c]	> 30 cm	104 cm	2
TOC [b]	> 20,000 ppm	67,500 ppm	3
Redox in sediment[c]	< 0	-76.8	2
O_2 in water column[c]	> 5 mg/L (summer)	6.75 mg/L (June)	2
Access from road	Yes	Yes	2
Site access	Accessible within proposed timeline and budget	Accessible within proposed timeline and budget	1
Geographic location	Close to lead PI institution	Close to lead PI institution	2

[a] Most important (1) to least important (3)

[b] Data from this study

[c] Data from Battelle 2012

The Marine Corps Combat Development Command (MCCDC) is a 56,000-acre military training facility located in Quantico, Virginia, 35 miles south of Washington, D.C. Specifically, Site 102 Abraham's Creek is located in the middle of the Officer Candidate School training area. Because of this, the Marine Corps is interested in identifying remedies that would minimize impacts to training. EPA Region 3 has recently recommended evaluating remedies that include sediment amendments. COCs in sediment at this site include DDx and PCBs. This site is currently in the Remedial Investigation/Feasibility Study phase under CERCLA and bench-scale treatability studies are currently being planned. Impediments to utilizing the site would be gaining access on specific days when training would prohibit access. The RPM indicated that in most cases, they have been able to work with OCS to gain access provided they are informed 3-4 weeks prior to needing access, and provide a range of dates rather than specific dates.

This demonstration was independent of other studies at MCB Quantico, so the timeline would be dependent on the ESTCP Project schedule only. The Success Criteria of achieving total PCB mass reduction of up to 50% was discussed with the RPM and he has indicated reduction of PCBs at this level would be sufficient to include bioremediation of PCBs in any future remediation plans for the site.

Analysis of available information for the site and discussion with site remediation project manager support the selection of Abraham's Creek, Marine Core Base Quantico, VA (Site 102) as the preferred location for technology demonstration.

5.3 TREATBILITY STUDY

Key aspects of planning the field application are determining the optimal inoculum loading on SediMite™, composition of SediMite™ with slow release carbon source (e.g., cellulose) and determining the appropriate loading rate of treatment material into the sediment. The efficacy of bioactive SediMite™ in degrading and reducing the overall concentration of PCBs for the selected site was determined in 2 L mesocosm test systems set up at IMET-UMBC under the supervision of K. Sowers.

5.3.1 Experimental Design for Treatability Study

Ten sediment mesocosms were tested for the effects of SediMite™ alone, SediMite™ with slow release electron donor, bioaugmentation with different concentrations of microorganisms and bioaugmentation with different halorespiring microorganisms. Maximum rates and treatment end points from these tests were used as baseline values to determine success of *in situ* treatment results with the goal of reducing total PCB levels by >50% and reduction of risk by >80% as a result of a combination of PCB degradation and reduction in bioavailability of residual PCBs. The study also demonstrated the ability to scale-up bioamendments volumes sufficient for the proposed demonstration plan and the tested delivery systems for adding the bioamendments to SediMite™. Treatment parameters were used to estimate quantities of SediMite™ and microorganisms required for the proposed demonstration plots in Abraham's Creek, MCBQ.

5.3.2 Sample Collection for Treatability Study

In order to assess the concentration of PCBs in the proposed test site for the Site Selection Memo four cores were extracted from across the length of the site between 38°29'49.37"N/ 77°18'57.16"W and 38°29'48.74"N/77°18'55.79"W on 18 October 2012 (**Figures 6&9**). The core samples were transferred to pre-cleaned glass sample jars sealed with Teflon lined lids for transport back to the lab. An additional 25 liters of sediment was also collected and transported to the lab in sealed 20 L polypropylene buckets for Treatability Studies. The grab sample for the treatability study was collected near site 15 described in the original assessment of the site containing 6.9 mg kg^{-1} total PCB (**Figure 8**). Water for the treatability study was collected in a 20 L carboy. All samples were transported to Sowers' lab in Baltimore and stored in the dark at 4 °C prior to use. Initial assessment of all samples indicates they contain an average of 5.1 ±1 mg kg^{-1} PCB, which exceeds the minimum target PCB concentration of 1 mg kg^{-1} proposed for this project.

5.3.3 Experimental Set Up

5.3.3.1 Sediment Homogenization

Sediment collected in four 20 L buckets was transferred to a 100-liter basin within an anaerobic glove bag under a nitrogen atmosphere to prevent oxidation of anaerobic zones in the sediment as described in the Treatability Study Report. The pooled sediment was thoroughly mixed with a 10 cm mud mixer (TBC Tools) mounted on a power drill. After homogenization three 5-mL subsamples were randomly collected for analysis.

5.3.3.2 Sediment Characterization

Sediment used in mesocosms was black to dark brown in color. Total PCB concentration in the pooled sediment samples was 3.53 ± 0.44 mg kg^{-1} with a mean of 3.5 ± 0.1 chlorines per biphenyl. The congener profile was consistent with that observed in the four core samples taken in October 2012 (Site Selection Memo). Total organic carbon (TOC) based on combustion and CO_2 measurement of the homogenized sediment was 6.7 % ± 0.5 (IMET-SOP-10), which is more than double the TOC value used in the Site Assessment. The prior value was based on a report by Battelle in 2012. The difference in values could be temporal: the 2012 samples were taken in June prior to organic loading over the summer months and samples used for treatability studies were taken in October 2012 after organic loading over the summer months. A thick matt of plant and algal growth was observed in October, which would support this conclusion. The sediment sample were also taken in different locations, which might have resulted in spatial variation. Since the sediment was homogenized prior to dispensing into mesocosms the TOC was not measured in individual mesocosms. TOC was not measured between treatments since AC added as SediMite™ was known for each treatment (1.5%) and the 0.1% cellulose would not cause a measurable increase in TOC (< 0.003%) and the microbial bioamendments in mineral buffer would account for less than a millionth of a percent dry weight. We did not perform black carbon measurements since we added precise amounts of black carbon as SediMite™ to measured volumes of sediment (3% SediMite™: 50% AC = 1.5% black carbon g^{-1}).

5.3.3.3 Preparation of Bioamended SediMite™

The PCB degrading aerobe LB400 was prepared as bioaugmentation inoculum as described in the Treatability Work Plan. Briefly, a 100 mL culture was grown to $O.D._{600}$ of 1.0 (*ca.* 4×10^8 cells/mL), harvested by centrifugation, and the cell pellet was suspended in 100 mL of sterile M9 medium without biphenyl (IMET-SOP-8). The PCB dehalogenating anaerobe DF1 was grown in ten 50 mL cultures grown until 50% of PCB 61 was dechlorinated. DF1 was then sub-cultured 1:10 two times with 100uM tetrachloroethene as the electron donor instead of PCB 61 to remove any residual PCBs. Likewise, the other dehalogenating organisms: *o*-17 (PCB 65), SF1 (Aroclor 1260) and DEH10 (Arolcor 1260) were grown in 500 mL with PCB as the electron acceptor before being sub-cultured twice with 100 uM tetrachlorethene and increasingly smaller amounts of PCB as the electron acceptor before being harvested. Residual tetrachloroethene was removed from cultures by sparging with N_2 prior to harvesting. The dehalogenating cultures were harvested separately and anaerobically by centrifugation. DF1 was suspended in 100 mL of ECl medium without PCB61 to a final concentration of 5×10^7 cells mL^{-1}. Cultures *o*-17 and SF1/DEH10 were resuspended in 50 mL of ECl medium without PCB61 to a final concentration of 5×10^7 cells mL^{-1}.

Concentrations of cells were estimated by qPCR (IMET-SOP-6). Immediately before addition to mesocosms the anaerobe and aerobe were combined in the reservoir of a manual pump sprayer (1.75 L Flo-Master 56HD, Root-Lowell Mfc Co.) and the concentrated cultures were sprayed onto a single layer of 70 grams SediMite™ in an aluminum tray at the final concentrations indicated.

5.3.3.4 Preparation of mesocosms

Aliquots of homogenized sediment (1.75 L) were transferred to glass 2 liter TLC tanks in the anaerobic glove box (**Figure 12**) and treated as shown in **Table 4**. Normally for in-situ treatment SediMite™ is mixed into sediments by natural benthic activity. Because the short timeline of the treatability study (120 days) would not allow for sufficient mixing by bioturbation and in order to ensure uniformity between treatments for this study, unamended and bioamended SediMite™ was manually mixed into mesocosms with a Teflon spoon.

Treatments 1-3 were control treatments without bioamendments to determine the effects of SediMite™ and cellulose on indigenous PCB dechlorinating/degrading activity. Treatments 4-6 compared the effects of bioamendments quantities. Treatments 6-7 compared the effect of cellulose on the bioamended treatment. Treatments 8-10 compared the effects of different PCB dechlorinating cultures. SediMite™ custom manufactured by U. Ghosh (Sediment Solutions) was amended with cellulose as a slow release electron donor to promote reductive dechlorination where indicated. Cellulose amendment was at the level of 1.0% by weight of SediMite™. After distribution into sediment, SediMite™ is designed to disaggregate into small particles that are gradually mixed into the lower sediment layer as a result of bioturbation by benthic organisms in the field. Three 6 by 1 cm polyoxymethylene (POM) strips were inserted into each mesocosm as describe in the Treatability Study Proposal (Section 6.4) for analysis after day 120. Although the goal of the study was to monitor loss of PCB mass after treatment, measuring bioavailability was intended to quantify any specific effects of microbial activity on bioavailability.

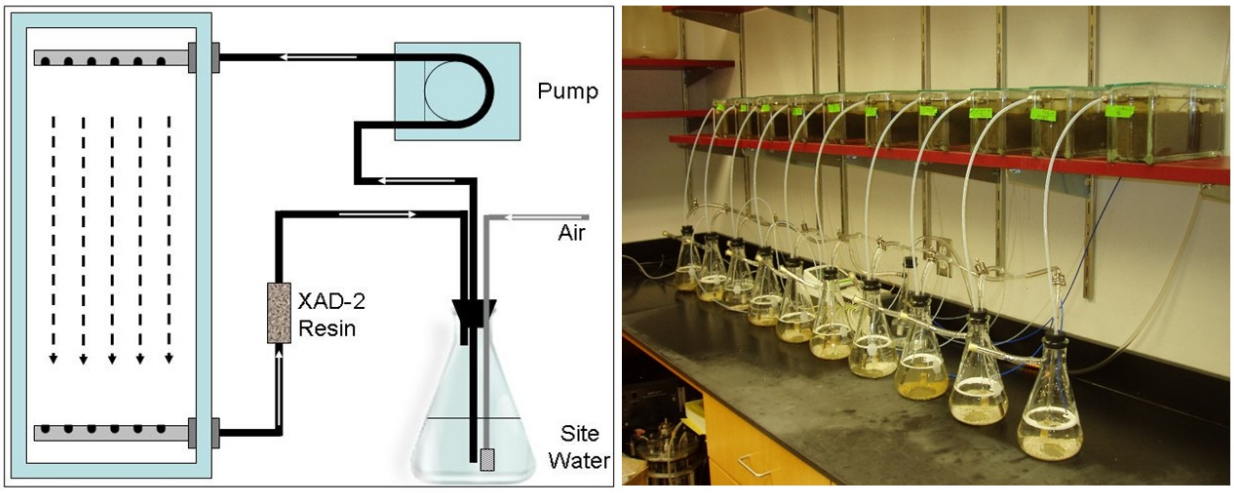


Figure 12. Illustration Showing Configuration of Recirculating Mesocosm Systems.

Table 4. Treatments Tested in Sediment Mesocosms.

Treatments 1-3 test abiotic effects of SediMite and cellulose, 4-5 test effects of bioamendments concentration, 7 tests effect of bioamendment without cellulose, 8-10 test effects of different PCB anaerobic halorespiring bacteria in bioamendments.

Treatment	SediMite™	Cellulose	Cells g^{-1} sediment[a]	Anaerobic Dechlorinator	Aerobic Degrader
1	-	-	-	-	-
2	3%	-	-	-	-
3	3%	0.03%	-	-	-
4	3%	0.03%	5×10^3	DF-1	LB400
5	3%	0.03%	5×10^4	DF-1	LB400
6	3%	0.03%	5×10^5	DF-1	LB400
7	3%	-	5×10^5	DF-1	LB400
8	3%	0.03%	5×10^5	SF1+DEH10	LB400
9	3%	0.03%	5×10^5	o-17	LB400
10	3%	0.03%	5×10^5	DF1+SF1+DEH10+ o-17	LB400

[a] Titer of each strain

After treatments the mesocosm tanks were sealed with glass plates and installed on recirculating system to simulate *in situ* conditions (**Table 3**). A peristaltic pump continuously circulated aerated water collected from the site at a retention time of one hour. Use of a recirculating system instead of sparging the mesocosms directly with air minimized the risk of PCB loss due to volatilization and minimized artificial sediment turbation. Continuous flow with oxygenated water was critical for: 1) maintaining aerophilic and microaerophilic activities of PCB degrading bacteria at the sediment surface, and 2) maintaining the bioturbation activity of the benthic community to promote distribution of the bioamended activated carbon into the sediment profile. Aerated water was pumped from a sparging flask containing a fritted glass gassing tube connected to an air supply. The dissolved oxygen concentration in the water column was maintained at 6.71 ± 0.27 mg kg^{-1} at the mesocosm inlets and 6.53 ± 0.27 mg kg^{-1} at the outlets, which is equivalent to the value of 6.75 mg kg^{-1} reported at the site in June 2012. Water flowing out of the mesocosm was passed through Amberlite XAD-2 polymeric absorbent (20-60 mesh) to remove any soluble PCBs and returned to the sparging flask. Each system was isolated using a 12 channel peristaltic pump (Watson-Marlow) to circulate the water. Mesocosms were operated at a room temperature of 22-24^{o}C. The room was maintained in the dark except during short periods for sampling and maintenance to prevent photosynthetic oxygen generation in the lower sediment column.

5.3.3.5 Analysis of mesocosms

After set up of the mesocosms and treatments sediment was allowed to settle for four hours, then three random core samples were taken from each mesocosm using random number generator and grid (Day 0 time point). Day 0 samples from each mesocosm were independently analyzed for PCB congeners and total PCBs. Mesocosms were periodically sampled at 30, 60 and 120 days by taking 6 cm deep cores using a 5 mL syringe barrel with the end cut off. An additional sampling time point at 375 days was added after the Treatability Report was submitted to assess long-term effects of treatments. Triplicate cores were sampled for each time-point using a sampling grid and random number generator. Cores were frozen prior to analysis. Frozen cores were extruded and sub-sectioned at 0-3 and 3-6, cm below the surface. Each subsection was homogenized by mixing.

One mL of each core was removed for PCB analysis and the remainder of the core was refrozen in case re-analysis was necessary. Monitoring included quantitative PCB congener analysis to determine efficacy and rate of total degradation with selected ratios of SediMite™ to biocatalysts and identification of any recalcitrant congeners. Statistical analyses of data include mean, standard deviation and statistical significance with a t-test when indicated.

5.3.3.6 Sediment characterization

Within one week an orange colored layer formed in the top 3 mm below the sediment surface and the sediment in the remainder of the sediment column retained the original dark coloration, indicating both aerobic and anaerobic zones (**Figure 13**). This zonation was observed for the duration of the 375-day period of the experiment. Within two weeks worms were observed in Treatments 5 and 6 and active burrowing was observed in these mesocosms for the remainder of the experiment. The active bioturbation indicated that oxygen levels in the water column were consistent throughout the experimental period. Bioturbation was not observed in the remaining mesocosms. It is likely that most of the benthic population was lost during storage of the sediment at 4°C for ten months prior to initiation of the mesocosms and only a few worms present in Treatments 5 and 6 survived and proliferated.

Figure 13. Mesocosm Showing Development of Distinct Oxic and Anoxic Zones (all treatments) and Bioturbation Due to Active Burrowing by Benthic Organisms (Treatments 5 & 6).

(See Figure SX in Appendix)

5.3.3.7 Effect of treatments on reduction of PCB levels in sediment

The efficacy of bioaugmentation for reducing the levels of Aroclor-contaminated sediment from Abraham's Creek is shown in **Table 5 and Figure 14**.

Table 5. Effect of Treatments on Reduction of Total PCB Levels in Mesocosms with Sediment from Abraham's Creek.

Treatment	Day 0		Day 30		Day 60		Day 120		Day 375		Significance ($\rho<0.05$)‡
	mg kg^{-1} ±SD	% decrease*	mg kg^{-1} ±SD	% decrease*	mg kg^{-1} ±SD	% decrease*	mg kg^{-1} ±SD	% decrease*	mg kg^{-1} ±SD	% decrease*	
1	3.9 ± 0.6	---	4.7 ± 1.8	0	4.5 ± 1.1	0	4.0 ± 0.7	0	3.1 ± 1.0	20	---
2	4.3 ± 0.7	---	5.6 ± 1.0	0	3.5 ± 0.7	18	3.6 ± 0.9	16	3.3 ± 0.6	23	---
3	3.6 ± 0.1	---	4.0 ± 0.3	0	3.7 ± 0.5	0	3.2 ± 0.4	10	4.0 ± 0.8	0	---
4	3.2 ± 1.0	---	4.5 ± 1.2	0	3.0 ± 0.5	7	2.8 ± 0.7	13	2.5 ± 0.9	22	---
5	2.8 ± 0.6	---	2.5 ± 0.3	12	2.0 ± 0.3	30	1.4 ± 0.3	51	1.2 ± 0.2	58	+
6	3.6 ± 1.5	---	1.2 ± 0.1	55	1.0 ± 0.2	63	0.8 ± 0.1	78	0.8 ± 0.1	78	+
7	3.5 ± 0.7	---	2.6 ± 0.5	26	2.4 ± 0.5	32	2.2 ± 0.4	38	1.8 ± 0.4	49	+
8	3.8 ± 0.3	---	3.2 ± 0.6	15	2.7 ± 0.4	28	2.4 ± 0.4	36	2.0 ± 0.6	47	+
9	3.2 ± 0.8	---	2.3 ± 0.3	28	2.1 ± 0.3	35	1.6 ± 0.3	50	1.8 ± 1.0	44	-
10	3.1 ± 0.3	---	2.5 ± 0.4	21	2.1 ± 0.3	32	1.8 ± 0.4	42	1.5 ± 0.6	52	+

† Day 30, 60 and 120 = mean and sd top and bottom of sediment cores (n-6); days 0 and 365 = mean and sd of combined core (n-3)

*% Decrease is compared to the Day 0 starting concentration

‡ T-test for day 0 vs. Day 375

The only apparent reduction in PCB levels was observed in mesocosms treated with bioamended SediMite™. The results in treatment's 1, 2 and 3 indicate that there was no significant biostimulation ($\rho>0.05$) of indigenous bacterial populations as a result of mixing, addition of SediMite™ or addition of cellulose as a carbon source. These results indicate that biostimulation of indigenous populations of PCB dechlorinating bacteria with an exogenous fermentable carbon source is not an effective treatment for stimulating PCB degradation.

All bioamended treatments showed significant degradation with the exception of treatments 4 and 9. Comparing the effect of cell titer Treatment 4 amended with 10^3 cells g^{-1} showed no significant effect, whereas Treatments 5 and 6 bioamended with 10^4, and 10^5 cells g^{-1} sediment, respectively, showed the most significant change of 58 and 78% reduction of total PCB levels, respectively. Eliminating cellulose as an additional slow release carbon source with 10^5 cells g^{-1} sediment in treatment 7 decreased the rate and extent of PCB level reduction in 375 days compared with treatment 6 that contained cellulose with the same amount of bioamendment. The extent of degradation with addition of *o*-17 in treatment 8, which preferentially attacks congeners in single flanked *ortho* substituted and double flanked *meta* with 10^5 cells g^{-1} sediment in treatment 7 decreased the extent of PCB level reduction in 375 days compared with treatment 6 that contained cellulose with the same amount of bioamendment. The extent of degradation with addition of *o*-17 in treatment 8, which preferentially attacks congeners in single flanked *ortho* substituted and double flanked *meta* chlorine substituted positions, and with DEH10 and SF-1 in treatment 9, which preferentially attack congeners in single and double flanked *meta* chlorine substituted positions, was less than that observed with a similar cell concentration of DF1 in treatment 6. Interestingly, although bioaugmentation with DF-1 and LB400 in treatments 5 and 6 yielded the highest rate and total degradation of PCB degradation, adding all three halorespiring cultures together with LB400 in treatment 10 was less efficient than using DF1 only. Although differences between Treatments 6 and 10 were not statistically significant ($\rho>0.05$), the results suggest that addition of DF-1 in combination with LB400 was the most robust treatment for reducing overall PCB levels.

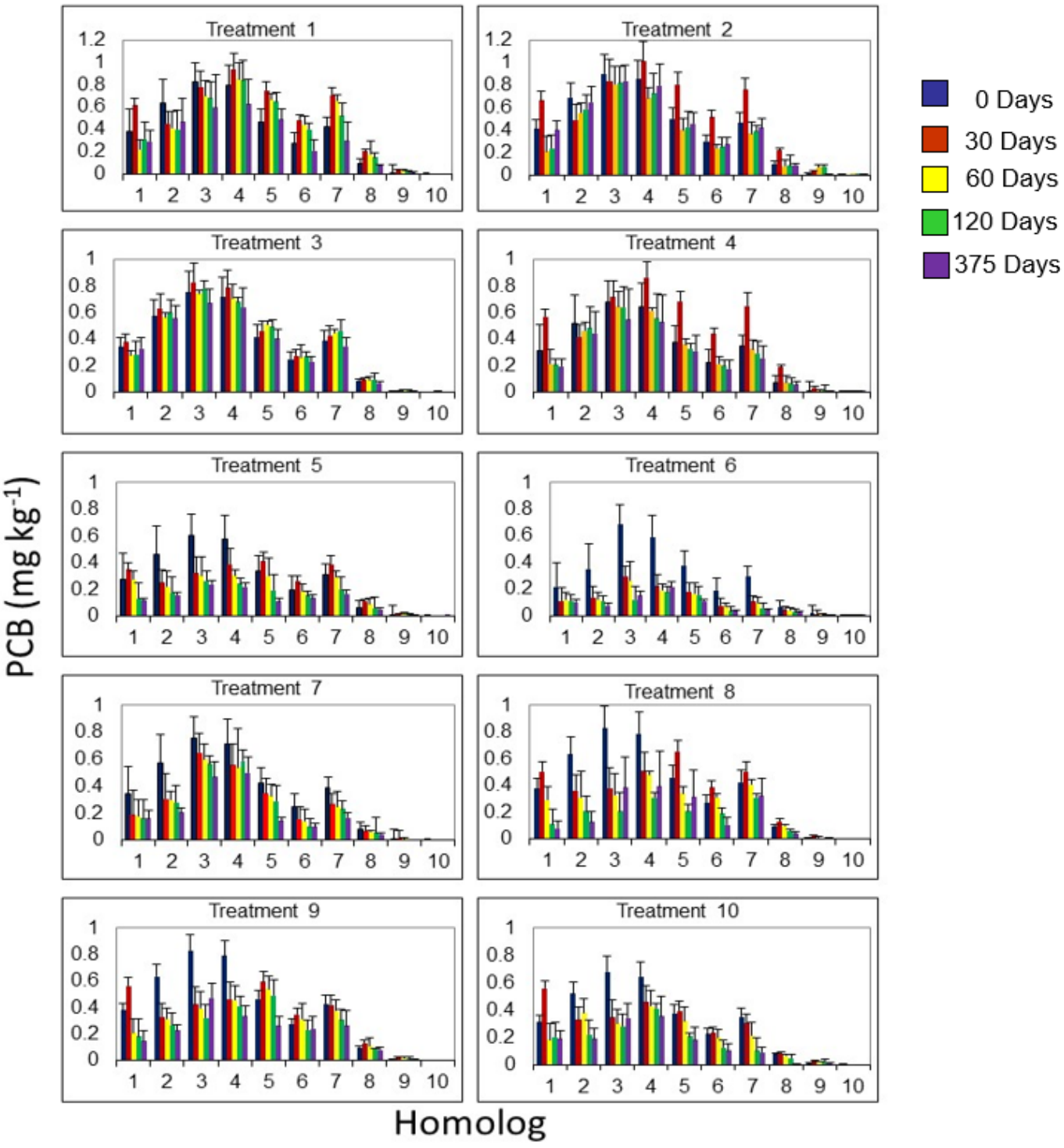


Figure 14. Effect of Treatments on PCB Homolog Concentrations with Time.

5.3.3.8 PCB degradation throughout depth profile

PCB concentrations were not significantly different ($\rho < 0.05$) for the upper and lower half of the sediment column in all mesocosms (**Figure 15**). The results indicate that bioaugmentation was effective within 10 cm below the sediment surface. Benthic activity (worms) was observed in only treatments 5 and 6, which also had the greatest extent of degradation. It is unlikely the benthic worms contributed significantly to the direct reduction in PCB levels as the fraction of total PCBs bioaccumulated by worms would be negligible (Sun, 2009). Furthermore, core samples included both sediment and biota, therefore, any PCB adsorbed by the worms would have been extracted from the core samples. It is possible that bioturbation promoted oxygenation of the lower sediment profile thereby contributing to greater degradation in Treatments 5 and 6. However, the results indicate that even in mesocosms where benthic activity was not observed there was sufficient diffusion of oxygen through the porewater to support aerobic degradation. The similarity of the homolog patterns between the top and bottom cores after 120 days further confirm that both anaerobic dechlorination and aerobic degradation occurred throughout the sediment column in treatments 5 to 10. Therefore, it is not possible to unequivocally conclude whether bioturbation contributed to PCB degradation based on the results.

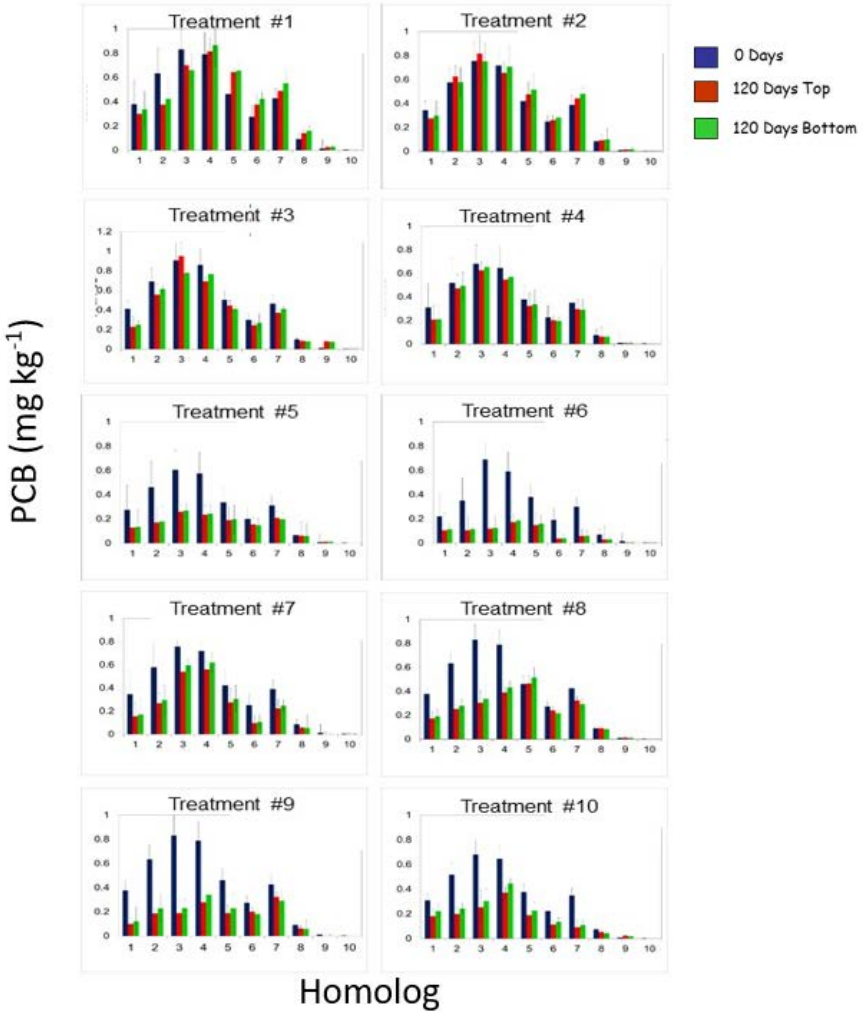


Figure 15. Effect of Treatments on PCB Homolog Concentrations in Upper and Lower Sediment Profile.

5.3.3.9 Effects of treatments on coplanar congeners

Most of the toxic effects of PCBs for humans are mediated through the aryl hydrocarbon receptor (AhR), a cytosolic receptor protein present in most vertebrate tissues with high affinity for 2,3,7,8-substituted PCDD/Fs and some coplanar PCB congeners. Three coplanar congeners were detected in Abraham's Creek sediment: 2,3,4,4'5-pentachlorobiphenyl (PCB 114), 2,3,3',4,4',5-hexachlorobiphenyl (PCB156) and 2,3,3',4,4',5'-hexachlorobiphenyl (PCB 157) (**Figure 16**). Treatment 6 showed the most significant reduction in concentration of these three congeners from 7.40 ng g^{-1}dw on day 0 to 1.13 and 0.75 ng g^{-1} dw 120 and 375 days after treatment, respectively. Factoring in the toxic equivalency factor (TEF) for each coplanar congener relative to 2,3,7,8-tetra dibenzo-p-dioxin [7], the total toxic equivalency (TEQ) was reduced by 90% from 220 to 23 ρg kg^{-1} 375 days after treatment. There appears to be less TEQ reduction in treatment 5 after day 375 compared with other bioamendment treatments even though the reduction of total PCBs (Table 5) in treatment 5 is not significantly different compared with treatments 7, 8, 9 and 10 ($P > 0.05$). However, the reduction of TEQ for treatment 5 was only significantly different from treatment 9 ($P > 0.05$), which suggests that the apparent difference in total PCB and TEQ reduction is likely due to sample variation or experimental error.

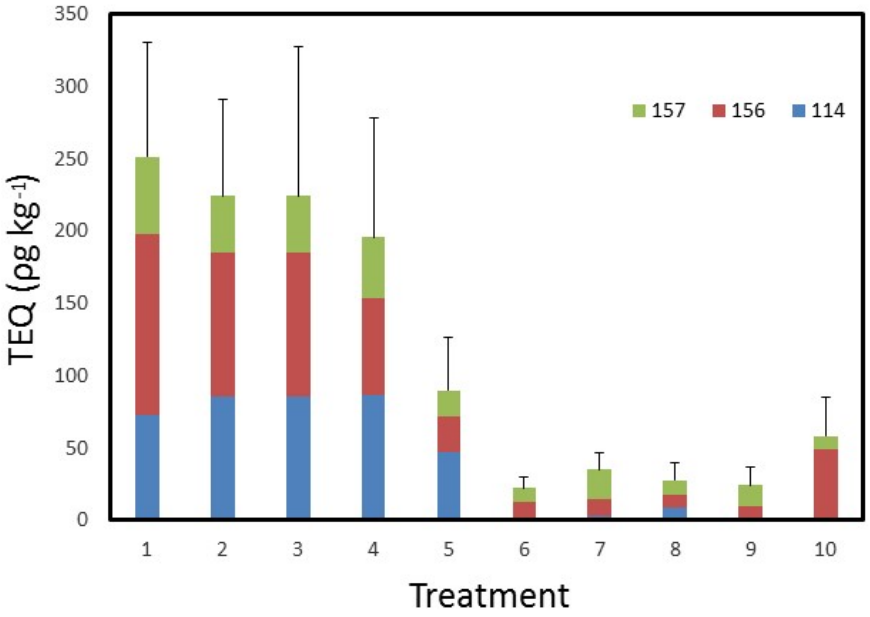


Figure 16. Effect of Treatments on Potential Toxicity of Co-planar Congeners after 375 Days.

TEQ methodology in human risk assessment in the context of this study is only intended for estimating relative changes in potential exposure to dioxin-like chemicals from consumption of aquatic food products as this approach does not take into account a number of factors such as bioavailability within an abiotic matrix and other factor affecting biomagnification within the food chain.

5.3.3.10 Effect of treatments on PCBs in porewater

The change in sediment porewater PCB concentrations in the treatability study was measured by passive equilibrium sampling using polyoxymethylene (POM) polymer strips. The passive samplers were removed from the mesocosms after 120 days of exposure, rinsed with water to remove any attached sediment, and analyzed for PCBs as described in Beckingham et al. [8]. PCB concentration in POM was converted to estimated PCB concentrations in the porewater phase based on equilibrium partitioning constants presented in Ghosh et al. [9]. Performance reference compounds were not used in this measurement.

Results of estimated porewater concentrations are presented in **Figure 17**. The greatest reductions in the sediment PCB concentrations do not match with the greatest reductions observed in the freely dissolved porewater concentrations. The sediment was homogenized well before aliquoting into each treatment tank and should have resulted in the same concentration of PCBs in each tank.

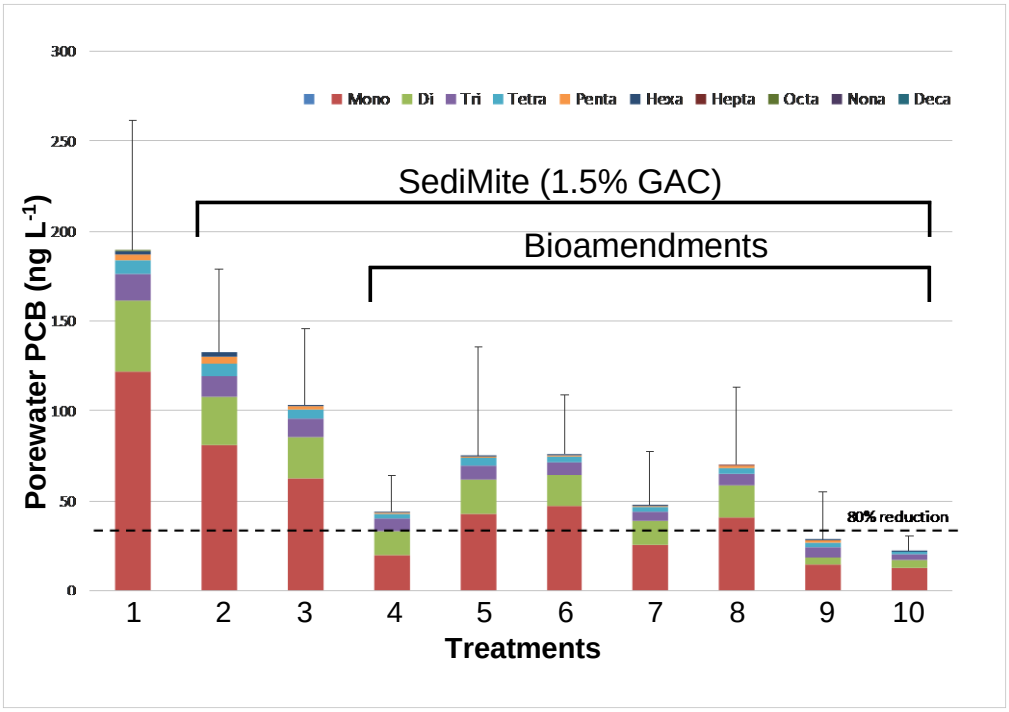


Figure 17. Effect of Treatments on Porewater Concentrations of PCB Homologs Estimated from Equilibrium Passive Sampling Exposed to the Sediment for the 120 Day Duration.

The measured initial total PCB concentrations in each tank at the start of the experiment are shown in **Table 5**. While, there are some differences in the average measured initial concentrations, (high in Treatment 3 and low in treatments 5, 6, and 10), the differences are not statistically significant except for the high concentration in Treatment 3. The porewater concentration measured in the control mesocosm after 120 days was 189 ± 73 ng L^{-1}. The addition of SediMite™ alone (at a dose of 1.5% AC to sediment) with or without cellulose had a small influence on porewater concentration. Addition of the various bioamendments along with SediMite™ had a strong influence on porewater concentration ranging from 60-88% reduction compared to the control sediment.

These reductions are based on the assumption that each treatment tank started with the same initial concentration of PCBs in sediment. A high level of bioturbation associated with native worm activity was observed in treatments 5 and 6. Since performance reference compounds were not used in the treatability study passive sampling, extent of non-equilibrium could not be corrected, and it is likely that the relatively higher porewater concentrations observed in treatments 5 and 6 (compared to the other bioamendment treatments) could be partly caused by higher mass transfer of the porewater PCBs to the passive sampler as a result of bioturbation.

Another consideration is that the reduction seen in the total sediment concentrations is reflective of the mass reduction seen for each PCB congener in sediment and the respective abundance of each of those congeners in the sediment. However, the total porewater concentrations are skewed by the higher solubility of the lower chlorinated PCB congeners (primarily dominated by the mono, di, and tri). As such we do not expect to see the same trends in total concentration reductions in the sediment and porewater. Also, it is important to remember that we are following an active microbial dechlorination process along with some aerobic degradation. While the higher chlorinated PCBs are being actively dechlorinated to lower chlorinated ones (which are more soluble), the aerobic organisms are also degrading the dechlorinated intermediates. The relative abundance of the individual PCB congeners will therefore depend on the relative rates of dechlorination and aerobic degradation in the various combinations of organisms in each treatment tank. In **Figure 17**, the trends associated with the higher chlorinated PCBs in porewater was being masked by the abundance of the mono and dichlorobiphenyls. When the trends of the mono-tetra and penta-octa homologs are viewed separately (**Figure 18**), the reductions in porewater PCB concentrations appear to be greater for the higher chlorinated PCBs compared to the reductions in the lower chlorinated PCBs, especially for the treatments containing 10^5 cells/ml of the DF1 culture. This observation may be an indication of active microbial dechlorination with formation of lower chlorinated products that persist for some time before degradation by the aerobic PCB degrader.

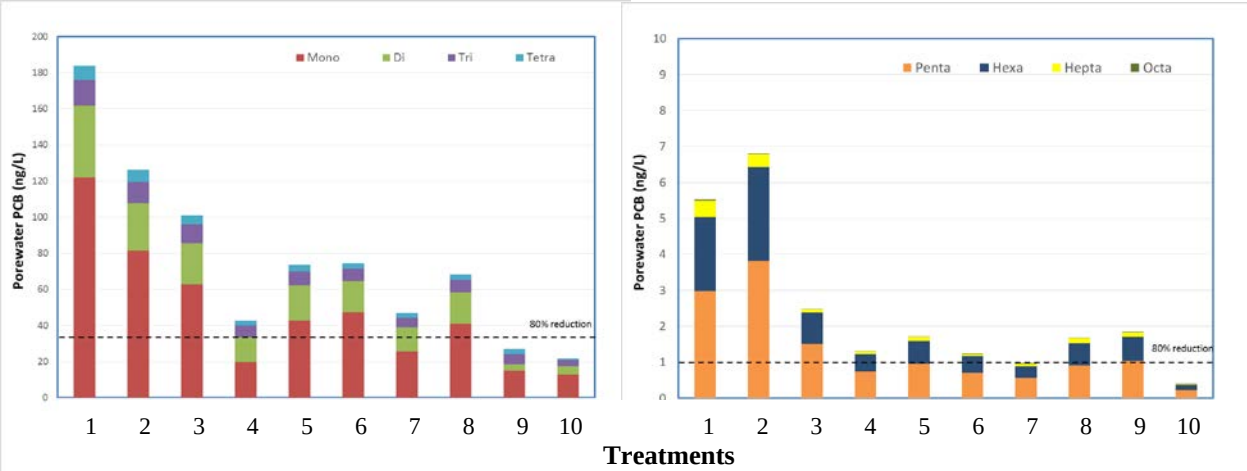


Figure 18. Effect of Treatments on Porewater Concentrations of Mono- to Tetra-Chlorinated (A) and Penta- to Octa-chlorinated Congeners (B) after 120 Days.

After the Treatability Study Report was submitted the change in sediment porewater PCB concentrations was measured again 375 days after treatments. Results of estimated porewater concentrations are presented in **Figure 19**. There was a 30-35% reduction in PCB porewater concentrations after treatment with non-bioamended SediMite in treatments two and three compared with untreated sediment. Porewater PCB was reduced 40% after treatment with SediMite bioamended with 10^3, in treatment 4, but the difference was not significant ($\rho<0.05$) from the abiotic SediMite treatments. All of the remaining bioamended treatments showed a significant reduction of PCBs in the porewater compared with untreated sediment ($\rho<0.05$) ranging from 94% in treatment 10 to the greatest reduction, 97%, in treatment 6. Over time all the treatments with 10^4 and higher level of bioamended reached a low porewater value with little difference in final concentrations among them. It is possible that kinetic differences among these bioamendment treatments are being masked over time as slower treatments are able to catch up with time. Coplanar PCB congeners in the aqueous phase were below our detection limit of 0.01 ng L^{-1} water in all of the mesocosms, including the untreated mesocosm.

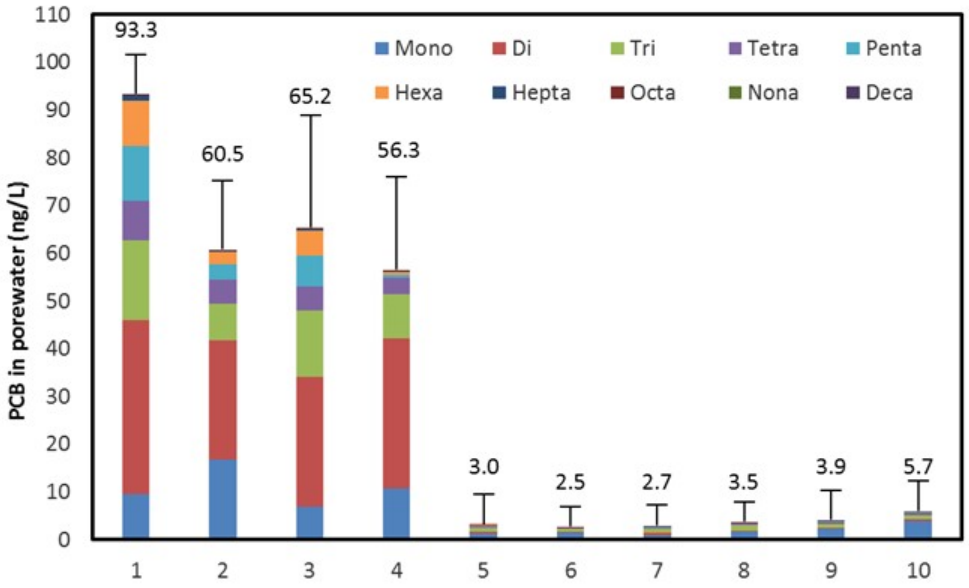


Figure 19. Effect of Treatments on Porewater Concentrations of PCB Homologs Estimated from Equilibrium Passive Sampling Exposed to the Sediment for the 375-day Duration.

5.3.3.11 Fate of bioamendments

The titer of DF1 and LB400 in the most active treatment 6 was approximately 8×10^5 gene copies/ g sediment (**Figure 20**). Both bioamendments were detected 375 days after treatment, although their titer decreased about 2-3 orders of magnitude. The titer of bioamendments in treatments that were not bioaugmented was below the theoretical detection limit of 102 gene copies/ g sediment. There was a total decrease in PCBs in treatment 5, but no significant increase in dechlorination products between days 0 and 60, which indicates degradation of dechlorination products.

The observed accumulation of dechlorination products on days 120 and 375 coincides with two orders of magnitude decrease in the titer of LB400. Overall, the results suggest that the aerobic degradation rate by LB400 was greater than the halorespiration rate of DF-1 in the first two months, but as the titer of LB400 decreased the net rate of degradation no longer exceeded that of anaerobic dechlorination. The titer of DF1 also decreased over the course of 375 days, but at a slower rate than LB400. Although halorespiration of PCBs supports growth of halorespiring bacteria [10] Lombard *et al.* [11] reported that the thermodynamic cell yield of halorespiring bacteria at environmentally soluble PCB concentrations in this study would be too low to maintain the high titer of DF1 added as bioamendment. Re-amending the mesocosm with 10^5 cells g^{-1} DF1 and LB400 after 375 days did not stimulate further degradation, which suggests the PCB concentration was no longer bioavailable to microbes. The conclusion is that the high cell titer in the initial treatment was sufficient to reduce the bioavailable portion of PCBs by 97% based on the porewater data and the remaining PCBs remained strongly adsorbed to the sediments and AC.

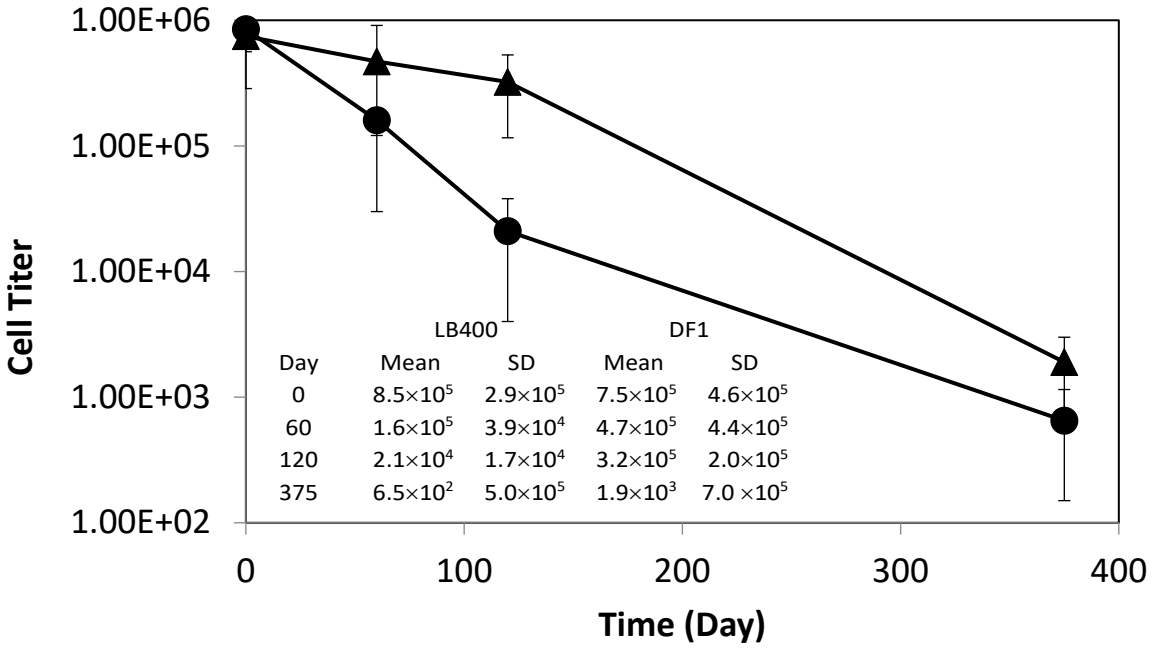


Figure 20. Change in Cell Titer of DF-1 (▲) and LB400 (●) after Bioamendment in Treatment 6 (SediMite, cellulose, and 10^5 cells of DF1 and LB400).

Tabular data shown in inset. Error bars indicate standard deviation of cell titer.

5.3.3.12 Effect of treatments on indigenous microbial populations

As is often the case with lab incubations, there was a significant decrease (paired t-test, $p<0.01$) in microbial diversity over time in the mesocosm reactors (**Figure 21**). The high-performing treatment 6 was an exception, where diversity increased between days 0 and 375. As dispersal is unlikely in these systems, the increase in diversity appears to be driven by increasing evenness.

OTUs assigned to Betaproteobacteria had the highest relative abundance in all treatments and increased from day 0 to day 375. (**Figure 22**). OTUs belonging to the genera *Caloramator*, *Pseudomonas*, *Clostridium*, *Desulfosporosinus*, *Geobacter*, and *Sulfuricurvum* were significantly more abundant on day 375 compared to day 0 (**Figure 23**). Ordination using non-metric multidimensional scaling (NMDS) on the weighted unifrac distance [12] demonstrated significant clustering of samples by time point (Anosim r=0.609, p=0.001) (**Figure 24**). However, no clustering by treatment type was observed. As was the case with the qPCR assay, Dehalococcoidaceae and Burkholderiaceae relative abundance declined in all mesocosms except in Treatment 6. Enhanced PCB degradation in treatment 6 may be the result of the increased diversity and relative abundance of Dehalococcoidaceae and Burkholderiaceae.

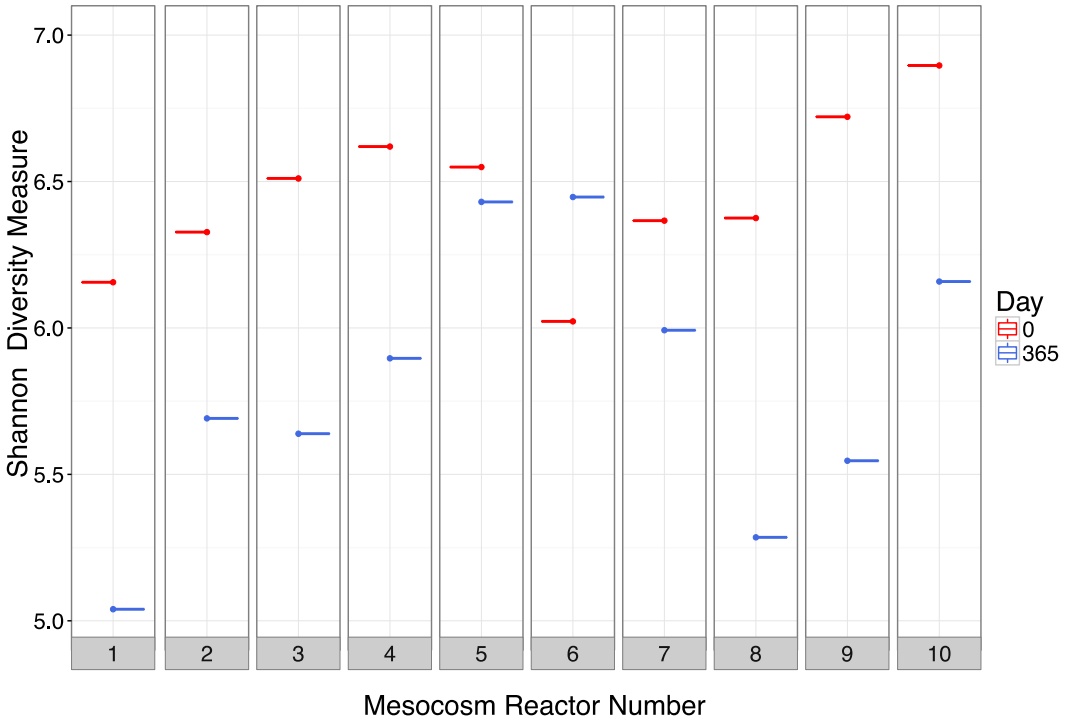


Figure 21. Microbial Community Changes in Response to Treatments.

The Shannon alpha diversity measure for each reactor. Day 0 in red and day 375 in blue.

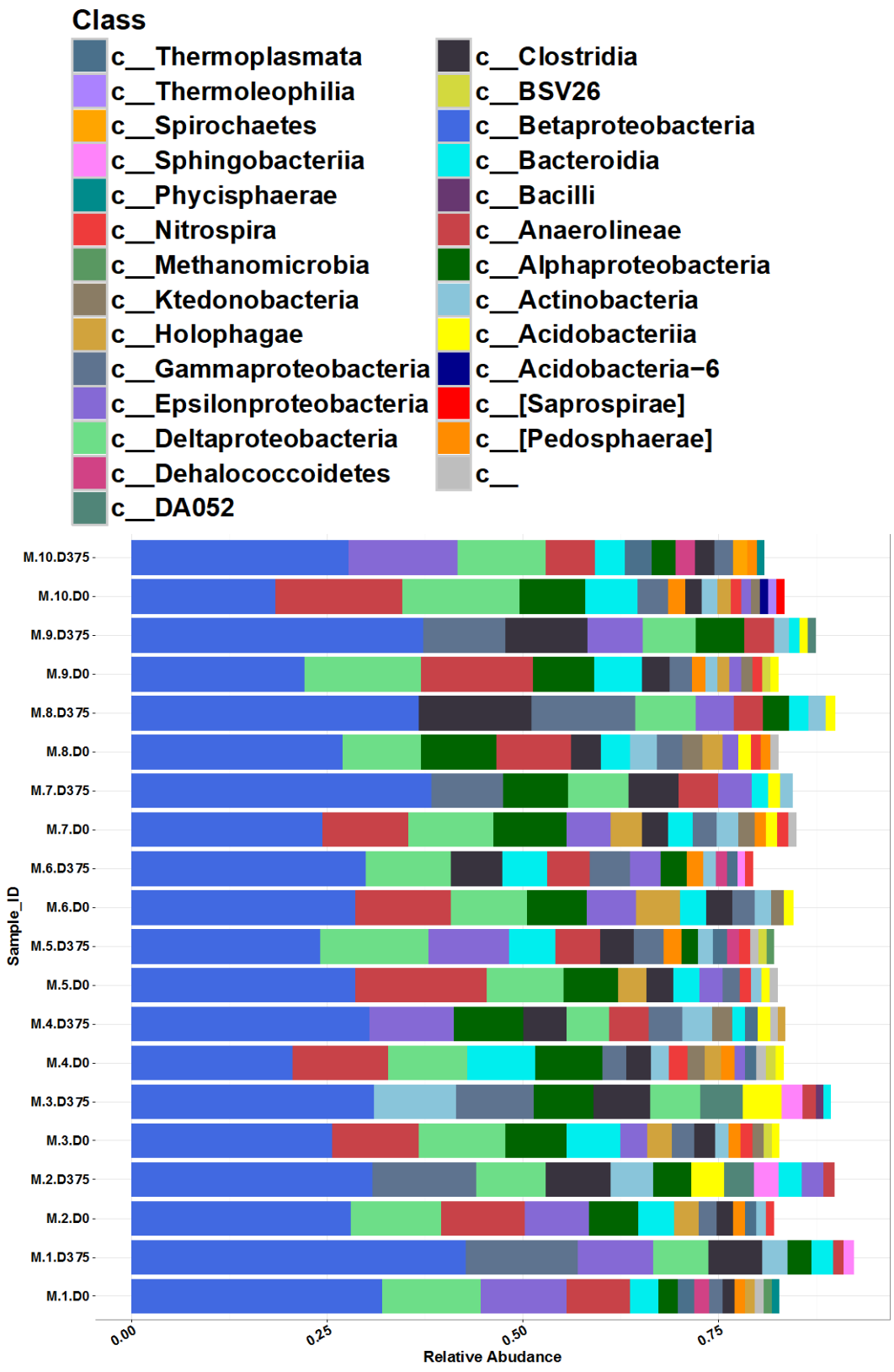


Figure 22. Class-level Resolution of Taxa in Each Mesocosm.

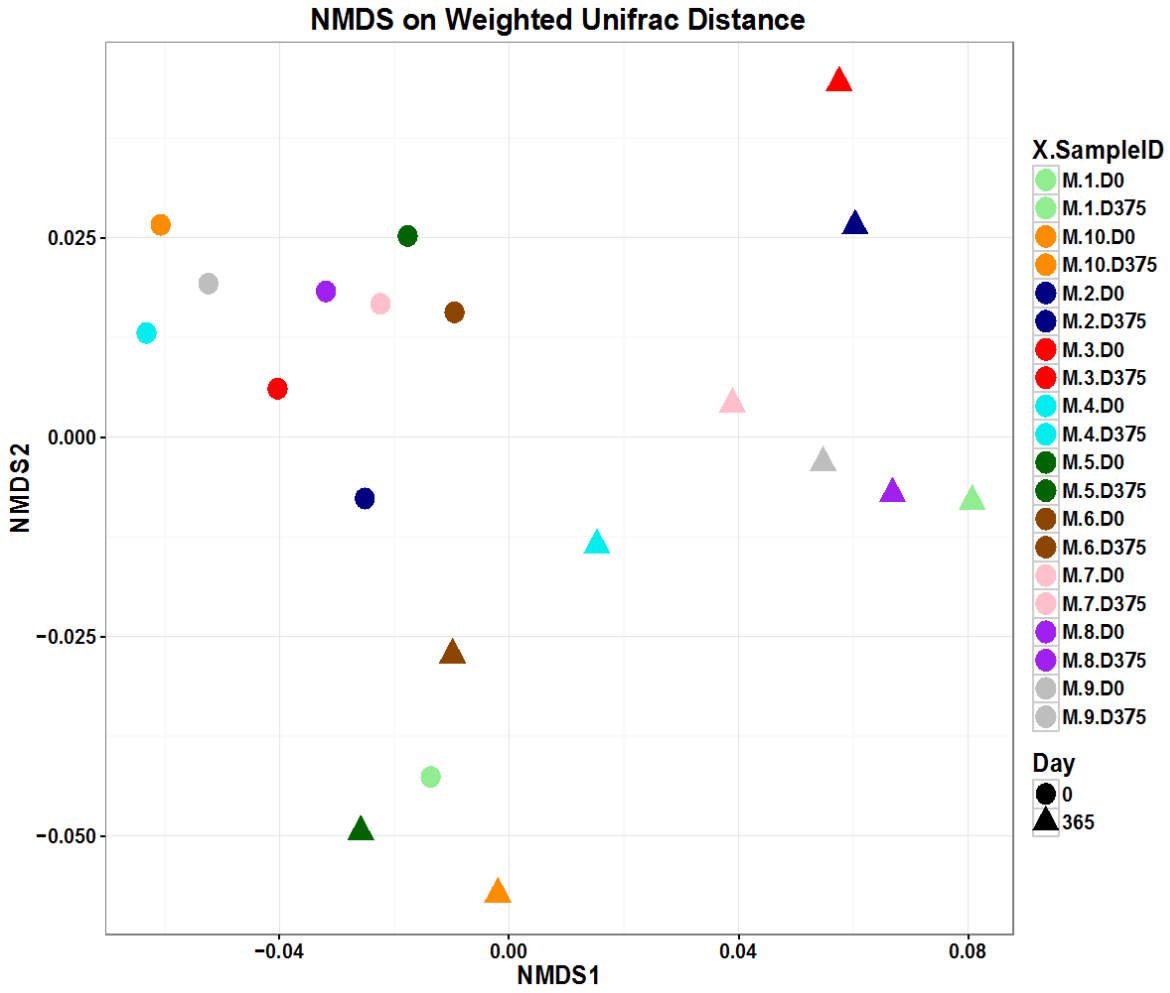


Figure 23. Nonmetric Multidimensional Scaling Using the Weighted Unifrac Distance between Each Sample.

Mesocosm treatments are indicated by different colors and timepoints are delineated by shapes (circles = 0 days, triangles = 375 days). Points that are closer together represent communities that are more similar.

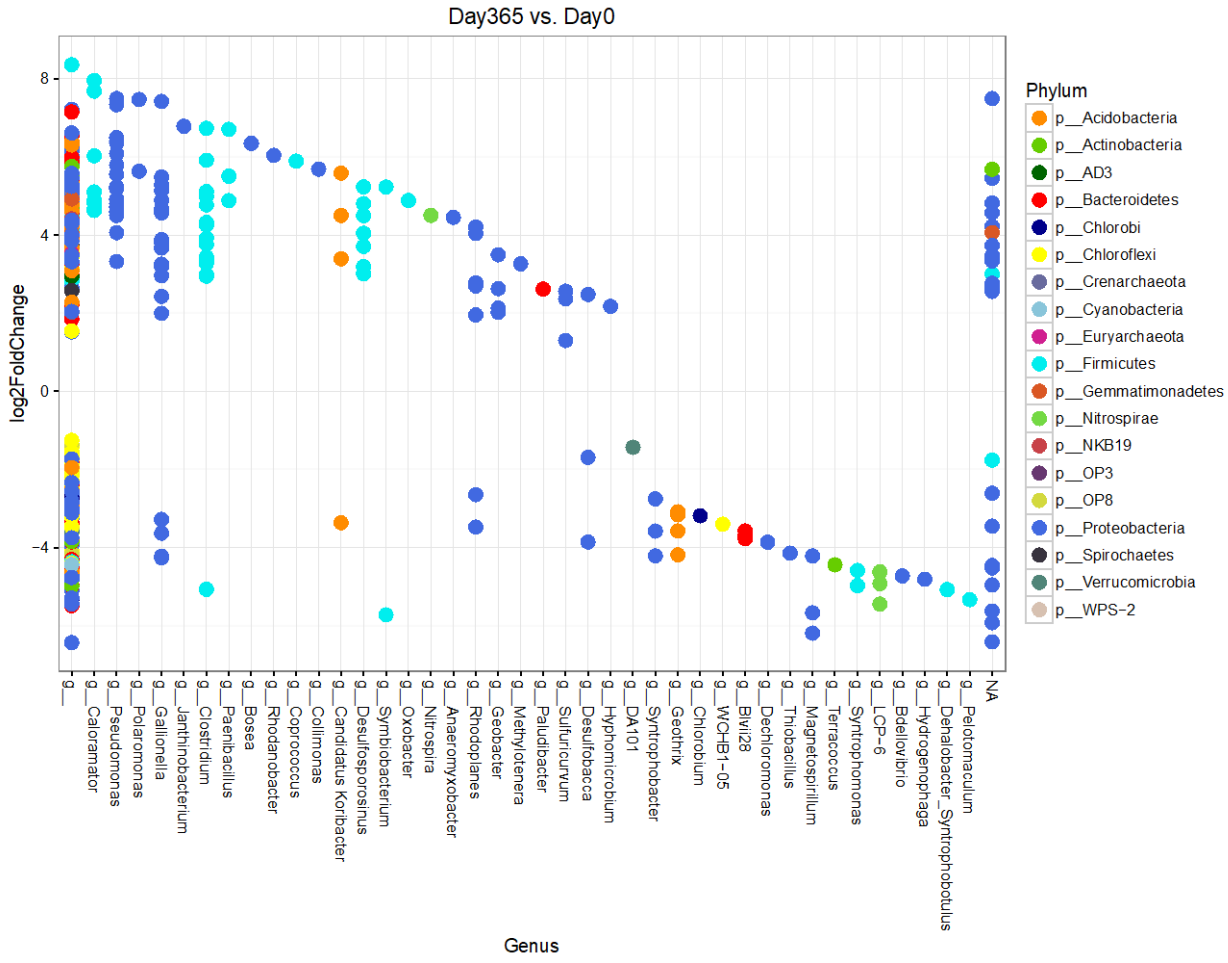


Figure 24. Significantly Different Taxa Enriched at Day 375 Compared to Day 0.

The log 2-fold difference is indicated on the y-axis with taxa more abundant at day 375 denoted by positive y values. Genera are named along the x-axis and the phyla corresponding to the listed genera are shaded in different colors.

5.3.4 Pilot Scale Delivery System Design

A major challenge in introducing microorganisms to an aquatic environment is the potential of the organisms to be washed away from the site. Delivery in the form of SediMite™ can address the challenge by embedding the microorganisms within the carbon matrix. However, the microbes cannot be incorporated during the manufacture of the pellets due to the final drying process involved in the current process that would kill the organisms. "First, tests were conducted to determine the effect of mixing the anaerobic halorespirer and aerobic degrader together prior to inoculation of the SediMite. Second, tests were conducted to determine the effect of exposure to the water column on viability of the anaerobe and aerobe after bioamended pellets are deployed. Finally, two deployment systems were tested to determine the most effective method for delivery of bioamended SediMite onto the test site. Results from these trials provided information for the development of SOPs for field deployment.

5.3.4.1 Viability of cells after mixing

The Treatability Study Proposal stated that the microorganisms would be inoculated onto SediMite™ by one of two possible approaches depending on the outcome of the system test described below. If the anaerobic and aerobic microorganisms are compatible together for the duration of application without significant loss of viability they will be combined in a polypropylene tank and applied to SediMite pellets prior to application. If there was an excessive loss of viability after mixing the microorganisms together prior to inoculation of the SediMite™, then we would deliver the aerobic and anaerobic microorganisms to the manifold from separate aerobic an anaerobic reservoirs, respectively. In order to determine whether the aerobe and anaerobe could be combined prior to absorption to SediMite™ or would need to be adsorbed form separate reservoirs, we investigated the viability of LB400 and DF-1 after combining the inocula in a single reservoir.

Ten mL of LB400 (10^9 cell mL^{-1}) was added to 990 ml DF-1 culture (10^7 cell mL^{-1}) in a 1 liter screw cap bottle and cells were enumerated and tested for viability over time (**Table 6**). The results show there was no significant decline in cells numbers or viability of either the aerobe or the anaerobe 1 week after mixing, indicating that the cultures could be mixed and pumped from a single reservoir while exposed to air during deployment in the field.

The results indicated that SediMite pellets could be inoculated 14 days prior to deployment with no significant loss of cell titer or viability.

Table 6. Effect of Mixing Cultures on Total Cell Counts and Viability

Bacterial strain		Initial mixture (sd)	14 days after mixing (sd)	N	Significance ($P < 0.05$) †
LB400	Total	1.9×10^7 (0.7)	2.9×10^7 (1.0)	3	No
	Viable	1.3×10^7 (0.3)	0.7×10^7 (0.3)	6	No
DF-1	Total	1.6×10^7 (0.3)	1.7×10^7 (0.2)	3	No
	Viable	2.3×10^7 (1.0)	1.8×10^7 (0.6)	3	No

† T-test comparing all replicates at times 0 versus 14 days after mixing

5.3.4.2 Viability of cells after delivery through a water column

Concentration of total and viable cells was compared for inocula adsorbed to SediMite™ before and after passage through a 1 m water column. This is a slight variation from our original methodology in which we proposed broadcasting the bioamended pellets into an inflatable pool containing 1 m of water. In this alternative approach we manually inoculated the pellets and applied the bioamended pellets through a 1-meter column of water to reduce the materials required and minimize the number of variables such as homogeneity of the inoculum on the pellets. SediMite™ pellets inoculated with microorganisms with a hand sprayer were passed through 110 x 4 cm glass column filled with water (**Figure 25**). The column was fitted with a no. 9 rubber stopper that had a 7 x 0.5 cm glass tube was inserted 5 cm through the stopper to serve as a drain. A short length of tubing was inserted onto the drain tube and it was sealed with a pair of hemostats. Water (1.2 L) was then added to the column.

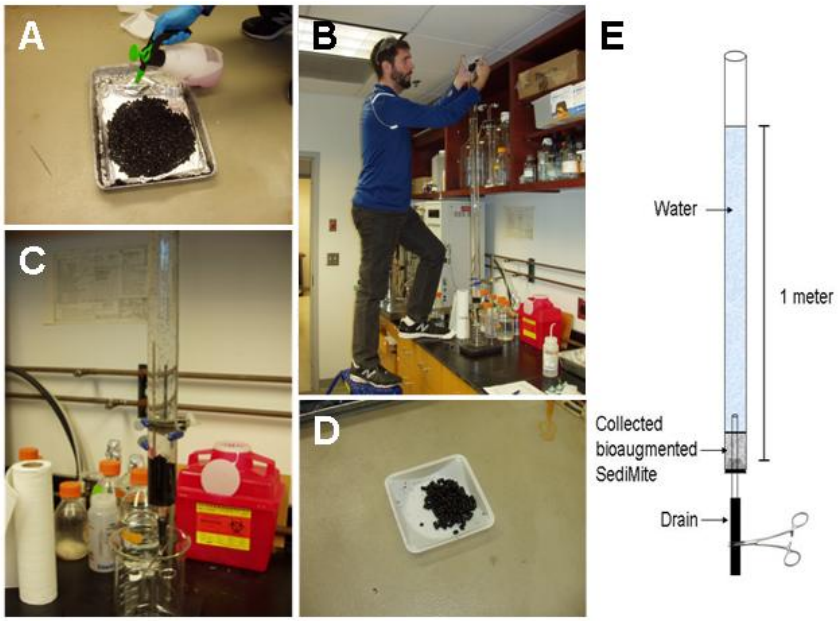


Figure 25. Viability of bioamended SediMite after passage through a water column.

SediMite was inoculated with DF-1 and LB400 (A); pellets were dropped through a 1 m column of water (B); pellets accumulated on the bottom of the column (C) and were collected after draining water and removing stopper (D). Panel E is a schematic diagram of the test column.

DF1 at 1.6×10^7 cells mL^{-1} (1000 mL) and LB400 at 2.03×10^9 cells mL^{-1} (100 mL) were combined in a 1.75L Hand Sprayer (Flo-Master). SediMite™ (375 g) was spread in a single layer in a 9x13 aluminum pan. Inoculum (50 mL) was sprayed onto the SediMite™ at a calibrated flow rate of 100 mL per min using a fine mist setting. It was observed that most of the 50 mL were absorbed into SediMite™. After inoculation, three pellets were immediately removed and transferred into microfuge tubes filled with 1 mL M9 media for the cell enumeration prior to passage through the water column. Approximately 30 grams of bioamended pellets were transferred to a large plastic weigh boat. Bioamended SediMite™ was added to the column of water and allowed to collect in the bottom. The water was drained from the column and the stopper was removed to collect the bioamended SediMite™. The SediMite™ collected below the top of the drain tube to minimize additional turbation of the SediMite™ as the water column was drained. Three random pellets were collected after passage through the column as described above. Cells absorbed to the SediMite™ were enumerated as described in IMET-SOP-8.

The results (**Table 7**) show there was no significant decrease in numbers or viability of cells absorbed to SediMite™ after passage through 1 m of water. The data indicate it will not be necessary to account for cell loss as a result of wash-off when calculating the concentration of inoculum required to achieve a final concentration of 10^7 cells g^{-1} SediMite™.

The results indicated that there was no significant loss of cell titer or viability after passing bioamended pellets through a one-meter length of water. Although the results show an increase in the titer of total and viable DF1 cells these values after passage through the water column, the differences were not statistically significant. The observed increase in values was likely due to precision error inherent in the MPN and QPCR assays used to monitor total and viable DF1 titer, respectively.

Table 7. Retention of Cells on SediMite™ after Passage through 1 m Water Column

Bacterial Strain		Cells/g SediMite™ before water column (sd)	Cells /g SediMite™ after water column (sd)	N	Significance (t-test, $P > 0.05$)†
LB400	Total	3.0×10^7 (2.3)	2.6×10^7 (0.46)	3	No
	Viable*	1.99×10^7 (0.59)	1.46×10^7 (0.75)	8	No
DF1	Total	0.677×10^7 (0.21)	0.85×10^7 (0.22)	3	No
	Viable*	0.99×10^7 (0.53)	2.43×10^7 (0.91)	3	No

† T-test comparing all replicates before passing through column versus after passing through column
*Reductive Dehalogenase mRNA copies per gram SediMite™

5.3.4.3 Deployment systems

Two approaches were developed and tested for adsorbing the microorganisms to the SediMite pellets and delivering the bioamended pellets to the sediments. One system, the Vortex, was designed to spray a mist of the active microorganisms into the nozzle to absorb into the SediMite™ pellets immediately before delivery onto the water surface. The second system, the venturi horn induction device (VHI), was designed to deliver bioamended pellets that were pre-inoculated with microorganisms. The goals of these studies were to test both systems for a consistent delivery ratio of microorganisms to SediMite™ and determine the viability of cells after delivery through a 1 m water column on SediMite™. Results from these trials provided information for the development of SOPs for field deployment.

The Modified Vortex

Sediment Solutions had extensive experience through three pilot-scale studies in SediMite™ delivery using an air-blown device manufactured by Vortex Granular Systems™ (**Figure 26**). For the project we utilized a modification that sprays a mist of the active microorganisms near the nozzle to absorb into the SediMite™ pellets immediately before delivery onto the water surface (**Figure 27**). The modification consists of a high-pressure TeeJet liquid spray nozzle located on the Vortex broadcasting nozzle perpendicular to SediMite™ flow. The spray nozzle was located near the nozzle exit port to prevent clogging that could result from accumulation of wetted SediMite™. Design of the nozzle for field inoculation was initiated by Sediment Solutions in collaboration with the vendor. The prototype nozzle (TeeJet 8003VS) saturated pellets 8% by weight with a dispersion distance of 30 ft. The pump and tank system from R & K pumps is designed to keep liquid amendments well mixed using a jet agitation system within the tank. The pressure at the delivery nozzle is adjustable and an analog pressure gauge on the pump line can be used to track the pressure. The pump was first calibrated with tap water to obtain water flow rates for individual nozzle types. The first nozzle tip to be tested was the TeeJet 8003 VS. It was found that the lowest consistent flow setting in the pump using this nozzle was at 10 psi or approximately, at 600 mL min^{-1} (**Figure 28**).

Figure 26. Vortex Granular Systems™ model TR.

The unit utilizes a positive airflow to propel SediMite to the desired area.

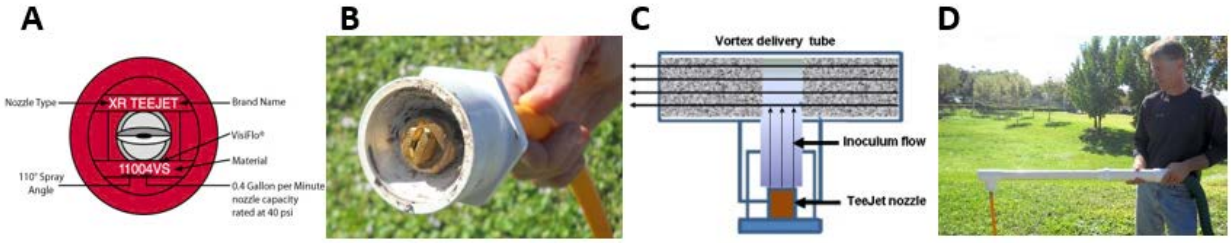


Figure 27. Vortex Modification with a XR TeeJet® Extended Range Flat Spray Tip Consistent Spray Distribution Over a Wide Range of Pressures (15-60 psi) Range of Pressures.

XR TeeJet (A) shown in removable Vortex cap fitting (B); schematic showing modified Vortex delivery tube with TeeJet installed perpendicular to flow of SediMite (C) and assembly shown on the end of the Vortex nozzle (D).

Rates of flow for the inoculum though the TeeJet were calibrated by measuring rate of water flow in collected a graduated cylinder. Two nozzles were calibrated: a TeeJet 8001VS (80º angle flat fan spray of fine droplets) which delivered 456 mL min^{-1} @ 60 psi; a TeeJet 8003VS (80º angle flat fan spray of medium droplets) which delivered 700 mL min^{-1} @ 10 psi. Rate of flow for the SediMite™ pellets was determined by timing the time to deliver 7 kg of SediMite™ pellets.

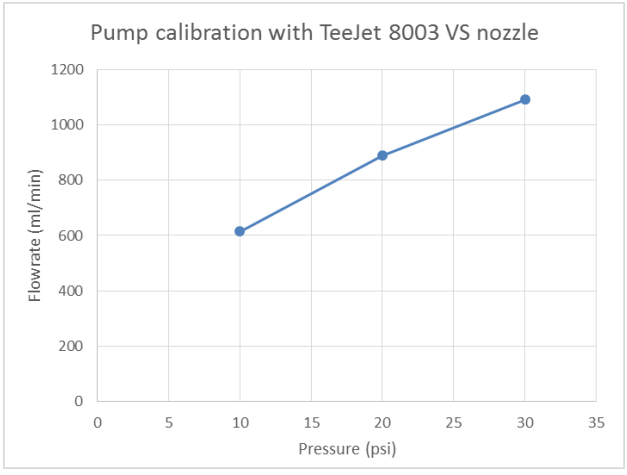


Figure 28. Calibration of Water Flow Rate Through TeeJet 8003VS Spray Nozzle

Inoculation efficiency was tested with the Vortex as shown in **Table 8**. LB400 (2 x 10^9 cell mL^{-1}) was diluted by 50% with water in the pump reservoir and one bucket of SediMite™ (11 kg) was added to the Vortex hopper. Pump and Vortex were run at rates indicted in **Table 8** and bioamended pellets were collected to determine the uniformity of inoculation based on qPCR and plating viability assays. Efficiency (unabsorbed inoculum) was estimated based on the volume of liquid collected at the outlet of the Vortex.

Table 8. Inoculation Rates and Efficiencies for TeeJet Nozzles Calibrated for Optimal Flow

Nozzle	Inoculation rate	Pellet velocity	Total cells/g (sd)	Viable cells/g (sd)	Inoculum loss
8003VS medium nozzle	700 mL min^{-1}	4.5 kg min^{-1}	5.9×10^7 (2.7)	2.1×10^8 (1.1)	40%
8001VS fine nozzle	456 mL min^{-1}	4.5 kg min^{-1}	5.5×10^7 (3.1)	2.4×10^8 (0.1)	25%

The results show that the system was effectively calibrated for consistent delivery of the target concentration of greater than 10^7 cells g^{-1} SediMite™. The 3-fold higher values of viable cells versus total cells reflects the presumptive nature of the qPCR assay when enumerating cells with the former representing the more accurate assessment. Both nozzles would achieve the target concentrations of cells, however, the fine nozzle (8001) reduced the loss of unabsorbed inoculum.

The modified Vortex was effective for combined inoculation and delivery of bioamended SediMite™. However, the system had the following limitations: 1) relatively low delivery rate; and 2) significant loss of inoculum. There was also an occasional problem with clogging in the hopper outlet, but this was attributed to inadequate vibration from the hopper vibrator, which could be easily adjusted to solve the issue.

The Modified Venturi Horn Induction Device

The second delivery system tested was a modified Venturi Horn Induction device (VHI); modified for delivery of SediMite™ by Brightfields, Inc. This pneumatic air blower uses high air pressure provided by a compressor to a venturi to create low pressure through the back end of the unit that draws in the pellets and entrains them in the air jet. For this project the air horn was modified with a 15 cm flexible tube at the vacuum intake to draw SediMite™ pellets into the unit from a tray and project them up to 30 feet (**Figure 29**).

Figure 29. Modified VHI Utilizes a Vacuum Generated by Compressed Air through a Venturi to Propel SediMite to the Desired Area.

The modified VHI was modified and tested by Brightfields. The unit successfully delivered SediMite™ at a rate of 45 kg min^{-1}, which is equivalent to coverage of 1 acre/day with a single unit. Dust generated by fines was an issue, but this could be controlled by increasing the moisture content of the SediMite™ prior to deployment or by modifying the formulation of binder in the SediMite™. The pellets were inoculated prior to deployment by spraying a premeasured volume of buffer containing a known concentration of microorganisms to the pellets with an electric pump. The buffered bioamendment was sprayed onto a known quantity of pellets in a cement mixer to ensure even distribution of the microorganisms (**Figure 30**). 27 kg of pellets sufficient for treating 14.8 m^2 were inoculated in 2 minutes.

The VHI device was effective for rapid delivery of bioamended SediMite™. The primary limitation of the system was requirement for inoculation of the pellets prior to deployment with the VHI. However, a method was developed utilizing a cement mixer and pumping cells with an electric mixer/pump system, which was calculated to be sufficient for the treatment 800 m^2 of sediment in this pilot study. This system is readily scalable for larger treatments by using a larger mixer or using a conveyor with a manifold to deliver the inoculum onto the pellets. Because of the consistency of the inoculum application on the pellets and minimal loss of inoculum compared with the Vortex, this approach was chosen for the pilot study.

Figure 30. Inoculation of SediMite™ Pellets Prior to Deployment with VHI Device.

Inoculum is sprayed onto SediMite pellets at a specified rate in a cement mixer.

5.3.5 Pilot Scale Biomass Production

Methodology was developed for scale-up of both the PCB halorespiring microorganisms and the aerobic degrading microorganisms in volumes sufficient for the proposed pilot-scale study. As stated in the Treatability Study Proposal applying 10^5 cells in the top 1 cm of a 0.25-acre plot would require 1.01×10^{12} cells. We estimated that this concentration of the anaerobe DF1 could be generated in the 250-liter scale-up vessel (10^7 mL^{-1} in 210 liters = 2×10^{12}) based on biomass production achieved in a 16 L working volume. The aerobic degrading bacterium is routinely grown to concentrations of 10^9 mL^{-1} in 1-liter culture bottle and we estimated that scale up in a 20-liter bioreactor (16 liter working volume) would yield 10^{13} cells, an order of magnitude greater than required for the field demonstration. The goal was to confirm our ability to grow both the anaerobe and the aerobe to a harvested concentration of 10^{12} viable cells in 20 L^{-1} and maintain viability during transport to the site.

5.3.5.1 Biomass scale-up of Anaerobic PCB halorespirer

The anaerobic halorespiring bacterium "*Dehalobium chlorocoercia"* DF-1 [6] was grown in co-culture with *Desufovibrio* sp. in E-Cl medium containing 200 μm perchloroethene (PCE). Inoculum was cultured in 10-160 mL serum vials containing 100 mL medium sealed under N_2-CO_2 gas mixture (4:1) with a butyl rubber septum. A carbonate buffer system is used to maintain a pH value of 6.8 for the duration of batch growth. Once the culture achieved 50% dechlorination it was transferred into 16 L of the same medium in a 20 L BioFlo IV bioreactor until 50% dechlorination of PCE, then into 210 L of medium in a 250-liter pilot-scale bioreactor (**Figure 31**). The culture was sparged with N_2-CO_2 and spiked with PCE three times after 50% dechlorination of PCE until a yield of 2.2×10^7 cells $mL^{-1}$was achieved. Once the culture had achieved 80% PCE degradation after the final spike of PCE it was chilled to 15ºC, then sparged with nitrogen to remove residual chlorinated ethenes (strain DF1 produces tri- and dichloroethene from PCE). Eighteen liters of culture was transferred to a 20 L Cornelius flask, which was then sealed. The remaining cells were harvested anaerobically from the fermentor with a continuous centrifuge (CEPA Z-41, 2-liter pellet capacity) at 17,000 × g and a rate of 2 L/min. The cell pellet was resuspended in the 18 L unconcentrated culture in the Cornelius flask. The flask was sealed under headspace of N_2-CO_2 and 200 μl PCE (100 μM) was added. The container was stored at room temperature (23-24 °C). The final yield was 1.3×10^8 cells mL^{-1} and cell loss during the harvesting process was 3.0×10^6 or approximately 14% of the total yield. The final yield of DF-1 harvested was 2.6×10^{12} cells, which was the yield required for the field demonstration.

Figure 31. BioFlo Bioreactor (20 L) and Pilot-scale Fermentor (250-liter) at IMET-UMBC for Scale Up of Microorganisms.

A 19 L SS Cornelius flask (A & B, front) will be used to transport concentrated inocula to site.

5.3.5.2 Biomass scale-up of Aerobic PCB degrader

Paraburkholderia xenovorans LB400 was grown aerobically in M9 minimal medium [13] with sodium benzoate as the carbon source and electron donor as described previously [14]. Inoculum was grown in five 1000 mL Erlenmeyer flasks containing 200 mL medium and incubated at 30 °C with shaking at 100 rpm. Once the culture has achieved an $O.D._{600}$ of 1.0 the cultures were transferred into 16 L of the same medium in a 20 L BioFlo IV bioreactor.

Once the culture achieved an $O.D._{600}$ of 1.0 in the BioFlo IV the culture was transferred to a 20-liter Cornelius flask that was stored at room temperature (23-24 °C). The final yield was 2.7×10^9 cells mL^{-1}, which is equal to a total yield of 4.3×10^{13} cells; an order of magnitude great than required for the field demonstration.

Prior mesocosm studies indicate that PCB degrading microorganisms maintain a steady state concentration of 10^5 cells mL^{-1} even when added in excess. Bench scale studies indicated that adding 10^5 each of anaerobic halorespirers to stimulate PCB degradation and when more cells are added the cells the numbers decline to a steady state concentration of 10^5 cells cm^{-1}. Applying 10^5 cells in the top 1 cm of a 0.25-acre plot will require 1×10^{12} cells. The results confirm that we could generate the required amounts of bioamendments with a single scale-up cycle of each bacterial strain for the proposed field demonstration.

5.3.5.3 Viability of concentrated cells for transport

Scaled up cultures of LB400 and DF-1 transferred to separate 20 L Cornelius flasks were monitored for total cell numbers and viability over time to determine the shelf life of the concentrated cell cultures and maximum allowable storage time for maintaining viability. Three 1 mL aliquots of concentrated cells were removed from containers on days 0, 1 and 7 or 8. Cells in triplicate aliquots were quantified by qPCR and final concentration was calculated as cells mL^{-1}. Viability of LB400 and DF-1 was determined as described in the Field Demonstration Work Plan.

The results shown in **Table 9** indicate that LB400 and DF-1 can be stored for a period of at least 7 days after harvesting and retain the required number of viable cells. These results would enable us to calculate the lead time required to prepare for the field demonstration.

Table 9. Effect of Storage on Total Cell Counts and Viability

Bacterial strain		Day 0	1 Day	7 Days	8 Days
LB400	Total	$(2.1 \pm 0.2) \times 10^9$	$(2.0 \pm 0.1) \times 10^9$	-	$(1.9 \pm 0.2) \times 10^9$
	Viable	$(2.2 \pm 1.5) \times 10^9$	$(2.7 \pm 0.7) \times 10^9$	-	$(1.3 \pm 0.3) \times 10^9$
DF-1	Total	$(1.3 \pm 0.2) \times 10^8$	$(6.3 \pm 3.1) \times 10^8$	$(1.5 \pm 0.5) \times 10^8$	-
	Viable*	$(2.7 \pm 3.5) \times 10^9$	$(2.8 \pm 1.5) \times 10^9$	$(0.7 \pm 0.5) \times 10^9$	-

* mRNA copies

The results indicate that we could generate the required amount of bioamendments for the proposed pilot filed study. The microorganisms have a shelf life of at least 1 week with acceptable loss of viability enabling us time to store and transport the bioamendments to the site after a single scale-up run.

5.3.6 Treatability Study Summary

The effectiveness of bioaugmentation in degrading and reducing the overall concentration of PCBs for the selected site was tested and optimized in 2 L sediment/water mesocosm systems as described in the Treatability Report. Sediment collected from the site was amended with several combinations of electron donor and biocatalyst concentrations adsorbed and delivered on SediMite™, hereafter referred to as bioamended SediMite™. As shown in **Table 9** below all performance objectives were achieved. PCB levels in sediment were most effectively reduced with 10^5 DF1 and LB400cells/g sediment and SediMite™ supplemented with 0.1 % cellulose.

The same level of degradation was observed throughout the 10 cm sediment bed. Porewater concentrations decreased to a greater degree in the presence of bioamendments compared to SediMite™ treatment alone. Scale up in bioreactors provided the required amount of cell mass for treatment of two 0.25-acre test plots proposed for Abraham's Creek that assumes 10^5 cells g^{-1} sediment at a depth of 1 cm. Key aspects of planning the field application was determining the optimal inoculum loading on SediMite™, the composition of SediMite™ with slow release carbon source (*e.g.*, cellulose) and the appropriate loading rate of treatment material into the sediment. Since this study was focused on bioaugmentation, the SediMite™ loading rate was kept constant at 3 % by dry weight of sediment in the bioactive zone. The venture air mover was chosen as the delivery method, demonstrated to deliver 45 kg min^{-1} of bioamended SediMite™, and cell viability was shown to be maintained both during the absorption process and after passing through a 1 m water column. The results demonstrated that the system was capable of deploying 10^5 cell/g sediment with application of 3 g SediMite™ 100 g^{-1} sediment within the bioactive zone. Successful completion of the treatability study based on quantitative performance assessment success criteria (**Table 10**) indicated that field testing could proceed.

The results Treatability Study are summarized below:

1) Levels of both higher and lower chlorinated congeners were reduced indicating that both anaerobic reductive dechlorination and aerobic degradation occurred concurrently

2) The only significant reductions in total levels of PCBs were observed in mesocosms bioamended with cell titers of 10^4 or 10^5 g^{-1} , with the most reduction, a mean of 78% after 120 days, in Treatment 6

3) The overall toxicity was reduced by up to 90% in Treatment 6 based on toxic equivalency of dioxin-like congeners in the sediments

4) Porewater concentrations of all PCB homologs were reduced after bioaugmentation by up to 88% after 120 days and up to 97% after 375 days

5) Replenishing the bioamendments after 375 days in Treatment 6 did not stimulate further reduction of PCB levels indicating the remaining PCB were no longer bioavailable

6) PCB levels were reduced throughout the 10 cm sediment column including both the aerobic and anaerobic zones as a result of bioturbation

7) Overall results indicate the SediMite™/cellulose with 10^5 DF1 and LB400 g^{-1} sediment will be most effective for PCB mass reduction for the field demonstration project

8) Sufficient amount of bioamendments were produced to complete the proposed pilot study.

9) The Vortex and VHI were successfully calibrated to deliver 10^7 cell g^{-1} SediMite™ with 25 % and 0% loss of inoculum during pellet inoculation, respectively.

10) There was no significant loss in viability during storage, transport and passage of bioamended SediMite through a 1m water column.

Table 10. Quantitative Performance Assessment for Treatability Studies

Performance Objective	Metric	Data Required	Success Criteria	Test Results
Quantitative Performance Objectives Treatability Studies				
Effect of treatment on total PCB degradation	Optimal ratio SediMite, microorganisms, cellulose/volume sediment	Rates/total depletion of PCBs in sediment treated in open flow mesocosms	>75% reduction of total PCB concentration within 120 days; reduction of PCB co-planer congener mass to less than 10 ppb	78% reduction of total PCB concentration with bioamendment at 120 days; reduction of PCB co-planer congener mass to 0.75 ppb
Effect of treatment on PCB concentration in porewater	Optimal ratio SediMite, microorganisms, cellulose/volume sediment porewater	Rates/total depletion of PCBs in sediment porewater treated in open flow mesocosms	>75% reduction of total PCB concentration within 120 days; reduction of PCB co-planer congener mass to less than 10 ppb	Up to 88% reduction in porewater concentration with bioamendment after 120 days; up to 97% reduction in porewater with bioamendments after 375 days; co-planar PCBs below detection limit
Effect of treatment on PCBs throughout sediment column	Compare effect of treatment on PCBs throughout depth of core	Rates/total depletion of PCBs in sediment + porewater in top 3 cm & bottom 3 cm	Mean removal of >75% PCBs within 120 days; reduction of coplanar PCBs to less than 10 ppb) from upper and lower core fractions	Same reduction levels of PCBs throughout 10 cm sediment bed
Quantitative Performance Objectives – Pilot Scale Delivery				
Delivery & Spatial Delivery of SediMite & Biocatalyst	Controlled rates of inoculum dispersion on sediments	Enumeration of aerobic and anaerobic cells on pellets using q-PCR	Modified Vortex and VHI calibrated for known inoculum flow rates	Calibrated for pellet inoculation at 1×10^7 cells/g SediMite
Delivery of viable catalysts through water column	Loss of cell viability of both aerobe and anaerobe at minimal to deliver target cell concentration without excessive cell material	Enumeration of aerobic and anaerobic cells on pellets after delivery through 1 m water column using q-PCR	Cell viability of aerobe & anaerobe maintained >50%	Cell viability of aerobe & anaerobe maintained at 100 %
Quantitative Performance Objectives – Scale-Up of Cell Material				
Biomass scale-up	Scale-up of anaerobe and aerobes to 10^{12} cells	Quantitative analysis of cells based on qPCR	Yield of 10^{12} viable cells resuspended in volume of medium required for dispersion	DF-1 yield 2.6 $\times$ 10^{12} viable cells LB400 yield 4.3 $\times$ 10^{13} viable cells
Biomass viability	Concentration and storage of anaerobe and aerobes at 10^{12} cells	Quantitative analysis of cells based on qPCR over time	Cell viability of aerobe & anaerobe maintained >50% for up to 7 days	No significant change in viability after 7 days

5.4 FIELD TRIAL: DESIGN AND LAYOUT OF TECHNOLOGY COMPONENTS

5.4.1 Loading Rate

Key aspects of planning the field application is determining the optimal inoculum loading on SediMite™, composition of SediMite™ with slow release carbon source (*e.g.*, cellulose) and determining the appropriate loading rate of treatment material into the sediment. Since this study is focused on bioaugmentation, the SediMite™ loading rate in the treatability study was kept low and constant at 3% SediMite™ by dry weight of sediment (equivalent to 1.5% AC by dry weight) in bioactive zone while the bioaugmentation dose was varied over 3 orders of magnitude (10^3 – 10^5 cells/g). Previous studies with AC used loading rates ranging from 3 – 5% AC in sediment [15]. We had proposed to determine site-specific loading rate of the microorganisms based on the results of the treatability study by choosing the lowest dose that would achieve the maximum extent of degradation in the period of the treatability study. As indicated in **Table 5** of the Treatability Study, the best performance of 78% reduction of total PCBs in 120 days was observed at an organism-loading rate of 1×10^5 cells/g sediment within the benthic zone (0.1 m). The loading rate for the pilot-scale application at Abrahams Creek was calculated based on the treatability results as summarized in **Table 11**.

Table 11. Loading Rate of SediMite™ and Cells in Abrahams Creek for Top 10cm and 50% Safety Factor

	Value	Units
Volume of sediment treated per square meter (1 sq. m. x 0.1m)	0.1	cu m
Dry mass of sed to be treated/sq. m. (dry bulk density of sed = 0.5 kg/L)	50	kg
Mass of native carbon/ sq m (at 6.7% by dry weight)	3.35	kg
Weight of SediMite™ per square m (3% SediMite™ by dry weight sediment)	1.50	kg
Mass of SediMite™/sq m plus 50% safety factor	2.25	kg
SediMite™ required for treatment area (3x400 sq. m)	2700	kg
Time to apply using 1 vortex (rate of application = 5 kg/min)	9.00	h
Number of aerobes and anaerobes (each) needed per sq. m.	5×10^9	# of cells
Total number of aerobes and anaerobes needed for one plot (400 sq. m.)	2×10^{12}	# of cells

5.4.2 Production of SediMite™

SediMite™ used for this pilot-scale demonstration contained 0.1% by weight of cellulose as an electron donor as described in the treatability study report. The remaining constituents of SediMite™ remained the same as the standard formulation (50% activated carbon, 30% sand, and 20% clay binder by dry weight). The residual moisture content ranged from 5-10%.

SediMite™ produced for this project was loaded into 500kg bulk bags and shipped on standard pallets loaded with two bags per pallet. Six bulk bags containing SediMite™ (three pallets) were delivered on site for the application. A fork-lift was used at the site to unload the pallets and position the bulk bags at the staging area near the access road adjacent to the proposed plots.

5.4.3 Production of Bioamendment

PCB organohalide respiring microorganisms and the aerobic degrading microorganisms were mass cultured in the scale-up facility at IMET-UMBC using a 250-liter pilot-scale bioreactor (210 liter working volume) and 20 liter bioreactor, respectively (**Figure 32**).

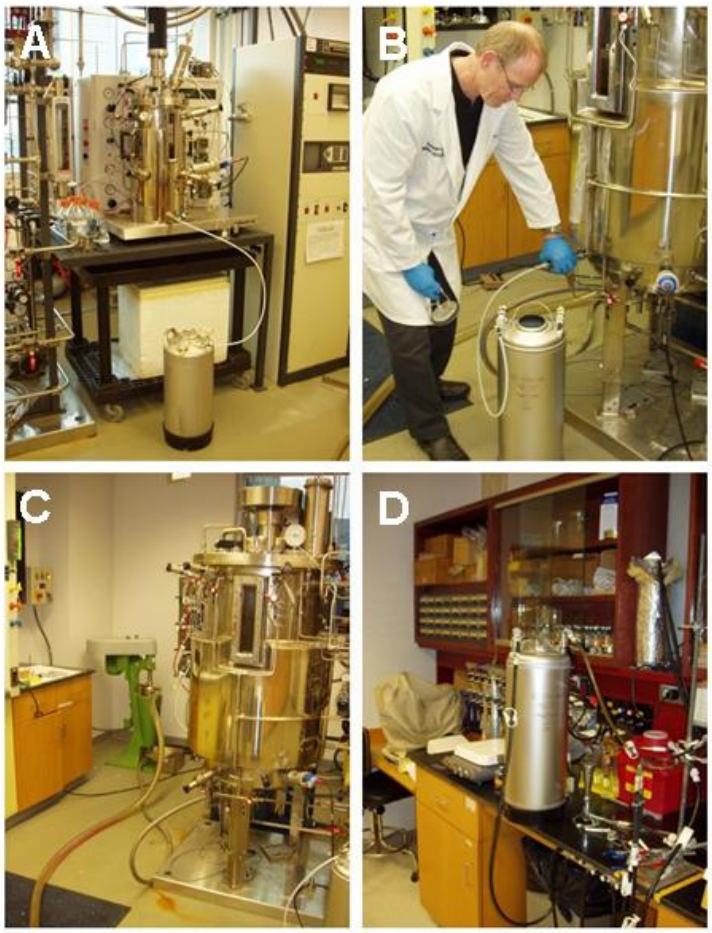


Figure 32. Scale-up of Bioamendments.

(A), The aerobic PCB degrader LB400 was grown in a 20 liter fermentor and harvested directly into a 20 L Cornelius flask; (B), anaerobic dechlorinator DF-1 was grown in a 250 liter fermentor and 18 L of culture was transferred to a 20 L Cornelius flask prior to harvesting; (C), remaining DF-1 culture was harvested with a continuous centrifuge, the cell pellet was washed from the bowl with 1 liter of medium and the concentrated cells were transferred to the medium in the Cornelius flask; (D) concentrated DF-1 culture was sparged with N_2-CO_2 prior to storage.

The anaerobic halorespiring bacterium "Dehalobium chlorocoercia" DF-1 [6] was grown in co-culture with Desulfovibrio sp. in E-Cl medium containing 200 μm perchloroethene (PCE). PCE was monitored throughout growth by GC-FID and replenished as required. The culture was grown statically at 20C. The 200 L DF1 culture was started 20 March 2015 by inoculation of 15L starter culture of DF1 grown in similar fashion in a 20L fermentor on PCE (cell density of starter culture of 2.2x107 cells per mL by qPCR). The 200 L culture of DF1 reached 50% dechlorination of PCE to trichloroethene (TCE), along with trace amounts of dichloroethenes (DCE), after 11 days (31 March 2015).

The 200 L culture was then spiked with an additional 200 uM PCE (second addition of PCE). The 200 L culture of DF1 reached 72% dechlorination of PCE to TCE, along with trace amounts of DCE after 7 days (1 April 2015). The headspace of the culture was sparged by N_2:CO_2 (80:20) along with stirring at 100 rpm for 5 mins to remove any chlorinated ethenes. The 200 L culture was then spiked with 400 uM PCE (third addition of PCE) and dechlorination was monitored. The 200 L DF1 culture was sparged and replenished with PCE as described above on days 25 (fourth addition of PCE) and 32 (fifth addition of PCE), then sparged without a re-spike of PCE on day 35 prior to harvesting (24 April 2015). Cells were anaerobically harvested with a CEPE Z41 continuous centrifuge and the cell pellet was backflushed with 20 liters of medium to obtain the desired concentration of cells for deployment. Cell density of the 200L DF1 culture before harvesting was $9.1 \pm 0.4 \times 10^6$ cells per mL (qPCR). DF1 cells were harvested by centrifugation and concentrated to about 10L in ECL media in a 20 L Cornelius flask. The headspace was N2:CO2 (80:20). Cell density of the concentrated DF1 culture was $1.7 \pm 0.2 \times 10^9$ cells per mL, for a total yield of 1.7×10^{13} cells in 20 L. The cell titer recovered after centrifugation and harvesting was 86% of the theoretical yield.

The aerobic degrading bacterium *P. xenovorans* LB400 [5] was grown in M9 mineral medium [13] supplemented with benzoate (5 mM) as the sole carbon and energy source at 30°C. The molality of the medium was adjusted with artificial sea salts [16] to be isotonic with the site water. LB400 was grown aerobically twice in a 20L fermentor (14L media). The first LB400 culture was started 20 April 2015 by inoculation of a 250 mL starter culture of LB400 grown on 10 mM biphenyl. The culture reached an $O.D._{600nm}$ of 1.06 after 48 hours, harvested by gravity into a 20 L Cornelius flask and stored at 20C. Most Probable Number (MPN) analysis of the first harvested LB400 culture confirmed 0.9×10^9 viable LB400 cells per mL. The second LB400 culture was started 22 April 2015. The culture reached an $O.D._{600nm}$ of 1.2 after 48 hours and was harvested by gravity into a 20 L Cornelius flask and stored at 20C. MPN analysis of second harvested LB400 culture confirmed 1.1×10^9 viable LB400 cells mL^{-1}. The average cell density of harvested LB400 was 1.0×10^9 cells per mL and a total yield of 2.8×10^{13} cells (in 20 L total).

Bench scale studies have indicated that adding 10^4 to 10^5 each of anaerobic halorespirers and aerobic degraders is sufficient to stimulate PCB degradation. Applying 10^5 cells in the top 1 cm of two plot areas of 0.1-acre plot required 10^{12} cells, which was generated in one batch for both the anaerobe (10^7 mL^{-1} in 210 liters = 2×10^{12}) and the aerobe (10^9 ml^{-1} in 20 liters = 10^{13}). Production time was three weeks for the anaerobe and one week for the aerobe. Based on treatability studies the maximum shelf life for no decrease in cell viability is 14 days for the aerobe and 2 months for the anaerobe, so both bioamendments were transported to the site within 14 days of harvesting.

5.4.4 Preparation of Biomended SediMite™

Concentrated DF1 and LB400 cells were taken to the site in Cornelius flasks and diluted into concentrated M9 media on site. The diluted cells:buffer mixture was then applied to pellets as described below. Concentrated M9 medium was made at 26.5x, which was the maximum solubility of the salts. Concentrated $MgSO_4$+$CaCl_2$ was made and added separately as they would precipitate in 26.5x concentrated M9. Water was obtained by collecting surface water from the Quantico Site and filtering it through a large funnel containing a steel mesh to remove particulates. This was done as chlorine in commercial water (tap water) is potentially bactericidal.

Cell:buffer mixture was made on the site in 19 Liter batches in a 20 L commercial sprayer tank as follows. Surface water from Quantico was added to the reservoir of the commercial sprayer using a graduated cylinder. Next, concentrated M9 Media was added using a graduated cylinder. After mixing concentrated $MgSO_4$+$CaCl_2$ was added using a graduated cylinder and mixed in the commercial sprayer by gentle rocking. Finally, the cells were added using a graduated cylinder and mixed by gentle rocking. Despite repeated opening and closing of the Cornelius flask containing DF1, the culture maintained adequate reducing potential to remain anaerobic throughout based on the redox indicator resazurin. The calculated concentration of DF1 in the cells:buffer mixture was $1.25x10^8$ cells mL^{-1}. The calculate concentration of LB400 in the cells:buffer mixture was $1.73x10^8$ cells mL^{-1}.

The bioamendments were applied to SediMite on-site using a 3.5 cu. ft. 1/2 HP cement mixer to ensure an even distribution on the pellets (Figure 33). The bioamended SediMite was sealed in 20 L buckets and transferred to the treatment site for application. The estimated concentration for DF1 and LB400 cells was $9.2x10^6$ and $1.3x10^7$ cells per gram pellet, respectively. This was close to the result observed from testing pellets: $9.2x10^6$ vs. $1.4x10^7$ (estimated vs. observed) cells $gram^{-1}$ pellet for DF1 and $1.3x10^7$ vs. $1.6x10^7$ (estimated vs. observed) cells $gram^{-1}$ pellet for LB400 (Table 12).

Figure 33. Staging Area at Abrahams Creek Used to Prepare Bioamended SediMite Prior to Deployment.

Table 12. Calculated Estimate Cell Concentrations in Cells:Buffer Mixture Added to Pellets

DF1 (cells mL^{-1}) in cells:buffer mixture	mL cells:buffer mixture 30 lbs^{-1} pellets	DF1 cells 30 lbs^{-1} pellets	grams/pound	Estimated DF1 cells $gram^{-1}$ pellet
1.25×10^8	1000	1.25×10^{11}	13,607	9.18×10^6
LB400 (cells mL^{-1}) in cells:buffer mixture	**mL cells:buffer mixture 30 lbs^{-1} pellets**	**LB400 cells per 30 lbs^{-1} pellets**	**grams/pound**	**Estimated LB400 cells $gram^{-1}$ pellet**
1.73×10^8	1000	$1.73\ 10^{11}$	13,607	$1.27\ 10^7$

5.4.5 Deployment of Bioamended SediMite™

Figure 34. Flat Bottom Boat Loaded with VHI System, Hoppers Filled with Bioamended SediMite and 20 L Buckets of Bioamended SediMite for Refilling Hoppers (left panel); Application of Bioamended SediMite™ at Abraham's Creek (right)

The test plots in Abrahams Creek were treated using the VHI system with a flexible duct connected to the inlet as shown in **Figure 34.** The VHI is designed specifically to disperse most granular or pelletized products used in an outdoor remediation and was modified for SediMite™ application on a previous remediation project. The VHI system uses compressed air to create a vacuum and draw the pelletized application material into an air stream and ejects the material to a distance up to 30 ft. The rate of application and distance of throw is controlled by the operator via a control valve. The VHI system used at Abraham's Creek horn was a-140 psi 6" horn fitted with a 20" intake hose (4" diameter). The compressor capacity was a 370 cfm to meet the requirements of the VHI. Due to the simple design there are no moving parts and clogging with bioamended SediMite was found not to be an issue. The unit is lightweight for use in small watercraft, in this case a 16' flat bottom boat. One person manually operated the VHI controlling the flow and dispersion of the pellets and a second person positioned the boat within the deployment quadrant based on GPS coordinates and ensured continuous flow of pelletized products from the hopper to the intake of the VHI. The 400 sq. m. areas (or a modified plot based on field observations) were subdivided into eight subplots and marked by stakes. Application began by positioning the shallow draft boat at the edge of the first strip to be treated. Application rate of SediMite™ and bioamendments were calibrated in advance and also monitored during application. The spreader nozzle was directed side to side to obtain an even application of SediMite™ in each sub-plot. SediMite™ application was continued until the required dose for the subplot was achieved. The hoppers were refilled once with buckets of bioamended SediMite pre-loaded onto the boat, then returned to shore for re-loading. All boating operations were performed according to the Health and Safety Plan (Field Demonstration Plan ER-201215).

5.5 FIELD TEST

Field testing was performed in close coordination with the RPMs and after approval of the demonstration plan by ESTCP and NAVFAC. The testing involved three phases: 1) initial baseline sampling, 2) application of treatment amendments in the field, and 3) subsequent monitoring visits to collect post-treatment samples. The application of treatment amendments was completed in 3 days and each sampling visit was performed in a day. Field activities are described below.

5.5.1 Initial Baseline Sampling and Marking of Test Plots

The main purpose of the baseline sampling was to characterize the pre-existing conditions at the site. Before collecting the baseline samples, the field crew surveyed the proposed locations and determined that the planned layout of the treatment plots was logistically feasible and appropriate. The crew also verified water depths along each treatment plot to ensure access with a boat. They did not exceed 305 cm during the highest water level in the spring. The corner of the treatment plots was identified using GPS coordinates and marked using a 1" polyvinyl chloride (PVC) pipe pushed into the sediment (**Figure 35**). Additional markers were inserted to indicate the five subplots.

Figure 35. Aerial View of Treatment Site along an Access Road at the Testing Site (A); Treatment Plots Marked Off with 1" PVC Pipe Prior to Treatment (B).

Samples of sediment cores were collected for PCB, black carbon, and microbial analysis as described below in Section 5.5, Sampling Plan, for baseline analysis. Three passive samplers were deployed in each plot to measure freely dissolved PCB concentration in the sediment porewater and overlying water. The initial baseline sampling was timed to occur at least 1-month before treatment application to allow retrieval of the passive samplers during the first day of mobilization for the application. The timing for each of these field events was coordinated with the site RPM. The placement and dimensions of the plots were not modified from the original Site Demonstration Plan.

5.5.2 Application of Bioactive SediMite™ in the Field

The field application of SediMite™ was conducted with assistance from Brightfields Inc., an environmental remediation company with prior experience with full-scale application of SediMite™ in the field. The field crew included personnel from UMBC-IMET (Dr. Sowers, and Dr. Payne), Sediment Solutions (Dr. Ghosh and additional personnel), and Brightfields Inc. (Mr. Jeff Vance and additional personnel). The deployment was completed within three days.

Day 1 (27 April 2015): Equipment, bioamendment and SediMite™ were mobilized at the staging and treatment areas. Passive samplers deployed during baseline monitoring (31 March 2015) were retrieved. SediMite™ without bioamendment was deployed in Plot 2. Unamended SediMite™ (2 x 500 kg bulk bags) was applied to the 400 m^2 plot for a final application at the dosing rate indicated in Table 11. Each 400 sq. m. treatment plot was divided into five 80 m^2 sub-plots. Based on the dosing calculation in **Table 11**, each subplot received 200 kg of SediMite™ transferred to the boat in 14 20 L buckets. This quantity of SediMite™ was loaded into ten 20 L buckets and placed on the boat. This activity was repeated five times to complete application in the entire treatment plot. Application of SediMite™ in the treatment plot was completed in approximately 4-5 hours.

Day 2: (28 April 2015): Bioamended SediMite (1000 kg) was prepared and deployed in Plot 3. 0.2 kg of bioamended SediMite was prepared and transferred to the staging area for loading onto the boat by the time treatment of the prior 80 m^2 subplot was completed. This resulted in an efficient, continuous operation. An additional 0.2 kg of bioamended SediMite was prepared for deployment the next morning and stored in airtight buckets stored in shade to protect it from heating by sunlight. In this way deployment could begin on day 3 as the next 0.2 kg of bioamended SediMite was prepared.

Day 3: Operation continued on day 3 to complete the application of SediMite™ with biological amendments in Plot 4, which was completed by 11:00. Equipment was packed and staging area was cleared by 13:00. A timeline for each phase of field testing is shown in **Table 13**.

Table 13. Schedule Showing Each Phase of Field Testing

Task	2015										2016										
	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11
Pre-sampling & plot layout																					
Passive sampler removal																					
Process samples																					
Bioamendment deployment																					
Post-treatment sampling 1																					
Process samples																					
Passive sampler removal																					
Post-treatment sampling 2																					
Passive sampler removal																					
Process samples																					

5.5.3 Sampling Methods

5.5.3.1 Spatial distribution of the amendment

One 4 L galvanized weighted steel buckets were randomly positioned within each of the five subplots. The buckets were attached to a nylon line and float for later retrieval. After the amendment was deployed the buckets were retrieved and excess water was carefully decanted. The containers were sealed with a metal lid and transported to the lab.

5.5.3.2 Sediment cores

The measure of performance involved comparing the treated plots to untreated plots. Data from these measurements was used to evaluate the reduction in total PCB levels and reduction in potential exposure based on PCB levels in porewater. Since DDx congeners are also reported Toxic Chemicals of Concern at the site and are co-assayed with PCBs we also monitored and report on the effects of treatments on DDx levels in porewater and sediment. Measurements based on differences between treated and untreated plots rather than relying solely on before and after measurements in the treatment plots accounted for temporal variability. Plots were randomly sampled at five locations at each time point to account for spatial variability of PCBs within and between plots. In each of the four plots, five sampling locations were randomly selected using a stratified random sampling strategy. This sampling strategy ensured that the sampling locations are more evenly dispersed within each plot, as opposed to spatially aggregated, which could occur if simple random sampling was used. This approach meets the criterion of random sampling for statistical tests. To obtain the five stratified random sampling locations, each plot was divided into five equal sub-areas containing the same number of possible sampling locations (**Figure 36**). The outside 3-foot edge of the plots was not included to ensure that the sampling locations are located within the actual treatment area. In each sub-area, a random sampling location was selected. These same sampling locations for the five plots will be used in all three sampling time points so that pair-wise comparisons could be made over time when appropriate. The total number and types of samples obtained from each plot at each sampling time point is in Data Analysis (Section 5.6).

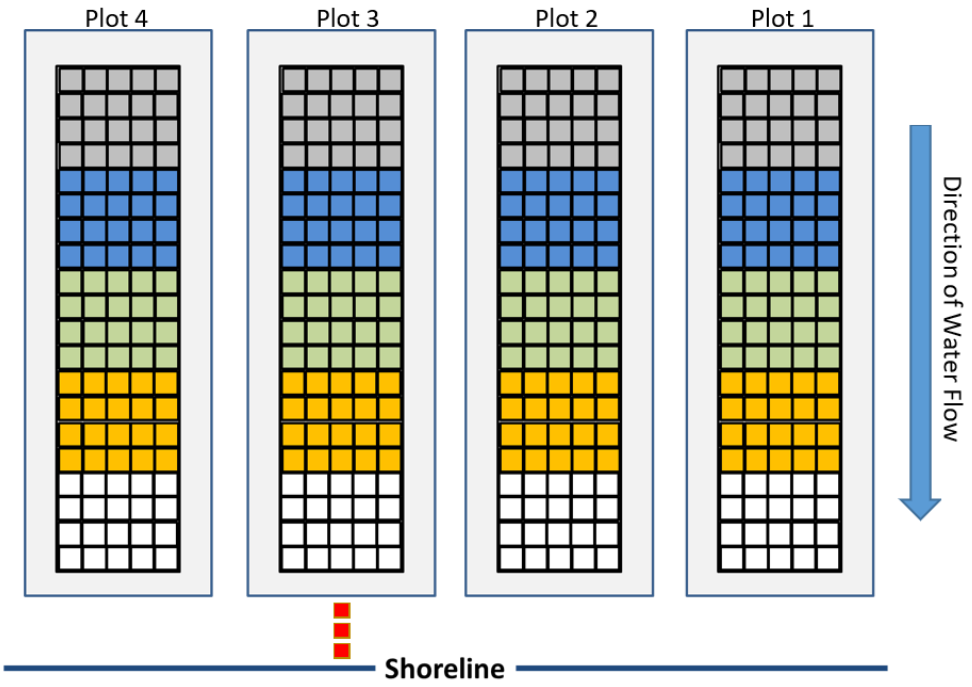


Figure 36. Schematic of the Five Sampling Locations in Each Plot.

Each of the five sub-areas (delineated by colors) has the same number of possible sampling locations. The outside edge of the plots was not included in the selection process to ensure that sampling locations are located within the actual treatment area. = downstream sampling locations. Water flowed into three 38 cm corrugated steel culverts located along the shoreline.

Sediment samples were also collected 0.6, 1.2 and 1.8 m downstream of plot 3 to measure potential drift of the amendment outside of the plot. The sediment samples were collected utilizing a Wildco® 5cm diameter hand core sediment sampler device. The hand core sediment sampler consists of a stainless-steel core body and cutting head, disposable eggshell-type core catcher, and an individual use 50 cm plastic sample liner tube. Samples were collected from a small boat.

5.5.3.3 PCBs, TOC and enumeration of bioamendments in sediments

The samples were collected by advancing the core device into the top 30 cm of sediment after contact with sediment was made (**Figure 37**). Care was taken to maintain the core device in a vertical position when advancing and removing the device so that the sediment disturbance is minimized. Immediately following the removal of the core device from the water, the 50 cm plastic sample liner tube was carefully removed from the core body maintaining the liner in the vertical position and caps were placed on each end to hold the sample within the liner. Liners were labeled and placed in a vertical position into an insulated cooler with ice for transfer to the lab where the individual sample transfer could be completed. Each core was logged with a physical description. In the lab, measured depths of 0-7.5, 7.5-15 and 15-30 cm from each sediment core were extruded and transferred into 250 or 500 mL I-CHEM borosilicate jars (**Figure 37**). Each sample within the top 0-7.5 and 7.5-15 cm was individually homogenized by mixing in the lab before subsampling for chemical and biological analyses. The portion of the core below 15 cm was archived since these samples are below the region of bioturbation. The intention was to process these samples if we found evidence that the bioamended AC had migrated below the lower portion of the 7.5-15 cm cores to determine the depth of mixing. All remaining samples were archived at -20 ^{o}C until completion of the project and approval of the final report.

Figure 37. Sediment Sampling with 5 cm Polycarbonate Lined Core Sampler (A); Extrusion and Subsampling of 7.5 cm Subcores Section in the Lab (B).

5.5.3.4 PCB in porewater

Freely dissolved PCBs in sediment porewater and overlying water immediately above the sediment were measured by passive sampling using polyethylene (PE) passive sampling. The preparation, deployment, removal, and extraction methods for the samplers are described in detail in the Field Demonstration Plan. Briefly, the field deployments consisted of a 15 x 15 cm 77 µm PE sheet encased in a stainless-steel mesh and frame for protection (**Figure 38**). The mesh and metal framed PE strips were embedded into the sediment using a 3 m pole designed to hold the frame.

The pole had a 15 x 15 cm metal platform perpendicular to the mesh frame that would prevent the frame from being pushed beyond the sediment surface. The PE strip was positioned in the sediment 0-15 cm below the surface. A second PE membrane encased in stainless steel screen was attached to the lead line near the surface of the water to measure PCBs in the water column. The samplers were attached with a nylon rope to a marked buoy placed on the water surface for ease of retrieval. After equilibrating in situ for at least 30 days the samplers were retrieved, rinsed with deionized water and sealed in I-CHEM Certified borosilicate jars. After retrieval the 0-15 cm strip of PE was sectioned into 0-7.5 and 7.5-15 cm depth intervals for measurement to correspond to the sections of sediment cores used for PCB and other analyses. The sample were transported on ice to the lab and stored at 4°C until they were processed.

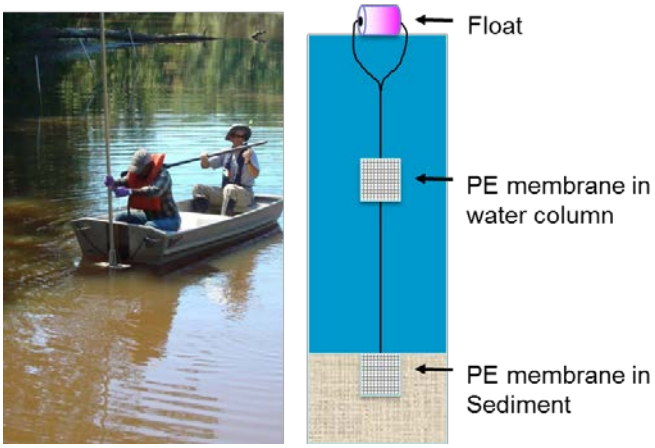


Figure 38. Assembly of 15 x 15 cm Passive Samplers Prior to Deployment (A); Insertion of Passive Samplers into Sediment (B).

Freely dissolved PCB concentration in the water phase was determined based upon the following equation:

$$C_w = \frac{C_{PE}}{K_{PE}}$$

where C_{PE} is the concentration of individual PCB congener in PE, C_w is the porewater concentration of the corresponding congener and K_{PE} is the PE-water distribution ratio. Values of K_{PE} presented in Ghosh *et al.* [9] were used for the calculations. Five performance reference PCB compounds were included in the PE samplers to correct for non-equilibrium conditions and assess porewater concentration using the first order non-equilibrium correction method as described in Oen et al., [17]. The five non-Aroclor congeners chosen for PRCs in this study were PCB 29, PCB 69, PCB 103, PCB 155, and PCB 192.

5.5.4 Analytical Methods

The Field Study included the following critical measurements: PCB concentrations in sediments or soil fractions, total organic carbon, and sediment or soil moisture content. In addition, the growth and survival of the microbial inoculum containing DF1 and LB400 was monitored with quantitative PCR (qPCR). Finally, growth of DF1 in the lab was monitored by GC-FID analysis of chloroethenes (CE) dechlorination. The acceptance criteria for data quality objectives of the different measurements are described in Appendix D of the Treatability Work Plan.

For analysis of PCBs, analytical precision were verified through the analysis of sample duplicates and accuracy were assessed through the analysis of laboratory blank samples, surrogate PCB spike samples, and matrix spike samples. For analysis of CE, analytical precision will be verified through the analysis of sample replicates and accuracy will be assessed through the analysis of laboratory blank samples, surrogate CE spike samples, and matrix spike samples. Details of the QA/QC plans are described in Appendix D of the Treatability Work Plan. Analytical methods are described briefly below. Detailed analytical methodology, calibration, QA and sample documentation are provided in the SOPs are provided in Field Demonstration Work Plan.

5.5.4.1 Total organic carbon and activated carbon

TOC in sediment was determined by carbon combustion and CO_2 analysis with a non-dispersive infrared gas analyzer (NDIR). The activated carbon analysis uses a chemical oxidation method to burn off a major portion of the natural organic carbon while preserving most of the activated carbon in the sample. This technique for measuring activated carbon in sediments is based on Grossman and Ghosh [18] as described in SOP-13 Appendix D of the Treatability Work Plan.

5.5.4.2 Temperature monitoring

A temperature data logger was placed in the top 5 cm of sediment in test plot 3 for the purpose of assessing the effects of temperature on PCB degradation rates. However, this data were not collected because the data logger was lost prior to the 140 day sampling event.

5.5.4.3 PCB extraction

Sediment samples were extracted using an Accelerated Solvent Extractor (Dionex) following EPA Method 3545 as previously described [2]. Briefly, approximately 5 g wet weight sediment is dried in a desiccator with pelletized diatomaceous earth (Dionex) at room temperature. The dried sediment (1 g) is extracted in an 11 ml stainless steel extraction cell containing 0.6 g Cu and 2.4 g Florosil on the bottom of the cell and anhydrous Na_2SO_4 in the remaining void volume. PCB 166 (10 µl stock of 400 µg l^{-1} hexane) is added as a surrogate to correct for extraction efficiency. Each sample is extracted with 20 ml of pesticide grade hexane (Acros Organics) at 100°C and purged with 1 MPa nitrogen. The extract is evaporated to a final volume of 1 ml at 30°C under nitrogen and 10 µl of PCB 30 and PCB 204 (400 µg l^{-1} each in acetone) are added as internal standards.

5.5.4.4 PCB analysis

PCB congeners were analyzed using a Hewlett-Packard 6890 series II gas chromatograph (GC) with a DB-1 capillary column (60 m by 0.25 mm by 0.25 µm; JW Scientific) and a ^{63}Ni electron capture detector by a modified method of EPA 8082 as previously described [2]. Briefly, PCB congeners in a mixture containing 209 congeners were quantified with a 10-point calibration curve using PCB 30 and PCB 204 as internal standards. Using this protocol 206 congeners are resolved in 150 individual peaks (excluding internal standards PCB 30 and PCB 204 and surrogate PCB166). Organochlorine pesticides have the potential to co-extract and co-elute with PCBs and DDx is present in ppm concentration at this site. However, we determined that only two DDx congeners co-elute with PCB congeners in our assay. Congener *p,p*-DDE co-elutes with PCB 145, however, this congener is tetra-*ortho*-chlorinated and does not occur in Aroclors or dechlorination products of Aroclors. Congener *p,p*-DDT co-elutes with co-eluting PCB congeners 130/137/176. All three congeners

make up no more than 0.6 % of some Aroclors and tandem GC-MS analysis confirmed that the co-eluting peak from Abraham's Creek detected at this retention time was composed only of *p,p*-DDT; no PCB is detected. Based on our GC and tandem GC-MS analysis DDx congeners do not interfere with the quantification of PCBs using the above extraction/ analysis protocol.

5.5.4.5 DNA extraction

DNA is extracted by adding 0.25 g of sediment from each sample core to a PowerBead microcentrifuge tube (Power Soil DNA Isolation Kit, MOBIO Laboratories, Inc.) as previously described [2]. Extracted DNA samples will have an A260/280 ratio of ≥ 1.6 and an A260/230 ratio of ≥ 2.0. All DNA samples are diluted to 2 ng/µl in TE buffer (10 mM Tris, 1 mM ethylenediamintetraacetic acid, pH 8.0) and stored at -20 ºC.

5.5.4.6 Enumeration of biocatalysts

Enumeration of putative halorespiring Chloroflexi in each subcore was performed by quantitative PCR (qPCR) using iQ SYBR Green Supermix (Bio-Rad Laboratories) and primers specific for the 16S rRNA gene of a deep branching, putative dechlorinating clade within the Chloroflexi (348F/884R) [19] as described previously. Alternately, primers SKFPat9F and SKFPat9R, targeting a putative reductive dehalogenase specific to DF1 are used to quantify DF1 only [3]. PCR with SKFPat9F/SKFPat9R is performed using the following program: initial denaturation at 95 ºC for 5 min; followed by 35 cycles of 95 ºC for 45 sec, 55 ºC for 25 sec, and 72 ºC for 25 sec. Enumeration of LB400 in each subcore is performed by quantitative PCR (qPCR) using iQ SYBR Green Supermix and primers specific for the upstream region of the LB400 *bphA* gene operon (CIOP0/CIOP1, Table S1) [20]. PCR with CIOP0/CIOP1 is performed using the following program: initial denaturation at 95 ºC for 5 min; followed by 35 cycles of 95 ºC for 45 sec, 58 ºC for 30 sec, and 72 ºC for 30 sec.

5.5.4.7 Community analysis of PCB dechlorinating bacteria

Genomic DNA was amplified by PCR using the 515F and 806R primers as detailed in the official protocol of the earth microbiome project (http://www.earthmicrobiome.org/). Amplicon pools were sequenced on an Illumina MiSeq and an average of 17,000 sequences per sample were recovered. For analysis, the QIIME (v1.9.1) bioinformatics pipeline and Phyloseq R package were used. OTUs were picked using open reference OTU picking (pick_open_reference_otus.py, default settings) against the Greengenes 13.8 database. For diversity analyses, sequences were rarefied to 9500 sequences per sample.

5.5.4.8 Special training needs/certification

All research personnel working in the laboratory underwent safety training from the University of Maryland Baltimore Environmental Health and Safety (UMB-EHS). General laboratory safety guidelines were followed by all researchers in the laboratory. Laboratory personnel handling PCB- and CE-containing solids or liquids used protection equipment such as nitrile gloves and safety goggles. Work using organic solvents was always conducted inside certified fume hoods. Solid and liquid wastes produced during the research was stored in marked containers and disposed according to the guidelines of the UMB-EHS. Proper logs were kept for all organohalide-containing materials used in the laboratory. All analysts performing organohalide analyses were directly trained and supervised by the PI. The analyst should be able to analyze and quantify a multi-point calibration and quantify a known organohalide concentration within established limits.

5.5.4.9 Documents and records

This Quality Assurance Project plan formed the basis for all experimental and analytical work carried out in this research project. Copies of the plan were provided to the lab personnel who will carry out the research in the laboratory. The PI was responsible to ensure that all lab personnel have carefully read this document and are following the document in carrying out the research project.

All experiments and analytical work carried out in the project were documented in detail in bound laboratory notebooks, which were routinely checked by the project principal investigator. In addition, chromatographic data from all PCB analysis are stored on computer hard disk and backup copies were stored on a cloud-based backup server and on DVD disks. All quantification data was stored in a spreadsheet file for further evaluation and calculations. In addition to test files and QC data, the spreadsheet data report includes the identification of outliers, details regarding the corrective actions taken, and discussion of any necessary deviation from the protocols established in the referenced methods. All hard copy documents and computer data records will be stored and archived by the principal investigator for no less than 5 years after completion of the project.

5.5.4.10 Sampling methods, handling, and custody

Sediment samples collected were preserved at 4^{o}C. The chain-of-custody record remained with the sample from the time of arrival through analysis, experimentation, and final disposition. Upon arrival, the sample custodian logged in the samples, checked for and resolved any discrepancies, and provided unique laboratory identifications. Samples were stored at or below 4°C upon arrival.

Organohalide-containing solid or liquid samples were stored in glass vials with Teflon-lined caps at or below 4°C in darkness until analysis. Holding time was less than 1 month for all samples generated in the laboratory. All organohalide waste materials generated in the analytical sample processing were disposed through UMB-EHS.

5.5.5 Sampling Plan

The field demonstration project was designed to evaluate the effect of treatment combinations on a set of established performance criteria. In statistical terms, this design contains three main factors (or main treatments), and a series of response variables (equivalent to performance criteria). Plot 1 did not receive SediMite™ or bioamendment and, therefore, is defined as the untreated control for evaluating treatment effects. Plot 2 received SediMite™ without bioamendment and, therefore, is defined as a treatment-specific control to differentiate any abiotic effects by SediMite™ alone from the bioamendments. Plots 3 and 4 were replicate plots treated with bioamendment to account for spatial variability and variability of treatment application. Measurements for the primary (e.g., total PCB and aqueous PCB concentrations) and secondary (e.g., effects on the indigenous microbial population) were made at four discrete time steps: 1 month before treatment application, 4.3 months post-treatment, and 13.6 months post-treatment (**Table 14**).

For the first sampling event (1-month pre-treatment), the objective of the data analysis was to define baseline conditions for all treatments. A principal question addressed at this step was whether sediment concentrations of PCBs were homogeneous across all plots. This was accomplished using parametric one-way analysis of variance (ANOVA). Samples across a transect of all four proposed plots were used to locate a large area with consistently elevated and relatively homogeneous concentrations of PCBs for the field study. Comparisons between treatments and controls were made using Student's t-test (t test).

Table 14. Numbers and Types of Samples Collected

Parameter	Matrix	No. of Samples	Analyte[1]	Location
Pre-treatment	Sediment core	20	TOC, black carbon, PCBs, bioamendment, indigenous microbial community	5 cores/Plot
	Porewater	12	PCBs, sediment temperature	Insert passive samplers 3/plot, temperature data logger
Post-treatment D140	Sediment core	20	PCB, bioamendment	5 cores/Plot
	Porewater	12	PCBs, sediment temperature	Retrieve/insert passive samplers 3/plot, temperature data logger[1]
Post-treatment D409	Sediment core	20	TOC, black carbon, PCBs, bioamendment, indigenous microbial community	5 cores/Plot
	Porewater	12	PCBs, sediment temperature	Retrieve/insert passive samplers 3/plot, temperature data logger[1]

[1] Sediment temperature was not monitored as proposed because the data logger was severed from the float and could not be retrieved.

Since the microbes used for bioamendment require a temperature range of 20 to 30^{o}C for maximum activity, bioamended SediMite™ was be deployed in late spring (April) when the sediment temperature would begin approaching 20 ^{o}C.

The first post-treatment sampling event immediately after deployment: 1) established immediate post-treatment effects, and 2) confirmed homogenous distribution of the SediMite and microorganisms.

The second and third post sampling events (4.3 and 13.6 months) determined the efficacy of bioamended SediMite™ for reducing PCB concentration as a function of time. This consisted of evaluating total PCBs in sediment and bioavailable PCB in the aqueous phase. We assessed the biological effects of the bioamendments by comparing with day 0 data using two-group (t-test) tests. We assessed the sustainability of the bioamendments by enumerating the remaining cells and perform a microbial community analysis to determine if there were any long-term changes in the indigenous microbial community as a result of treatment. Sorenson's similarity coefficient [21] were used as a diversity index for comparisons of community profiles between treatments. If an index of 1 was obtained the samples were considered identical, while 0 indicates that the samples have no similarity [22]. Alpha values of 5% were set to determine significance in all applicable statistical analyses. The analytical methods for sample analyses are summarized in **Table 15**.

Table 15. Analytical Methods for Sample Analysis [a]

Matrix	Analyte	Method	Container	Storage conditions	Storage time
Sediment	PCBs	EPA 3545/8082	Analytical grade borosilicate jar w/ Teflon-lined lid	4 ^{o}C	30 days
	TOC	SOP-10		4 ^{o}C	30 days
	Black carbon	SOP-13		4 ^{o}C	30 days
	Bioamendment enumeration	SOP-6		-20 ^{o}C	14 days
	Indigenous microbial community	SOP-11		-20 ^{o}C	14 days
Porewater	PCBs	SOP-12/EPA 8082		4 ^{o}C	30 days

[a] For each sampling event and each treatment plot, sediment will be archived for PCBs and TOC/black carbon in either an 235 or 470 mL borosilicate jar with a Teflon-lined lid stored at -20 ^{o}C.

5.6 SAMPLING RESULTS

5.6.1 Distribution of Amendment in Test plots

Plot 2 was treated with 1000 kg (1 Ton) of SediMite™ and Plots 3 and 4 were treated with 1000 kg (1 Ton) each of bioamended SediMite™ (**Figure 39**). Two types of SediMite™ were prepared for the study (**Figure 40**). Extruded pellets (3000 kg) were prepared by an international manufacturer, but because there was the possibility of delay in shipment a second SediMite™ preparation synthesized with a cold press by a local US manufacturer was ordered to assure arrival within the timeline. By the time the deployment date arrived the pelleted SediMite™ was still in shipment and the decision was made to use the available cold press form. Non-bioamended cold-press type SediMite was deployed in treatment plot 2 on Day 1. This form of SediMite™ generated a great deal of dust during distribution with the VHI. The dust could have been somewhat mitigated once the pellets were inoculated with bioamendment, which would have increased the water contents of the pellets. However, the extruded-type SediMite™ arrived later that day and the decision was made to treat plots 3 and 4 with the extruded pellets as originally planned. The achieved loading of amendments based on sediment collection trays is shown in **Figure 41**. The range of achieved loadings was similar in all three plots, but the median and mean loading of amendment in plot 2 was significantly less than the other two plots. A mean loading value of 0.71 Tons (SD 0.6) indicated an average loss of 30% amendment as a result of the loss of SediMite™ observed in the form of dust during the application in Plot 2. In contrast, Plots 3 and 4 treated with pelleted amendment had achieved loading values of 0.95 Tons (SD 0.65 and 0.58, respectively) indicating an average loss of only 5%. Converting the data to kg m^2 (**Table 16**), only Plot 4 met spatial distribution target of 70% of samples within ± 50%. This variation indicated uneven distribution with VHI. Overall, the results indicate although the pelleted form of SediMite™ was successfully delivered at the desired mean dose within the targeted area of plots 3 and 4, there was considerable variation in the application amount within the plots.

Figure 39. Application of Amendment with a VHI from a Flat Bottom Boat.

High pressure air compressor is visible on shore in upper right of figure.

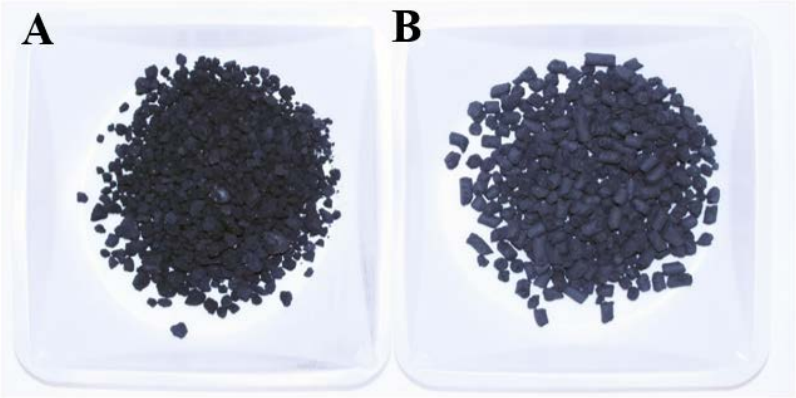


Figure 40. Image Showing Two Types of SediMite Used in the Study: Cold Press Granules (A) and Extruded Pellets (B).

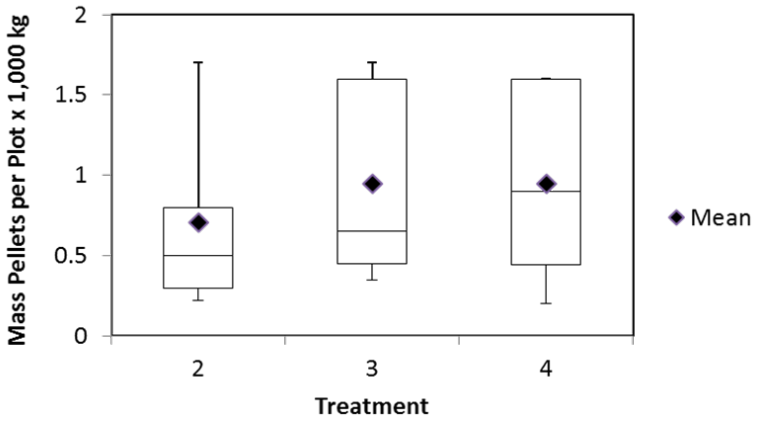


Figure 41. Distribution of SediMite in Plot 2 and Bioamended SediMite in Plots 3 and 4 Based on Five Collection Trays Placed in Each Plot.

Table 16. Distribution of SediMite in Plot 2 and Bioamended SediMite in Plots 3 and 4 Based on Five Collection Trays Placed in Each Plot.

	Plot 2	Plot 3	Plot 4
Subplot	Mass (kg m^2)	Mass (kg m^2)	Mass (kg m^2)
1	.89	3.74	1.2
2	1.57	.66	3.14
3	.40	.84	1.15
4	4.40	4.41	nd
5	1.17	2.29	4.04
Mean	1.69	2.39	2.38
SD	1.6	1.7	1.4
% of samples that met target 1.5 ± 50% kg m^2	40%	20%	75%

5.6.2 Total Organic Carbon and Black Carbon Analyses

Pre-treatment sediment cores were taken on 30 March 2015 and post-treatment sediment cores were taken on 15 September 2015 and 14 June 2016 (**Figure 42**). Sediment cores were retrieved from randomly selected locations within each of the five subplots located by GPS. Winds >15 k hr^{-1} on pre-treatment cores made accurate placement of the boat difficult during the coring process, but all samples were successfully retrieved from within the designated treatment subplots.

Figure 42. Sediment Sample Locations on Days 0 (pre-treatment), 140 and 409 (post-treatment).

Sample locations are numbered as follows: plot 1, SED1-5; plot 2, SED 6-10; plot 3, SED 11-15 and plot 4, SED 16-20. Sediment samples taken 0.6, 1.2 and 1.2 m downstream of plot 3 are indicated by triangles. Sampling dates for individual sediment cores are indicated in the figure legend.

Total organic carbon (TOC) was analyzed to determine the amount organic carbon in the plots, which includes both native carbon and carbon as SediMite™. As illustrated in **Table 17** and **Figure 43** the mean TOC ranged from 2.2 to 3.2% native carbon all plots prior to treatments.

As expected the mean values increased in treated plots 2, 3 and 4. The mean values in plot 4 after treatment was considerably greater than in plot 3 and there was a greater range on day 409 in plot 4. This observation indicates that the application was not homogenous between the selected sample locations.

Table 17. Total Organic Carbon Detected in the Upper 7.5 cm of Core Samples from Each Treatment Plot.

	Plot 1		Plot 2		Plot 3		Plot 4		Downstream	
Day	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
-30	3.2	1.1	2.6	1.0	2.3	1.8	2.2	0.9	ND	ND
140	3.4	0.7	4.7	0.5	4.8	1.9	7.6	1.9	2.0	0.5
409	3.3	1.4	5.9	2.8	3.2	1.7	7.6	7.4	1.3	0.3

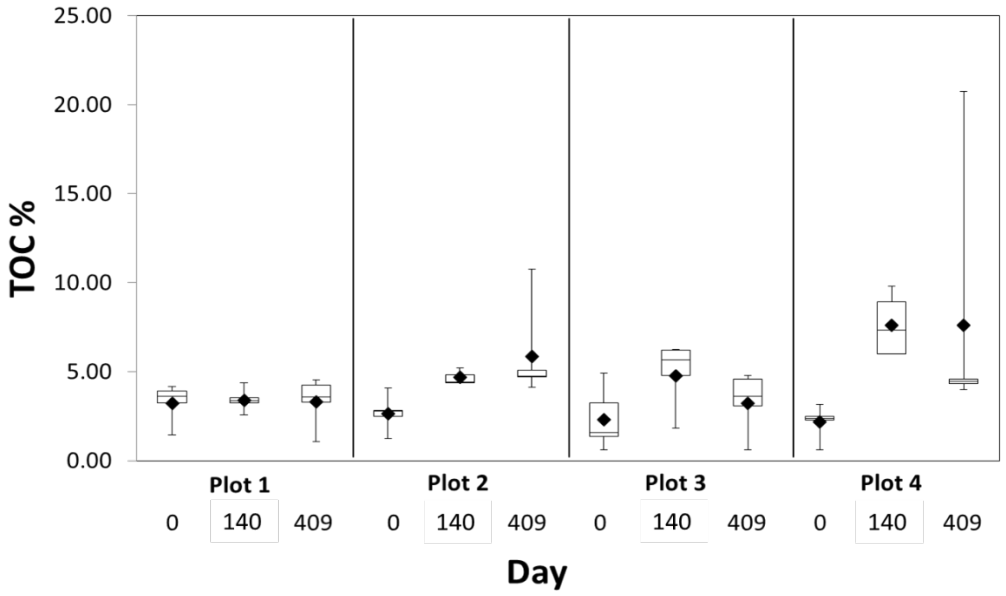


Figure 43. Percent Total Organic Carbon Detected in the Upper 7.5 cm of Core Samples from Each Treatment Plot.

Black carbon (BC) was analyzed to determine the background levels and distribution of BC were SediMite™ within sample cores form all four plots. As illustrated in **Table 18** and **Figure 44**, background levels of BC ranged from 0.2 to 0.4% in all four plots. As expected higher levels of detected in plots 2, treated with SediMite™, and plots 3 and 4, treated with bioamended SediMite™. As we observed with the sediment collection trays the amount of BC varied between sample locations in each plot. However, the mean values observed in plot 4 were greater than those observed in plot 3. Although the same amount of measured amendment was applied to each plot (1000 kg) the wide variation reflects the amount of BC reflects the amount detected in cores from five locations within the plot. The overall results indicate that the bioamended BC was not evenly distributed within each plot, which would result in the wide range of concentrations observed among the five-sample core in each plot.

Table 18. Black Carbon Detected in the Upper 7.5 cm of Core Samples from Each Treatment Plot.

	Plot 1		Plot 2		Plot 3		Plot 4		Downstream	
Day	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
-30	0.4	0.2	0.3	0.2	0.2	0.2	0.4	0.4	ND	ND
140	0.4	0.2	0.6	0.1	1.6	1.3	3.9	2.3	0.3	0.2
409	0.6	0.3	2.1	2.8	0.7	0.4	7.0	8.2	0.4	0.2

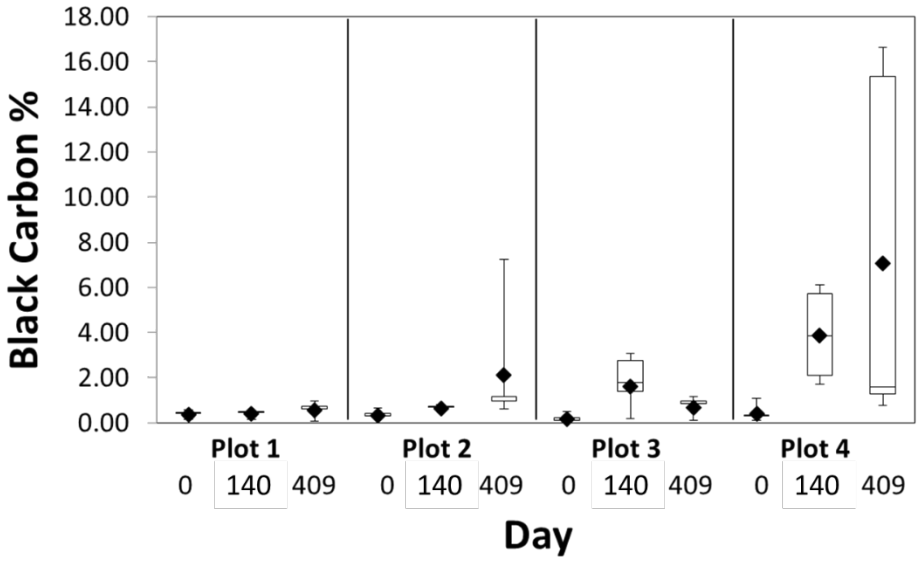


Figure 44. Percent Black Carbon Detected in the Upper 7.5 cm of Core Samples from Each Treatment Plot.

The mean concentrations of BC 0.6, 1.2 and 1.8 m downstream of plot 3 was at background level, which indicates that bioamended BC remained in place throughout the 409-day post-treatment period.

5.6.3 Effect of Treatments on PCB Concentrations in Sediments

Table 19 and **Figure 45** show the effect of treatments on total PCB concentrations in the sediments. The only significant decrease in PCB concentration is observed in the top 7.5 cm of bioamended treatments 3 and 4 ($p<0.05$). The mean rate of degradation for treatments 3 and 4 was 1.7 (r^2=0.0.29) and 3.2 (r^2=0.0.54) µg kg^{-1} day^{-1}, respectively (**Figure 46)**. The estimated rate in the field is lower than that observed in treatment 6 of the mesocosm study, where 65% of the PCB was degraded in the first 30 days at a rate of 73 µg kg^{-1} day^{-1}. However, unlike the mesocosm study, the field treatment was not artificially mixed and the temperatures ranged from sub-zero to approximately 20°C over the course of the 409 day incubation period rather than a constant 20°C. Despite the disparity in rates the data shows that the bioamendment reduced the PCB concentration at a slower but significant overall rate.

Table 19. Effect of Treatments on Reduction of Total PCB Levels after 409 Days.

	Day 0		Day 140		Day 409		
Treatment (core depth)	**mg/kg**	**Percent decrease***	**mg/kg**	**Percent decrease***	**mg/kg**	**Percent decrease***	**Significance (p<0.05)‡**
1 (0-7.5 cm)	2.6 ± 0.9	—	3.1 ± 0.6	—	2.4 ± 0.5	8	—
2 (0-7.5 cm)	2.5 ± 0.5	—	2.9 ± 0.6	—	2.6 ± 0.8	—	—
3 (0-7.5 cm)	2.3 ± 0.5	—	1.9 ± 0.8	17	1.6 ± 0.3	30	yes
4 (0-7.5 cm)	2.5 ± 0.3	—	1.8 ± 0.7	28	1.2 ± 0.3	52	yes
1 (7.5-15 cm)	2.9 ± 1.1	—	3.3 ± 1.0	—	3.7 ± 0.8	—	—
2 (7.5-15 cm)	3.0 ± 0.5	—	3.6 ± 0.7	—	3.3 ± 0.8	—	—
3 (7.5-15 cm)	2.7 ± 0.8	—	4.2 ± 1.2	—	3.5 ± 0.6	—	—
4 (7.5-15 cm)	2.5 ± 0.6	—	3.4 ± 1.0	—	3.0 ± 1.1	—	—

*Percent decrease of mean value compared to day 0

‡T-test for day 0 vs. day 409

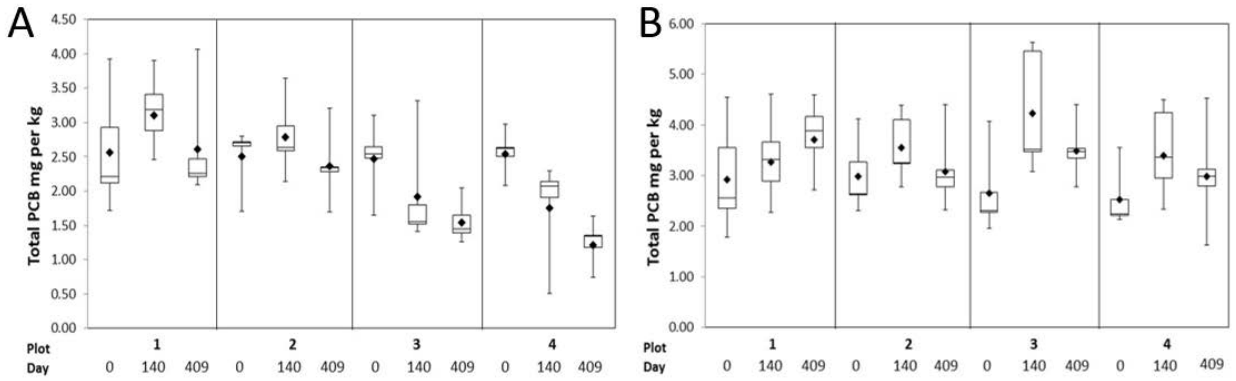


Figure 45. Total PCB Concentrations in 0-7.5 cm (A) and 7.5-15 cm (B) Sediment Cores.

Calculations based on five sample cores for each plot at locations shown in Figure 42. Mean values represented by (♦).

The continuity of degradation over time in Treatments 3 and 4 suggests that this trend will continue, but this can only be confirmed by continued monitoring to obtain more than three datum points. There was no detectable decrease in PCB concentration below 7.5 cm. This is not unexpected since the sediment below 7.5 cm is likely below the benthic zone where natural mixing of the bioamendment by bioturbation is expected to occur. There was also no detectable change in PCB concentration observed in three sediment cores taken downstream of plot 3 (data not shown), indicating there was no movement of the bioamended SediMite™ outside of the plot.

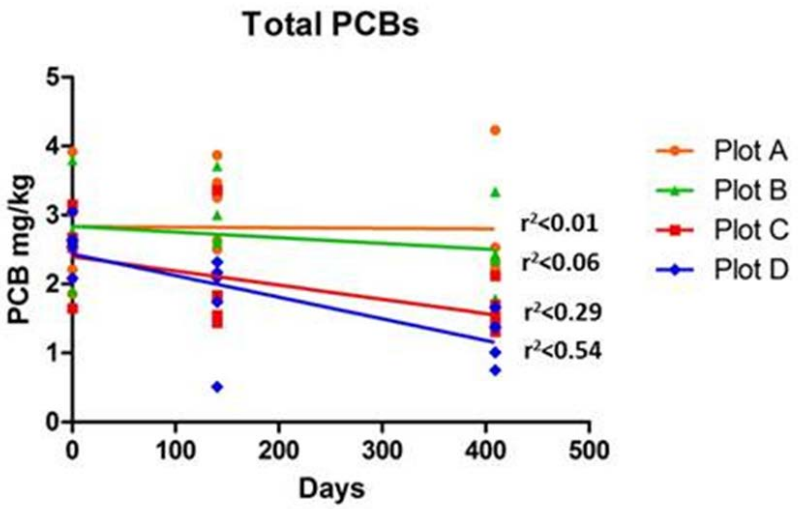


Figure 46. Linear Regression Analysis of Total PCB Concentrations in 0-7.5 cm of Sediment Cores.

R^2 values are shown adjacent to each line of best fit. Calculations based on five sample cores for each treatment plot indicated in legend from locations shown in Figure 42.

Although treatment of the two bioamended plots 3 and 4 was identical, plot 3 showed less PCB reduction then plot 4. In order to explain the range of PCB degradation levels within and between bioamended plots 3 and 4, the amounts of BC in the individual sample cores were compared with the reduction of PCB levels. As shown in the **Figure 47,** there was a direct relationship between the amount of BC in an individual sample and the extent of PCB degradation. Four lines of evidence indicate that the decrease in PCB was due to microbial activity and not the black carbon. 1) There was no significant difference in PCB levels between the untreated control and plot 2 treated with SediMite. 2) A significant decrease in total PCB levels was only observed in bioamended plots 3 and 4. 3) Extraction efficiency using methods described in this study is not affected significantly ($p>0.05$) by different amounts of black carbon (**See Appendix**). 4) Even after excluding the two outliers in plot 4 (**Figure 47**) that had exceptionally high concentrations of black carbon (15.3 and 16.6%), the decrease in total PCB concentration was 43% instead of 52% compared with Day 0 and was still statistically significant ($p<0.05$). Furthermore, reduction of PCB levels in Abraham's Creek sediment was already confirmed to occur by bioaugmentation in the mesocosm treatability study using the same dosage of cells under controlled conditions and uniform AC application, whereas no significant reduction was observed in controls treated with black carbon without bioamendment. The results indicate that increasing the amount of bioamended carbon to 3-5% (1.5% was used in this study) combined with a more homogenous application, would achieve maximum degradation and homogeneity of PCB degradation throughout the plot.

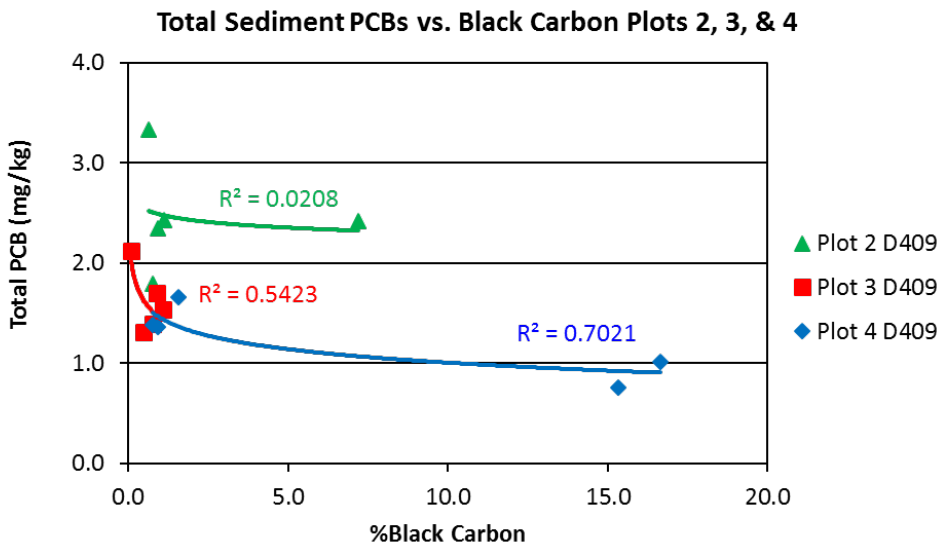


Figure 47. Total PCBs Detected in Sediment versus Percent Black Carbon in Sample Cores from Abiotic Plot 2 and Bioamended Plots 3 and 4 409 Days After Treatment

An analysis of the homolog distribution showed a trend of decreasing mono- to nona-chlorobiphenyls in the bioamended plots (**Figure 48**). An examination of the individual congeners after 409 days (**Figure 49**) confirms net reduction of most mono- to nona-chlorobiphenyls indicating both anaerobic halorespiration and aerobic degradation both occurred in the bioamended plots. This uniform decrease indicates that any congener products resulting from anaerobic dechlorination were subject to aerobic degradation preventing their accumulation. Differences observed between the congener patterns of plots 3 and 4 are possibly the result of different indigenous microbial communities, which might influence the degradation pattern. Payne et al showed previously that the dechlorination pattern in sediments bioamended with DF1 is influenced by the indigenous community (Payne, 2011; Payne. 2013). DF1 is only capable of attacking chlorines that are flanked by two other chlorines, however, in sediments bioamended with DF1 additional dechlorination patterns are observed, including singly flanked chlorine positions. This pattern shift is presumably the result of indigenous halorespiring bacteria that are stimulated by the activity of DF1. Some pattern changes are observed in the non-bioamended plot, which might be attributed to natural attenuation by indigenous bacteria.

As stated above in Section 4.3 this site was also contaminated with three congeners of DDT, but there was no significant reduction (>0.05) of this POP after treatment with bioamended AC (See Appendix). This was not surprising as there are no reports of either DF1 or LB400 attacking DDT congeners.

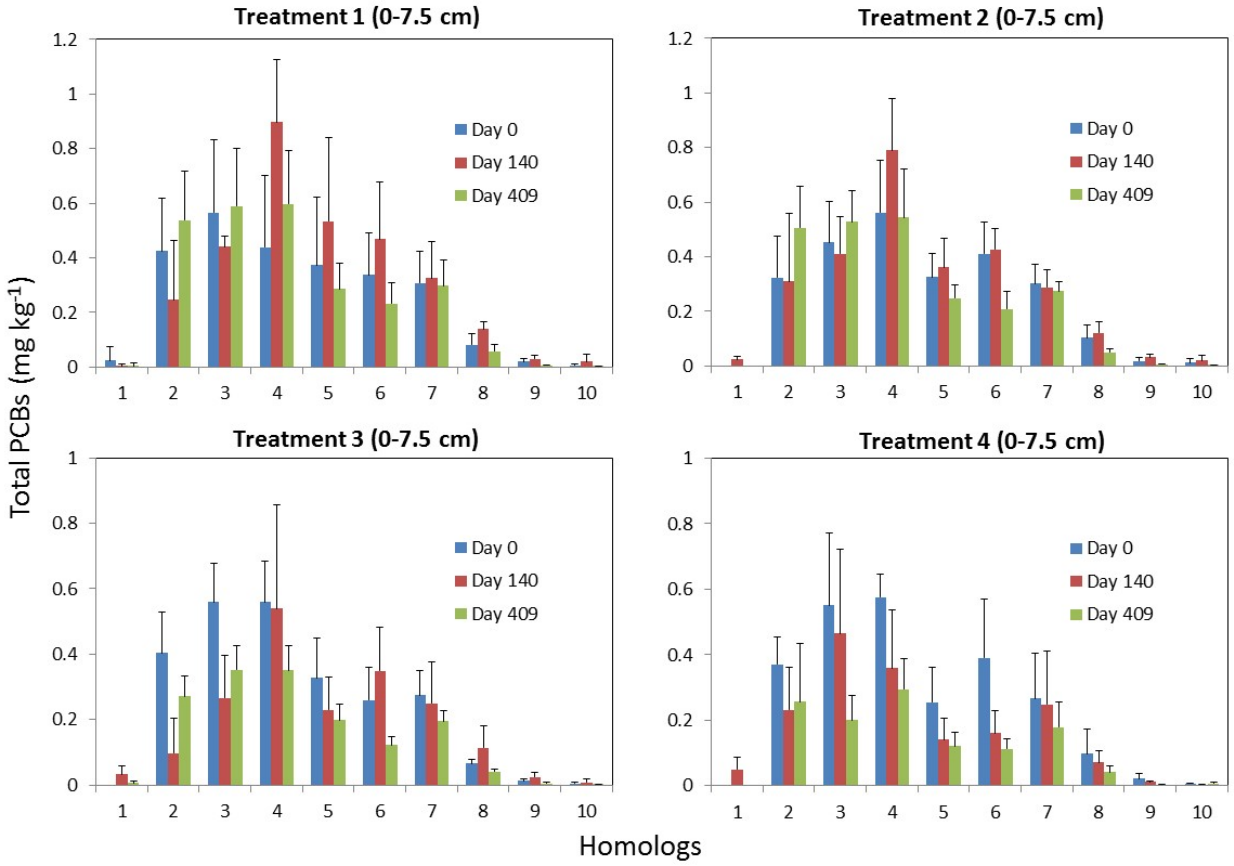


Figure 48. Effect of Treatments on PCB Homolog Concentrations in Upper (0-7.5 cm) Sediment Profile of Treatment Plots 1-4.

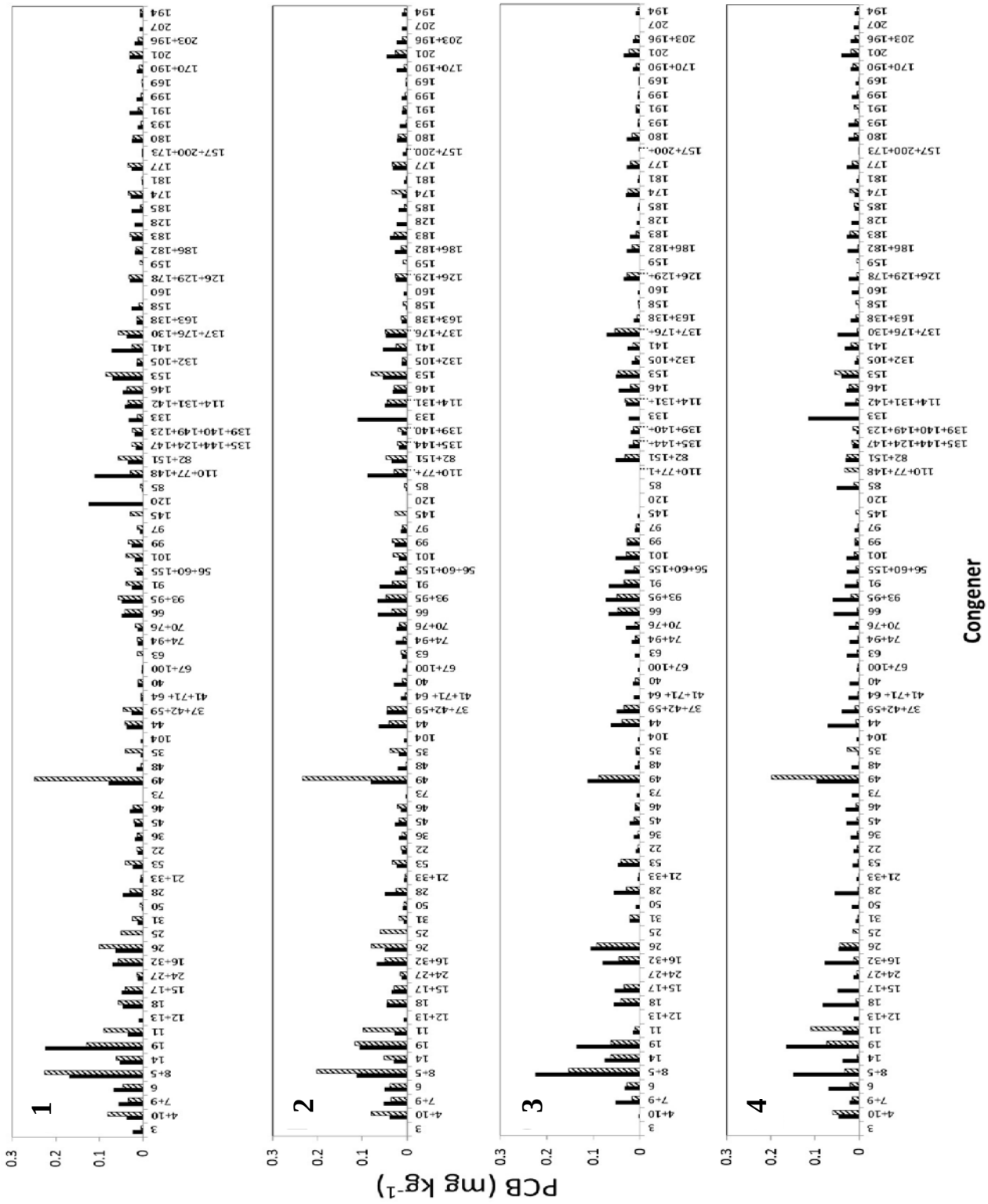


Figure 49. Effect of Treatments on PCB Congener Concentrations in Upper (0-7.5 cm) Sediment Profile of Treatment Plots 1-4.

Each plot is the mean values of five sediment cores.

We also examined the reduction in toxicity by reduction in the levels of coplanar PCBs. Only three coplanar PCBs (114, 156 and 157) were detected in sediment samples from Abraham's Creek. As shown in **Table 20 and Figure 50** significant reduction was only detected in plot 4 409 days after treatment, which resulted in an 80% reduction in TEQ.

Table 20. Effect of Treatments on Coplanar PCB 114, 156 and 157 Levels (µg kg^{-1}) in Sediment (0-7.5 cm)

	Plot 1		Plot 2		Plot 3		Plot 4	
Day	Mean	SD	Mean	SD	Mean	SD	Mean	SD
-30	66.2	45.5	105.8	83.8	45.8	36.0	64.5	41.3
140	70.8	13.0	60.84	37.0	58.0	17.7	29.5	21.3
409	52.4	31.6	74.6	17.6	46.4	12.3	13.1	10.8

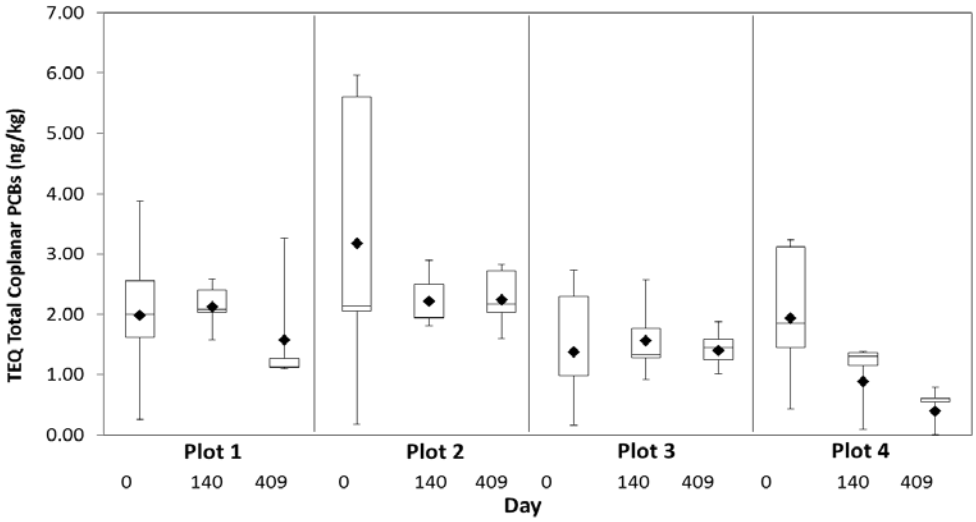


Figure 50. Effect of Treatments on Levels of Coplanar PCB 114, 156 and 157 in Toxic Equivalents in Sediment (0-7.5 cm).

5.6.4 Effect of Treatments on PCB Concentrations in Porewater

Freely dissolved concentrations of PCBs were measured in-situ in the sediment and overlying water of the treatment and control plots at each sampling event. The passive sampler locations are shown in **Figure 51**. As evident in the figure, most of the locations based on GPS coordinates fell within or very close to the boundary of each plot.

Figure 51. Passive Sampler Locations 140 and 409 Days Post-treatment.

Sampler locations are numbered as follows: plot 1, A1-A3; plot 2, B1-B3; plot 3, C1-C3 and plot 4, D1-D3. Recovery dates for individual samplers are indicated in the figure legend. Note: pre-treatment passive sampler locations were not determined; samplers A3 and D3 were lost and could not be retrieved in September 2016.

The coordinates of the passive samplers deployed 30 days prior to treatment were not determined, but they approximated the positions of the post-treatment samplers in three trisects along the length of the plots.

As shown in **Figure 52**, the total freely dissolved PCB concentration in the overlying water ranged from 4-8 ng L^{-1} across the four plots over three sampling events. Thus, the overlying water concentration appears to remain relatively constant spatially, which is expected based on mixing in the water column from flow in the creek and wind-associated disturbances. There is no obvious trend with time in the overlying water concentrations. The overlying water concentrations exceed USEPA human health criteria for 10^{-5} cancer risk (0.64 ng L^{-1}) by nearly an order of magnitude. The freely dissolved concentration of PCBs were also measured at two depths in the sediment.

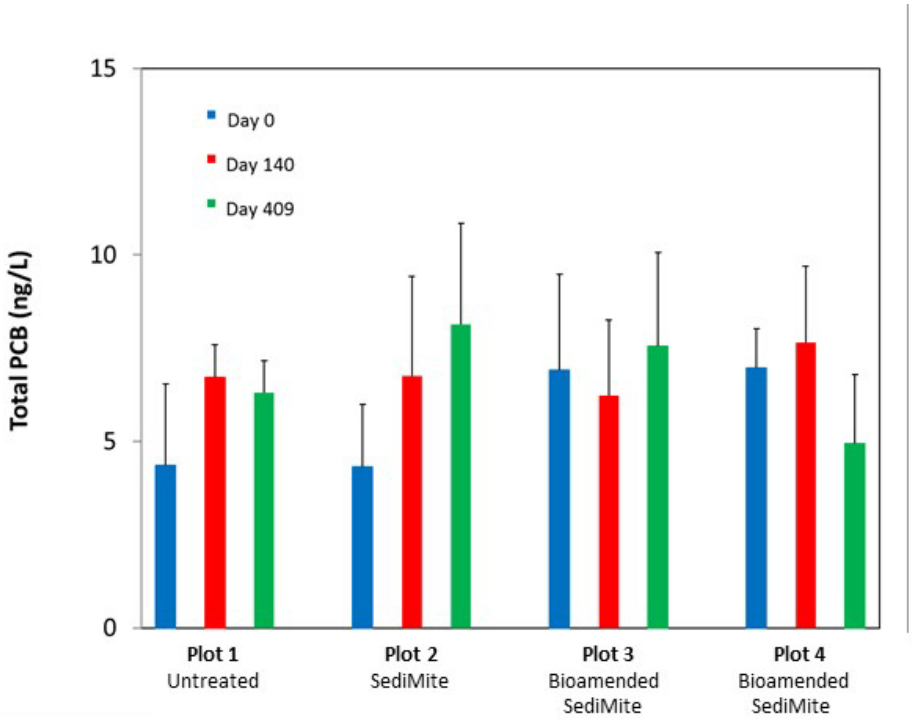


Figure 52. Freely Dissolved Total PCB Concentrations in the Overlying Water

The concentrations in the top bioactive zone (0-3") are shown in **Table 21** and **Figure 53a**. The average porewater concentration of total PCBs in the untreated plot remained close to 130 ng/L over the first 140 days and then decreased to 70 ng/L. However, most of this decrease was due to a decrease in the dichlorobiphenyls. For the untreated site, tri+ PCB congeners was at 36 ng/L before and after 409 days with an apparent increase in day 140 (**Figure 53b**). Tri+ PCBs are a good representation of the PCBs that bioaccumulate in fish and has been used for assessing dissolved PCB concentrations in the Hudson River remedial investigations (USEPA, 2000. Revised Baseline Modeling Report EXECUTIVE SUMMARY, January 2000: https://www3.epa.gov/hudson/rbmr-exsum.htm).

Table 21. Freely Dissolved Concentration of Total PCBs in Sediment Porewater in the 0-7.5 cm Surface Sediments

Treatment	Day 0			Day 140			Day 409			
	ng/L	STDEV	% Change*	ng/L	STDEV	% Change*	ng/L	STDEV	% Change*	Significance‡
1	127	111	—	129	132	1	70	51	-45	no
2	164	134	—	174	67	6	112	73	-32	no
3	155	44	—	105	70	-32	41	48	-73	yes
4	156	68	—	97	31	-38	38	42	-76	no†
*Percent change compared to Day 0 amounts										
‡$p \leq 0.05$										
† N=2 for Day 409										

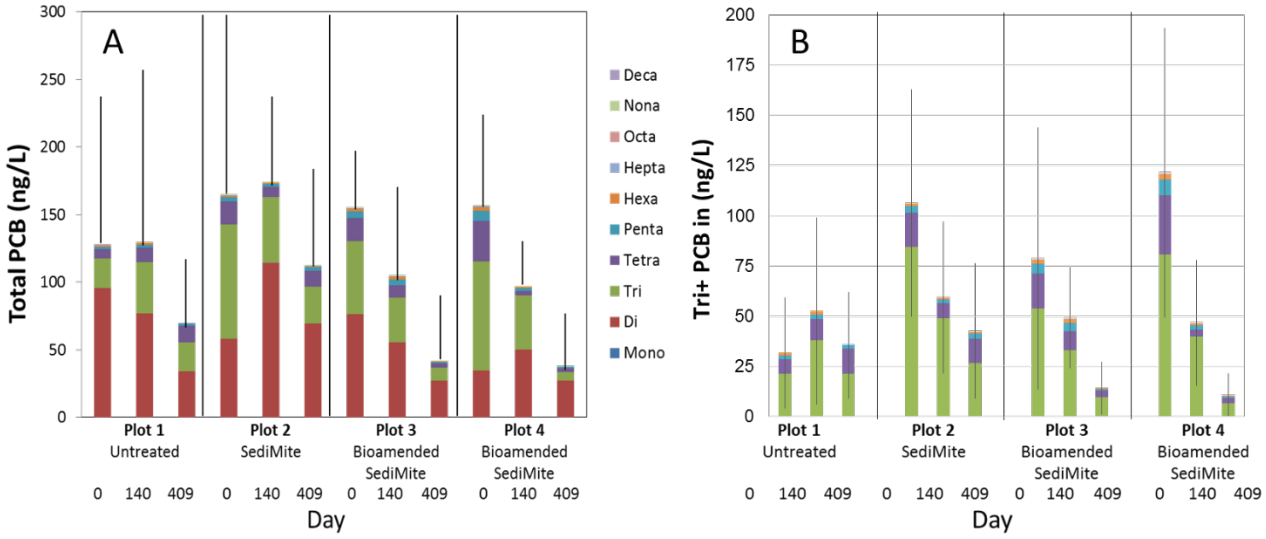


Figure 53. Freely Dissolved Concentration of Total PCBs in Sediment Porewater in the 0-7.5 cm Surface Sediments for di- to deca-chlorobiphenyls (A) and tri- to deca-chlorobiphenyls (B).

The starting porewater concentration of total PCBs in the other three treatment plots were slightly higher at about 160 ng/L at day 0. The freely dissolved concentration in porewater is more than an order of magnitude higher than the concentrations observed in the overlying water. Thus, there appears to be a strong gradient for PCB transport from the sediments into the overlying water at this site. The treatment effect appears to manifest into reductions in porewater PCBs over time. At day 140, moderate reductions of porewater PCBs are observed in the two bioamended plots. At day 409, the mean percent reductions in plots 3 and 4 were 73% and 76% respectively for total PCBs including dichloro congeners. For the tri+ PCB congeners the reductions in plots 3 and 4 after 409 days were 84% and 95% compared to 64% reduction in plot 2 after the same time. Plots 1 & 2 show a mean reduction of porewater total PCB concentration, but the change was not significant ($p<0.05$). Note that the target dose of AC for this demonstration was kept at a very low value of 1.5% AC (as percent dry sediment) so as to not overwhelm the treatment with the effect of the AC.

There is some spatial variability in measurements of the porewater concentration across the plots and over time making some of the observed differences in mean concentrations not statistically significant especially when the dichlorobiphenyls are included. However, the reductions are statistically significant for plots 3 and 4 after 409 days when looking at either all congeners or tri+ PCBs. These observed reductions in tri+ PCB congeners in the porewater in conjunction with observed reductions of these congeners in the sediment phase (Figure 46) provides strong indication of the effectiveness of the treatments in reducing both the mass and bioavailability of the tri+ PCBs at the site.

The freely dissolved concentration in the deeper sediments 7.5-15 cm) did not show a significant change with treatment or over time. Thus, it appears that in the one year period after treatment, the effect of the treatment is observable in the top 7.5 cm of sediment, perhaps because of slow penetration of the amendments to the deeper zone of sediments. The porewater concentrations in the deeper zone is consistent with the observation of no change in sediment PCB concentrations in this zone as shown earlier in **Figure 54**.

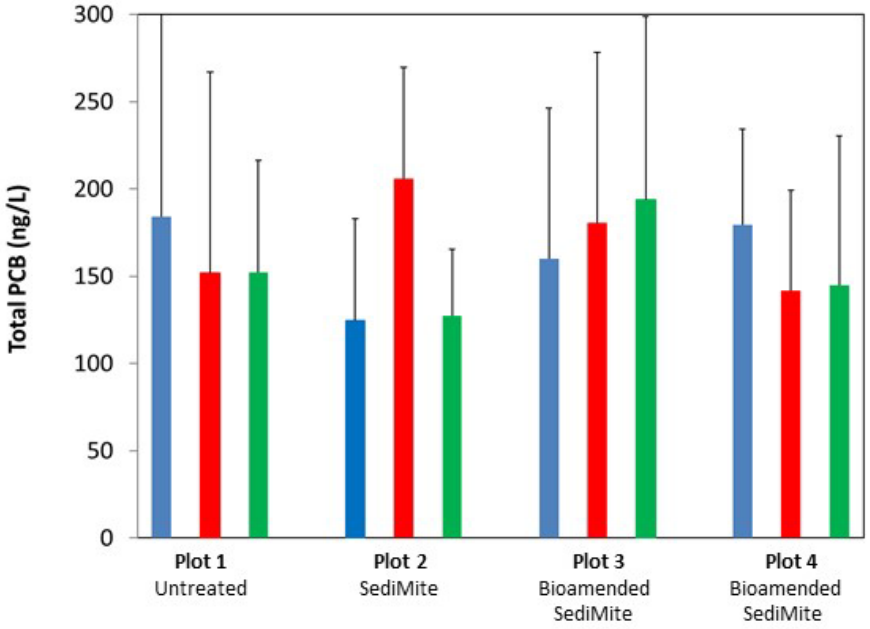


Figure 54. Freely Dissolved Concentration of Total PCBs in Sediment Porewater in the 7.5-15 cm Depth Below Surface.

5.6.5 Fate of Bioamendments After Treatment

The titer of the bioamendments LB400 and DF1 were monitored after deployment into the test plots (**Table 22** and **Figure 55**). The combined distribution of DF1 and LB400 was 2.4±1.9 cell g^{-1} AC with 88% distributed onto AC at target titer of $>1\times10^{7}$ cell g^{-1} based on 16 SediMite pellets randomly sampled after inoculation. Sediment was not sampled immediately after treatment (Day 0), but was estimated as 3.4×10^{5} cells g^{-1} dw sediment from the mean titer of the sampled pellets and mean distribution of SediMite in Plots 3 and 4.

The titer of the anaerobic halorespirer and aerobic degrader decreased by 2 to 3 orders of magnitude after 409 days in plots 3 and 4. This is similar to the decrease in cell numbers observed in the mesocosm treatability study after a similar period of time. Despite the decrease the titer was greater than background levels, which suggests that the bioamendments were maintained in the sediments albeit at a lower titer. Some background signal was detected in Plots 1 and 2 at low levels between 10^1 and 10^2, which could have resulted from indigenous microorganisms or cross contamination. Signal from indigenous microorganisms was not detected in untreated mesocosm studies in which the sediment was separated in individual tanks. Although this suggests that the signal observed in the site plots not treated with bioamendment resulted from low level cross contamination, we cannot draw any firm conclusions since the signal was near the detection limit of the assay. Bioamendment added at a titer of 10^3 in the mesocosm study had no significant effect on PCB levels. The titer of 10^2 or less gene copies detected in the non-bioamended treatments is consistent with the negligible reduction of PCB concentrations observed in Plots 1 and 2. Overall, the results indicate that the bioamendment titer decreased but was still retained at in sediment after 409 days. This suggests that the microbes were still viable and dechlorination and degradation activity would continue after 409 days. However, additional monitoring would be necessary to confirm this conclusion.

Table 22. Titer of Bioamendments LB400 and DF1 Deployed in Test Plots Based on Quantitative PCR Enumeration of 16S rRNA Gene Copies.

Day 0 (*) values were estimated from the cell titer measured on randomly sampled pellets and amount of bioamended SediMite deployed into plot.

DF1 Cell Numbers

Treatment	Day 0*		Day 140		Day 409	
	AVG	STDEV	AVG	STDEV	AVG	STDEV
1	0.0E+00	0.0E+00	0.0E+00	0.0E+00	8.0E+01	1.8E+02
2	3.3E+00	1.2E+01	2.8E+01	1.7E+02	0.0E+00	0.0E+00
3	1.2E+05	9.9E+04	1.2E+05	3.9E+04	5.8E+02	1.3E+03
4	8.9E+04	1.0E+05	4.9E+03	5.7E+03	2.3E+02	4.3E+02

LB400 Cell Numbers

Treatment	Day 0*		Day 140		Day 409	
	AVG	STDEV	AVG	STDEV	AVG	STDEV
1	0.0E+00	0.0E+00	7.0E+00	2.0E+02	1.6E+02	3.6E+02
2	6.0E+00	1.0E+02	2.0E+02	1.3E+02	0.0E+00	0.0E+00
3	9.5E+04	1.0E+05	3.9E+03	9.0E+03	1.5E+02	3.3E+02
4	1.0E+05	1.3E+05	7.4E+03	5.7E+03	1.2E+02	2.7E+02

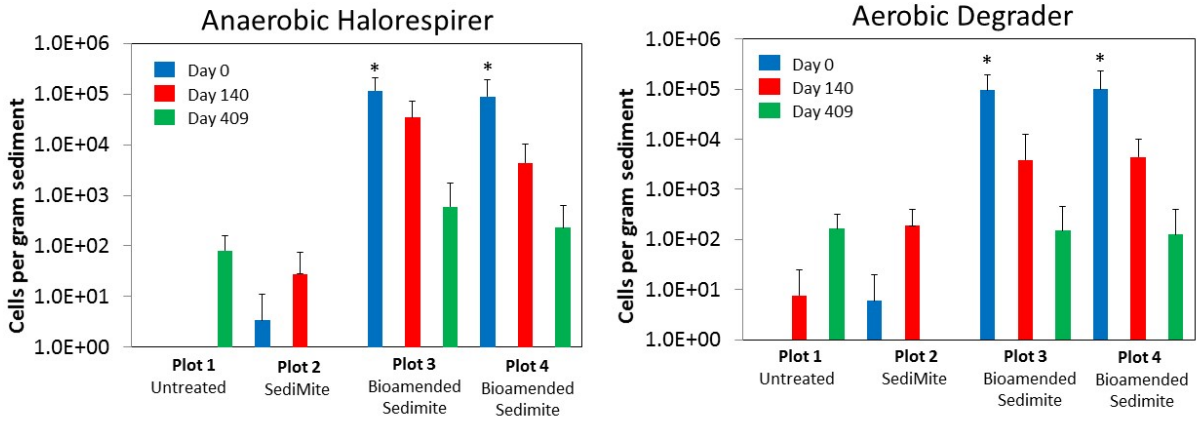


Figure 55. Titer of Bioamendments LB400 and DF1 Before and After Deployment Into Test Plots Based on Quantitative PCR Enumeration of 16S rRNA Gene Copies.

Asterisks indicate estimated cell titer based on amount of bioamended SediMite deployed into plot.

As indicated in Section 5.6.1 the amount of BC was not consistent throughout the treatment plots. In order to determine how variation in SediMite distribution would affect the distribution of bioamendment, the titer of the bioamendment was also examined relative to BC (**Figure 56**). Although the r^2 was low for both days 140 and 409, there was a correlation between the amount of BC and the cell titer detected in the sediment. The results indicate that distribution of the bioamended AC had a direct effect on the distribution of bioamendment, which could contribute to post-treatment differences in PCB concentrations observed within treatment plots 3 and 4.

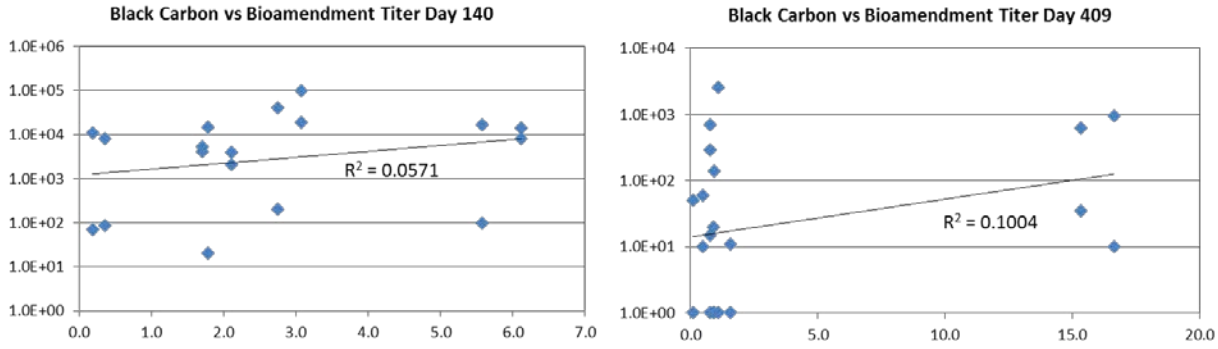


Figure 56. Bioamendment Titer Versus Black Carbon in Sample Cores from Abiotic Plot 2 and Bioamended Plots 3 and 4 140 and 409 Days After Treatment.

5.6.6 Effects of Treatments on the Indigenous Microbial Community

In addition to monitoring the titer of the bioamendments we examined the overall microbial diversity before and 140 days after treatment at each test plot. Plot 4 began the experiment significantly more diverse than all other sites and remained significantly more diverse over 140 days (**Figure 57**).

The microbial diversity was not significantly different between any other sites, time points, or depths. Therefore, bioaugmentation and the addition of activated carbon did not significantly alter total microbial diversity on a macroscale.

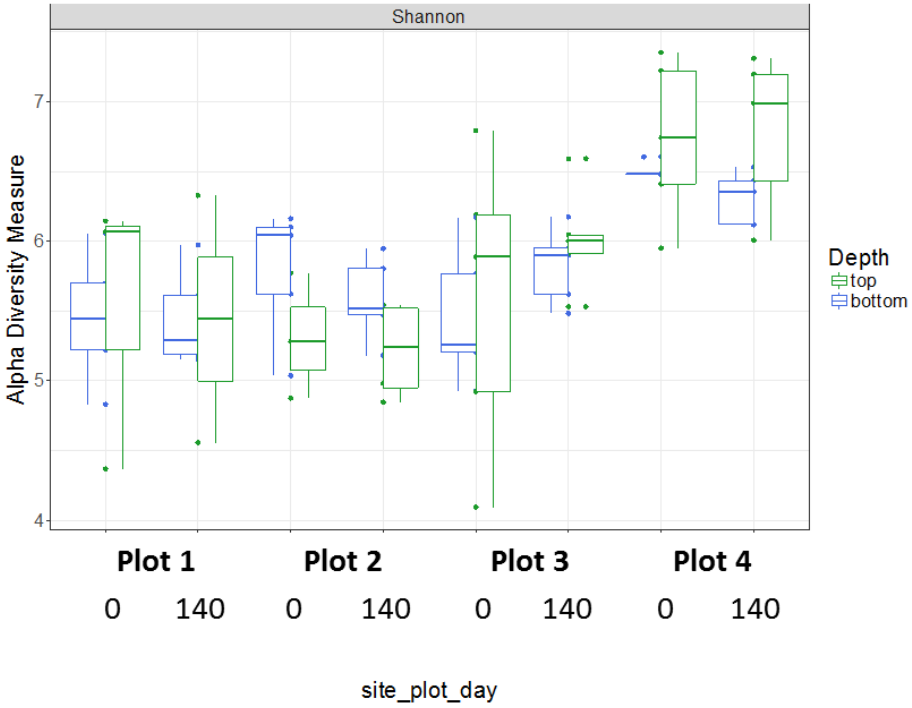


Figure 57. Shannon Alpha Diversity Measure for 0-7.5 cm and 7.5-15 cm Sediment Depth in Each Plot Before and 140 Days After Treatment.

However, non-metric multidimensional scaling ordination using the weighted unifrac distance between samples demonstrated significant differences in microbial community composition along sample plot (permutational manova, R^2=0.299, p=0.001), the treatment type at each plot (none/SediMite/bioamended, R^2=0.07, p=0.002), and depth (R^2=0.03, p=0.007) (**Figure 58**). This diversity metric measures the presence, absence, abundance, and phylogenetic relationships between samples, thus considering relationships between OTUs as a factor in determining distance between samples. 30% of the variation observed in the microbial community structure and composition was due to between plot diversity, largely driven by treatment plot D, as seen above. Additionally, the type of treatment and depth combined explained 10 % of the variation between samples.

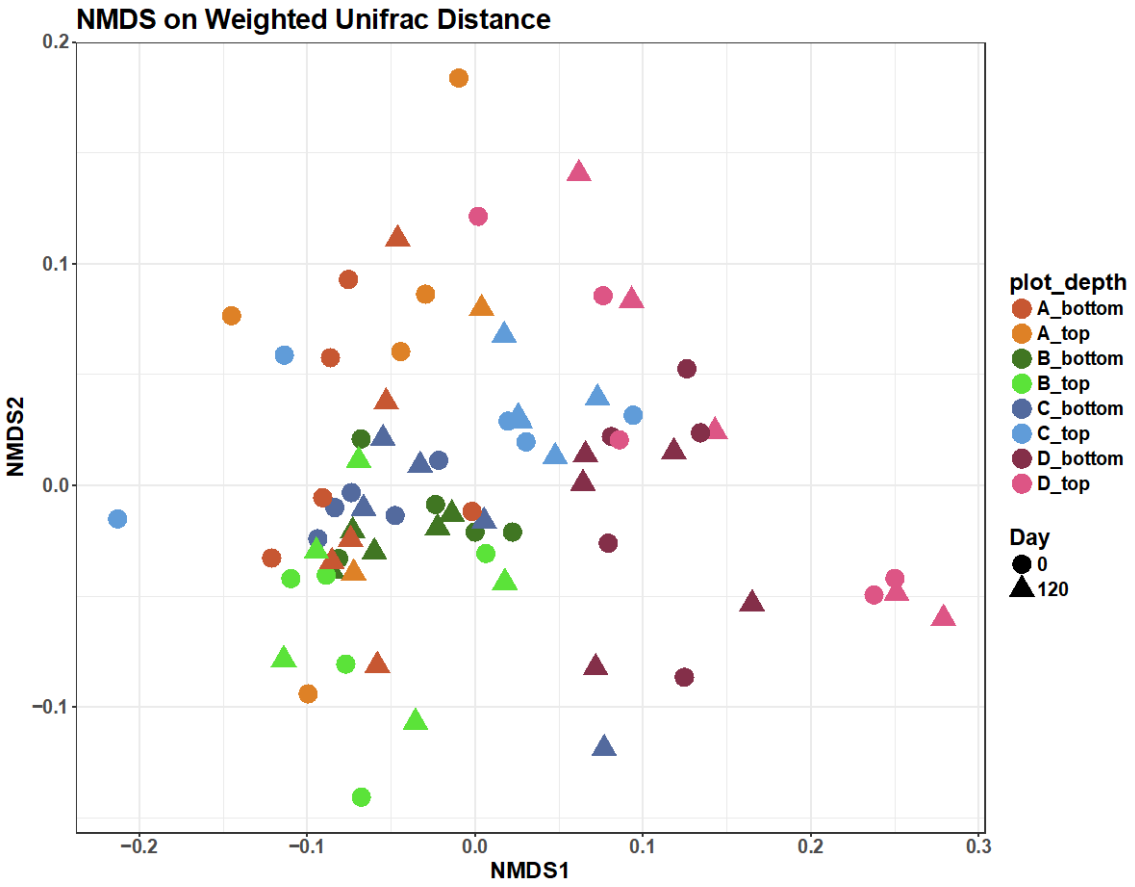


Figure 58. NMDS Using the Weighted Unifrac Metric to Determine the Dissimilarity Between Communities in Each Sample.

In plots C and D, the relative abundance of the Pseudomonadaceae family significantly decreased in the top sediment from day 0 to day 140 (**Figure 59**). No other taxa significantly increased over that time in those plots, indicating a negative selective pressure on Pseudomonadaceae resulting from the addition of activated carbon. However, a large percentage of sequences (~20%) could not be classified beyond the phylum or class level indicating a significant amount of novel or poorly characterized microorganisms in these plots. Future studies should rely on whole genome analysis (metagenomics) to assign taxonomy and metabolic function to this 'microbial dark matter'.

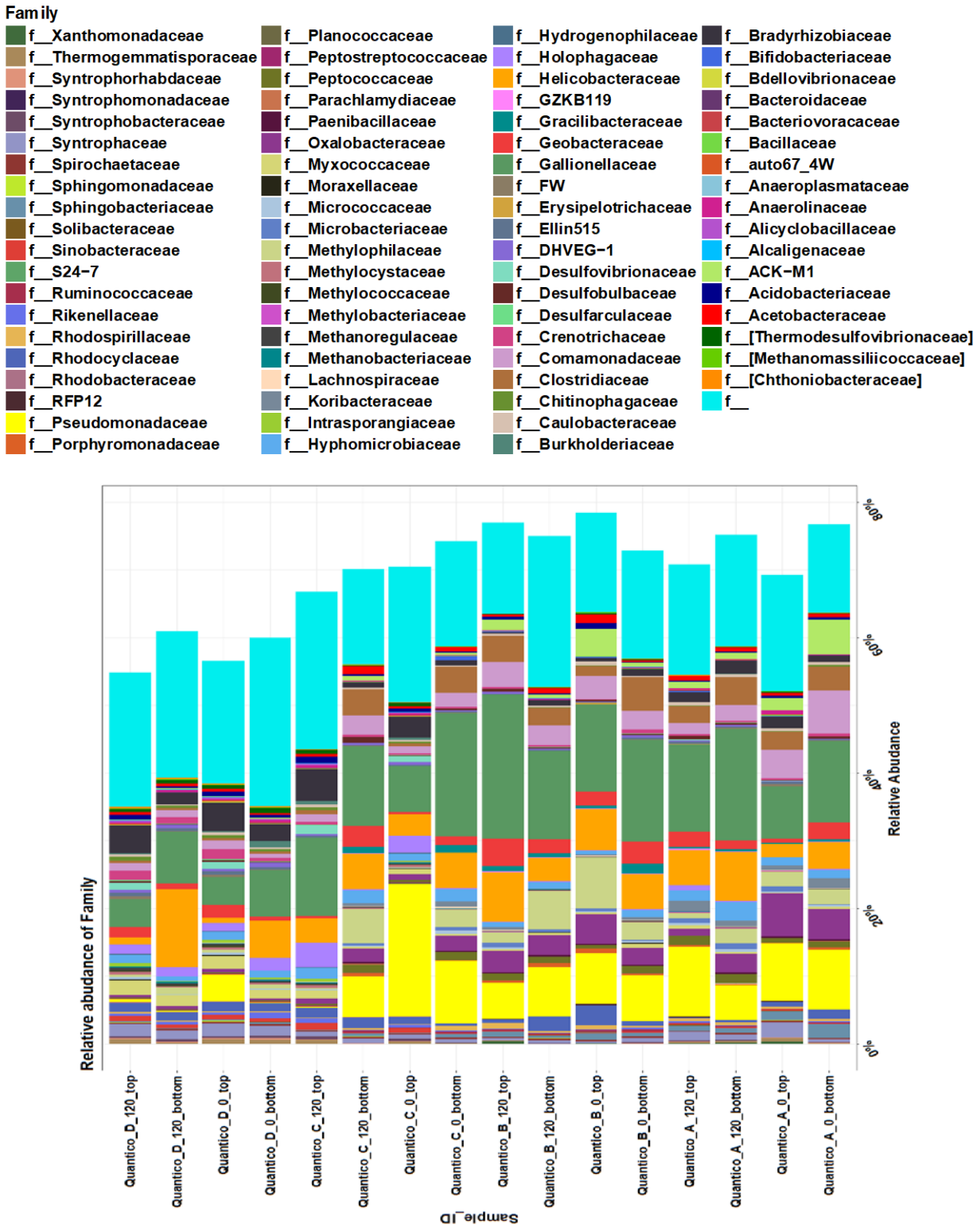


Figure 59. Relative Abundance of Each Plot/Day/Depth at the Family Taxonomic Level.

Page Intentionally Left Blank

6.0 PERFORMANCE ASSESSMENT

6.1 FIELD STUDY SUMMARY

A comparison of the test results with the success criteria is provided in **Table 23**

Table 23. Performance Objectives and Test Results

Performance Objective	Data Requirements	Success Criteria	Test Results
Delivery & spatial uniformity of application	Analysis of sediment cores to evaluate the uniformity of SediMite™ & biocatalyst delivery in the top 6-inch of sediment	Uniform spatial distribution: More than 70% of the samples should have ± 50% of target dose of carbon; 60% of the sampling sites should indicate presence of biocatalysts at an abundance of at least 1 x 10^5 cells/g	Extruded pellet delivery (95%) compared with cold press (71%); only Plot 4 met spatial distribution target due to uneven distribution with VHI. Cell distribution was 2.4 E7 ±1.9 cells g^{-1} AC; titer in sediment correlated with distribution of AC
Persistence of AC and viable biocatalysts in sediments	Stability of AC and sustainability of viable inoculated anaerobic and aerobic biocatalysts in the sediments	Minimal mobilization of AC from site of application and sustained viability of bioamendment for duration of test	No detection of AC or microbial activity 60, 120 and 180 cm downstream of plot 3
Effect of treatment on PCB degradation	PCB depletion rate, change in risk factors including bioavailability & formation/depletion of toxic congeners, effect on indigenous community	>50% reduction of total PCB concentration and >80% combined risk reduction resulting from reduction of total PCBs, no accumulation of coplanar congeners & reduction in bioavailability; insignificant effect on indigenous bacteria	>50% reduction of total PCB concentration and >80% risk reduction with no accumulation of coplanar congeners in plot 4, but not plot 3. The difference attributed to uneven deployment of the amendment. Insignificant effect on indigenous bacteria
Effect of treatment on reduction of porewater PCB concentration in surficial sediment	Measurement of porewater PCB concentration in the top 3" of surficial sediment using passive samplers	> 80% reduction in porewater PCB concentration resulting from a combination of PCB degradation and sequestration in carbon	Tri+ PCB congeners in sediment porewater was reduced by 84% and 95% in the two bioamended field plots after 409 days-only 64% reduction with non-bioamended GAC
Performance of biocatalyst deployment system	Delivery system monitored for ease of operation, any issues of clogging of the nozzle during delivery, rates of application,	Consistent delivery of SediMite™ & microorganisms, no clogging of delivery nozzle, no clogging of TeeJet nozzle	The VHI system was found to better meet the proposed performance criteria. However, even distribution was difficult to achieve in large open areas. The VHI met all other performance criteria
Health and safety	Potential for hazards during application will be evaluated and documented. Potential formation of airborne dust or mist during application will be monitored.	Minimal dust during delivery of SediMite™, minimal aerosols during delivery of SediMite™	Cold press granules generated excessive dust with 29% loss of SediMite; extruded pellets generated minor dust with 5% loss of SediMite. For future applications dust can be reduced by increasing water content of extruded pellets.

Table 23. Performance Objectives and Test Results (Continued)

Performance Objective	Data Requirements	Success Criteria	Test Results
Scalability of technology	Any scale up issues related to microbes or field application will be documented	Successful scale-up, concentration, transport of viable biocatalysts to field and field distribution	Both microorganisms scaled-up, transported and 88% distributed onto AC at target titer of $>1\times10^{7}$ cells g^{-1}
Technology transfer/regulatory acceptance	The demonstrated technology should receive favorable feedback from the regulatory and industry community.	Feedback will be sought and documented from representatives in the regulatory community and industry representatives	Results to be included in CERCLA process document on clean-up of MCBQ site by NAVFAC, results reported to EPA reps in regions 2,3,5 & 10, three treatability studies conducted for industry for possible full scale treatment, data at various stages presented to regulators & industry at national meetings.

The results support the following summary conclusions regarding the performance of bioamended SediMite™ as a method to treat sediments by reducing total and soluble fractions of PCBs:

1. Both anaerobic halorespiring and aerobic biphenyl degrading bioamendments could be mass cultured, transported to a site and delivered through a water column to sediments without loss of viability. Scale up of microorganisms for treating greater than an acre will require higher volume bioreactors (> 250 L).

2. Treatment with the bioamendment mixture on 3% bioamended SediMite™ reduced the mean total PCB concentration by 30% in Plot 3 and 52% in Plot 4 based on 5 sediment cores. Even after excluding two outliers in plot 4 that had exceptionally high concentrations of black carbon (15.3 and 16.6%) due to variability in application, the decrease in total PCB concentration was 43% instead of 52% compared with Day 0 and was still statistically significant ($p>0.05$). The tri+ PCB congeners in sediment porewater were reduced by 84% and 95% in the two bioamended field plots after 409 days. Co-planer congener levels were reduced by up to 80% in the sediment and were undetectable in the porewater.

3. All homolog groups were reduced in sediment and porewater indicating that both anaerobic halorespiration and aerobic degradation occurred within the benthic zone of the field sediments

4. The effectiveness of bioamended SediMite™ for reducing concentrations of total and soluble PCBs was affected by the homogeneity of the application. Although the mean values in plot 4 met the performance objectives, this was not the case for identically treated Plot 3. However, there was also wide variation within each bioamended plot and maximum values exceeded the performance objectives for total and porewater concentrations of PCBs in both bioamended plots. For full-scale treatment more consistent application would be required to achieve maximum degradation and homogeneity of PCB degradation throughout the plot. The VHI is appropriate for application in water margin areas, wetlands and difficult to access areas such as below piers and under overhanging trees.

However, for large areas, methods that ensure even distribution such as a boat mounted belt spreader or land based telebelt are required to evenly distribute the bioamendments. Dust was an issue with granulated SediMite™, but less so with pelleted SediMite™. However, this could be controlled by the addition of more water or binder into SediMite™.

5. The titer of the bioamendments decreased over two orders of magnitude, but were still detectable after 409 days in the field, which suggests PCB degradation could continue beyond the timeline of this project. Indigenous microbial diversity was not significantly different between sites, time points, or depths. Therefore, bioaugmentation and the addition of activated carbon did not significantly alter total microbial diversity on a macroscale.

6. The bioamended SediMite™ was stable and did not migrate downstream of the treatment area.

7. The study was limited to two post-assessments 140 and 409 days after treatment. Multi-year post-treatment assessments would be necessary to fully validate the long-term effectiveness of the bioamended AC to reduce total and porewater concentrations of PCBs in sediments. In addition, we observed a gradual reduction in the abundance of the bioamendments over time in the field. Future work should explore the feasibility of a second application to further accelerate the rate of PCB degradation. Our experience with the field application suggests that two applications may be helpful not just in further reducing the total PCB concentrations, but also potentially reduce the spatial heterogeneity in application observed after a single application.

6.2 LESSON LEARNED

1. To achieve more even application and optimal results for a full-scale application, it is advisable to increase the target dose of SediMite to 6% by dry weight of target sediment depth which would be twice the dose applied in the pilot study. The subsequent cost assessment takes into account this recommended increased dose.

2. Amendment application method needs to be fine tunes further for a more even application of the amendments in the field. As indicated earlier, a telebelt or a mechanical spreader may be better suited for a full-scale application.

3. To overcome the small-scale spatial variability encountered during monitoring, it is recommended that future monitoring involve compositing of multiple cores from each location.

4. The achieved rates of biotransformation was much smaller than those achieved in the laboratory treatability studies. The slower rates in the field are likely due to: 1) slow mixing and lack of uniformity of the applied amendments, 2) low seasonal temperatures.

5. Longer (>1 year) monitoring periods are needed in the field to fully assess the effectiveness of the bioamendments.

Page Intentionally Left Blank

7.0 COST ASSESSMENT

7.1 COST MODEL

The general approach to addressing contaminated sediment is dredging, removal from the site and disposal with or without treatment. Additional costs associated with the technology often include containment of treated area to prevent release of resuspended PCB contaminated sediments downstream, dewatering of the dredge material before transport and backfill of clean fill material to restore the site back to the original condition. Development of a tractable microbial *in situ* treatment system would provide a more cost-effective and environmentally sustainable means of treating persistent pollutants. The initial costs for dredging and off-site disposal are very high; however, the long-term monitoring, oversight and management expenses are low. In situ remediation with bioamended SediMite™ will require post-treatment monitoring to ensure the proper functioning of the bioamendment in reduction of PCB concentrations. In terms of the life cycle assessment, use of bioamended SediMite™ would have a significantly reduced impact compared with dredging by reducing the health risks associated with sediment disruption, reducing overall energy use (reduced carbon footprint), effectively negating the requirement for extensive waste management and obviating the requirement for substantial habitat restoration. A cost model for treatment of PCB impacted sediments with bioamended SediMite™ is shown in **Table 24**.

Table 24. Cost Model for Bioremediation of PCBs in Sediments with Bioamended SediMite™

Cost element	Data Requirements
Treatability Study	Personnel & labor Materials (Bioamendment, SediMite™, disposable labware) Analytical laboratory costs
Baseline characterization	Pre-treatment assessment of concentrations and distribution of PCBs Post-treatment assessment of bioamendment distribution Post-treatment assessment of bioamendment viability
Materials cost	Unit: cost/lb bioamended SediMite™ Production of SediMite™ Production of bioamendment
Installation	Unit: cost/treatment area Scale-dependent installation method Mobilization cost Personnel and labor
Post-treatment monitoring	Personnel & labor for sampling Analytical laboratory costs

7.2 COST DRIVERS

7.2.1 Treatability Study

A treatability study was required to determine the site-specific requirements for implementation of the technology. The effectiveness of bioaugmentation in degrading and reducing the overall concentration of PCBs for the selected site was tested and optimized in 2 L sediment/water mesocosm systems for a minimum period of three months (described in the Treatability Report).

Data were tracked in an Excel spreadsheet and will include the following cost parameters: labor, materials, and analytical testing. Labor was tracked according to the type of personnel required to conduct the treatability study (e.g., field technician to collect sediment samples, lab technician to prepare, monitor and analyze samples from mesocosms, etc.) and their associated labor hours. In addition, all material purchases and analytical laboratory costs were recorded in the spreadsheet. Costs will vary with number of mesocosms and test period for a selected site.

7.2.2 Baseline Characterization

Pre-treatment assessment was conducted to determine the PCB concentrations, spatial distribution of TOC and sediment characteristics. Post treatment assessment included spatial uniformity of application will be tested by placing collection trays on the sediment bed within the treatment area and retrieving the trays immediately after application to measure the dose of SediMite™ achieved at the different locations. The top layer of the sediment core was assessed for depth of the activated carbon layer and distribution of viable bioamendment. Data were tracked in an Excel spreadsheet and will include the following cost parameters: labor, materials, and analytical testing. Labor was tracked according to the type of personnel required to conduct the treatability study (e.g., field technician to collect sediment samples, lab technician to analyze samples for PCBs and bioamendment viability) and their associated labor hours. In addition, all material purchases and analytical laboratory costs were recorded in the spreadsheet. Costs will vary with number of samples collected from the site.

7.2.3 Cost Element: Materials Cost

Production cost for both the SediMite™ and bioamendment were determined in units of cost/kg bioamended material. Data were tracked in an Excel spreadsheet and included the following cost parameters: labor, materials, and production costs by third parties. SediMite™ production cost is based on materials and bulk production costs. Bioamendment production is based on two levels: production for up to an acre in a pilot-scale reactor (200 L) and production-scale for larger areas (up to 4000 L) by a third party. All material purchases and analytical laboratory costs were recorded in the spreadsheet. Costs will vary with the area at the treatment at a selected site.

7.2.4 Installation

Production cost for installation of bioamended SediMite™ was determined in units of cost/treatment area. Data were tracked in an Excel spreadsheet and will include the following cost parameters: labor, equipment mobilization and materials. One-time small specialized equipment costs and rental costs of larger equipment were recorded in the spreadsheet. Costs will vary with the area at the treatment at a selected site.

7.2.5 Post-treatment Monitoring

Data were tracked in an Excel spreadsheet and included the following cost parameters: labor, materials, and analytical testing. Labor was tracked according to the type of personnel required to conduct the monitoring (e.g., field technician to collect sediment samples, lab technician to assay the samples for PCBs and bioamendment enumeration and AC, etc.) and their associated labor hours. In addition, all material purchases and analytical laboratory costs were calculated.

7.3 COST ANALYSIS

7.3.1 Cost of Treatability Study

The costs of treatment with bioamended SediMite™ for treating PCB impacted sediments are described in this section using Abraham's Creek as a model (**Table 25**). The cost analysis for the treatability study is described separately since the number of samples tested in this study included a number of different treatments to identify critical parameters (e.g., cell titer, bacterial strains and slow release carbon) for optimal efficiency. Since these parameters would be used in subsequent treatments the primary cost driver would be the number of sediment samples tested for any given site. In the case of Abraham's Creek we determined the costs for 8 samples, which would include 7 treated and 1 untreated samples monitored for 180 days.

Table 25. Cost Analysis for Treatability Studies Based on Abraham's Creek

Treatability Assay	**$ 50,364.00**
Bench-scale treatability studies set up (8 tanks @ $240/tank)	1,920.00
Bench-scale treatability studies maintenance (6 months @ $200/week)	5,200.00
Congener analysis (8 tanks & 3 sample times @ $600/sample)	14,400.00
DNA analysis (8 tanks & 3 sample times @ $380/sample)	9,120.00
Inoculum preparation	300.00
Passive sampler deployment and extraction (24 samplers)	16,400.00
Data analysis & final report (36 hr @ $84/hr)	3,024.00
TOTAL	50,364.00

If the congener distribution and co-contaminants are similar throughout the site, multiple sediment samples could be combined and homogenized, then distributed into four tanks: three treated replicates and one untreated control. This would reduce the costs by nearly half.

7.3.2 Cost of Full-scale Implementation

To develop a realistic cost estimate for a full-scale project, the field contractor for this project, Brightfields Inc. was asked to prepare an estimate for the treatment application in the entire impacted area of Abraham's Creek. The 2008 Feasibility study for this site prepared by Battelle identifies a total of 7.8 acres of pond and wetland areas that would potentially need to be treated [23]. Brightfields has implemented several sediment remediation projects around the country including the largest SediMite™ project to date at Mirror Lake, DE (a 5-acre treatment area). Brightfields assisted us in this ESTCP project to apply the treatment and also for the sampling events and is familiar with the site logistics. The cost estimate for treatment in the field assumes characteristics similar to those at Abraham's Creek, which are as follows:

- The sites have already been characterized for PCBs congeners and distribution
- The site is accessible for deployment of boat(s) and equipment
- The site has a staging area that is accessible to the water
- The site is deep enough to be navigable by boat

Material Staging

In order to adequately treat the Site, approximately 160 tons of SediMite™ will be needed at an application rate of approximately 20 tons per acre. This application rate is twice the rate used for the pilot-scale demonstration. In order to reduce logistical complications Brightfields will plan to ship and stage bulk quantities of SediMite™ at an offsite storage facility prior to inoculation. Once the project has commenced bulk materials will be shipped from the storage facility to the Site staging areas as needed for daily/weekly activities. Bulk materials will be moved with a fork lift and transported with a trailer. Everyday tools and materials needed at the Site will remain staged in an onsite job trailer.

Implementation of Treatment

The treatment for the Site will include the application of inoculated SediMite™ across the 7.8 acres through a variety of methods dependent upon Site conditions and application areas. Application methods will include pneumatic distribution and mechanical spreading. Site access and treatment locations will determine the application method. The two application areas include upland wetland areas and in-water areas. For all treatment locations the SediMite™ will need to be inoculated with the microbes prior to application. Inoculation will be done through mechanical mixing and/or spraying of the SediMite™ with the prescribed dosage of microbes to ensure adequate coverage. Typically, inoculation of Sedimite™ will be done on land at the on-site staging location and transported to the distribution locations; however, certain application methods can be equipped with a sprayer to perform the inoculation during application procedures.

Application of inoculated Sedimite™ in the in-water locations will be done through the use of pneumatic broadcast spraying and mechanical spreading from a barge. In-water application will be done with grids to delineate application areas. Pneumatic broadcast spraying consists of using an air compressor and VHI device to evenly distribute inoculated Sedimite™ into the water. The pneumatic blower is most useful to get coverage in hard to reach locations especially near the banks and around any obstructions that may be in the water. Mechanical spreading will involve the use of a “shaker table” and/or a poly salt spreader. The “shaker table” and poly salt spreader allow the inoculated SediMite™ to be loaded onto the barge and continuously distributed in a uniform fashion while keeping the barge in motion. The “shaker table” can also have a sprayer set so that Sedimite™ can be inoculated during the application.

Inoculated SediMite™ will be applied to upland wetland areas through the use of mass material moving equipment such as a telebelt truck. A telebelt truck will have the ability to apply a large amount of material over a wide area with minimal disturbance once set in place. In order to properly stage the telebelt truck HD composite mats will be used to build access roads to application areas. Alternately haul roads can be built using geotextile and single shredded mulch when HD mats are not suitable. In order to install access roads some brush clearing and grubbing may be performed through the use of a Cat 259 CTL with a brush cutting attachment. The telebelt truck also allows for SediMite™ to be inoculated prior to application or during application by using sprayers mounted over the conveyor belt. In the upland wetland areas there may also be the need for pneumatic spraying of inoculated SediMite™ in the more sensitive and hard to reach locations.

The costs provided below (**Table 26**) are for a 7.8 acre site similar in characteristics to the project site at Abraham’s Creek. The costs are expected to be lower for larger sites due to economies of scale and lower burden of mobilization and other fixed costs.

Table 26. Estimated Full-scale Costs for Bioamended SediMite Application at Abraham's Creek.

Site prep				**$ 18,240.00**
Environmental Analyst	**30**	**hrs.**	**$ 87.00**	**$ 2,610.00**
Project Manager	**10**	**hrs.**	**$ 123.00**	**$ 1,230.00**
Vehicle	**3**	**day**	**$ 75.00**	**$ 225.00**
Machinery (off road fork lift)	**1.5**	**lot**	**$ 4,200.00**	**$ 6,300.00**
PPE per day	**3**	**at**	**$ 25.00**	**$ 75.00**
E&S Controls	**1**	**@**	**$ 7,800.00**	**$ 7,800.00**
Bioamended SediMite product				**$ 1,371,200.00**
SediMite product (including purchasing/handing costs)	**160**	**tons**	**$ 4,370.00**	**$ 699,200.00**
Microorganism	**8400**	**liters**	**$ 80.00**	**$ 672,000.00**
SediMite application wetland				**$ 218,440.00**
Telebelt (15 day rental)	**1**	**lot**	**$ 28,960.00**	**$ 28,960.00**
Fuel	**30**	**days**	**$ 186.00**	**$ 5,580.00**
Swamp Matts (mat rental or mulch delivered	**6**	**weeks**	**$ 8,400.00**	**$ 50,400.00**
Carbon Storage (Offsite or onsite containers)	**6**	**weeks**	**$ 1,500.00**	**$ 9,000.00**
Environmental Analyst	**300**	**hrs.**	**$ 87.00**	**$ 26,100.00**
Project Manager	**200**	**hrs.**	**$ 123.00**	**$ 24,600.00**
Environmental Analyst	**300**	**hrs.**	**$ 87.00**	**$ 26,100.00**
Environmental Analyst	**300**	**hrs.**	**$ 87.00**	**$ 26,100.00**
Vehicle	**30**	**day**	**$ 75.00**	**$ 2,250.00**
Per diem	**120**	**days**	**$ 155.00**	**$ 18,600.00**
PPE per day	**30**	**at**	**$ 25.00**	**$ 750.00**
SediMite application open water				**$ 145,165.00**
MTL	**4**	**weeks**	**$ 1,875.00**	**$ 7,500.00**
Fuel	**20**	**days**	**$ 97.00**	**$ 1,940.00**
Mixing of microorganism and SediMite	**4**	**weeks**	**$ 4,200.00**	**$ 16,800.00**
Carbon Storage (Offsite or onsite containers)	**4**	**weeks**	**$ 1,500.00**	**$ 6,000.00**
Environmental Analyst	**225**	**hrs.**	**$ 87.00**	**$ 19,575.00**
Project Manager	**200**	**hrs.**	**$ 123.00**	**$ 24,600.00**
Compressor/ salt spreader (rental)	**4**	**weeks**	**$ 2,100.00**	**$ 8,400.00**
Boat (rental)	**4**	**weeks**	**$ 1,700.00**	**$ 6,800.00**
Environmental Analyst	**225**	**hrs.**	**$ 87.00**	**$ 19,575.00**
Environmental Analyst	**225**	**hrs.**	**$ 87.00**	**$ 19,575.00**
Vehicle	**20**	**day**	**$ 75.00**	**$ 1,500.00**
Per diem	**80**	**days**	**$ 155.00**	**$ 12,400.00**
PPE per day	**20**	**at**	**$ 25.00**	**$ 500.00**
Project Coordination				**$ 14,875.00**
HASP	**1**	**@**	**$ 3,800.00**	**$ 3,800.00**
Environmental Analyst	**40**	**hrs.**	**$ 87.00**	**$ 3,480.00**
Project Manager	**45**	**hrs.**	**$ 123.00**	**$ 5,535.00**
Financial Manager	**20**	**hrs.**	**$ 103.00**	**$ 2,060.00**
Contingency	**1**	**@**	**$ 1,518,680.00**	**$ 151,868.00**
Total Fee				**$ 1,767,920.00**
			per Acre	**$ 226,656.41**

7.4 COST COMPARISON TO OTHER TECHNOLOGIES

The costs determined for the bioamended SediMite™ technology were compared with costs estimated for other technologies evaluated in the 2008 Feasibility Study conducted for this site. The construction cost of each of the 8 alternatives presented in the Feasibility report are compared to the cost of the construction of the bioamended SediMite™ in **Table 27**. The costs presented in the 2008 Feasibility report were escalated to present value (2017) using a 3% inflation rate. As shown in **Table 27**, the net present cost for the 8 technologies evaluated in the Feasibility study ranged from $0 for no further action scenario to $25M for full excavation and disposal off-site. Implementation of an isolation cap with or without reactive media is estimated at $4M. In comparison, the estimated cost of bioamended SediMite application is $1.8M. The capping design involves an 18" sand cap with a 6" topsoil habitat layer creating a total of 24" of cap thickness. This can have a major effect of altering the nature of the wetland/pond system. In comparison the amount of material to be added as bioamended SediMite™ will barely alter the bathymetry of the pond and wetland system with minimal impact on the existing ecosystem. We anticipate the annual monitoring and maintenance costs for bioamended SediMite™ to be in the range of costs for Monitored Natural Attenuation or capping which are estimated at about $100k/year for the first 5 years. The total annualized cost per acre for each of the alternatives are shown in the last column of Table 27.

Table 27. Comparison of Total Capital Costs of Implementation of Remediation Technologies at Abraham's Creek.

Costs for Alt 1-8 are based on 2008 Feasibility Study for the site (Battelle 2008).

Treatment Alternative	Total Capital Cost (2017 dollars)	O&M Cost	Total Annualized cost ($/acre)
Alt 1: No further action	0	**0**	**0**
Alt 2: Monitored Natural Attenuation	130,000	**520,000**	**83,333**
Alt 3: Isolation cap	4,030,000	**910,000**	**633,333**
Alt 4: Excavation & on-site CDF	17,030,000	**910,000**	**2,300,000**
Alt 5: Excavate & off-site disposal	25,090,000	**0**	**3,216,000**
Alt 6: Partial excavation & off-site disposal	11,570,000	**260,000**	**1,516,666**
Alt 7: Capping and wetland creation	5,850,000	**910,000**	**866,666**
Alt 8: Reactive cap	4,030,000	**910,000**	**633,333**
Alt 9: Bioamended SediMite™ (present study)	**1,767,920**	**910,000**	**343,323**

8.0 IMPLEMENTATION ISSUES

This section describes implementation issues experienced during the performance period of this field study.

The draft and final Field Demonstration Work Plan was provided to the remedial project manager at NAVFAC and the Environmental Restoration Project Manager at MBQ prior to implementation. Although a permit was not required for the pilot scale field test, full-scale implementation would need to comply with the substantive requirements of environmental regulations at this CERCLA site. Any regulations would be identified in planning documents prior to fieldwork, which would then be reviewed by state and federal regulators. The only restrictions encountered during the field implementation and post-treatment sampling was obtaining permission to access the site between training sessions and ensuring that all equipment did not exceed the maximum height requirement due to proximity of the site to an airfield.

The biggest factor is that the technology is cost effective and capable of producing the desired results. A major concern would be site implementation without impacting training, so staging area(s), traffic, and availability to be on site would need planned. The approval process for possible full scale treatment would need to fit in to the training schedule of the site. A tele belt or other equipment would need to get approval from the airfield. This is a multi-step process if certain conditions exist but would generally follow: submit plan/equipment to airfield; airfield conducts an Obstacle Evaluation to submit to the FAA; waiver granted by FAA; prior to equipment being used in the field a Notice to Airman (NOTAM) is issued. We do not believe extensive examination of the technology itself would be required for the purpose of obtaining or exemption from a permit since regulatory agencies are familiar with use of SediMite™ in several other projects and the microbes used are not GMO nor pathogenic and are ubiquitous in the environment. Finally, before full scale implementation would be approved there would be comparison to other technologies during the feasibility stage of process.

In terms of procurement issues SediMite™ is available for order from Sediment Solutions, requiring a lead time of 6 months (for 160 tons) for production and shipment. The microorganisms used as bioamendments are available from either commercial culture collections and/or individual university labs. Scale-up of the microorganisms requires outsourcing to companies with large volume bioreactors with the ability to grow anaerobes in the case of the halorespiring microorganism. However, plans are under way to develop a commercial source for these microorganisms. The VHI used in this study requires slight modification, but other deployment methods (*e.g.*, telebelt, broadcaster) could be used directly without modification. All other equipment used in the study is available commercially. Commercial contractors such as Brightfields Inc. are familiar with the use and application of the material.

Page Intentionally Left Blank

9.0 REFERENCES

1. Kjellerup, B.V., Sun, X., Ghosh, U., May, H.D., and Sowers, K.R. (2008). Site-specific microbial communities in three PCB-impacted sediments are associated with different *in situ* dechlorinating activities. Environ. Microbiol. *10*, 1296-1309.
2. Payne, R.B., Chun, C., May, H.D., and Sowers, K.R. (2011). Enhanced reductive dechlorination of polychlorinated biphenyl impacted sediment by bioaugmentation with a dehalorespiring bacterium. Environ. Sci Technol. *45*, 8772–8779.
3. Payne, R.B., Fagervold, S.K., May, H.D., and Sowers, K.R. (2013). Remediation of polychlorinated biphenyl impacted sediment by concurrent bioaugmentation with anaerobic halorespiring and aerobic degrading bacteria. Env. Sci Technol. *47*, 3807−3815.
4. USEPA (2013). Use of Amendments for In Situ Remediation at Superfund Sediment Sites. (Office of Superfund Remediation and Technology Innovation).
5. Bedard, D.L., Unterman, R., Bopp, L.H., Brennan, M.J., Haberl, M.L., and Johnson, C. (1986). Rapid assay for screening and characterizing microorganisms for the ability to degrade polychlorinated biphenyls. Appl. Environ. Microbiol. *51*, 761-768.
6. May, H.D., Miller, G.S., Kjellerup, B.V., and Sowers, K.R. (2008). Dehalorespiration with polychlorinated biphenyls by an anaerobic ultramicrobacterium. Appl. Environ. Microbiol. *74*, 2089–2094.
7. van den Berg, M., Birnbaum, L.S., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., et al. (2006). The 2005 World Health Organization Re-evaluation of Human and Mammalian Toxic Equivalency Factors for Dioxins and Dioxin-like Compounds. Toxicol Sci *93*, 223-241.
8. Beckingham, B., and Ghosh, U. (2013). Polyoxymethylene passive samplers to monitor changes in bioavailability and flux of PCBs after activated carbon amendment to sediment in the field. Chemosphere *91*, 1401-1407.
9. Ghosh, U., Kane Driscoll, S., Burgess, R., Gobas, F., Maruya, K., Jonker, C., Gala, W., Choi, Y., Beegan, C., Apitz, S., et al. (2014). Passive Sampling Methods for Contaminated Sediments: Practical Guidance for Selection, Calibration and Implementation. Integr Environ Assess Manage *10*, 210-223.
10. Fagervold, S.K., May, H.D., and Sowers, K.R. (2007). Microbial reductive dechlorination of Aroclor 1260 in Baltimore Harbor sediment microcosms is catalyzed by three phylotypes within the phylum Chloroflexi. Appl. Environ. Microbiol. *73*, 3009-3018.
11. Lombard, N.J., Ghosh, U., Kjellerup, B.V., and Sowers, K.R. (2014). Kinetics and threshold level of 2,3,4,5-tetrachlorobiphenyl dechlorination by an organohalide respiring bacterium. Environ. Sci Technol. *48*, 4353-4360.
12. Lozupone, C., and Knight, R. (2005). UniFrac: a New Phylogenetic Method for Comparing Microbial Communities. Applied and environmental microbiology *71*, 8228-8235.
13. Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). Molecular cloning- a laboratory manual., (N.Y.: Cold Spring Harbour Press).

14. Denef, V.J., Patrauchan, M.A., Florizone, C., Park, J., Tsoi, T.V., Verstraete, W., Tiedje, J.M., and Eltis, L.D. (2005). Growth substrate- and phase-specific expression of biphenyl, benzoate, and C1 metabolic pathways in *Burkholderia xenovorans* LB400. J. Bacteriol. *187*, 7996-8005.

15. Ghosh, U., Luthy, R.G., Cornelissen, G., Werner, D., and Manzie, C.A. (2011). *In-situ* Sorbent Amendments: A New Direction in Contaminated Sediment Management. Environ. Sci. Technol. *45*, 1163-1168.

16. Sowers, K.R., Boone, J.E., and Gunsalus, R.P. (1993). Disaggregation of *Methanosarcina* spp. and growth as single cells at elevated osmolarity. Appl. Environ. Microbiol. *59*, 3832-3839.

17. Oen, A.M.P., Janssen, E.M.L., Cornelissen, G., Breedveld, G.D., Eek, E., and Luthy, R.G. (2011). *In situ* measurement of PCB pore water concentration profiles in activated carbon-amended sediment using passive samplers. Env. Sci Technol. *45*, 4053-4059.

18. Grossman, A., and Ghosh, U. (2009). Measurement of activated carbon and other black carbons in sediments. Chemosphere *75*, 469–475.

19. Fagervold, S.K., Watts, J.E.M., May, H.D., and Sowers, K.R. (2005). Sequential reductive dechlorination of *meta*-chlorinated polychlorinated biphenyl congeners in sediment microcosms by two different Chloroflexi phylotypes. Appl. Environ. Microbiol. *71*, 8085-8090.

20. Beltrametti, F., Reniero, D., Backhaus, S., and Hofer, B. (2001). Analysis of transcription of the bph locus of *Burkholderia* sp. strain LB400 and evidence that the ORF0 gene product acts as a regulator of the *bph*A1 promoter. Microbio. *147*, 2169–2182.

21. Southwood, T.R.E. (1978). Ecological Methods, (London,: Chapman and Hall).

22. Magurran, A.E. (1988). Ecological Diversity and its Measurement, (New Jersey: Princeton University Press).

23. Battelle (2008). Feasibility Study for Site 100 Chopawamsic Creek Including Abraham's Creek MCB Quantico VA. Volume M00264.AR.001381, MCB Quantico, VA 5090.3a.

APPENDIX A POINTS OF CONTACT

Point of Contact Name	Organization Name	Phone Fax Email	Role in Project
Kevin Sowers	Institute of Marine & Environmental Technology – UMBC	Phone: 410.234.8878 FAX: 410.234.8896 E-mail: sowers@umbc.edu	Principal Investigator/Project Manager
Upal Ghosh	Sediment Solutions	Phone: 410-455-8665 FAX: 410-455-6500 E-mail: ughosh@umbc.edu	Co-Investigator/ SediMite™ Production Leader
Harold May	Medical University of So. Carolina	Phone: 843.725.4856 E-mail: mayh@musc.edu	Co-Investigator/ Bioamendment Development Leader
Rayford Payne	Institute of Marine & Environmental Technology – UMBC	Phone: 410.234.8852 FAX: 410.234.8896 E-mail: payner@umbc.edu	Bioamendment Scale up & Analytical Leader

Page Intentionally Left Blank

APPENDIX B SUPPORTING DATA

Figure 14. Effect of Treatments on PCB homolog concentrations with time.

	Treatment 1		Treatment 2		Treatment 3		Treatment 4		Treatment 5		Treatment 6		Treatment 7		Treatment 8		Treatment 9		Treatment 10	
PCB	Avg	Stdev	Avg	Stdev	Avg	Stdev	Avg	Stdev	Avg	Stdev	Avg	Stdev	Avg	Stdev	Avg	Stdev	Avg	Stdev	Avg	Stdev
Mono	9.50	5.69	16.80	5.83	6.93	6.21	10.78	12.17	1.20	0.65	1.46	0.72	0.89	0.55	1.63	1.40	2.25	1.39	3.94	3.19
Di	36.42	3.22	24.93	12.33	27.11	5.89	31.30	13.58	0.43	0.34	0.25	0.10	0.66	0.52	0.16	0.12	0.25	0.15	0.40	0.33
Tri	16.78	4.75	7.68	4.23	13.91	7.90	9.36	2.40	0.85	0.24	0.56	0.07	0.66	0.24	1.32	0.81	0.83	0.31	0.80	0.61
Tetra	8.30	2.23	5.02	2.20	5.14	2.61	3.39	1.57	0.32	0.19	0.17	0.03	0.30	0.10	0.33	0.06	0.42	0.12	0.43	0.32
Penta	11.58	3.21	3.29	0.53	6.46	5.60	0.66	0.27	0.11	0.15	0.04	0.00	0.12	0.02	0.02	0.00	0.05	0.02	0.07	0.06
Hexa	9.34	2.87	2.52	0.26	5.15	6.17	0.59	0.36	0.06	0.08	0.03	0.01	0.03	0.02	0.01	0.01	0.03	0.02	0.05	0.04
Hepta	1.38	0.29	0.33	0.11	0.57	0.47	0.21	0.05	0.01	0.02	0.01	0.00	0.01	0.01	0.02	0.01	0.02	0.01	0.01	0.01
Octa	0.09	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.00	0.00
Nona	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Deca	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
TOTAL	93.38	7.68	60.56	14.02	65.28	22.68	56.28	18.67	2.99	0.91	2.51	0.79	2.67	1.36	3.50	1.08	3.85	1.53	5.69	4.44

Table 17 and Figure 43. Total organic carbon detected in the upper 7.5 cm of core samples from each treatment

Top Core	1 Day 0	1 Day120	1 Day409	2 Day 0	2 Day120	2 Day409	3 Day 0	3 Day120	3 Day409	4 Day 0	4 Day120	4 Day409
1	4.2	2.58	4.25	2.82	5.22	4.72	3.22	5.67	3.62	2.34	9.81	4.24
2	2.8	3.08	2.99	2.78	4.42	5.08	1.15	1.83	4.56	2.50	6.01	4.44
3	1.5	3.36	3.58	1.23	nd	4.12	0.63	3.93	2.57	0.60	6.00	4.00
4	3.9	4.35	4.54	4.09	nd	10.75	1.56	6.21	0.60	3.17	nd	20.75
5	3.6	3.53	1.08	2.16	4.35	4.65	4.92	6.26	4.78	2.19	8.63	4.58
Total												
CALCULATIONS												
	1 Day 0	1 Day120	1 Day409	2 Day 0	2 Day120	2 Day409	3 Day 0	3 Day120	3 Day409	4 Day 0	4 Day120	4 Day409
Count	5.00	5.00	5.00	5.00	3.00	5.00	5.00	5.00	5.00	5.00	4.00	5.00
Mean	3.20	3.38	3.29	2.62	4.66	5.86	2.30	4.78	3.23	2.16	7.61	7.60
SD	1.10	0.65	1.37	1.04	0.49	2.75	1.76	1.90	1.71	0.95	1.92	7.35
Max	4.18	4.35	4.54	4.09	5.22	10.75	4.92	6.26	4.78	3.17	9.81	20.75
Q3	3.91	3.53	4.25	2.82	4.82	5.08	3.22	6.21	4.56	2.50	8.93	4.58
Median	3.63	3.36	3.58	2.78	4.42	4.72	1.56	5.67	3.62	2.34	7.32	4.44
Q1	3.23	3.22	3.29	2.47	4.38	4.68	1.35	4.80	3.09	2.27	6.01	4.34
Min	1.45	2.58	1.08	1.23	4.35	4.12	0.63	1.83	0.60	0.60	6.00	4.00

Table 18 and Figure 44. Black carbon detected in the upper 7.5 cm of core samples from each treatment plot.

0-7.5 cm % Black Carbon												
Top Core	1 Day 0	1 Day120	1 Day409	2 Day 0	2 Day120	2 Day409	3 Day 0	3 Day120	3 Day409	4 Day 0	4 Day120	4 Day409
1	0.4	0.15	0.58	0.28	0.76	1.11	0.48	2.75	0.77	1.08	1.70	0.94
2	0.3	nd	0.50	0.32	0.60	0.78	0.06	0.18	0.49	0.31	6.12	1.57
3	0.1	0.45	0.67	0.63	0.54	0.64	0.06	0.36	0.10	0.09	2.11	0.76
4	0.6	0.52	0.93	0.13	nd	7.19	0.10	3.07	1.09	0.30	nd	15.34
5	0.4	0.43	0.07	nd	0.68	0.92	nd	1.78	0.90	0.14	5.58	16.63
Total												
CALCULATIONS												
	1 Day 0	1 Day120	1 Day409	2 Day 0	2 Day120	2 Day409	3 Day 0	3 Day120	3 Day409	4 Day 0	4 Day120	4 Day409
Count	5.00	4.00	5.00	4.00	4.00	5.00	4.00	5.00	5.00	5.00	4.00	5.00
Mean	0.37	0.39	0.55	0.34	0.64	2.13	0.17	1.63	0.67	0.38	3.88	7.05
SD	0.17	0.16	0.32	0.21	0.09	2.83	0.20	1.33	0.39	0.40	2.29	8.18
Max	0.58	0.52	0.93	0.63	0.76	7.19	0.48	3.07	1.09	1.08	6.12	16.63
Q3	0.42	0.47	0.67	0.40	0.70	1.11	0.20	2.75	0.90	0.31	5.71	15.34
Median	0.41	0.44	0.58	0.30	0.64	0.92	0.08	1.78	0.77	0.30	3.85	1.57
Q1	0.42	0.45	0.63	0.32	0.68	0.96	0.10	1.39	0.84	0.31	2.11	1.29
Min	0.14	0.15	0.07	0.13	0.54	0.64	0.06	0.18	0.10	0.09	1.70	0.76

nd = not determined (sample lost due to instrument error)

Figure 19. Effect of treatments after 375 days on porewater concentrations of PCB homologs estimated from equilibrium passive sampling.

	Treatment 1		Treatment 2		Treatment 3		Treatment 4		Treatment 5		Treatment 6		Treatment 7		Treatment 8		Treatment 9		Treatment 10	
PCB	Avg	Stdev	Avg	Stdev	Avg	Stdev	Avg	Stdev	Avg	Stdev	Avg	Stdev	Avg	Stdev	Avg	Stdev	Avg	Stdev	Avg	Stdev
Mono	9.50	5.69	16.80	5.83	6.93	6.21	10.78	12.17	1.20	0.65	1.46	0.72	0.89	0.55	1.63	1.40	2.25	1.39	3.94	3.19
Di	36.42	3.22	24.93	12.33	27.11	5.89	31.30	13.58	0.43	0.34	0.25	0.10	0.66	0.52	0.16	0.12	0.25	0.15	0.40	0.33
Tri	16.78	4.75	7.68	4.23	13.91	7.90	9.36	2.40	0.85	0.24	0.56	0.07	0.66	0.24	1.32	0.81	0.83	0.31	0.80	0.61
Tetra	8.30	2.23	5.02	2.20	5.14	2.61	3.39	1.57	0.32	0.19	0.17	0.03	0.30	0.10	0.33	0.06	0.42	0.12	0.43	0.32
Penta	11.58	3.21	3.29	0.53	6.46	5.60	0.66	0.27	0.11	0.15	0.04	0.00	0.12	0.02	0.02	0.00	0.05	0.02	0.07	0.06
Hexa	9.34	2.87	2.52	0.26	5.15	6.17	0.59	0.36	0.06	0.08	0.03	0.01	0.03	0.02	0.01	0.01	0.03	0.02	0.05	0.04
Hepta	1.38	0.29	0.33	0.11	0.57	0.47	0.21	0.05	0.01	0.02	0.01	0.00	0.01	0.01	0.02	0.01	0.02	0.01	0.01	0.01
Octa	0.09	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.00	0.00
Nona	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Deca	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
TOTAL	93.38	7.68	60.56	14.02	65.28	22.68	56.28	18.67	2.99	0.91	2.51	0.79	2.67	1.36	3.50	1.08	3.85	1.53	5.69	4.44

Figure 45. Total PCB concentrations in 0-7.5 cm (A) and 7.5-15 cm (B) sediment cores.

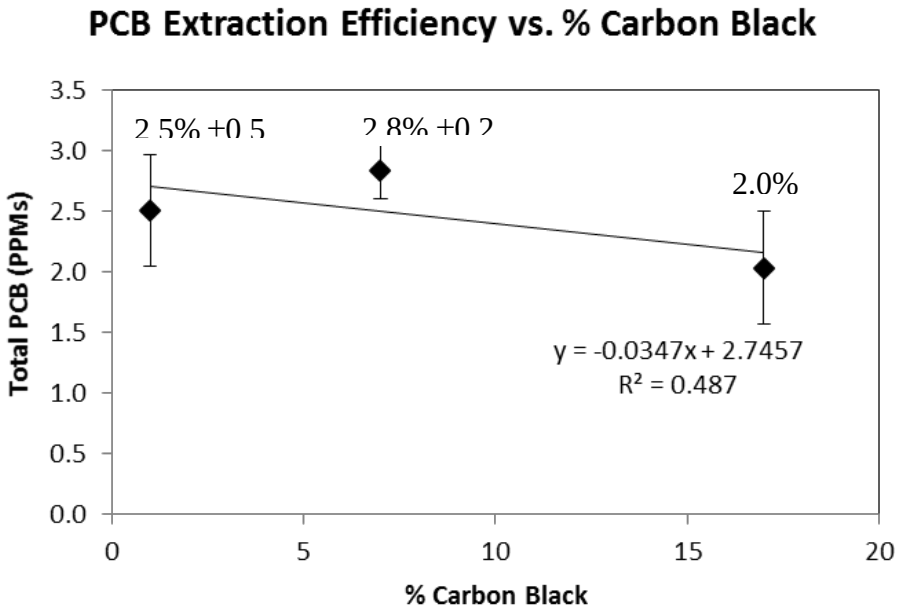
7.5-15 cm												
Top Core	1 Day 0	1 Day120	1 Day409	2 Day 0	2 Day120	2 Day409	3 Day 0	3 Day120	3 Day409	4 Day 0	4 Day120	4 Day409
1	4.60	4.71	3.26	2.36	4.18	3.20	2.00	3.47	2.78	2.25	4.34	2.65
2	2.63	3.72	3.99	2.64	2.85	2.36	2.67	3.49	3.52	2.27	2.57	1.67
3	3.65	3.41	4.24	2.69	3.30	3.04	4.08	3.11	3.58	2.21	2.37	4.62
4	1.84	2.30	4.70	4.17	4.18	2.61	2.36	5.50	3.25	2.58	4.53	3.21
5	2.19	2.50	2.72	3.26	3.26	4.40	2.30	5.74	4.56	3.65	3.46	3.05
Total												
CALCULATIONS 7.5 to 15 cm												
	1 Day 0	1 Day120	1 Day409	2 Day 0	2 Day120	2 Day409	3 Day 0	3 Day120	3 Day409	4 Day 0	4 Day120	4 Day409
Count	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Mean	2.98	3.33	3.78	3.02	3.55	3.12	2.68	4.26	3.54	2.59	3.45	3.04
SD	1.13	0.98	0.79	0.72	0.60	0.79	0.82	1.25	0.65	0.61	0.99	1.07
Max	4.60	4.71	4.70	4.17	4.18	4.40	4.08	5.74	4.56	3.65	4.53	4.62
Q3	3.65	3.72	4.24	3.26	4.18	3.20	2.67	5.50	3.58	2.58	4.34	3.21
Median	2.63	3.41	3.99	2.69	3.30	3.04	2.36	3.49	3.52	2.27	3.46	3.05
Q1	2.41	2.96	3.63	2.66	3.28	2.83	2.33	3.48	3.39	2.26	3.02	2.85
Min	1.84	2.30	2.72	2.36	2.85	2.36	2.00	3.11	2.78	2.21	2.37	1.67

Figure 46. Linear regression analysis of total PCB concentrations in 0-7.5 cm of sediment cores. R^2 values are shown adjacent to each line of best fit. Calculations based on five sample cores for each treatment plot indicated in legend from locations shown in Figure 42.

	Plot A	Plot B	Plot C	Plot D
Best-fit values				
Slope	-0.00008290 ± 0.001200	-0.0008207 ± 0.0008925	-0.002064 ± 0.0008887	-0.003132 ± 0.0007950
Y-intercept when X=0.0	2.833 ± 0.2994	2.836 ± 0.2228	2.398 ± 0.2218	2.436 ± 0.1984
X-intercept when Y=0.0	34170	3456	1162	777.7
1/slope	-12060	-1219	-484.5	-319.3
95% Confidence Intervals				
Slope	-0.002674 to 0.002508	-0.002748 to 0.001107	-0.003984 to -0.0001443	-0.004849 to -0.001415
Y-intercept when X=0.0	2.186 to 3.479	2.355 to 3.317	1.919 to 2.877	2.007 to 2.864
X-intercept when Y=0.0	1168 to +infinity	1108 to +infinity	622.0 to 14250	504.9 to 1562
Goodness of Fit				
r^2	0.0003673	0.06107	0.2932	0.5442
Sy.x	0.7885	0.5866	0.5842	0.5226
Is slope significantly non-zero?				
F	0.004777	0.8456	5.393	15.52
DFn, DFd	1.000, 13.00	1.000, 13.00	1.000, 13.00	1.000, 13.00
P value	0.9460	0.3746	0.0371	0.0017
Deviation from zero?	Not Significant	Not Significant	Significant	Significant
Data				
Number of X values	3	3	3	3
Maximum number of Y replicates	5	5	5	5
Total number of values	15	15	15	15
Number of missing values	0	0	0	0

.

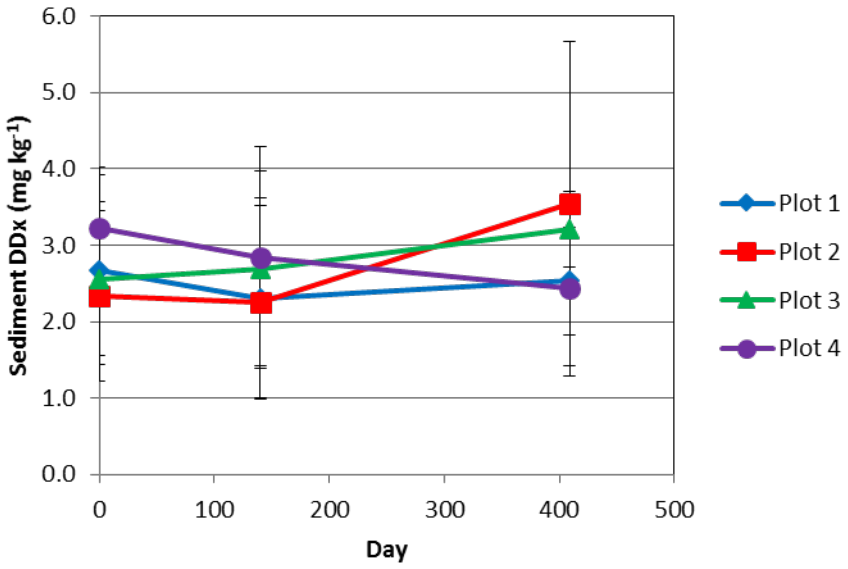
Page 73. PCB extraction efficiency with accelerated solvent extraction vs % black carbon



Sample	Time 0 Black Carbon[a]	Time Final Black Carbon[b]	Time 0 PCB[c]	Time Final PCB[d]
1%-1				2.4
1%-2				3.1
1%-3		0.43		2.0
7%-1				2.6
7%-2				2.8
7%-3		6.43		3.1
17%-1				2.6
17%-2				1.9
17%-3	0.43	16.43	2.6	1.7

[a] Measured for homogenized sediment sample from Abraham's Creek.
[b] Based on measures native BC in homogenized sediment and mass of BC added and homogenized in three subsamples.
[c] Measured for homogenized sediment sample.
[d] Measured in triplicate samples.

Page 74. Effect of treatments on concentration of DDx congeners in in upper (0-7.5 cm) sediment profile of treatment plots 1-4.



Day 0	1-1	1-2	1-3	1-4	1-5		
DDx Congener	ug / g					AVG	STDEV
o,p-DDE	0	0	0	0	0	0	0
p,p-DDE	4.24736	1.48181	2.80625	1.15	1.825	2.30208	1.25160
o,p-DDD	0.18421	0.30909	0.12718	0.261	0.1585	0.20799	0.07512
p,p-DDD	0.21947	0.16	0.18031	0.162	0.1025	0.16485	0.04225
p,p-DDT	0	0	0	0	0	0	0
	4.65105	1.95090	3.11375	1.573	2.086	2.67494	1.24327

Day 0	2-1	2-2	2-3	2-4	2-5		
DDx Congener	ug / g					AVG	STDEV
o,p-DDE	0	0	0	0	0	0	0
p,p-DDE	1.26086	1.87948	2.72727	3.63333	1.01296	2.102785	1.081545
o,p-DDD	0.16	0.15461	0.12878	0.09461	0.03055	0.113715	0.053204
p,p-DDD	0.13782	0.06897	0.17151	0.13102	0.05833	0.113535	0.048194
p,p-DDT	0	0	0	0	0	0	0
	1.55869	2.10307	3.02757	3.85897	1.10185	2.330035	1.115698

Day 0	3-1	3-2	3-3	3-4	3-5		
DDx Congener	ug / g					AVG	STDEV
o,p-DDE	0	0	0	0	0	0	0
p,p-DDE	3.02222	1.68490	3.46666	1.392	1.66	2.245159	0.932749
o,p-DDD	0.22814	0.13452	0.17622	0.1376	0.0594	0.14718	0.061961
p,p-DDD	0.2	0.16943	0.19866	0.232	0.0426	0.16854	0.073801
p,p-DDT	0	0	0	0	0	0	0
	3.4503	1.98886	3.84155	1.7616	1.762	2.560879	1.004437

Day 0	4-1	4-2	4-3	4-4	4-5		
DDx Congener						AVG	STDEV
o,p-DDE	0	0	0	0	0	0	0
p,p-DDE	2.30689	2.55	4.15714	3.13571	2.4	2.90995	0.76807
o,p-DDD	0.18896	0.263125	0.16476	0.19214	0.08517	0.17883	0.06394
p,p-DDD	0.22068	0.04375	0.20428	0.1125	0.075	0.13124	0.07827
p,p-DDT	0	0	0	0	0	0	0
	2.71655	2.856875	4.52619	3.44035	2.56017	3.22003	0.80239

Day 140	1-1	1-2	1-3	1-4	1-5		
DDx Congener	ug / g					AVG	STDEV
o,p-DDE	0	0	0	0	0	0	0
p,p-DDE	2.13125	1.14	1.31034	3.575	0.68	1.76731	1.13850
o,p-DDD	0.44187	0.199	0.11551	0.26333	0.201	0.24414	0.12238
p,p-DDD	0.49875	0.2865	0.25862	0.36041	0.07966	0.29679	0.15292
p,p-DDT	0	0	0	0	0	0	0
	3.07187	1.6255	1.68448	4.19875	0.96066	2.30825	1.30655

D 140	2-1	2-2	2-3	2-4	2-5		
DDx Congener	ug / g					AVG	STDEV
o,p-DDE	0	0	0	0	0	0	0
p,p-DDE	2.81034	2.59062	2.64838	0.56666	0.32727	1.78865	1.23034
o,p-DDD	0.39793	0.279062	0.23225	0.19733	0.18393	0.25810	0.08638
p,p-DDD	0.31034	0.14187	0.054838	0.21966	0.27151	0.19964	0.10262
p,p-DDT	0	0	0	0	0	0	0
	3.51862	3.01156	2.93548	0.98366	0.78272	2.24641	1.266461

D140	3-1	3-2	3-3	3-4	3-5		
DDx Congener	ug / g					AVG	STDEV
o,p-DDE	0	0	0	0	0	0	0
p,p-DDE	3.644444	3.5	0.685	2.00285	1.80882	2.32822	1.24311
o,p-DDD	0.407777	0.0785	0.347	0.07142	0.09117	0.19917	0.16425
p,p-DDD	0.24388	0.1565	0.2035	0.11542	0.11382	0.16662	0.05665
p,p-DDT	0	0	0	0	0	0	0
	4.29611	3.735	1.2355	2.18971	2.01382	2.44693	1.68224

D 140	4-1	4-2	4-3	4-4	4-5		
DDx Congener	ug / g					AVG	STDEV
o,p-DDE	0	0	0	0	0	0	0
p,p-DDE	1.89642	1.55476	4.63	0.92580	3.235	2.448399	1.483212
o,p-DDD	0.31785	0.18738	0.29133	0.13225	0.16	0.217766	0.082162
p,p-DDD	0.19	0.18976	0.08	0.28354	0.1535	0.179362	0.073515
p,p-DDT	0	0	0	0	0	0	0
	2.40428	1.93190	5.00133	1.34161	3.5485	2.845527	1.451967

D409	1-1	1-2	1-3	1-4	1-5		
DDx Congener	ug / g					AVG	STDEV
o,p-DDE	0	0	0	0	0	0	0
p,p-DDE	3.21973	2.01866	1.38817	2.01545	1.73157	2.074722	0.690437
o,p-DDD	0.15789	0.27733	0.06494	0.14509	0.19631	0.168316	0.077463
p,p-DDD	0.22631	0.37066	0.21397	0.12063	0.50508	0.287337	0.151041
p,p-DDT	0	0	0	0	0	0	0
	3.60394	2.66666	1.66709	2.28118	2.43298	2.530375	0.704976

D 409	2-1	2-2	2-3	2-4	2-5		
DDx Congener	ug / g					AVG	STDEV
o,p-DDE	0	0	0	0	0	0	0
p,p-DDE	2.46636	0.88723	5.85205	5.33437	2.04594	3.317195	2.164441
o,p-DDD	0.13436	0.0128	0.05342	0.02864	0.22567	0.090996	0.088631
p,p-DDD	0.23781	0.09861	0.07904	0.13208	0.145	0.138512	0.061387
p,p-DDT	0	0	0	0	0	0	0
	2.83854	0.99872	5.98452	5.49510	2.41662	3.546703	2.121909

D 409	3-2	3-2	3-3	3-4	3-5		
DDx Congener	ug / g					AVG	STDEV
o,p-DDE	0	0	0	0	0	0	0
p,p-DDE	3.26493	2.45061	2.73424	2.23037	1.62808	2.461654	0.605806
o,p-DDD	0.21688	0.06419	0.75068	0.22784	0.51797	0.355518	0.275198
p,p-DDD	0.13896	0.31975	0.39041	0.44683	0.66516	0.392226	0.191624
p,p-DDT	0	0	0	0	0	0	0
	3.62077	2.83456	3.87534	2.90506	2.81123	3.209398	0.501091

D 409	4-1	4-2	4-3	4-4	4-5		
DDx Congener	ug / g					AVG	STDEV
o,p-DDE	0	0	0	0	0	0	0
p,p-DDE	2.91883	1.44015	0.93157	0.96823	2.57441	1.766643	0.924854
o,p-DDD	0.36753	0.43636	0.13007	0.16882	0.41937	0.304435	0.144387
p,p-DDD	0.30454	0.3053	0.33909	0.19529	0.72945	0.37474	0.205575
p,p-DDT	0	0	0	0	0	0	0
	3.59090	2.18181	1.4007	1.33235	3.72325	2.445818	1.155935

Figure 48. Effect of treatments on PCB homolog concentrations in upper (0-7.5 cm)

Day 0	1-1	1-2	1-3	1-4	1-5	2-1	2-2	2-3	2-4
Homolog	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g
1	0.117075105	0	0	0	0	0	0	0	0
2	0.156029853	0.467304909	0.702329013	0.38242195	0.4158982	0.169118913	0.396181103	0.161968576	0.382002769
3	0.301560816	0.325623768	0.920286913	0.547015525	0.73696211	0.320477774	0.378850456	0.345022955	0.558778115
4	0.406822974	0.184972095	0.876124586	0.296454483	0.427676825	0.304278509	0.662212026	0.782767561	0.429756033
5	0.257822842	0.738664658	0.517937295	0.194743373	0.258538038	0.328663138	0.258956532	0.443142766	0.349120688
6	0.234530975	0.240414999	0.30351001	0.35585408	0.653546013	0.538366638	0.399159056	0.518298742	0.518503444
7	0.30842537	0.168130415	0.472894837	0.24150336	0.350623558	0.178300755	0.319717066	0.345630858	0.346789855
8	0.075250886	0.046783509	0.098083309	0.04785908	0.141509855	0.0516151	0.171019192	0.10980441	0.130481256
9	0.005874658	0.025171139	0.024557898	0.01069216	0.03269335	0.003103704	0.020868526	0.02308	0.032138513
10	0	0.015324818	0.005380922	0	0.00611695	0.007703913	0.003946487	0.036762667	0.00622741
total	1.863393479	2.212390311	3.921104782	2.07654401	3.0235649	1.901628443	2.610910444	2.766478533	2.753798085

Day 0	2-5	3-1	3-2	3-3	3-4	3-5	4-1	4-2	4-3
Homolog	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g			ug/g
1	0	0	0	0	0	0	0	0	0
2	0.504184967	0.233178963	0.455965275	0.558344372	0.343430533	0.432158544	0.360684483	0.300328	0.443597714
3	0.660651659	0.415950881	0.597467665	0.73161867	0.485311787	0.566272851	0.509977879	0.482804463	0.878460095
4	0.628948595	0.362597756	0.568796647	0.696510072	0.626016139	0.539098796	0.515180469	0.558142469	0.689890195
5	0.371814624	0.127518623	0.336254685	0.433977067	0.460132004	0.34869825	0.425246132	0.135990342	0.259873865
6	0.217882476	0.239739127	0.197044437	0.263973423	0.451609571	0.276756408	0.588476789	0.37883826	0.28344157
7	0.339479736	0.210704323	0.30701227	0.376410404	0.193422331	0.290982631	0.117433495	0.12822761	0.353418479
8	0.070411629	0.050559333	0.063677539	0.078439078	0.07842035	0.060352825	0.029757585	0.066855313	0.100835119
9	0.007259149	0.003944025	0.015943451	0.009745499	0.024032573	0.011111016	0.001875954	0.024895394	0.017116738
10	0.001270241	0.004699052	0.003493396	0.005388889	0.012139253	0.002111	0.004806276	0.007264625	0.004837714
total	2.801903078	1.648892084	2.545655367	3.154407474	2.674514541	2.527542321	2.553439062	2.083346475	3.03147149

Day 0	4-4	4-5

Homolog	ug/g	ug/g
1	0	0
2	0.283482607	0.469063656
3	0.269321311	0.614630944
4	0.533172964	0.585136303
5	0.187956145	0.350378461
6	0.452992505	0.215350709
7	0.419862696	0.316870412
8	0.223914571	0.066545475
9	0.045219643	0.016401454
10	0.010271429	0.00359375
total	2.426193871	2.637971165

Day 140	**1-1**	**1-2**	**1-3**	**1-4**	**1-5**	**2-1**	**2-2**	**2-3**	**2-4**
Homolog	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g
1	0	0	0.010689655	0	0.0136	0.030344828	0.0209375	0.03483871	0.0122
2	0.422457813	0	0.398325138	0.0225	0.3900325	0.655291207	0.141668281	0	0.345872867
3	0.446306563	0.423461975	0.467234259	0.482482417	0.382711087	0.47606119	0.302208578	0.31398129	0.343924097
4	0.693283775	0.8678663	1.151748517	1.110161271	0.661876123	1.096124707	0.698387672	0.708707758	0.610473843
5	1.013093109	0.281887203	0.541104732	0.658746621	0.280903838	0.528511859	0.41658618	0.281582191	0.276248913
6	0.324148626	0.425570795	0.465796661	0.788233938	0.411280578	0.478774534	0.540330849	0.388511204	0.460269939
7	0.213299439	0.315477735	0.249045075	0.552148042	0.301473941	0.256209817	0.355360852	0.29251014	0.340025326
8	0.124198214	0.153053667	0.10221177	0.154530396	0.163348656	0.104637655	0.0996044	0.139214145	0.183304928
9	0.013250009	0.03160495	0.047936517	0.038474917	0.01953522	0.049250448	0.024955297	0.029100758	0.022094183
10	0.002878756	0	0.031692655	0.0608295	0.003769667	0.032534103	0.039671844	0	0.0042282
total	3.252916304	2.498922625	3.465784979	3.8681071	2.628531609	3.707740348	2.639711453	2.188446197	2.598642295

Day 140	2-5	3-1	3-2	3-3	3-4	3-5	4-1	4-2	4-3
Homolog	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g
1	0.023151515	0.010333333	0.05935	0.054	0.007714286	0.037894737	0.054722911	0.010214943	0.038546956
2	0.402507515	0.068500778	0	0	0.212841114	0.208194789	0.363512377	0.083778957	0.310819629
3	0.620310212	0.149783017	0.2195695	0.486671	0.221344471	0.253142355	0.676599831	0.147469053	0.649473689
4	0.832215258	0.356192528	0.49891998	1.098497025	0.386088034	0.358075276	0.495923158	0.110777653	0.48753482
5	0.403099598	0.227380796	0.223281233	0.419544897	0.14791965	0.184990154	0.178835981	0.038220607	0.161264448
6	0.376233955	0.338087852	0.386099518	0.5858627	0.254142058	0.23479987	0.201619368	0.045024079	0.191625618
7	0.198560509	0.190510063	0.28394462	0.45396855	0.196858061	0.125360265	0.246170587	0.05124613	0.223525006
8	0.082424293	0.058859508	0.138326575	0.216359758	0.107885414	0.053666145	0.077331429	0.019471383	0.076453784
9	0.038549515	0.013647058	0.028587025	0.045106175	0.012695797	0.025224671	0.013216129	0.003591256	0.015161054
10	0.025560758	0.023671556	0	0	0.002505623	0.016912632	0.003690587	0.000918546	0.004244107
total	3.002613127	1.436966489	1.83807845	3.360010105	1.549994509	1.498260895	2.311622357	0.510712608	2.15864911

Day 140	4-4	4-5
Homolog	ug/g	ug/g
1	0.037303506	0.111563792
2	0.300793189	0.102552393
3	0.628522925	0.221890088
4	0.47180789	0.231093465
5	0.15444624	0.216729275
6	0.183698888	0.227214091
7	0.216185393	0.505413563
8	0.073858404	0.113183156
9	0.014671987	0.008591811
10	0.004107201	0.002480884
total	2.085395622	1.74071252

Day 409	**1-1**	**1-2**	**1-3**	**1-4**	**1-5**	**2-1**	**2-2**	**2-3**	**B-4**	**2-5**
Homolog	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g
1	0	0	0.02107	0	0	0	0	0	0	0
2	0.419503	0.455564	0.491912	0.469764	0.853269	0.414007	0.370561	0.734355	0.588569	0.421735
3	0.536099	0.473092	0.571823	0.414293	0.952963	0.551127	0.377926	0.687784	0.481068	0.542107
4	0.464101	0.466328	0.555591	0.578915	0.939487	0.526742	0.337544	0.831876	0.55296	0.485616
5	0.276916	0.297969	0.289551	0.176272	0.458446	0.320969	0.219791	0.279102	0.205938	0.305438
6	0.233475	0.237159	0.294228	0.256124	0.48795	0.251546	0.213333	0.409708	0.290562	0.26697
7	0.300536	0.254906	0.271674	0.211253	0.456949	0.294073	0.231129	0.325974	0.255173	0.264429
8	0.093145	0.044328	0.046343	0.032487	0.072865	0.064757	0.03536	0.061598	0.037092	0.052523
9	0.007168	0.009544	0.004166	0.005412	0.005829	0.004952	0.007615	0.005307	0.006201	0.00449
10	0.001453	0.002054	0.000792	0.001035	0.001001	0.001004	0.001639	0.001009	0.001186	0.000771
total	2.332397	2.240943	2.547149	2.145555	4.228758	2.429178	1.794898	3.336714	2.41875	2.344079

Day 409	**3-1**	**3-2**	**3-3**	**3-4**	**3-5**	**4-1**	**4-2**	**4-3**	**4-4**	**4-5**
Homolog	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g
1	0	0.008745	0.013933	0	0.010234	0	0	0	0	0
2	0.214218	0.208387	0.356286	0.269893	0.309176	0.495486	0.320301	0.30898	0.036053	0.052702
3	0.282189	0.295447	0.455805	0.333672	0.395488	0.164024	0.274791	0.287569	0.142999	0.201911
4	0.329777	0.272964	0.475433	0.31621	0.35645	0.268639	0.408177	0.335286	0.309606	0.149761
5	0.190926	0.153892	0.29273	0.207761	0.219271	0.097798	0.188113	0.113539	0.087367	0.140653
6	0.163852	0.134471	0.218187	0.186984	0.166146	0.120175	0.153253	0.148261	0.075104	0.14236
7	0.162747	0.186263	0.251579	0.181207	0.197182	0.162977	0.254675	0.144025	0.077433	0.252974
8	0.037598	0.040661	0.053715	0.0362	0.044046	0.042342	0.051979	0.037534	0.012751	0.062901
9	0.004632	0.008837	0.005307	0.007535	0.003733	0.002694	0.002869	0.00248	0.000218	0.004329
10	0.002919	0.001902	0.001009	0.001442	0.000641	0.012544	0	0.006322	0.010917	0.001374
total	1.388858	1.311568	2.123984	1.540904	1.702367	1.366678	1.654157	1.383995	0.752448	1.008965

Figure 49. Effect of treatments on PCB congener concentrations in upper (0-7.5 cm) sediment profile of treatment plots 1-4

1

Change >.007 mg/kg

Plot A 0-7.5 cm

PCB	Homolog	day0	day 409	change
3	1	0.023415	0.003688	-0.01973
4+10	2	0.03726	0.080122	0.042862
7+9	2	0.055241	0.034198	-0.02104
6	2	0.066982	0.046167	-0.02081
8+5	2	0.169057	0.225832	0.056775
14	2	0.052427	0.061312	0.008885
19	3	0.224242	0.128704	-0.09554
11	2	0.034022	0.09037	0.056349
12+13	2	0.009808	0	-0.00981
18	3	0.045987	0.056899	0.010912
15+17	4-3	0.049261	0.040274	-0.00899
24+27	3	0.012514	0.014449	0.001935
16+32	3	0.069784	0.057164	-0.01262
26	3	0.064248	0.099691	0.035443
25	3	0	0.050328	0.050328
31	3	0.011848	0.023273	0.011425
50	4	0.001274	0.007185	0.005911
28	3	0.046399	0.029624	-0.01678
21+33	3	0.006189	0.00505	-0.00114
53	4	0.022954	0.040321	0.017367
22	3	0.01158	0.014959	0.003378
36	3	0.018265	0.013194	-0.00507
45	4	0.019562	0.020462	0.0009
46	4	0.029809	0.022296	-0.00751
73	4	0	0	0
49	4	0.077495	0.247678	0.170183
48	4	0.014672	0.004257	-0.01041
35	3	0.004425	0.039615	0.03519
104	5	0.006022	0	-0.00602
44	4	0.038335	0.041976	0.003642
37+42+59	3-4	0.026598	0.045344	0.018746
41+71+ 64	4	0.005482	0.004375	-0.00111
40	4	0.011801	0.011689	-0.00011
67+100	**4-5**	0.0031	0.001446	-0.00165
63	4	0	0.012246	0.012246
74+94	4-5	0.011398	0.012118	0.00072
70+76	4	0.011983	0.018139	0.006156
66	4	0.049739	0.041768	-0.00797
93+95	5	0.048409	0.056739	0.00833
91	5	0.024059	0.038049	0.013991
56+60+1	**4**	0.014787	0.018558	0.003771
101	5	0.017405	0.039105	0.0217
99	5	0.025614	0.034269	0.008655
97	5	0.006741	0.012554	0.005814
145	**6**	0	0.029097	0.029097
120	5	0.123836	0	-0.12384
110+77	**4-5**	0.110411	0.028845	-0.08157
82+151	5-6	0.03462	0.056686	0.022067
135+144+	5-6	0.017349	0.025519	0.00817
139+140+	5-6	0.019901	0.023743	0.003842
133	6	0.032814	0.012233	-0.02058
114+131	5-6	0.041555	0.035433	-0.00612
146	6	0.046191	0.03674	-0.00945
153	7	0.070417	0.085815	0.015399
132+105	5-6	0.014734	0.011826	-0.00291
141	6	0.072062	0.026012	-0.04605
137+176	**6-7**	0.038178	0.056798	0.01862
163+138	6	0.014543	0.014028	-0.00051
158	6	0.026856	0.009295	-0.01756
160	6	0	0	0
126+129	5-6-7	0.02932	0.032299	0.002978
159	6	0	0.007686	0.007686
186+182	7	0.018299	0.01601	-0.00229
183	7	0.025112	0.031044	0.005932
128	6	0.019982	0	-0.01998
185	7	0.026321	0.005809	-0.02051
174	7	0.028253	0.033417	0.005164
181	7	0.001524	0.002239	0.000715
177	7	0.025938	0.034793	0.008855
157+200	6-7-8	0.000978	0.001761	0.000784
180	7	0.024031	0.022793	-0.00124
193	7	0.012322	0.003955	-0.00837
191	7	0.0307	0.01046	-0.02024
199	8	0.015394	0.004198	-0.0112
170+190	7	0.012633	0.009343	-0.00329
201	8	0.031613	0.030048	-0.00157
203+196	8	0.020085	0.011506	-0.00858
207	9	0.009218	0	-0.00922
194	8	0.006825	0.006226	-0.0006
SUM		2.620489	2.69896	

2

Plot B 0-7.5 cm

PCB	Homolog	day0	day 409 c	change
-3	1	0	0	0
(4+10)	2	0.040422	0.079728	0.039306
(7+9)	2	0.053127	0.035532	-0.01759
-6	2	0.050454	0.039741	-0.01071
(8+5)	2	0.112656	0.201051	0.088396
14	2	0.030321	0.052073	0.021752
-19	3	0.106912	0.11713	0.010219
11	2	0.028167	0.09772	0.069554
(12+13)	2	0.007545	0	-0.00755
-18	3	0.046309	0.043215	-0.00309
15 (+17)	4-3	0.03547	0.02941	-0.00606
(24+27)	3	0.012916	0.015845	0.00293
(16+32)	3	0.068039	0.050619	-0.01742
-26	3	0.048968	0.080502	0.031533
-25	3	0	0.058962	0.058962
-31	3	0.008075	0.019264	0.011189
50	4	0.009238	0.00755	-0.00169
-28	3	0.048936	0.024091	-0.02484
21(+33)	3	0.006256	0.004751	-0.00151
-53	4	0.024681	0.033214	0.008533
-22	3	0.010685	0.013272	0.002588
36	3	0.017774	0.011489	-0.00629
-45	4	0.027051	0.018297	-0.00875
-46	4	0.014228	0.021304	0.007077
73	4	0.003937	0	-0.00394
-49	4	0.080364	0.232798	0.152435
-48	4	0.020218	0.003427	-0.01679
35	3	0.017798	0.039006	0.021207
104	5	0.007341	0	-0.00734
-44	4	0.062898	0.040013	-0.02288
(37+42)+5	3-4	0.04581	0.042983	-0.00283
(41+71)mi	4	0.0146	0.004489	-0.01011
-40	4	0.029629	0.010769	-0.01886
67 + (10	**4-5**	0.009131	0.001476	-0.00766
-63	4	0.011498	0.013284	0.001786
(74) + 94	4-5	0.024883	0.0097	-0.01518
(70 +76)	4	0.023241	0.016233	-0.00701
-66	4	0.06476	0.034699	-0.03006
93 + (95)	5	0.066911	0.046608	-0.0203
-91	5	0.061718	0.032308	-0.02941
(56+60)+1	**4**	0.027674	0.014933	-0.01274
-101	5	0.017897	0.031497	0.0136
99	5	0.028353	0.032992	0.004639
-97	5	0.014165	0.010241	-0.00392
145	**6**	0	0.027373	0.027373
120	5	0	0	0
(110+77	**4-5**	0.0884	0.029774	-0.05863
(82+151)	5-6	0.0345	0.047397	0.012898
(135+144+	5-6	0.017868	0.022077	0.004209
139 +140 (	5-6	0.013482	0.020741	0.007258
133	6	0.1109	0	-0.1109
(114 + 1	5-6	0.049147	0.044333	-0.00481
-146	6	0.032136	0.029159	-0.00298
153	7	0.053554	0.079499	0.025945
(132+105	5-6	0.013268	0.010989	-0.00228
-141	6	0.053585	0.024732	-0.02885
(137+17	**6-7**	0.047684	0.048826	0.001141
(163+138)	6	0.013595	0.014525	0.00093
-158	6	0.002551	0.00934	0.006789
160	6	0.00865	0	-0.00865
126 + (1	5-6-7	0.025141	0.027149	0.002008
159	6	0	0.0084	0.0084
(186+182)	7	0.027256	0.013188	-0.01407
-183	7	0.037564	0.029599	-0.00797
-128	6	0.024241	0	-0.02424
-185	7	0.019662	0.005945	-0.01372
-174	7	0.012078	0.034382	0.022304
181	7	0.005879	0.001729	-0.00415
-177	7	0.032389	0.032076	-0.00031
(157+20	6-7-8	0.010515	0.000909	-0.00961
-180	7	0.022672	0.019463	-0.00321
-193	7	0.015879	0.00339	-0.01249
-191	7	0.011358	0.009002	-0.00236
-199	8	0.0125	0.003576	-0.00892
(170+190)	7	0.024436	0.008092	-0.01634
-201	8	0.046336	0.025881	-0.02045
(203+196)	8	0.023778	0.00981	-0.01397
-207	9	0.011865	0	-0.01187
-194	8	0.010617	0.005355	-0.00526
		2.566944	2.464724	-0.10222

3

Plot 0-7.5 cm

PCB	Homolog	day0	day 409 c	change
-3	1	0	0	0
(4+10)	2	0.002522	0	-0.00252
(7+9)	2	0.053184	0.016915	-0.03627
-6	2	0.033506	0.028058	-0.00545
(8+5)	2	0.225172	0.15296	-0.07221
14	2	0.07585	0.06304	-0.01281
-19	3	0.136702	0.062831	-0.07387
11	2	0.014381	0.010619	-0.00376
(12+13)	2	0	0	0
-18	3	0.055847	0.041898	-0.01395
15 (+17)	4-3	0.054726	0.034528	-0.0202
(24+27)	3	0	0	0
(16+32)	3	0.080235	0.044553	-0.03568
-26	3	0.105692	0.093755	-0.01194
-25	3	0	0	0
-31	3	0.023165	0.021919	-0.00125
50	4	0.008553	0	-0.00855
-28	3	0.055425	0.028714	-0.02671
21(+33)	3	0.005733	0.002666	-0.00307
-53	4	0.046961	0.041113	-0.00585
-22	3	0.008098	0.005633	-0.00247
36	3	0.013222	0.005432	-0.00779
-45	4	0.02208	0.01186	-0.01022
-46	4	0.01068	0.009813	-0.00087
73	4	0.006627	0	-0.00663
-49	4	0.112651	0.088599	-0.02405
-48	4	0.010285	0.00396	-0.00633
35	3	0.009114	0.008654	-0.00046
104	5	0.003601	0	-0.0036
-44	4	0.06129	0.038133	-0.02316
(37+42)+5(	3-4	0.05009	0.033391	-0.0167
(41+71)mir	4	0.013015	0	-0.01302
-40	4	0.014504	0.009284	-0.00522
67 + (10	**4-5**	0.003601	0	-0.0036
-63	4	0.010605	0	-0.01061
(74) + 94	4-5	0.017518	0.00916	-0.00836
(70 +76)	4	0.029873	0.010887	-0.01899
-66	4	0.068412	0.046941	-0.02147
93 + (95)	5	0.073301	0.048504	-0.0248
-91	5	0.066023	0.033161	-0.03286
(56+60)+	**4**	0.032378	0.01334	-0.01904
-101	5	0.052544	0.029619	-0.02293
99	5	0.028073	0.028475	0.000402
-97	5	0.010013	0.008197	-0.00182
145	**6**	0.005727	0	-0.00573
120	5	0	0	0
(110+77	**4-5**	0	0	0
(82+151)	5-6	0.053588	0.032081	-0.02151
(135+144+	5-6	0.023693	0.013664	-0.01003
139 +140 (	5-6	0.018937	0.014245	-0.00469
133	6	0.023561	0	-0.02356
(114 + 1	5-6	0.030074	0.032201	0.002127
-146	6	0.044774	0.020702	-0.02407
153	7	0.051023	0.048168	-0.00286
(132+105	5-6	0.018537	0.009615	-0.00892
-141	6	0.027734	0.016297	-0.01144
(137+17	**6-7**	0.070885	0.053135	-0.01775
(163+138)	6	0.012688	0.006469	-0.00622
-158	6	0.003087	0.003185	9.84E-05
160	6	0.003588	0	-0.00359
126 + (1	5-6-7	0.034453	0.028434	-0.00602
159	6	0	0	0
(186+182)	7	0.029053	0.017406	-0.01165
-183	7	0.02087	0.008205	-0.01266
-128	6	0.006559	0	-0.00656
-185	7	0.005377	0.001437	-0.00394
-174	7	0.029929	0.026323	-0.00361
181	7	0.006077	0.002115	-0.00396
-177	7	0.027911	0.020226	-0.00769
(157+20(	6-7-8	0.000337	0.000768	0.000432
-180	7	0.028757	0.016828	-0.01193
-193	7	0.005841	0.002995	-0.00285
-191	7	0.008085	0.008513	0.000429
-199	8	0.005008	0.002863	-0.00215
(170+190)	7	0.015068	0.007278	-0.00779
-201	8	0.033945	0.023439	-0.01051
(203+196)	8	0.01441	0.008947	-0.00546
-207	9	0.00094	0	-0.00094
-194	8	0.008575	0.004683	-0.00389
		2.510202	1.613536	-0.89667

4

Plot 0-7.5 cm

PCB	Homolog	day0	day 409 c	change
-3	1	0	0	0
(4+10)	2	0.046457	0.059869	0.013412
(7+9)	2	0.020636	0.015352	-0.00528
-6	2	0.069393	0.020032	-0.04936
(8+5)	2	0.149356	0.033871	-0.11549
14	2	0.037473	0.003562	-0.03391
-19	3	0.166303	0.073815	-0.09249
11	2	0.037178	0.110018	0.07284
(12+13)	2	0.010938	0	-0.01094
-18	3	0.081899	0.008191	-0.07371
15 (+17)	4-3	0.049089	0	-0.04909
(24+27)	3	0.013415	0.005489	-0.00793
(16+32)	3	0.078074	0.012156	-0.06592
-26	3	0.046718	0.044169	-0.00255
-25	3	0	0.013808	0.013808
-31	3	0.007512	0.002097	-0.00541
50	4	0.016774	0.00161	-0.01516
-28	3	0.054409	0.002113	-0.0523
21(+33)	3	0.007024	0.001601	-0.00542
-53	4	0.014364	0.002102	-0.01226
-22	3	0.012867	0.004592	-0.00827
36	3	0.019373	0.004736	-0.01464
-45	4	0.029149	0.006205	-0.02294
-46	4	0.030898	0.006838	-0.02406
73	4	0.017046	0	-0.01705
-49	4	0.096269	0.19824	0.101971
-48	4	0.018501	0.001675	-0.01683
35	3	0.002956	0.026984	0.024028
104	5	0.007027	0	-0.00703
-44	4	0.070215	0.00694	-0.06327
(37+42)+5(	3-4	0.0387	0.009704	-0.029
(41+71)mir	4	0.024802	0.002411	-0.02239
-40	4	0.020936	0.00146	-0.01948
67 + (10	**4-5**	0.005133	0.002835	-0.0023
-63	4	0.027353	0.004544	-0.02281
(74) + 94	4-5	0.02042	0.001868	-0.01855
(70 +76)	4	0.022696	0.007386	-0.01531
-66	4	0.057427	0.00392	-0.05351
93 + (95)	5	0.059224	0.018771	-0.04045
-91	5	0.032969	0.006583	-0.02639
(56+60)+	**4**	0.02729	0.00744	-0.01985
-101	5	0.028979	0.010855	-0.01812
99	5	0.009103	0.009318	0.000215
-97	5	0.010044	0.002093	-0.00795
145	**6**	0	0.007161	0.007161
120	5	0	0	0
(110+77	**4-5**	0	0.032959	0.032959
(82+151)	5-6	0.031267	0.027546	-0.00372
(135+144+	5-6	0.014005	0.01771	0.003705
139 +140 (	5-6	0.006972	0.013806	0.006834
133	6	0.116578	0	-0.11658
(114 + 1	5-6	0.032498	0.007712	-0.02479
-146	6	0.029161	0.021876	-0.00728
153	7	0.040509	0.055809	0.0153
(132+105	5-6	0.009203	0.005252	-0.00395
-141	6	0.031818	0.018031	-0.01379
(137+17	**6-7**	0.048968	0.004839	-0.04413
(163+138)	6	0.019654	0.00809	-0.01156
-158	6	0.001001	0.007151	0.00615
160	6	0.015966	0.000289	-0.01568
126 + (1	5-6-7	0.023434	0.005436	-0.018
159	6	0	0.005594	0.005594
(186+182)	7	0.026613	0.002721	-0.02389
-183	7	0.027917	0.020077	-0.00784
-128	6	0.018329	0	-0.01833
-185	7	0.010154	0.010111	-4.2E-05
-174	7	0.010192	0.019654	0.009462
181	7	0.007267	0.000549	-0.00672
-177	7	0.028197	0.015346	-0.01285
(157+20(	6-7-8	0.000858	0.000807	-5.1E-05
-180	7	0.024804	0.010315	-0.01449
-193	7	0.023862	0.009168	-0.01469
-191	7	0.002622	0.011429	0.008807
-199	8	0.016961	0.004532	-0.01243
(170+190)	7	0.018896	0.013908	-0.00499
-201	8	0.039597	0.017962	-0.02163
(203+196)	8	0.020056	0.009856	-0.0102
-207	9	0.011259	0.000514	-0.01075
-194	8	0.010662	0.003506	-0.00716
		2.549412	1.23407	-1.31534

Table 20. Effect of treatments on coplanar PCB 114, 156 and 157 levels (µg kg^{-1}) in sediment (0-7.5 cm)

0-0.75 cm Sediment Core Coplanar Congeners												
Top Core	1 Day 0	1 Day140	1 Day409	2 Day 0	2 Day140	2 Day409	3 Day 0	3 Day140	3 Day409	4 Day 0	4 Day140	4 Day409
1	0.0665	0.0693	0.0371	0.1869	0.0832	0.0629	0.0317	0.0444	0.0481	0.1041	0.0453	0.0256
2	0.0416	0.0659	0.0372	0.0661	0.0604	0.0725	0.0053	0.0586	0.0337	0.0344	0.0099	0.0191
3	0.0085	0.0800	0.0425	0.0711	0.0640	0.0942	0.0255	0.0857	0.0530	0.0616	0.0461	0.0174
4	0.0854	0.0526	0.0364	0.1991	0.0966	0.0532	0.0759	0.0410	0.0626	0.1079	0.0435	0.0032
5	0.1292	0.0861	0.1088	0.0058	0.0649	0.0906	0.0901	0.0305	0.0348	0.0144	0.0029	0.0004
Total												
CALCULATIONS												
	1 Day 0	1 Day140	1 Day409	2 Day 0	2 Day140	2 Day409	3 Day 0	3 Day140	3 Day409	4 Day 0	4 Day140	4 Day409
Count	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Mean	0.07	0.07	0.05	0.11	0.07	0.07	0.05	0.05	0.05	0.06	0.03	0.01
SD	0.05	0.01	0.03	0.08	0.02	0.02	0.04	0.02	0.01	0.04	0.02	0.01
Max	0.13	0.09	0.11	0.20	0.10	0.09	0.09	0.09	0.06	0.11	0.05	0.03
Q3	0.09	0.08	0.04	0.19	0.08	0.09	0.08	0.06	0.05	0.10	0.05	0.02
Median	0.07	0.07	0.04	0.07	0.06	0.07	0.03	0.04	0.05	0.06	0.04	0.02
Q1	0.08	0.07	0.04	0.13	0.07	0.08	0.05	0.05	0.05	0.08	0.04	0.02
Min	0.01	0.05	0.04	0.01	0.06	0.05	0.01	0.03	0.03	0.01	0.00	0.00

Figure 50. Effect of treatments on levels of coplanar PCB 114, 156 and 157 in toxic equivalents in sediment (0-7.5 cm).

0-0.75 cm Sediment Core Coplanar TEQ												
Top Core	1 Day 0	1 Day140	1 Day409	2 Day 0	2 Day140	2 Day409	3 Day 0	3 Day140	3 Day409	4 Day 0	4 Day140	4 Day409
1	1.9954418	2.0777091	1.1125597	5.6062683	2.4958766	1.8873129	0.9502711	1.3334624	1.4425446	3.1221574	1.3596825	0.7692919
2	1.2477474	1.9756999	1.1166333	1.9816877	1.8106125	2.1742665	0.1580383	1.7565960	1.0123876	1.0312800	0.2964269	0.5734504
3	0.2549635	2.4007777	1.2751031	2.1338774	1.9213703	2.8258443	0.7647972	2.5696250	1.5893590	1.8492357	1.3821123	0.5229613
4	2.5612595	1.5772675	1.0910803	5.9739045	2.8973115	1.5964952	2.2757572	1.2312943	1.8791441	3.2367021	1.3060008	0.0958249
5	3.8759862	2.5820629	3.2625849	0.1729865	1.9484525	2.7169730	2.7039868	0.9143005	1.0445462	0.4331158	0.0878206	0.0112145
Total												
CALCULATIONS Top Core												
	1 Day 0	1 Day140	1 Day409	2 Day 0	2 Day140	2 Day409	3 Day 0	3 Day140	3 Day409	4 Day 0	4 Day140	4 Day409
Count	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Mean	1.99	2.12	1.57	3.17	2.21	2.24	1.37	1.56	1.39	1.93	0.89	0.39
SD	1.36	0.39	0.95	2.51	0.47	0.53	1.07	0.64	0.37	1.24	0.64	0.33
Max	3.88	2.58	3.26	5.97	2.90	2.83	2.70	2.57	1.88	3.24	1.38	0.77
Q3	2.56	2.40	1.28	5.61	2.50	2.72	2.28	1.76	1.59	3.12	1.36	0.57
Median	2.00	2.08	1.12	2.13	1.95	2.17	0.95	1.33	1.44	1.85	1.31	0.52
Q1	1.62	2.03	1.11	2.06	1.93	2.03	0.98	1.28	1.24	1.44	1.15	0.55
Min	0.25	1.58	1.09	0.17	1.81	1.60	0.16	0.91	1.01	0.43	0.09	0.01

Figure 52. Freely dissolved total PCB concentrations in the overlying water

Day 0	1-1	1-2	1-3			2-1	2-2	2-3		
Homolog	ng/L			AVG	STDEV	ng/L			AVG	STDEV
1	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0
2	4.69679	1.82242	2.73726	4.37613	1.00796	1.61344	3.61237	4.10554	4.82304	0.94285
3	1.01594	0.36797	0.30474	0.74304	0.42561	0.17758	0.67239	0.84163	0.92575	0.38132
4	0.87369	0.21596	0.58524	0.66963	0.11955	0.31417	0.60170	0.42603	0.95506	0.52166
5	0.18570	0.03855	0.08335	0.40918	0.42512	0.24123	0.11530	0.08064	0.79591	1.09359
6	0.04698	0.01896	0.03432	0.06155	0.01181	0.02539	0.02927	0.03860	0.14314	0.08623
7	0.02415	0.01068	0.01737	0.03653	0.00368	0.01338	0.01337	0.02134	0.44239	0.27682
8	0.00001	0.00021	0.00000	0.00066	6.09E-06	0.00022	0.00059	0.00034	0.04418	0.03392
9	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00057	0.00000	0.01549	0.02683
10	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0
total	6.84328	2.47475	3.76230	6.29673	0.87340	2.38541	5.04557	5.51413	8.14497	2.69917

Day 0	3-1	3-2	3-3			4-1	4-2	4-3		
Homolog	ng/L			AVG	STDEV				AVG	STDEV
1	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0
2	7.35514	3.95011	3.33487	5.06254	1.31418	4.27863	5.50833	5.28233	3.42690	1.19202
3	0.73754	0.46586	0.81600	0.79341	0.39727	0.77156	0.64144	0.51634	0.62223	0.43135
4	1.43579	0.79596	0.67249	0.97718	0.41240	0.54210	1.08095	0.93111	0.69029	0.14737
5	0.21047	0.53683	0.16143	0.57342	0.75865	0.08698	0.16768	0.80575	0.13472	0.06013
6	0.07071	0.06521	0.03737	0.08883	0.04511	0.04394	0.06360	0.07309	0.04402	0.00613
7	0.03438	0.03323	0.01769	0.05806	0.03004	0.02114	0.02888	0.03953	0.02952	0.00484
8	0.00000	0.00115	0.00032	0.00141	0.00071	0.00034	0.00037	0.00061	0.00035	0.00025
9	0.00000	0.00049	0.00030	0.00037	0.00032	0.00003	0.00035	0.00000	0.00028	0.00017
10	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00003	0.00000	0.00001	8.13E-06
total	9.84403	5.84885	5.04047	7.55521	2.51288	5.74472	7.49163	7.64876	4.94831	1.84144

Day 140	1-1	1-2	1-3			2-1	2-2	2-3		
Homolog	ng/L			AVG	STDEV	ng/L			AVG	STDEV
1	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0
2	3.66411	5.04340	5.08982	4.59911	1.008131	1.61344	3.47290	6.43682	4.95486	2.095806
3	0.44217	0.71496	1.04433	0.73382	0.425793	0.17758	0.60224	0.60394	0.60309	0.001202
4	0.75367	1.44479	0.58501	0.92782	0.11926	0.31417	0.62600	1.29542	0.96071	0.473351
5	0.70341	0.21723	0.10820	0.34295	0.420875	0.24123	0.12127	0.19615	0.15871	0.052952
6	0.06932	0.08441	0.05272	0.06882	0.011744	0.02539	0.03029	0.07167	0.05098	0.029261
7	0.03838	0.03778	0.03329	0.03648	0.003599	0.01338	0.01525	0.03204	0.02365	0.011872
8	0.00064	0.00000	0.00064	0.00043	6.05E-06	0.00022	0.00068	0.00000	0.00034	0.000482
9	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00065	0.00000	0.00032	0.00046
10	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0
total	5.67169	7.54257	6.91401	6.70942	0.878451	2.38541	4.86928	8.63604	6.75266	2.663502

Day 140	3-1	3-2	3-3			4-1	4-2	4-3		
Homolog	ng/L			AVG	STDEV	ng/L			AVG	STDEV
1	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0
2	7.35514	5.18285	3.13971	4.16128	1.44472	3.79812	5.94244	6.05693	5.26583	1.27236
3	0.73754	0.53419	0.74560	0.63989	0.14948	0.85507	1.09794	0.68355	0.87885	0.20821
4	1.43579	1.00999	0.70577	0.85788	0.21511	0.49371	0.89581	1.23289	0.87414	0.37006
5	0.21047	0.79640	0.15247	0.47444	0.45532	0.09634	0.20509	1.08128	0.46090	0.54000
6	0.07071	0.09549	0.03700	0.06624	0.04135	0.05775	0.06132	0.14107	0.08671	0.04711
7	0.03438	0.04473	0.01685	0.03079	0.01971	0.04754	0.05574	0.11471	0.07266	0.03664
8	0.00000	0.00176	0.00035	0.00106	0.00099	0.00170	0.00274	0.00258	0.00234	0.00056
9	0.00000	0.00080	0.00032	0.00056	0.00033	0.00038	0.00170	0.00000	0.00069	0.00089
10	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00003	0.00000	0.00001	1.65E-05
total	9.84403	7.66621	4.79807	6.23214	2.02807	5.35061	8.26280	9.31301	7.64214	

Day 409	1-1	1-2	1-3			2-1	2-2	2-3		
Homolog	ng/L			AVG	STDEV	ng/L			AVG	STDEV
1	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0
2	3.66340	5.04340	5.08887	4.37613	1.00796	4.92921	3.83160	5.70833	4.82304	0.94285
3	0.44209	0.71496	1.04400	0.74304	0.42561	1.36577	0.71978	0.69170	0.92575	0.38132
4	0.75417	1.44479	0.58510	0.66963	0.11955	1.20125	0.35586	1.30808	0.95506	0.52166
5	0.70979	0.21723	0.10857	0.40918	0.42512	0.25413	0.07897	2.05463	0.79591	1.09359
6	0.06991	0.08441	0.05320	0.06155	0.01181	0.10562	0.08203	0.24178	0.14314	0.08623
7	0.03914	0.03778	0.03392	0.03653	0.00368	0.39097	0.19488	0.74131	0.44239	0.27681
8	0.00066	0.00000	0.00066	0.00066	6.09E-06	0.01323	0.08045	0.03887	0.04418	0.03392
9	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.04648	0.00000	0.01549	0.02683
10	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0
total	5.67915	7.54257	6.91432	6.29673	0.87340	8.26018	5.39005	10.7847	8.14497	2.69916

Day 409	3-1	3-2	3-3			4-1	4-2	4-3		
Homolog	ng/L			AVG	STDEV	ng/L			AVG	STDEV
1	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0
2	6.24645	3.64849	5.29268	5.06254	1.31418	4.26979	2.58401	6.05693	3.42690	1.19202
3	0.71251	0.44281	1.22491	0.79341	0.39727	0.92724	0.31722	0.68355	0.62223	0.43135
4	1.45255	0.76389	0.71509	0.97718	0.41240	0.79450	0.58608	1.23289	0.69029	0.14737
5	1.44939	0.12668	0.14420	0.57342	0.75865	0.17724	0.09219	1.08128	0.13472	0.06013
6	0.13944	0.05283	0.07420	0.08883	0.04511	0.04836	0.03968	0.14107	0.04402	0.00613
7	0.09122	0.03265	0.05029	0.05806	0.03004	0.03295	0.02609	0.11471	0.02952	0.00484
8	0.00185	0.00059	0.00180	0.00141	0.00071	0.00017	0.00053	0.00258		
9	0.00000	0.00049	0.00061	0.00037	0.00032	0.00016	0.00040	0.00000		
10	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00001	0.00000		
total	10.0934	5.06843	7.50379	7.55521	2.51288	6.25041	3.64621	9.31301		

Table 21 and Figure 53a. Freely dissolved concentration of PCBs in sediment porewater 0-7.5 cm sediments

Day 0	1-1	1-2	1-3			2-1	2	2-3		
Homolog	ng/L			AVG	STDEV	ng/L			AVG	STDEV
1	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0
2	4.53859	167.5787	115.0264	95.71445	83.21793	122.5698	17.03199	34.33780	57.97989	56.601886
3	1.02000	37.66157	25.85090	21.51082	18.70236	152.0434	18.95107	82.57774	84.52410	66.56755
4	0.33954	12.53675	8.60522	7.16050	6.22562	26.92752	4.20670	19.78718	16.97380	11.61874
5	0.07821	2.88789	1.98224	1.64945	1.43409	4.86957	1.55148	3.68016	3.36707	1.68105
6	0.06177	2.28070	1.56547	1.30264	1.13257	1.16677	0.88871	1.50028	1.18525	0.306206
7	0.01060	0.08915	0.05532	0.05169	0.03939	0.38347	0.29879	0.43475	0.37234	0.068664
8	0.00826	0.02473	0.01096	0.01465	0.00883	0.01031	0.01413	0.01675	0.01373	0.00323
9	0.00000	0.00000	0.00003	0.00001	1.49E-05	0.01050	0.00128	0.00000	0.00393	0.00573
10	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0
total	6.05697	223.0595	153.0969	127.4042	110.7591	307.9815	42.94414	142.3346	164.4201	

Day 0	3-1	3-2	3-3			4-1	4-1	4-3		
Homolog	ng/L			AVG	STDEV	ng/L			AVG	STDEV
1	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0
2	77.94179	105.0870	45.18361	76.07081	29.9955	10.01190	29.44938	64.64728	34.70285	27.69396
3	21.99392	31.10929	109.1163	54.07320	47.88618	139.8440	29.46321	72.40947	80.57225	55.64132
4	4.88375	7.52495	39.19067	17.19979	19.09039	25.23231	17.31016	46.85367	29.79872	15.29195
5	3.28241	8.03777	3.01823	4.77947	2.82486	18.98619	0.89125	3.33973	7.73906	9.81693
6	1.42738	3.35244	1.49627	2.09203	1.09208	6.45092	0.36459	1.73706	2.85086	3.19237
7	0.54884	0.94394	0.40334	0.63204	0.27973	1.21703	0.10272	0.46402	0.59459	0.56851
8	0.16995	0.03099	0.03314	0.07802	0.07961	0.07203	0.00779	0.07314	0.05099	0.03741
9	0.00000	0.00000	0.00938	0.00313	0.00541	0.00000	0.00219	0.04492	0.01570	0.02532
10	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	0.00001	0.00000	3.89E-06
total	110.2480	156.0864	198.4510	154.9284		201.81445	77.59130	189.56929	156.3250	

Day 140	1-1	1-2	1-3			2-1	2-2	2-3		
Homolog	ng/L			AVG	STDEV	ng/L			AVG	STDEV
1	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0
2	1.52408	28.80150	199.6979	76.67452	107.4109	202.9231	101.54192	38.05684	114.1739	83.15587
3	0.42583	69.30108	44.88004	38.20232	34.91982	34.34922	22.74521	89.66124	48.91856	35.75804
4	0.29456	16.60443	14.93962	10.61287	8.974608	12.24073	4.80054	5.69235	7.57787	4.062695
5	0.10766	3.08292	3.44140	2.21066	1.830052	0.91354	0.93466	4.02477	1.95766	1.790204
6	0.05039	1.27057	2.71783	1.34626	1.335331	0.43177	0.22896	1.62888	0.76320	0.756528
7	0.04149	0.36837	0.09605	0.16863	0.175112	0.10288	0.06600	0.49901	0.22263	0.240064
8	0.01822	0.01650	0.01903	0.01791	0.001293	0.00952	0.00198	0.01821	0.00990	0.008125
9	0.00002	0.00054	0.00005	0.00020	0.000294	0.00268	0.00201	0.00000	0.00156	0.001396
10	0.00000	0.00024	0.00000	0.00008	0.000137	0.00000	0.00000	0.00000	0.00000	0
total	2.46225	119.4461	265.7919	129.2334	131.9374	250.9734	130.32128	139.58131	173.6253	

Day 140	3-1	3-2	3-3			4-1	4-2	4-3		
Homolog	ng/L			AVG	STDEV	ng/L			AVG	STDEV
1	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0
2	49.93147	0.00000	115.6608	55.19744	58.00997	51.18702	33.89574	65.24589	50.10955	15.70282
3	56.43343	8.27037	35.21765	33.30715	24.1383	67.74299	36.46314	15.70414	39.97009	26.19608
4	11.88430	9.39670	6.92656	9.40252	2.478876	5.62441	1.93243	2.14744	3.23476	2.072287
5	4.49824	4.76213	2.64248	3.96761	1.155164	5.03683	0.28682	1.39674	2.24013	2.48478
6	2.44932	2.56559	1.09015	2.03502	0.820341	2.17738	0.16728	0.62770	0.99079	1.053092
7	0.71416	1.07600	0.37833	0.72283	0.348919	0.57466	0.02895	0.22610	0.27657	0.276334
8	0.03866	0.08779	0.04695	0.05780	0.0263	0.01947	0.00129	0.00896	0.00991	0.009125
9	0.00343	0.03716	0.00061	0.01373	0.020335	0.00000	0.00130	0.00028	0.00053	0.000685
10	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0
total	125.9530	26.19574	161.9635	104.7041		132.3627	72.77696	85.35726	96.83233	

Day 409	1-1	1-2	1-3			2-1	2-2	2-3		
Homolog	ng/L			AVG	STDEV	ng/L			AVG	STDEV
1	0.00000	nd	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0
2	16.50214	nd	51.21804	33.86009	24.54785	58.80008	114.59223	35.37952	69.59061	40.69386
3	10.03329	nd	32.60913	21.32121	15.96353	9.79692	53.83903	16.71423	26.78339	23.68476
4	6.06504	nd	19.31217	12.68860	9.367136	8.56458	20.97614	6.66844	12.06972	7.771235
5	0.81659	nd	2.49224	1.65442	1.184864	1.42157	4.42801	1.65741	2.50233	1.671851
6	0.00988	nd	0.03100	0.02044	0.014934	0.31517	1.43447	0.70808	0.81924	0.567871
7	0.00244	nd	0.00689	0.00466	0.003146	0.25918	0.75388	0.37544	0.46283	0.25867
8	0.00000	nd	0.00000	0.00000	0	0.09316	0.03421	0.01689	0.04809	0.039982
9	0.00000	nd	0.00000	0.00000	0	0.00019	0.00114	0.00056	0.00063	0.000477
10	0.00000	nd	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0
total	33.42938	nd	105.66946	69.54942	51.08145	79.25084	196.05910	61.52056	112.27683	73.09714

D 409	3-1	3-2	3-3			4-1	4-2	4-3		
Homolog				AVG	STDEV				AVG	STDEV
1	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	nd	0.00000	0
2	12.76739	1.04714	67.37442	27.06298	35.39915	5.21834	48.46144	nd	17.89326	26.60109
3	7.30060	1.12101	21.16490	9.86217	10.26453	1.52626	11.94648	nd	4.49091	6.501648
4	4.16320	0.80621	4.32905	3.09949	1.987765	0.85891	4.58246	nd	1.81379	2.435894
5	0.77363	0.16197	1.70245	0.87935	0.775663	0.29887	1.30849	nd	0.53579	0.685663
6	0.46259	0.10808	0.71828	0.42965	0.306429	0.11351	0.44600	nd	0.18650	0.231786
7	0.02112	0.05159	0.27154	0.11475	0.136637	0.10584	0.22003	nd	0.10862	0.110041
8	0.00017	0.01819	0.03583	0.01806	0.017828	0.05437	0.00992	nd	0.02143	0.028955
9	0.00000	0.00000	0.00050	0.00017	0.000289	0.00006	0.00076	nd	0.00027	0.000425
10	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	nd	0.00000	0
total	25.48869	3.31419	95.59697	41.46662	48.17155	8.17617	66.97558	nd	25.05058	

nd = not determined due to loss of sampler

Figure 53b. Freely dissolved concentration of total PCBs in sediment porewater in the 0-7.5 cm surface sediments for tri- to deca-chlorobiphenyls

	1-1	1-2	1-3		2-1	2-2	2-3		3-1	3-2	3-3		~~4-1~~	~~4-2~~	4-3
PCB															
Tri	21.5	38.2	21.3		84.5	48.9	26.8		54.1	33.3	9.9		80.6	40.0	6.7
Tetra	7.2	10.6	12.7		17.0	7.6	12.1		17.2	9.4	3.1		29.8	3.2	2.7
Penta	1.6	2.2	1.7		3.4	2.0	2.5		4.8	4.0	0.9		7.7	2.2	0.8
Hexa	1.3	1.3	0.0		1.2	0.8	0.8		2.1	2.0	0.4		2.9	1.0	0.3
Hepta	0.1	0.2	0.0		0.4	0.2	0.5		0.6	0.7	0.1		0.6	0.3	0.2
Octa	0.0	0.0	0.0		0.0	0.0	0.0		0.1	0.1	0.0		0.1	0.0	0.0
TOTAL	31.7	52.6	35.7		106.4	59.4	42.7		78.9	49.5	14.4		121.6	46.7	10.7
	STDEV														
	DAY 0	**DAY 140**	**DAY 409**		**DAY 0**	**DAY 140**	**DAY 409**		**DAY 0**	**DAY 140**	**DAY 409**		**DAY 0**	**DAY 140**	**DAY 409**
Tri	18.7	34.9	16.0		66.6	35.8	23.7		47.9	24.1	10.3		55.6	26.2	7.4
Tetra	6.2	9.0	9.4		11.6	4.1	7.8		19.1	2.5	2.0		15.3	2.1	2.6
Penta	1.4	1.8	1.2		1.7	1.8	1.7		2.8	1.2	0.8		9.8	2.5	0.7
Hexa	1.1	1.3	0.0		0.3	0.8	0.6		1.1	0.8	0.3		3.2	1.1	0.2
Hepta	0.0	0.2	0.0		0.1	0.2	0.3		0.3	0.3	0.1		0.6	0.3	0.1
Octa	0.0	0.0	0.0		0.0	0.0	0.0		0.1	0.0	0.0		0.0	0.0	0.0
TOTAL	27.5	46.4	26.5		56.4	37.7	33.7		65.1	25.1	13.1		71.9	31.3	11.0

Figure 54. Freely dissolved concentration of total PCBs in sediment porewater in the 7.5-15 cm depth below surface.

Day 0	1-1	1-2	1-3			2-1	2-2	2-3		
Homolog	ng/L			AVG	STDEV	ng/L			AVG	STDEV
1	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0
2	64.62953	103.70853	222.0248	130.1209	81.95449	15.23282	26.71393	25.12138	22.35604	6.22007
3	14.52481	36.39474	69.18956	40.03637	27.51372	57.47875	43.20225	107.6807	69.45391	33.86623
4	4.83501	9.46578	21.51751	11.93943	8.611949	15.62240	15.84621	38.16503	23.21121	12.95087
5	1.11376	0.55245	2.57926	1.41516	1.046481	4.32037	0.61602	20.59959	8.51199	10.63076
6	0.87959	0.01864	0.90494	0.60106	0.504547	1.29035	1.25983	0.22462	0.92493	0.606683
7	0.03109	0.00110	0.14485	0.05901	0.075836	0.27045	0.24350	0.05408	0.18934	0.117913
8	0.00595	0.00000	0.00000	0.00198	0.003436	0.00270	0.00000	0.00000	0.00090	0.001558
9	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0
10	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0
total	86.01973	150.1412	316.3610	184.1740	118.8821	94.21783	87.88173	191.8454	124.64833	58.28056

Day 0	3-1	3-2	3-3			4-1	4-2	4-3		
Homolog	ng/L			AVG	STDEV	ng/L			AVG	STDEV
1	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0
2	5.25979	0.00000	31.70353	12.32111	16.99044	43.32140	41.05638	38.05074	40.80951	2.64398
3	27.76510	82.69940	130.7885	80.41767	51.54958	147.4776	79.74585	53.97505	93.73286	48.29505
4	39.09549	54.23003	44.19236	45.83929	7.70051	38.85762	22.85010	48.38427	36.69733	12.90343
5	5.00416	8.38796	32.39754	15.26322	14.93489	9.84679	3.42205	3.82289	5.69725	3.59919
6	0.84080	5.58870	10.59331	5.67427	4.87681	2.74960	1.56596	1.84714	2.05423	0.61839
7	0.42487	0.30295	0.61836	0.44873	0.15905	0.61916	0.29213	0.41961	0.44363	0.16483
8	0.00000	0.00000	0.00000	0.00000	5.28E-07	0.00592	0.00000	0.00000	0.00197	0.00341
9	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0
10	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0
total	78.39021	151.2090	250.2936	159.9642	86.28548	242.8781	148.9324	146.4997	179.4367	54.95531

Day 140	1-1	1-2	1-3			2-1	2-2	2-3		
Homolog	ng/L			AVG	STDEV	ng/L			AVG	STDEV
1	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0
2	137.7814	232.8333	11.31807	127.3109	111.1282	122.1163	40.74021	115.0642	92.64029	45.08489
3	24.12869	16.28938	12.84564	17.7545	5.782465	91.64671	24.68635	77.72185	64.68497	35.3326
4	7.75030	3.64343	2.69643	4.69672	2.686535	41.08017	14.87811	45.71587	33.89138	16.62832
5	0.41769	2.33794	1.14722	1.30095	0.969312	8.82491	13.27256	4.90884	9.00210	4.184673
6	0.28325	1.20241	0.68964	0.72510	0.460602	5.03533	10.93339	0.05353	5.34075	5.446361
7	0.06621	0.32097	0.20406	0.19708	0.127521	0.19904	0.63772	0.01289	0.28322	0.32081
8	0.00236	0.01740	0.01089	0.01022	0.00754	0.00199	0.00000	0.00000	0.00066	0.001147
9	0.00240	0.00056	0.00097	0.00131	0.000967	0.00000	0.00000	0.00000	0.00000	0
10	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0
total	170.4323	256.6453	28.91292	151.9968	114.9801	268.9045	105.1483	243.4772	205.8433	88.12636

Day 140	3-1	3-2	3-3			4-1	4-2	4-3		
Homolog	ng/L			AVG	STDEV	ng/L			AVG	STDEV
1	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0
2	133.2178	46.17136	57.91865	79.10263	47.23182	35.67862	71.48010	58.10162	55.08678	18.09015
3	99.97823	55.93224	38.71905	64.87651	31.59385	27.16248	86.59140	71.46953	61.74114	30.88576
4	44.81473	16.47402	22.86617	28.05164	14.86492	11.95439	25.50422	20.57643	19.34502	6.858336
5	9.62718	2.54810	4.17604	5.45044	3.707614	2.22575	3.94483	3.53634	3.23564	0.898126
6	5.49309	1.69774	1.36548	2.85210	2.293188	1.24724	2.62835	2.69611	2.19057	0.817648
7	0.21713	0.09203	0.07971	0.12962	0.076036	0.05346	0.14248	0.17845	0.12479	0.064344
8	0.00217	0.00003	0.00000	0.00073	0.001243	0.00051	0.00004	0.00004	0.00020	0.000269
9	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0
10	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0
total	293.3504	122.9155	125.1251	180.4636	97.76902	78.32245	190.2914	156.5585	141.72413	57.4396

Day 409	1-1	1-2	1-3			2-1	2-2	2-3		
Homolog	ng/L			AVG	STDEV	ng/L			AVG	STDEV
1	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0
2	97.21326	232.83331	85.99137	91.60232	81.73257	127.10710	41.80667	60.81473	82.97625	49.67972
3	6.46231	16.28938	70.27147	38.36689	34.35658	28.60226	26.28654	42.07922	70.19033	34.00583
4	1.48169	3.64343	29.95059	15.71614	15.84939	9.93279	16.13762	25.09565	30.73954	16.22107
5	1.03447	2.33794	7.17442	4.10444	3.234953	2.52948	15.70090	4.91926	6.45313	4.393259
6	0.57782	1.20241	3.92033	2.24908	1.777151	2.02657	13.04205	1.62540	3.39344	2.721656
7	0.18635	0.32097	0.21092	0.19863	0.071692	0.09373	0.94098	0.10979	0.17987	0.105219
8	0.01242	0.01740	0.00353	0.00798	0.007023	0.01836	0.00000	0.00000	0.00136	0.002284
9	0.00049	0.00056	0.00000	0.00024	0.000304	0.00005	0.00000	0.00000	0.00000	0
10	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0
total	106.9688	256.64539	197.52264	152.24572	64.03123	170.31036	113.91476	134.64407	193.93390	104.9733

Day 409	3-1	3-2	3-3			4-1	4-2	4-3		
Homolog	ng/L			AVG	STDEV				AVG	STDEV
1	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0.00000
2	139.87977	48.23426	60.81473	82.97625	49.67972	37.83571	75.45483	58.10162	56.64527	56.64527
3	107.98868	60.50307	42.07922	70.19033	34.00583	29.46708	94.11700	71.46953	61.79204	61.79204
4	49.02864	18.09431	25.09565	30.73954	16.22107	13.10905	28.04270	20.57643	20.57587	20.57587
5	11.40768	3.03244	4.91926	6.45313	4.393259	2.58779	4.60380	3.53634	3.59579	3.59579
6	6.52756	2.02736	1.62540	3.39344	2.721656	1.45351	3.07311	2.69611	2.26331	2.26331
7	0.30086	0.12894	0.10979	0.17987	0.105219	0.06946	0.18632	0.17845	0.12789	0.12789
8	0.00399	0.00007	0.00000	0.00136	0.002284	0.00080	0.00009	0.00004	0.00045	0.00045
9	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0.00000
10	0.00000	0.00000	0.00000	0.00000	0	0.00000	0.00000	0.00000	0.00000	0.00000
total	315.13717	132.02047	134.64407	193.93390	104.9733	84.52341	205.47784	156.55853	145.00063	85.5277

Table 22 and Figure 55. Titer of bioamendments LB400 and DF1 deployed in test plots based on quantitative PCR enumeration of 16S rRNA gene copies.

DF1	Cells
1-1-T D0	0
1-2-T D0	0
1-3-T D0	0
1-4-T D0	0
1-5-T D0	0
2-1-T D0	0
2-2-T D0	0
2-3-T D0	17
2-4-T D0	0
2-5-T D0	0
LB400	Cells
1-1-T D0	0
1-2-T D0	0
1-3-T D0	0
1-4-T D0	0
1-5-T D0	0
2-1-T D0	0
2-2-T D0	30
2-3-T D0	0
2-4-T D0	0
2-5-T D0	0
DF1	Cells
1-1-T D140	0
1-2-T D140	0
1-3-T D140	0
1-4-T D140	0
1-5-T D140	0
2-1-T D140	7.9
2-2-T D140	110
2-3-T D140	2.1
2-4-T D140	0
2-5-T D140	19
LB400	
1-1-T D140	0
1-2-T D140	0
1-3-T D140	38
1-4-T D140	0
1-5-T D140	0
2-1-T D140	9
2-2-T D140	0
2-3-T D140	129
2-4-T D140	333
2-5-T D140	462

DF1	Cells
3-1-T D0	ND
3-2-T D0	ND
3-3-T D0	ND
3-4-T D0	ND
3-5-T D0	ND
4-1-T D0	ND
4-2-T D0	ND
4-3-T D0	ND
4-4-T D0	ND
4-5-T D0	ND
LB400	Cells
3-1-T D0	ND
3-2-T D0	ND
3-3-T D0	ND
3-4-T D0	ND
3-5-T D0	ND
4-1-T D0	ND
4-2-T D0	ND
4-3-T D0	ND
4-4-T D0	ND
4-5-T D0	ND
DF1	Cells
3-1-T D140	40000
3-2-T D140	11000
3-3-T D140	8000
3-4-T D140	100000
3-5-T D140	15000
4-1-T D140	5200
4-2-T D140	14000
4-3-T D140	2100
4-4-T D140	510
4-5-T D140	100
LB400	
3-1-T D140	200
3-2-T D140	70
3-3-T D140	85
3-4-T D140	19110
3-5-T D140	20
4-1-T D140	4085
4-2-T D140	8237
4-3-T D140	3997
4-4-T D140	2023
4-5-T D140	16500

DF1	
1-1-T D409	178
1-2-T D409	103
1-3-T D409	9
1-4-T D409	115
1-5-T D409	0
2-1-T D409	0
2-2-T D409	0
2-3-T D409	0
2-4-T D409	0
2-5-T D409	0
LB400	
1-1-T D409	209
1-2-T D409	0
1-3-T D409	0
1-4-T D409	352
1-5-T D409	258
2-1-T D409	0
2-2-T D409	0
2-3-T D409	0
2-4-T D409	0
2-5-T D409	0

DF1	
3-1-T D409	290
3-2-T D409	10
3-3-T D409	50
3-4-T D409	2600
3-5-T D409	20
4-1-T D409	140
4-2-T D409	11
4-3-T D409	15
4-4-T D409	35
4-5-T D409	950
LB400	
3-1-T D409	690
3-2-T D409	60
3-3-T D409	1
3-4-T D409	1
3-5-T D409	1
4-1-T D409	1
4-2-T D409	1
4-3-T D409	1
4-4-T D409	620
4-5-T D409	10

Figure 56. Bioamendment titer versus black carbon in sample cores from abiotic plot 2 and bioamended plots 3 and 4 140 and 409 days after treatment

Carbon vs cell numbers DF1			% Carbon	Cells
3-1-T D140	499	13.7	2.7	4.0E+04
3-2-T D140	502	0.92	0.2	1.1E+04
3-3-T D140	506	1.8	0.4	8.0E+03
3-4-T D140	501	15.4	3.1	1.0E+05
3-5-T D140	500	8.9	1.8	1.5E+04
4-1-T D140	501	8.5	1.7	5.2E+03
4-2-T D140	502	30.7	6.1	1.4E+04
4-3-T D140	507	10.7	2.1	2.1E+03
4-4-T D140	NA	NA		5.1E+02
4-5-T D140	500	27.9	5.6	1.0E+02
Carbon vs cell numbers LB400				
3-1-T D140	499	13.7	2.7	2.0E+02
3-2-T D140	502	0.92	0.2	7.0E+01
3-3-T D140	506	1.8	0.4	8.5E+01
3-4-T D140	501	15.4	3.1	1.9E+04
3-5-T D140	500	8.9	1.8	2.0E+01
4-1-T D140	501	8.5	1.7	4.1E+03
4-2-T D140	502	30.7	6.1	8.2E+03
4-3-T D140	507	10.7	2.1	4.0E+03
4-4-T D140	NA	NA		2.0E+03
4-5-T D140	500	27.9	5.6	1.7E+04

Carbon vs cell numbers DF1				
3-1-T D409	544	4.2	0.8	2.9E+02
3-2-T D409	553	2.7	0.5	1.0E+01
3-3-T D409	605	0.6	0.1	5.0E+01
3-4-T D409	485	5.4	1.1	2.6E+03
3-5-T D409	487	4.4	0.9	2.0E+01
4-1-T D409	520	4.9	0.9	1.4E+02
4-2-T D409	534	8.4	1.6	1.1E+01
4-3-T D409	502	3.8	0.8	1.5E+01
4-4-T D409	554	85	15.3	3.5E+01
4-5-T D409	511	85	16.6	9.5E+02
Carbon vs cell numbers LB400				
3-1-T D409	544	4.2	0.8	6.9E+02
3-2-T D409	553	2.7	0.5	6.0E+01
3-3-T D409	605	0.6	0.1	1.0E+00
3-4-T D409	485	5.4	1.1	1.0E+00
3-5-T D409	487	4.4	0.9	1.0E+00
4-1-T D409	520	4.9	0.9	1.0E+00
4-2-T D409	534	8.4	1.6	1.0E+00
4-3-T D409	502	3.8	0.8	1.0E+00
4-4-T D409	554	85	15.3	6.2E+02
4-5-T D409	511	85	16.6	1.0E+01